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POSTER PRESENTATION

Bioenergetics

P001

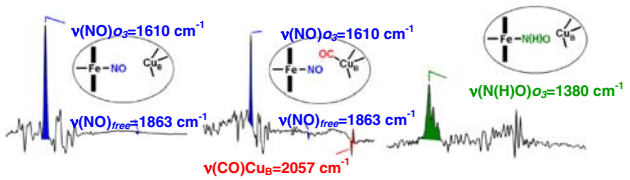
Low temperature photolysis study of NO, NO/CO and HNO complexes in cytochrome bo_3 from *Escherichia coli*

Takahiro Hayashi[1], Myat T. Lin[2], Robert B. Gennis[2], Pierre Moënne-Loccoz[1]

[1]Department of Science and Engineering, School of Medicine, Oregon Health and Science University, 20,000 NW Walker Road, Beaverton, OR 97006, USA.

[2]Department of Biochemistry, University of Illinois, 600 S Mathews Avenue, Urbana, IL 61801, USA. hayashi@ebs.ogi.edu

Bacterial heme–copper terminal oxidases react with NO to form heme–nitrosyl complexes, which, in some of these enzymes, can further react with a second NO to produce N_2O ($2NO + 2e^- + 2H^+ \rightarrow N_2O + H_2O$). It is unknown whether the formation of Cu_B–NO is an obligate step of this catalytic reaction. Here, we present low-temperature photolysis experiments on NO, NO/CO and HNO complexes of reduced cytochrome bo_3, which is a quinol oxidase with NO reductase activity. Upon illumination of bo_3–NO, NO dissociates from heme-o_3 to dock into a proteinaceous pocket and shows ν(NO) at 1,863 cm^{-1}. When the same experiment is carried out in the presence of CO, an [o_3–NO·OC–Cu_B] tertiary complex is formed at the active site. We also examined the reaction of bo_3 with Angeli's salt, a HNO donor, and characterized an o_3–HNO complex. These data suggest that in bo_3, NO is activated at the heme–o_3 and that the role of the Cu_B is limited to promoting the formation of a heme iron-hyponitrite species through electrostatic interaction.



P002

Multi-step electron tunneling in a transmembrane protein

Bert T. Lai, Jay R. Winkler, Harry B. Gray

Beckman Institute, California Institute of Technology, 1200 East California Boulevard, Pasadena, CA 91125, USA. bert@caltech.edu

Multi-step electron tunneling has been demonstrated experimentally using a rhenium-labeled metalloprotein in solution. However, in natural redox systems, such as those involved in photosynthesis and respiration, multi-step electron tunneling must occur across a membrane. The research here involves studying electron tunneling through a transmembrane protein, namely outer membrane protein A (OmpA). A rhenium sensitizer complex will be covalently bound to a cysteine residue folded inside a small unilamellar vesicle. It will be photochemically excited and oxidized. Following electronic excitation and oxidation of the Re(I) sensitizer, electron transfer from a distant Ru(II) donor, which is attached to another cysteine residue positioned outside the vesicle when folded, through intermediate trytophans to the Re(I)* acceptor will be monitored by transient absorption.

P003

Hydrogen bond network around the non-heme iron in photosystem II

Ryouta Takahashi[1], Miwa Sugiura[2], Alain Boussac[3], Takumi Noguchi[1]

[1]Institute of Materials Science, University of Tsukuba, Tsukuba 305-8573, Japan.

[2]Cell-Free Science and Technology Research Center, Ehime University, Matsuyama 790-8577, Japan.

[3]iBiTec-S, URA CNRS 2096, CEA Saclay 91191 Gif sur Yvette, France. s-takahashi@ims.tsukuba.ac.jp

In photosystem II (PSII), a non-heme iron which is located between the primary (Q_A) and secondary (Q_B) quinone acceptors controls the electron transfer from Q_A to Q_B. The recent X-ray structures showed that D1-Y246, D2-Y244 and D1-E244 are located near the bicarbonate molecule coordinating to the non-heme iron. However, the details of their H-bond structures and the roles in the quinone reactions have not yet been clarified. In this study, we have studied the molecular interactions around the non-heme iron by means of Fourier transform infrared (FTIR) spectroscopy. A light-induced Fe^{2+}/Fe^{3+} difference spectrum in the PS II core complexes showed peaks at 1,256/1,232 cm^{-1}, which downshifted by 16 cm^{-1} upon labeling of Tyr side chains with 4-^{13}C-Tyr. These peaks were assigned to the COH vibrations of a Tyr side chain(s), which may be attributed to D1-Y246 and/or D2-Y244. The C=O/COH bands of bicarbonate and the C=O band of a COOH group were also detected. From these data, it is proposed that D1-Y246, D2-Y244, and D1-E244 interact with the bicarbonate to form an H-bond network around the non-heme iron, which can be involved in a proton transfer pathway to Q_B (Fig. 1).

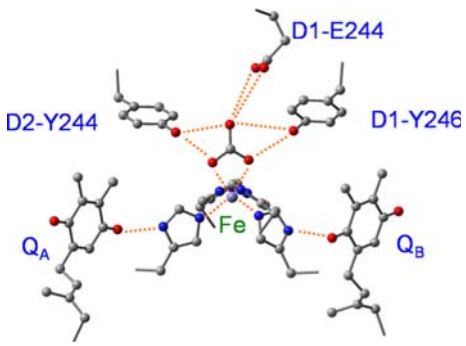


Fig. 1 H-bond network around the non-heme iron

P004

FTIR Detection of the DOD bending vibrations of water molecules involved in the photosynthetic water oxidation

Hiroyuki Suzuki[1], Miwa Sugiura[2], Takumi Noguchi[1]
[1]Institute of Materials Science, University of Tsukuba, Ibaraki 305-8573, Japan.
[2]Cell-Free Science and Technology Research Center, Ehime University, Matsuyama 790-8577, Japan. hisuzuki@ims.tsukuba.ac.jp

Photosynthetic water oxidation takes place at the water oxidizing center (WOC) in photosystem II (PSII). The reaction proceeds through a light-driven cycle of five intermediates called S_n state ($n = 0$–4). During the S-state cycle, two water molecules are oxidized into one O_2 molecule and four protons. To clarify the mechanism of water oxidation, it is of utmost importance to detect water molecules in WOC and monitor their reactions at the molecular level. In previous studies [1, 2], we detected water molecules as OH stretching vibrations using Fourier transform infrared (FTIR) spectroscopy. However, the OH vibrations of strongly H-bonded water have been difficult to detect because of significant band broadening. In this study, we have detected the DOD bending vibrations of heavy water involved in photosynthetic water oxidation [3]. Flash-induced FTIR difference spectra during the S-state cycle were measured with PSII core films of *T. elongatus* moderately deuterated with $D_2^{16}O$ or $D_2^{18}O$. The $D_2^{18}O$-minus-$D_2^{16}O$ double difference spectra at individual S-state transitions ($S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_0$, and $S_0 \rightarrow S_1$) showed six to eight DOD peaks in the 1,150–1,250 cm^{-1} region, indicating that at least two water molecules, not in any deprotonated forms, participate in the reaction at each transition throughout the cycle. The negative bands at $\sim$1,240 cm^{-1} in the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions did not have corresponding counter bands in other transitions. This result suggests that substrate water molecules are inserted into the catalytic site in the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions.

References

1. Noguchi T, Sugiura M (2000) Biochemistry 39:10943-10949
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P005

Influence of D1-H332, a ligand to the Mn_4Ca-cluster, in the water oxidation mechanism of the oxygen-evolving photosystem II enzyme

Miwa Sugiura[1], Yohei Ohno[1], Fabrice Rappaport[2], Hiroyuki Suzuki[3], Takumi Noguchi[3], Hidenori Hayashi[1], Alain Boussac[4]
[1]Cell-Free Science and Technology Research Center, Ehime University, Matsuyama 790-8577, Japan.
[2]Université P. et M. Curie, CNRS UMR 7141, 13 rue P. et M. Curie, 75005 Paris, France.
[3]Institute of Materials Science, University of Tsukuba, Ibaraki 305-8573, JP.
[4]iBiTec-S, CNRS URA 2096, CEA Saclay 91191 Gif/Yvette, France. msugiura@chem.sci.ehime-u.ac.jp

The active site for water oxidation in photosystem II goes through five oxidation states (S_0–S_4). The light-induced O_2 evolution involves a Mn_4Ca-cluster bound to at least seven amino acids of the D1 and CP43 polypeptides. The nature of the interactions of the Mn_4Ca cluster with the surrounding amino acids is a key point to preserve the high driven force required to oxidize water. To study the role of one of these ligands, the D1-H332, we analyzed purified PSII from *T. elongatus* in which this amino acid residue was replaced by either Gln or Ser. The effect of the mutation was investigated by a range of techniques like oxymetry, thermoluminescence measurements, time-resolved UV–visible absorption change, EPR and FTIR spectroscopies. Although the S_3–S_0 transition seemed kinetically unaffected, the O_2 evolution activity was found to be 70–80% of that in the WT* (the WT* is a strain which expresses only one of the three variant copies encoding D1 protein [1]). Thermoluminescence measurements indicated that the thermodynamic properties of the S_3 state were affected, i.e. the redox potential of S_3 was decreased. This decrease could originate from the structural change detected by EPR in the S_3 state of the H332 mutants. All the results will be discussed in the frame of the relationships between structural, kinetics and thermodynamic properties involved in the $S_3Tyr_Z^{\cdot} + 2H_2O$ to $S_0 + O_2$ transition.

Reference

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P006

Potent inhibitors of nitrous oxide reductase from *Achromobacter cycloclastes*

Koyu Fujita, David Dooley
Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717 USA. koyu@montana.edu

Nitrous oxide reductase (N_2OR) catalyzes the 2-electron reduction of N_2O to N_2 and H_2O in the terminal step of denitrification. The crystallographic data show that N_2OR is comprised two identical subunits and has two copper groups, a so-called dinuclear Cu_A and tetranuclear Cu_Z. Recent studies have demonstrated that fully reduced Cu_Z plays a key role in the catalytic cycle [1]; however, details of the mechanism of N_2O reduction are not well understood. Although inhibition studies using small anions are a powerful approach to understand enzymatic mechanisms, few such investigations have been reported with N_2OR.

Turnover kinetics and spectroscopic studies of *Achromobacter cycloclastes* N_2OR ($Ac N_2OR$) in the presence of azide, cyanide, fluoride and chloride, respectively, have been performed to help elucidate the catalytic mechanism. Data show that azide and cyanide are potent inhibitors of $Ac N_2OR$, and a slow, tight-binding model best described the inhibition. The data are consistent with some of these anions binding at the Cu_Z site (Fig. 1).

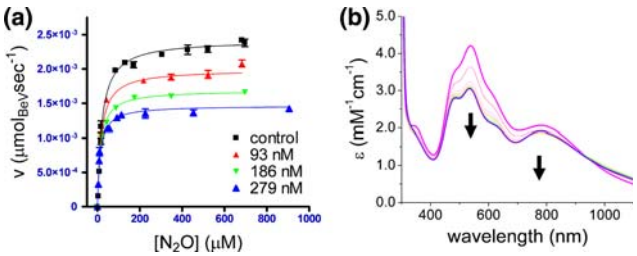

Fig. 1 **a** Kinetics behavior of N_2OR with respect to [N_2O] in the presence of NaN_3. **b** UV–vis titration of oxidized $Ac N_2OR$ with NaN_3

Reference

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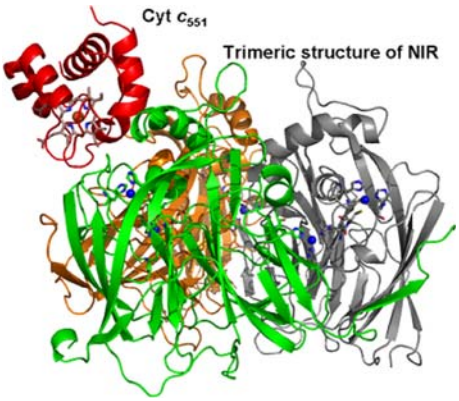
P007

A single methionine residue at the docking interface dramatically affects the interprotein electron transfer from cytochrome *c* to Cu-containing nitrite reductase

Hiroyasu Koteishi, Masaki Nojiri, Kazuya Yamaguchi, Shinnichiro Suzuki
Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan.
koteishi@chem.sci.osaka-u.ac.jp

Dissimilatory copper-containing nitrite reductase (CuNIR) catalyzes a one-electron reduction of NO_2^- to NO. CuNIR folds a trimeric structure and has two distinct copper centers in each monomer which are Type 1 Cu (T1Cu) and Type 2 Cu (T2Cu). T1Cu relays an electron from a donor protein to T2Cu, in which NO_2^- is reduced to NO. Recently, we have succeeded in determining the crystal structure of a protein–protein binary complex of CuNIR with its redox partner protein, cytochrome c_{551} (Cyt c_{551}) from *Achromobacter xylosoxidans*, at 1.7 Å resolution. In the protein–protein docking interface between CuNIR and Cyt c_{551}, Met135 of CuNIR locates at the center of the hydrophobic core region. To investigate the role of Met135 residue, site-directed mutagenesis was performed and kinetic analysis of electron transfer reaction between M135S mutant and Cyt c_{551} was carried out by stopped-flow techniques. The Ser mutation of Met135 dramatically affected the electron transfer rate constant. It was suggested that Met135 is important for the electron transfer between these proteins. Furthermore, we have determined the crystal structure of M135S CuNIR at 2.3 Å. The structural comparison between native and mutant NIR will be also discussed.



P008

Systematic synthesis of heptadecanuclear manganese oxo clusters involving mixed valence Mn_{13} supercubane cores

Ryoko Kubota, Yayoi Okui, Florina Catusanu, Takayuki Nakajima, Tomoaki Tanase*
Department of Chemistry, Faculty of Science, Nara Women's University, Nara 630-8506, Japan. tanase@cc.nara-wu.ac.jp

Recently, manganese oxo clusters have attracted increasing attention with relevance to PSII OEC as well as nano-structured molecular devices such as single-molecule magnet (SMM). Using triscarboxylate Kemp's triacid (H_3kta) and terminal ligands L, we have systematically prepared novel heptadecanuclear manganese oxo clusters formulated as $[Mn_{17}O_{14}(kta)_6(L)_4]$ [L = bpy (**1**), phen (**2**), 4,7-Ph_2phen (**3**), dmf (**4**), etc.] (Fig. 1a), in which a mixed valence Mn_{13} supercubane core, $\{Mn^{IV}Mn^{III}_{10}Mn^{II}_2(\mu_3\text{-}O)_8(\mu_5\text{-}O)_6(kta)_6\}^{8+}$, is surrounded by four Mn(II) units terminated by *L*. A similar procedure with methanol used as *L* and solvent gave another type of Mn_{17} oxo cluster, $[Mn_{17}O_{12}(OMe)_2(kta)_6(MeOH)_4]$ (**5**), which involves a two-electron reduced $\{Mn^{IV}Mn^{III}_8Mn^{II}_4(\mu_3\text{-}OMe)_2(\mu_3\text{-}O)_6(\mu_5\text{-}O)_6(kta)_6\}^{8+}$ supercubane core (Fig. 1b).

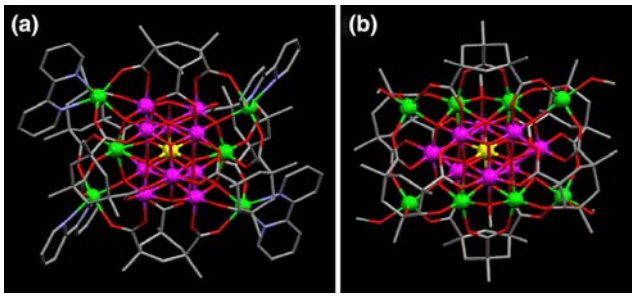


Fig. 1 Structures of Mn_{17} clusters **a 1** and **b 5**; Mn^{IV} (*yellow*), Mn^{III} (violet), Mn^{II} (*green*)

P009

Structure–function analysis of synthetic complexes obtained through inter-molecular disulfide bond between ferredoxin and ferredoxin-NADP+ reductase

Yoko Kimata-Ariga, Yukiko Sakakibara, Takahisa Ikegami and Toshiharu Hase
Institute for Protein Research, Osaka University, Osaka, Japan.
a-yoko@protein.osaka-u.ac.jp

Ferredoxin-NADP+ reductase (FNR) catalyses the conversion of NADP+ to NADPH using reduced ferredoxin (Fd) as an electron donor. Fd and FNR form a 1:1 complex, stabilized mainly by electrostatic interactions, for efficient electron transfer between their redox centers (2Fe–2S cluster and FAD, respectively) [1]. The relationship between electron transfer function and the interaction mode of Fd and FNR was investigated using Zea mays leaf Fd tethered to FNR by a disulfide bond in various configurations. The resulting Fd-FNR heterodimers showed a variety of efficiency for the intra-molecular electron transfer between Fd- and FNR domains. In order to investigate the interaction mode of Fd- and FNR domains of the heterodimers, NMR chemical shift perturbation analysis of Fd domain and absorption spectral analysis of flavin component of FNR domain were performed. Each two heterodimers with higher electron transfer efficiency (Fd21–FNR19 and Fd59–FNR19) and with lower efficiency (Fd21–FNR36 and Fd59–FNR36) were selected for the analyses. Both analyses showed that the interaction of Fd- and FNR domains in Fd21–FNR19 and Fd59–FNR19 is more similar to that of wild-type Fd:FNR complex than that of Fd21–FNR36 and Fd59–FNR36. However, small but significant differences in the FAD spectral changes and NMR chemical shift changes were observed between Fd21–FNR19 and Fd59–FNR19, although their electron transfer efficiency was almost the same, comparable to that of wild-type FNR with saturating concentration of Fd. Therefore, there seems to be a certain range of structural flexibility for attaining the maximum level of electron transfer between Fd and FNR.

Reference

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P010

Electron tunneling through mutant azurins on mixed-SAM gold electrodes

Keiko Yokoyama[1], Kyle M. Lancaster[1], Yuling Sheng[1], Nobuhumi Nakamura[2], Hiroyuki Ohno[2], Brian S. Leigh[1], Katsumi Niki[1], Jay R. Winkler[1], John H. Richards[1], Harry B. Gray[1]

[1]Beckman Institute, California Institute of Technology, Pasadena California 91125, USA. [2]Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan. yokoyama@caltech.edu

We have demonstrated that azurin (*P. aeruginosa*) exhibits particularly strong voltammetric responses on (alkanethiol + ω-hydroxyl-alkanethiol) mixed-SAM electrodes. We report results for mutant azurins in which asparagine-47 was replaced by alanine, aspartic acid, lysine, arginine, leucine, threonine, serine, and glutamine. The N47D mutant on a mixed SAM exhibited a well-defined electrochemical response; N47T and N47S gave a weak signal; but the other five mutants showed no response. It is likely that the N47 side-chain carbonyl docks to the mixed SAM surface, providing a very favorable electron tunneling pathway to the copper via the N47–C112 hydrogen bond. We also examined the effect on the ET rates by mutation of the copper cysteine and methionine ligands. The ET rate constants for M121L azurin are similar to those of wild type; those for C112D are three orders of magnitude smaller; and those for C112D/M121L are two orders of magnitude smaller than those of wild-type at pH 7 (Fig. 1).

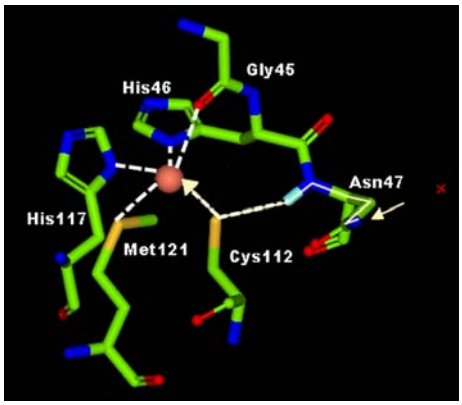

Fig. 1 The proposed ET pathway and active site of *P. aeruginosa* azurin. (PDB code: 4AZU)

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P011

Heme peptides for catalytic hydrogen evolution from water

Gretchen E. Keller, Jay R. Winkler, Harry B. Gray

Beckman Institute, California Institute of Technology, Pasadena, CA 91125. gkeller@caltech.edu

Cytochrome *c* is a small, well-characterized heme protein involved in electron transport. The wild-type protein has a reduction potential of +260 mV (vs. NHE); however, this potential is tunable via axial ligand (Met80) mutation and through metal substitution. We are investigating a series of cytochrome *c* variants where Met80 has been removed. The cytochrome *c* protein architecture can be stripped down by proteolysis with pepsin/trypsin or chemically cleaved by cyanogen bromide to afford microperoxidases MPX ($X = 8, 9, 11$) and cyt *c*-65. Cobalt substitution into these frameworks provides another route to altering redox properties, and both iron and cobalt systems are being examined spectroscopically and electrochemically. Further, we are investigating the efficiency of these protein-based cobalt porphyrins as proton-reduction catalysts for solar-driven water splitting.

P012

Construction of an artificial enzyme for olefin metathesis

Clemens Mayer, Dennis Gillingham, Donald Hilvert

Laboratory of Organic Chemistry, ETH Hönggerberg, Zürich, Switzerland. gillingham@org.chem.ethz.ch

Olefin metathesis is a fundamentally new reaction in synthetic chemistry and it has quickly become established as one of the cornerstones in synthetic analysis and polymer chemistry. An underappreciated feature of olefin metathesis is its bioorthogonality. Current efforts to utilize this property are mired in the struggle to create an active and reliable water-soluble catalyst [1]. We describe our efforts to create an artificial metalloenzyme by covalently linking a metathesis catalyst to a protein scaffold. The new artificial metalloenzyme is optimized by mutagenesis to address selectivity problems in current olefin metathesis technology.

This work is supported by a Marie-Curie International Incoming Fellowship to D.G. (IIF-AEOM).

Reference

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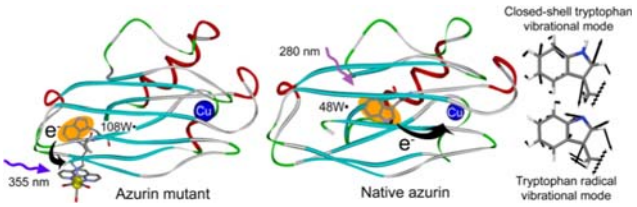
P013

Resonance Raman investigations of structure and dynamics of amino acid radicals as electron transfer intermediates in azurin

Hannah S. Shafaat, Brian S. Leigh, Michael J. Tauber, Judy E. Kim

Department of Chemistry and Biochemistry, University of California at San Diego, 9500 Gilman Dr., La Jolla, CA 92093 USA. judyk@ucsd.edu

Tryptophan residues play a significant role in mediating biological electron transfer (ET) reactions, both as crucial elements of electron tunneling pathways through protein matrices and as radical intermediates that facilitate long-range electron transfer processes. Azurin, a blue copper electron transfer protein, contains a single native tryptophan residue in a hydrophobic pocket that may play a role in the natural ET pathway. Other non-native tryptophan residues in azurin have been shown to modulate ET rates through the protein. Here, we report on the direct reduction of the copper center from the natural tryptophan residue and present resonance Raman spectra of tryptophan radicals in the hydrophobic protein environment and in a solvent-accessible region. Our spectra show differential effects of pH and deuteration on the structures of these radicals in these distinct microenvironments. Additionally, the dynamics and quantitative mode displacements of the tryptophan excited state have been determined from resonance Raman scattering intensities, providing information on the internal reorganization energy and nuclear motions coupled to the electron transfer reaction.



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P014

Time-resolved Resonance Raman study on structural relaxation process of cytochrome *c* oxidase following photolysis of carbonmonoxide

Izumi Ishigami[1], Satoru Nakashima[2], Kyoko Shinzawa-Itoh[1], Shinya Yoshikawa[1,2], Takashi Ogura[1,2]
[1]Department of Life Science.
[2]Picobiology Institute, Graduate School of Life Science, University of Hyogo, Hyogo 678-1297, Japan. rj07p002@stkt.u-hyogo.ac.jp

Mitochondrial cytochrome *c* oxidase (C*c*O) reduces dioxygen to water, and transports protons from the negative to the positive spaces. C*c*O has four redox active centers. His376 and His378, ligating to hemes a_3 and *a*, respectively, are the residues of helix X. According to X-ray crystallography, helix X exhibits conformational change upon redox-change and ligand binding. Conformational change of helix X must be important in the function of C*c*O, since it links heme a_3 (the dioxygen reducing site) with heme *a* (the proton pump site). In the present study, we examined structural relaxation process at heme a_3 from the carbonmonoxide (CO)-bound to the equilibrium fully reduced states following photolysis of CO using time-resolved resonance Raman spectroscopy.

The ν_{Fe-His} mode appeared at 220 cm^{-1} at 10 ns after CO-photolysis, which was 6 cm^{-1} higher than that of equilibrium reduced state of 214 cm^{-1}. The frequency remained unchanged until 100 ns and then showed downshift exponentially with time with a rate constant of 1.3×10^6 s^{-1}. The porphyrin ν_9 mode at 230 cm^{-1} of CO-bound heme a_3 reflects the planarity of porphyrin. It exhibited intensity reduction of 20% at 10 ns and further 40% at 100 ns, and stayed constant at larger delay times, of the original intensity before CO-photolysis. The bending mode of vinyl or formyl substituent of heme a_3 exhibited upshift from 435 to 436 cm^{-1} later than 5.3 ms after CO-photolysis. These results lead us to conclude that a sequential protein dynamics occurs as follows after CO-photolysis; (1) planarity of heme a_3 decreases within 100 ns, (2) Fe–His band becomes weaker in about 1 μs and (3) heme a_3 shifts horizontally so that the peripheral group suffers steric hindrance.

P015

The role of type1 copper-containing N-terminal domain of hexameric nitrite reductase from a methylotrophic denitrifying bacterium, *Hyphomicrobium denitrificans*.

Saori Ikebuchi, Masaki Nojiri, Felicia Shirota, Daisuke Hira, Kazuya Yamaguchi, Shinnichiro Suzuki.
Department of Chemistry, Graduate School of Science, Osaka University, Osaka 560-0043, Japan. ikebuchi@chem.sci.osaka-u.ac.jp

Copper-containing nitrite reductase (CuNIR) catalyzes a one-electron reduction of NO_2^- to NO. CuNIR folds a trimeric structure with two distinct Cu sites per a ca. 37-kDa monomer unit. The type 1 Cu site (T1Cu) buried within each monomer relays an electron from the redox partner protein to the catalytic type 2 Cu site (T2Cu), where NO_2^- is reduced to NO. Recently, the structure of a CuNIR from the methylotrophic denitrifying bacterium *Hyphomicrobium denitrificans* (HdNIR) has been reported, establishing the existence of a new family of CuNIR where an additional T1Cu ($T1Cu_N$) containing cupredoxin domain is located at the N-terminus [1]. To understand the role of this N-terminal domain and $T1Cu_N$, mutagenesis of the histidine ligand residue for $T1Cu_N$ to glycine (H119G) has been performed and characterized spectroscopically and biochemically. In the UV–vis spectrum of H119G, the absorption band at 600 nm [that is characteristic of oxidation state $T1Cu_N$ (Cu^{2+})] is lower than that of wild-type (WT), so it is thought that $T1Cu_N$ of H119G is in the reduction state (Cu^+). The NO_2^--reducing enzyme assays and stopped-flow experiments using cytochrome c_{550} (Cytc_{550}) as a electron donor protein were also carried out, and the results show that H119G has higher activity than WT. These results suggest that the reduction of $T1Cu_N$ affects the interaction between HdNIR and Cytc_{550} (Fig. 1).

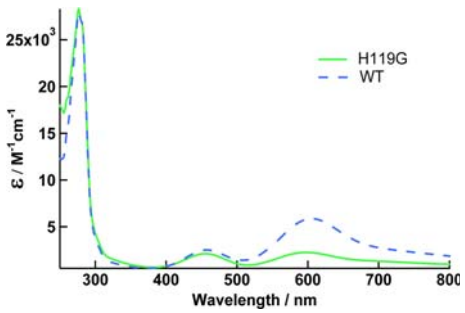

Fig. 1 UV–vis spectra of H119G and WT

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P016

A new resonance raman marker band of cytochrome *c* oxidase

Miyuki Sakaguchi[1], Kyoko Shinzawa-Itoh[1], Shinya Yoshikawa[1,2], Hiroshi Fujii[3], Takashi Ogura[1,2]
[1]Department of Life Science.
[2]Picobiology Institute, Graduate School of Life Science, University of Hyogo, Hyogo 678-1297, Japan.
[3]Okazaki Institute Integrative Bioscience, Okazaki 444-8787, Japan. rj08w013@stkt.u-hyogo.ac.jp

The redox change of heme *a* was proposed to drive proton pump in cytochrome *c* oxidase (C*c*O) [1]. The OH group of the hydroxyfarnesylethyl (HFE) substituent of heme *a* is hydrogen-bonded to S382 in its oxidized state. Upon reduction, the hydrogen-bond is cleaved and the OH group of S382 rotates 110° to make a cavity for proton collection. At the same time, the OH group of HFE substituent rotates 120°, which might cause a frequency shift of the vibrational mode associated with the HFE group.

C*c*O in the reduced state gave a resonance Raman (RR) band at 1,247 cm^{-1} upon Soret excitation and it showed an upshift to 1,250 cm^{-1} in the oxidized state. It showed deuterium sensitivity. It

also showed an upshift to 1,253 cm^{-1} upon freezing at −15 °C. No other porphyrin in-plane vibrational mode showed such a significant shift upon freezing. RR spectra of bis-imidazole complex of Fe^{2+}-2-vinyl-4-hydroxymethyl deuteroporphyrin gave a Raman band at 1,249 cm^{-1}, which exhibited deuterium sensitivity. The pattern of Raman difference spectrum (H_2O–D_2O) was similar to that of C*c*O. The results suggest that the band at 1,247 cm^{-1} of C*c*O is assignable to a vibrational mode associated with the OH group of HFE substituent probably of heme *a*. It could be a marker band to monitor the conformational change of the HFE group during proton pumping.

Reference

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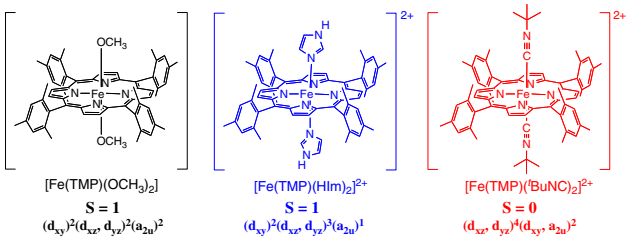
P017

One-electron oxidized product of iron(III) porphyrinate that is diamagnetic

Akira Ikezaki[1], Hideyuki Tukada[2], Mikio Nakamura[1,3]
[1]Department of Chemistry, School of Medicine, Toho University, Tokyo 143-8540, Japan.
[2]Graduate School of Integrated Science, Yokohama City University, Yokohama 236-0027, Japan.
[3]Division of Chemistry, Graduate School of Science, Toho University, Funabashi 274-8510. Japan. ikezaki@med.toho-u.ac.jp

Elucidation of the electronic structures of high-valent iron porphyrins is quite important because they play important roles in a biological system. One-electron oxidation of iron(III) porphyrinates gives three possible products, (1) iron(IV) porphyrin complexes such as $Fe(TMP)(OCH_3)_2$ and (Fe=O)(TMP), (2) high-spin or mixed high- and intermediate-spin iron(III) porphyrin radical cations such as $[Fe(TPP\cdot)Cl]^+$ and $[Fe(TPP\cdot)]^{2+}$, and (3) low-spin iron(III) porphyrin radical cations such as $[Fe(TPP\cdot)(HIm)_2]^{2+}$. In this paper, we present the fourth case. Namely, a novel low-spin iron(III) porphyrin radical cation, $[Fe(TMP\cdot)(^tBuNC)_2]^{2+}$, that is diamagnetic. The spectroscopic and magnetic data of this complex will be presented.



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P018

Spectroelectrochemistry of electron transfer components in photosystem II

Tadao Shibamoto[1], Yoshinori Kuroiwa[1], Yuki Kato[1], Miwa Sugiura[2], Tadashi Watanabe[1]
[1]Institute of Industrial Science, University of Tokyo, Japan.
[2]Cell-Free Science and Technology Research Center, Ehime University. Japan. watanabe@iis.u-tokyo.ac.jp

Photosynthesis proceeds with an overall quantum yield of ca. 1.0 through many energy and electron transfer steps, and this must be backed up by exquisite redox potential tuning among the molecular components in photosystem (PS) I and II. This aspect, however, is still far from being unraveled. Only the redox potentials of cytochrome (Cyt) *b*559, consisting of two protein subunits and a heme iron, and a plastoquinone Q_A have been measured among the electron transfer components of PS II (Fig. 1). The reported values of both Cyt *b*559 and Q_A redox potentials, however, exhibit heavy scatters of more than 100 mV probably due to the low accuracy of chemical titration that has traditionally been used. In the present study, we have tried to measure the redox potentials of Cyt *b*559 and Q_A precisely by spectroelectrochemistry using an optically transparent thin-layer electrode cell. Comparison of the redox potentials thus determined [1] with reported values sheds light to the redox properties which are buried in the previous measurement.

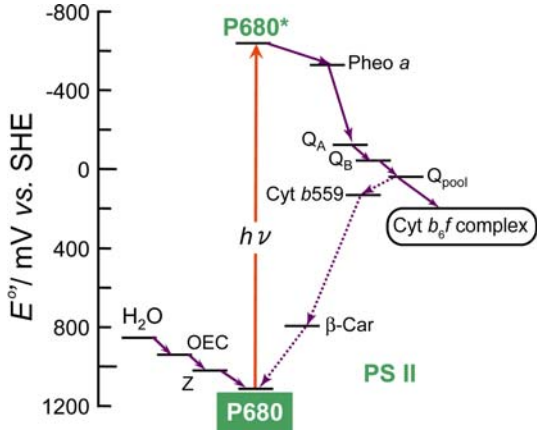

Fig. 1 Electron transfer scheme of PS II

Reference

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P019

Redox potentials of chlorophyll *a* and pheophytin *a* in the electron transfer chain of oxygenic photosynthesis determined by spectroelectrochemistry

Yuki Kato[1], Akimasa Nakamura[1], Miwa Sugiura[3], Tadashi Watanabe[1]
[1]Institute of Industrial Science, University of Tokyo, Japan.
[2]Cell-Free Science and Technology Research Center, Ehime University. Japan. yukikato@iis.u-tokyo.ac.jp

Oxygenic photosynthetic organisms convert photon energy to chemical free energy very efficiently in the primary process through light-induced charge separation and subsequent electron transfers in photosystems (PS) I and II. Chlorophyll (Chl) *a* is a major constituent in PSs (ca. 40–200 depending on the type of PS and species), while a small number of Chl *a* and its derivatives play key roles in the reaction center. In PS I, a heterodimer of Chl *a* and its $C13^2$ epimer, Chl *a*′, constitutes P700 that works as the primary electron donor, and monomeric Chl *a* works as the primary acceptor; in PS II, P680 that is considered as a homodimer of Chl *a* and pheophytin (Pheo) *a*, a Mg depleted derivative of Chl *a*, work as the primary electron donor and acceptor, respectively. Among them, the redox potentials of P700 and Pheo *a* have traditionally been investigated by chemical redox titration, and have been used to estimate those of the other components, since the P680 and Chl *a* potentials are too high and low to measure

directly, respectively. However, heavy scatters and non-negligible experimental errors exist in the reported potentials of P700 and Pheo *a*, rendering the electron transfer mechanism ambiguous. To overcome the drawbacks inherent to the titration, we have applied spectroelectrochemistry to potential determination. The precise values of P700 and Pheo *a* potentials thus determined are discussed in relation to the electron transfer mechanism (Fig. 1).

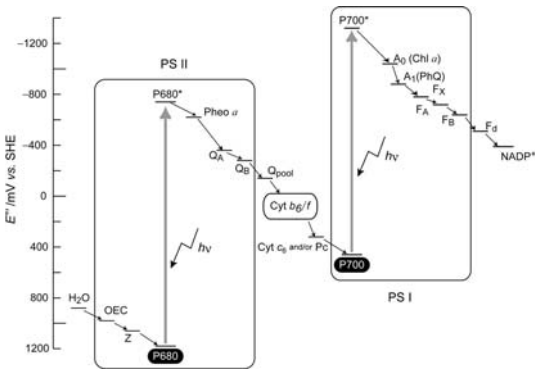

Fig. 1 Electron transfer scheme based on the redox potentials of the chain components

P020

Computational study for the radical scavenging effects of carotenes

Ching-Han Hu
Department of Chemistry, National Changhua University of Education, Changhua, Taiwan. chingkth@cc.ncue.edu.tw

Carotenes are an important type of molecules that occur naturally in plants and photosynthetic organisms. They are known to perform free radical scavenging and singlet oxygen quenching effects in biological tissues. Carotenes are also important plant pigments. They act as light harvesting antenna in the photosynthetic system, and protect the plant from the harmful effects caused by singlet oxygen and triplet chlorophyll. Recent progresses of our research group on the understanding of properties and reactivities of carotene species will be presented. In our research, the radical scavenging effects of carotenes were examined. Hydrogen abstraction and radical addition mechanisms were explored with quantum chemistry approaches. The addition mechanism was shown by our data to be the more thermodynamically favorable reaction path.

P021

Intermolecular electron transfer reaction of *c*-type heme-containing copper nitrite reductase from *Pseudoalteromonas haloplanktis* TAC125

Ryosuke Ishikawa, Masaki Nojiri, Kazuya Yamaguchi, Shinnichiro Suzuki
Department of Chemistry, Graduate School of Science, Osaka University, Osaka 560-0043, Japan. ryosuke@chem.sci.osaka-u.ac.jp

The *c*-type heme-containing copper nitrite reductase(PhNIR), isolated from a psychrophilic bacterium, *Pseudoalteromonas haloplanktis* TAC125, has been structurally characterized. PhNIR folds a trimeric structure and has one *c*-type heme and two distinct copper centers in each monomer which are Type1Cu and Type2Cu. PhNIR accepts one electron from an external electron-donor protein and catalyzes the one-electron reduction of nitrite ion to nitrogen monoxide.

Recently, cytochrome c_{4A} (PhCyt c_{4A}, ca. 9kDa) was isolated from *P. haloplanktis* and spectroscopically characterized. Moreover, the intermolecular electron transfer from this heme–protein to PhNIR has been observed. Electron-transfer rate constants of PhNIR with PhCyt c_{4A} were determined to be 3.3×10^4 $M^{-1}s^{-1}$ at pH 6.5 (25 °C) by the cyclic voltammetry method (Fig. 1).

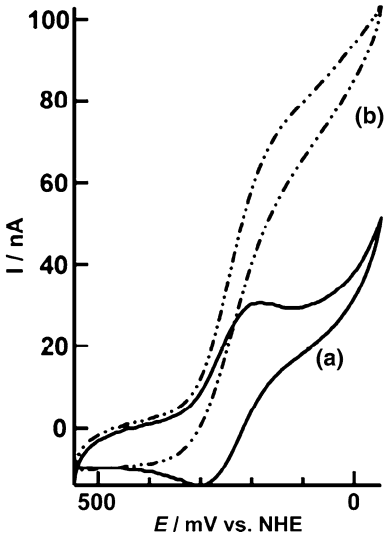

Fig. 1 Cyclic voltammograms of PhCyt c_{4A} (**a**) and after addition of PhNIR and 50 mM NO_2^- (**b**)

P022

Low-frequency distortion modes of heme detected by femtosecond coherence spectroscopy

Minoru Kubo[1,3], Flaviu Gruia[1], Abdelkrim Benabbas[1], Alexander Barabanschikov[1], William R. Montfort[2], Estelle M. Maes[2], Paul M. Champion[1]
[1]Department of Physics, Northeastern University, Boston, MA 02115, USA.
[2]Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ 8572, USA.
[3]Present Address: Graduate School of Life Science, University of Hyogo, Hyogo 678-1297, Japan. mkubo@sci.u-hyogo.ac.jp

Resonance Raman spectroscopy has yielded significant information about the heme structures and dynamics of a variety of heme proteins through the Fe-ligand stretching bands and oxidation/spin marker bands of heme. This conventional spectroscopy, however, has the shortcoming that the low frequency region (<200 cm^{-1}) is difficult to access because it is obscured by Rayleigh and quasi-elastic scattering. Femtosecond coherence spectroscopy (FCS), which is the recently advanced time-domain version of Raman spectroscopy, enables us to monitor low frequency Raman-active modes of a resonant chromophore in a protein. In this study [1], low frequency mode assignments have been made for iron porphine model compounds using FCS in combination with DFT calculations. FCS has also been applied to NP4, a NO-transport heme protein. We find a mode near 60 cm^{-1} that is conspicuously sensitive to the ruffling out-of-plane distortion of heme. Importantly, the band intensity of this mode depends quadratically on the magnitude of the ruffling distortion. To quantitatively account for this correlation, a new "distortion-induced" Raman enhancement mechanism that is uniquely relevant to low frequency soft modes is presented [1].

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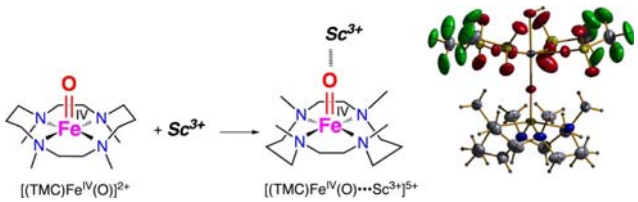
P023

Structure and reactivity of metal ion complexes of non-heme iron(IV)-oxo species

Yuma Morimoto[1], Hiroaki Kotani[1], Pance Naumov[1], Yong-Min Lee[2], Wonwoo Nam[2], Shunichi Fukuzumi[1]
[1]Department of Material and Life Science, Graduate School of Engineering, Osaka University, SORST, JST, Osaka 565-0871, Japan.
[2]Department Chemistry, Division of NanoSciences, Ewha Womans University, Seoul 120-750, Korea.
y-morimoto@chem.eng.osaka-u.ac.jp

Metal ions play pivotal roles in biological electron-transfer (ET) systems such as photosynthesis and respiration. For example, the oxidation of water to dioxygen is catalyzed by Mn–oxo clusters with Ca^{2+} ion at the oxygen-evolving center (OEC) in photosystem II. We report herein the effects of redox-inactive metal ions on the structure and ET reactivity of non-heme iron(IV)–oxo complexes for the first time.

Addition of redox inactive metal ions such as Sc^{3+} to a non-heme oxoiron(IV) complex ($[(TMC)Fe^{IV}(O)]^{2+}$) resulted in formation of Sc^{3+} binding iron(IV)–oxo complex, which is confirmed by the X-ray crystallography as shown in Scheme 1. The ET properties of Sc^{3+} binding iron(IV)–oxo complex was compared to that of $[(TMC)Fe^{IV}(O)]^{2+}$ [1]. The binding of Sc^{3+} enabled the two-electron reduction of $[(TMC)Fe^{IV}(O)(Sc^{3+})]^{5+}$ by ferrocene, whereas only the one-electron reduction of $[(TMC)Fe^{IV}(O)]^{2}$ occurred without Sc^{3+}.



Scheme 1

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P024

A systematic screening of polynuclear manganese complexes for water oxidation catalysis

Denys Shevchenko, Anders Thapper, Magnus Anderlund, Stenbjörn Styring
Department of Photochemistry and Molecular Science, Uppsala University, 751 20 Uppsala, Sweden.
denys.shevchenko@fotomol.uu.se

We have carried out a systematic screening of a series of known polynuclear manganese complexes, most of which are tetranuclear, for their ability to catalyze water oxidation and evolve oxygen. The compounds can be divided on four groups based on ligand types. The first group is complexes with mono- and bidentate ligands. The second and third ones consist of complexes based on tri- and more than tridentate ligands, respectively. The fourth group includes polyoxometalate complexes. $KHSO_5$ (oxone) and $[Ru(bpy)_3](ClO_4)_3$ were used as oxidants. Oxygen evolution has been detected in four cases in the reaction with oxone and in one case in the reaction with $[Ru(bpy)_3](ClO_4)_3$. In most cases formation of a fine brown precipitate was observed after or even before oxidant addition. Therefore, it is most likely that the starting complexes act as precursors for the in situ formation of the active species as result of solvolysis or oxidation reactions. It has been found that active species in the case of $(n\text{-}Bu)_4N[Mn_4O_2(PhCOO)_7(pic)_2]$ [1] (Hpic-2-picolinic acid) is a product of hydrolysis suggested to be hydrated manganese oxide. It shows a maximum oxygen evolution rate one order of magnitude higher ($64.3\ mM_{O_2}\cdot s^{-1}\cdot M_{metal}^{-1}$) than commercial manganese dioxide at the same conditions.

Acknowledgment: This research was supported by the Swedish Energy Agency, the Knut and Alice Wallenberg Foundation and a Marie Curie International Incoming Fellowship (No. 236511) within the 7th European Community Framework Programme.

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P025

Glutathione transferase: GSH activation mechanism proposal

Daniel F.A.R. Dourado[1], Pedro Alexandrino Fernandes[1], Bengt Mannervik[2], Maria João Ramos[1]
[1]REQUIMTE/Departamento de Química, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal.
[2]Department of Biochemistry and Organic Chemistry, Uppsala University, BMC, Box 576, 75123 Uppsala, Sweden

The cell detoxification mechanism of xenobiotic and endobiotic compounds follows a series of different steps. To begin with, toxic compounds are converted into strong electrophiles, by the mixed-function oxidation activity of cytochrome *P*-450. Those electrophiles are subsequently transformed into more soluble and less toxic substrates, by conjugation with glutathione (GSH) due to the catalytic activity of glutathione transferases (GSTs), which are recognized by ATP-dependent transmembrane pumps such as P-glycoproteins and MRP family proteins, and consequently expelled from the cell. GSTs studies are of great importance since they have been implicated in the development of drug resistance in tumoral cells and are related to human diseases such as Parkinson's, Alzheimer's, atherosclerosis, liver cirrhosis, aging and cataract formation. In terms of structure GSTs can be homodimers or heterodimers having each monomer two active centers, a G-site pocket for glutathione (GSH) and an H-site pocket for the electrophilic substrate. When GSH binds to the G-site, the pKa of its thiol group drops 1.5 units promoting its deprotonation. This strong nucleophilic thiolate is now able to react with the electrophilic substrate, bounded in the H-site, building up a more soluble and less toxic compound. The nature of the residue that, behaving as a base, deprotonates the GSH thiol group is still unknown. Based on QM/MM calculations we propose a mechanism for GSH activation with an overall free energy barrier consistent with the enzyme kinetics experimental studies.

Reference
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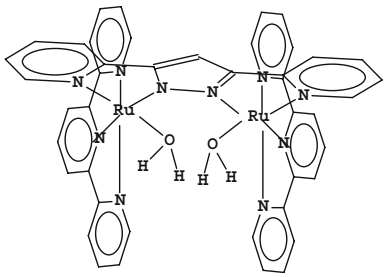
P026

Oxygen–oxygen bond formation pathways induced by Ru–Hbpp complexes

Sophie Romain, Fernando Bozoglian, Xavier Sala, Antoni Llobet
Institute of Chemical Research of Catalonia (ICIQ), Avinguda Països Catalans 16, 43007 Tarragona, Spain, Departament de Química Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain. allobet@iciq.es

The oxidation of water to molecular dioxygen is a reaction that takes place in the dark at the OEC-PSII. It is a very interesting reaction to be modeled from a bioinorganic perspective since it can give some hints regarding the potential mechanisms that operate in this natural system. On the other hand, it is of tremendous importance from an energetic perspective, since it is recognized to be the bottleneck for the development of commercial light harvesting devices for the photo production of H_2 from water [1]. In 2004, we reported a new water oxidation catalyst $\{[Ru^{II}(trpy)(H_2O)]_2(\mu\text{-bpp})\}^{3+}$ [2] (trpy is 2,2′:6′,2″-terpyridine, bpp is 2,6-bis (pyridyl)-pyrazolate) containing a pyrazolate bridging unit, whose structure is shown in the figure. We now report a thorough kinetic analysis combined with O^{18} labeling experiments that allows us to clearly elucidate the reaction mechanism, showing that is only intramolecular [3].



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P027

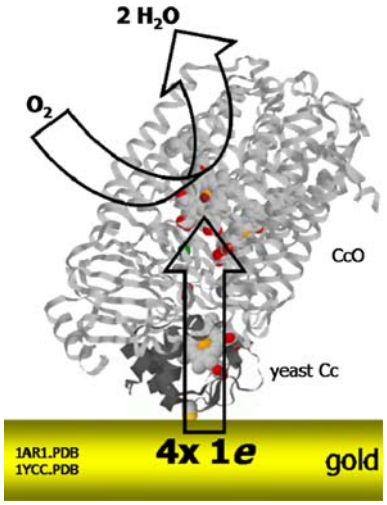
Yeast cytochrome *c* as sticky substrate for *P. denitrificans* cytochrome *c* oxidase: application as Co-immobilized mediator for voltammetry

F.G.M. Wiertz[1], O.-M. Richter[2], B. Ludwig[2], H.A. Heering[1]
[1]Leiden University, Leiden Institute of Chemistry, Einsteinweg 55, 2333 CC Leiden, The Netherlands.
[2]Goethe Universität, Institute of Biochemistry, Max-von Laue-Str. 9, 60438, Frankfurt am Main, Germany. f.wiertz@chem.leidenuniv.nl

Paracoccus denitrificans cytochrome *c* oxidase (C*c*O) catalyses the oxidation of four cytochrome *c* (C*c*) by oxygen. In addition, four protons are translocated from the cytoplasm to the periplasm, generating a proton electrochemical gradient across the cytoplasmic membrane:

$$4Cc^{2+} + O_2 + 8H^+_{cytoplasm} \rightarrow 4Cc^{3+} + 2H_2O + 4H^+_{periplasm}$$

C*c*O is a four-subunit membrane enzyme, containing two heme groups (*a*, a_3) and two copper centers (Cu_A, Cu_B). The binuclear Cu_A centre is the primary electron acceptor from cytochromes c_{552} or c_{550}. Heme a_3 and Cu_B form the site for oxygen reduction. Our activity assays and binding studies reveal that oxidized yeast iso-1 C*c* (YC*c*) remains bound to C*c*O. Moreover, YC*c* contains an exposed Cys opposite the docking site for C*c*O. The thiol can be used to anchor the protein directly to gold, promoting fast electron transfer [1]. YC*c* thus allows for the formation of a permanent, oriented substrate/enzyme complex on the electrode. Direct electrochemical control of the redox states of both C*c* and C*c*O facilitates steady state as well as pre-steady-state electroenzymology.



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P028

Storage of solar energy in chemical bonds using horseradish peroxidase

Matthew R. Hartings[1], Maraia Ener[1], Jay R. Winkler[1], Harry B. Gray[1]
[1]Beckman Institute, California Institute of Technology, Pasadena CA 91125, USA. hartings@caltech.edu

Horseradish peroxidase is a heme protein capable of oxidizing many small molecule substrates. During turnover, a ferryl species is generated (compound II) followed by the generation of a ferryl radical cation species (compound I), where the cation is localized on the porphyrin. Previous studies have found that it is possible to create compound II upon iron oxidation by a photoexcited $Ru(bpy)_3$ molecule in solution [1]. This study focused on understanding the dynamic processes leading to the observation of compound II. Our current work is exploiting the photogeneration of compound II in order to turn over the substrate into product in the absence of molecular oxygen. These experiments are a very real attempt to mimic photosynthetic systems in their ability to convert solar energy into chemical bonds.

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P029

Synthesis, characterization, and oxygen-evolving activity of dinuclear ruthenium complex with a Bis-tpa type ligand (6-hpa)

Ryoko Sugiyama, Yutaka Hitomi, Takuzo Funabiki, and Masahito Kodera
Doshisha University, Graduate School of Engineering, Kyoto 610-0321, Japan. dti0563@mail4.doshisha.ac.jp

Green plants have developed complex catalysts for the dioxygen evolution via a four-electron oxidation of two water molecules. Development of catalysts capable of achieving the reaction is important in terms of modeling photosynthesis in green plants. Ruthenium-based catalysts show activity of water oxidation. The first example of this catalyst is $[\{Ru(bpy)_2(H_2O)\}_2O]$ $(ClO_4)_4$ [1] (bpy = 2,2′-bipyridine) in which two ruthenium ions are linked by a μ-oxo bridge. Its turnover number is quite low due to the low stability. A practical water oxidation catalyst must have much better activity than this complex. This problem has been addressed by the design of ligands that increase the stability of the catalyst. In this work, We have synthesized a new dinuclear ruthenium complex $[Ru_2(H_2O)_2(OH)_2(6\text{-hpa})](ClO_4)_2$ **(1)** to investigate dioxygen-evolving activity of this complex. The complex **1** was obtained as follows. A mixture of 6-hpa and $[RuCl_2(DMSO)_4]$ in MeOH was refluxed for 8 h under Ar atmosphere. After concentration, $NaClO_4$ was added to give a yellow precipitate. The solid was dissolved in an aqueous acetone, and $AgClO_4$ was added to remove coordinated chloride ion, resulting in a deep green solution with white solid of AgCl. After filtration of the solid and concentration of the filtrate, **1** was isolated as a green solid.

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P030

Redox function of pseudo-tetrahedral copper-dithiocarbamato complexes as a progressive analogue to type I copper center

Tomoaki Toyama, Kotaro Yoshii, Takanori Inazumi, Tomohiko Inomata, Tomohiro Ozawa, Yasuhiro Funahashi, Hideki Masuda
Department of Frontier Materials, Graduate School of Engineering, Nagoya Institute of Technology, Nagoya 466-8555, Japan.
cgp11094@stn.nitech.ac.jp

In blue copper proteins, type I copper sites are redox-active centers having pseudo-tetrahedral coordination with two His-imidazoles and Cys/Met-sulfur ligands. In this study, we synthesized new copper-dithiocarbamato (DTC) complexes with a natural alkaroid, (−)-sparteine (**Sp**), and its stereo isomer, α-isosparteine (**αSp**), as biomimetic model compounds of type I copper sites. **Sp** is able to enforce tetrahedral distortion around the metal center. In this report, we describe about the details of crystal structures and electrochemical properties of these copper complexes.

First, we synthesized $[Cu^{II}(\mathbf{Sp})DTC]PF_6$ (**1**) and $[Cu^{II}(\mathbf{\alpha Sp})DTC]ClO_4$ (**2**), and performed them X-ray single crystal structural analysis. When compared with these coordination structures, we found that the torsion angle of N_2 and S_2 coordination planes in **2** is larger and more approximate to orthogonal in ***Td*** than that of **1**. Second, the cyclic voltammogram of **1** and **2** exhibited quasi-reversible redox processes of $Cu^{II/III}$ and $Cu^{I/II}$, respectively. Taking into account these results, we discussed about formation of low-valent (1+) and high-valent (3+) copper centers in S-containing ligand fields with tetrahedral distortion (Fig. 1).

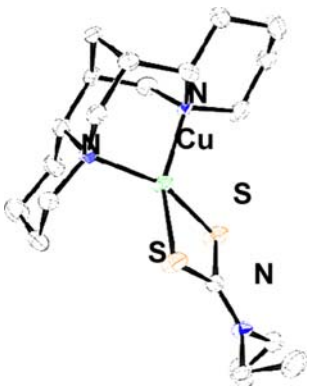

Fig. 1 Crystal structure of $[Cu^{II}(\mathbf{Sp})DTC]^+$

P031

Fine-tuning of functional and structural model complexes for the active site of copper-containing nitrite reductase

Makoto Misoo[1], Akiko Minami[1], Yasushi Kai[2], Shinnichiro Suzuki[1], Shinobu Itoh[3], Kazuya Yamagichi[1]
[1]Department of Chemistry, Graduate School of Science, Osaka University, Osaka 560-0043, Japan.
[2]Department of Environmental and Biotechnological Frontier Engineering, Fukui University of Technology, Fukui 910-8505, Japan.
[3]Department of Material and Life Science, Graduate School of Engineering, Osaka University, Osaka 565-0871, Japan.
kazu@ch.wani.osaka-u.ac.jp

Nitrite reductase (NIR), a key enzyme of denitrification, catalyzes the reduction of nitrite to nitrogen monoxide. Generally, copper-containing NIR possesses each of type 1 Cu (blue copper) and type 2 Cu (nonblue copper) per a monomer. The type 2 Cu site is bound by three His residues and one solvent water, which results in a distored tetrahedral geometry. We have reported that $[Cu(Me_2bpa)]^{2+}$ complex (Me_2bpa: bis(6-methyl 2-pyridyl methyl) amine) is a good functional and structural model for the type 2 active site of NIR [1]. In this study, we prepared $[Cu(X_nbpaY)]^{2+}$ complexes with several substituents (*X*: H_2, Me_1H_1, Me_2; *Y*: H, Et, Bz, EtPh) and investigated the spectroscopic and electrochemical characterization, X-ray crystal structural analysis, and the catalytic activities of the NIR model complexes. The redox potentials and catalytic activities were obviously affected with the substituents. Moreover, the cation-π interaction between Cu^{2+} and phenyl ethyl substituent was observed (Fig. 1). These substituents are available for the fine-tuning of the catalytic activities and the electronic structures of NIR model complexes.

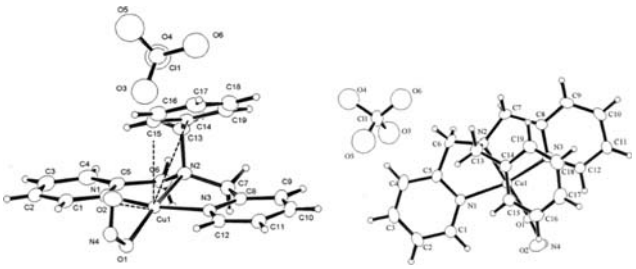

Fig. 1

Reference

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P032

Electron transfer reaction of porphyrin and porphycene complexes of Cu(II) and Zn(II) in acetonitrile

Masahiko Inamo[1], Kaori Aoki[1], Toshimitsu Goshima[1], Yohei Kozuka[1], Yukiko Kawamori[1], Noboru Ono[2], Yoshio Hisaeda[3], Hideo D. Takagi[4]

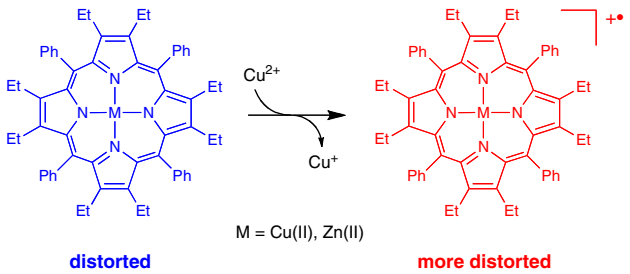
[1]Department of Chemistry, Aichi University of Education, Kariya 448-8542, Japan.

[2]Department of Chemistry, Ehime University, Matsuyama 790-8577, Japan.

[3]Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 819-0395, Japan.

[4]Research Centre for Materials Science, Nagoya University, Nagoya 464-8602, Japan. minamo@auecc.aichi-edu.ac.jp

The outer-sphere one-electron oxidation reaction of the Cu(II) and Zn(II) complexes of nonplanar 2,3,7,8,12,13,17,18-octaethyl-5,10,15,20-*tetra*-phenylporphyrin (EOTPP) as well as the planar porphyrins and porphycenes by Cu^{2+} giving corresponding π-cation radicals was investigated in acetonitrile. The electron self-exchange rate constants between the parent porphyrin and porphycene complexes and their π-cation radicals were determined using the Marcus cross relation for the electron transfer reaction. The obtained rate constants are in the order of 10^9–10^{11} M^{-1} s^{-1} for the planar porphyrin and porphycene complexes and 10^4–10^6 M^{-1} s^{-1} for the nonplanar OETPP complexes at $T = 25.0$ °C. The relatively slow self-exchange reaction of the distorted porphyrin complexes was ascribed to the significant deformation of the complex associated with the oxidation reaction from the parent complex to the corresponding π-cation radical.



P033

X-ray structure and function of peroxide bridge between Fe and Cu in the O_2 reduction site of the fully oxidized cytochrome *c* oxidase

Hiroshi Aoyama[1,2], Kazumasa Muramoto[3], Kyoko Shinzawa-Itoh[3], Kunio Hirata[4], Eiki Yamashita[4], Tomitake Tsukihara[3,4], Takashi Ogura[3], Shinya Yoshikawa[3]

[1]RIKEN SPring-8 Center, Sayo, Sayo 679-5148, Japan.

[2]Graduate School of Pharmaceutical Science, Osaka University, Suita 565-0871, Japan.

[3]Picobiology Institute, Department of Life Science, University of Hyogo, Kamighori, Akoh 678-1297, Japan.

[4]Institute for Protein Research, Osaka University, Suita 565-0871, Japan. muramoto@sci.u-hyogo.ac.jp

Cytochrome *c* oxidase (C*c*O) is the terminal oxidase of the respiratory chain embedded in mitochondrial and bacterial membrane. C*c*O catalyzes O_2 reduction to H_2O coupled to a proton pump across the membrane. The O_2 reduction site consists of heme and copper atom (heme a_3 and Cu_B). Electrons for O_2 reduction are transferred from cytochrome *c* in the positive side space to the O_2 reduction site via other metal centers (Cu_A and heme *a*). The protons for H_2O formation are transferred from the negative side space via two hydrogen-bond network. The proton pumping pathway is proposed differently between mammalian and bacterial C*c*O. Recent X-ray structural analysis improved the resolution of bovine C*c*O structure. However, strong X-ray irradiation to C*c*O crystal induces reduction of the metal centers. Therefore, we performed the X-ray diffraction experiment minimizing X-ray irradiation effects by shortening irradiation time and using many crystals to examine the structure of the fully oxidized "as isolated" (without any reduction/oxidation treatment) bovine heart C*c*O. The X-ray structure showed a peroxide group bridging the two metal sites in the O_2 reduction site (Fe^{3+}–O^-–O^-–Cu^{2+}). Physiological relevance of the present X-ray structural results will be discussed.

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P034

Contrasting catalytic properties of the two uptake hydrogenases of *Escherichia coli*

Michael Lukey[1], Alison Parkin[1], Frank Sargent[2], Fraser Armstrong[2]

[1]Department of Inorganic Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QR, England, UK.

[2]College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, UK. michael.lukey@balliol.ox.ac.uk

The catalytic properties of the isoenzymes hydrogenase-1 (Hyd-1) and hydrogenase-2 (Hyd-2) of *E. coli* have been investigated by electrochemical methods. These proteins are known to be membrane-bound and to function in H_2-uptake. Protein film voltammetry (PFV) experiments reveal that both enzymes are highly active in H_2 oxidation, but only Hyd-2 also shows significant H_2 production activity. While Hyd-1 can function in 20% O_2, H_2 oxidation by Hyd-2 is completely inhibited by much lower O_2 concentrations. Furthermore, after exposure to a transient O_2 burst, Hyd-1 recovers rapidly and completely at a relatively high potential, whereas Hyd-2 recovers only partially and at a lower potential. H_2 production by Hyd-2 is much less affected than H_2 oxidation by the presence of O_2, and H_2 production activity can be

sustained for long periods of time in the presence of this gas. Hyd-2 is shown to be more sensitive than Hyd-1 to CO, a molecule known to be inhibitory to many other hydrogenase enzymes. The results are discussed in relation to the possible physiological roles of Hyd-1 and Hyd-2, and the varying conditions under which *E. coli* utilises H_2.

P035

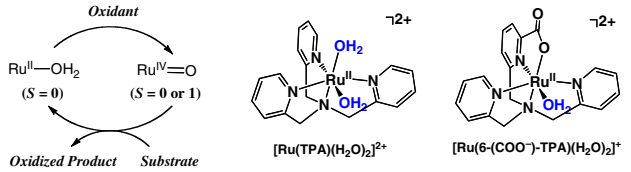
Reactivity of Ru(IV)–oxo complexes derived from proton-coupled electron transfer of Ru(II)–aqua complexes

Takahiko Kojima[1], Yuichirou Hirai[2], Yasuhisa Mizutani[3], Kenichiro Ikemura[4], Takashi Ogura[4], Yoshihito Shiota[5], Kazunari Yoshizawa[5], Shunichi Fukuzumi[2]

[1]Department of Chemistry, University of Tsukuba, Ibaraki 305-8751, Japan.
[2]Department of Material Life Science, Osaka University, SORST(JST), Suita, Osaka 565-0871, Japan.
[3]Department of Chemistry, Osaka University, Toyonaka, Osaka 560-0043, Japan.
[4]Graduate School of Life Sciece, University of Hyogo, Kouto, Hyogo 678-1297, Japan.
[5]Institute for Materials Chemistry and Physics, Kyushu University, Moto-oka, Fukuoka 819-0395, Japan. kojima@chem.tsukuba.ac.jp

Proton-coupled electron transfer (PCET) in which proton and electron are transferred simultaneously plays indispensable roles in biological redox reactions. In the course of water oxidation in photosynthesis, a high-valent manganese–oxo complex is proposed to be formed from a Mn–aqua complex via multistep PCET as a responsible species for the dioxygen evolution.

Inspired by this process, Ru–aqua complexes have been converted to be isolable high-valent Ru–oxo complexes which can oxidize organic substrates. We synthesized novel Ru(II)–aqua complexes with use of tris(2-pyridyl- methyl)amine (TPA) [1] and its derivatives. They allowed us to have a series of Ru(IV)–oxo complexes bearing different spin states. Those complexes exhibited highly efficient and highly selective catalysis in oxidative conversion of organic substrates with similar rate constants and activation parameters. Details of mechanistic insights will be discussed.



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P036

Effects of axial ligands on the electron-transfer vs. proton-coupled electron-transfer reactions of non-heme oxoiron(IV) complexes

Shunichi Fukuzumi[1], Hiroaki Kotani[1], Tomoyoshi Suenobu[1], Seungwoo Hong[2], Yong-Min Lee[2], Wonwoo Nam[2]

[1]Department of Material and Life Science, Graduate School of Engineering, Osaka University, SORST, JST, Osaka 565-0871, Japan.
[2]Department of Chemistry, Division of NanoSciences, Ewha Womans University, Seoul 120-750, Korea. fukuzumi@chem.eng.osaka-u.ac.jp

The effects of axial ligands on the electron-transfer reduction and the proton-coupled electron-transfer reduction of mononuclear non-heme oxoiron(IV) complexes were investigated using $[Fe^{IV}(O)(TMC)]^{2+}$ (**1**) with various axial ligands *X* (**1**-*X*), where TMC is 1,4,8,11-tetramethyl- 1,4,8,11-tetraazacyclotetradecane and *X* is CH_3CN (**1**-$NCCH_3$), CF_3COO^- (**1**-$OOCCF_3$), or N_3^- (**1**-N_3), and ferrocene derivatives as one-electron reductants.

As the binding strength of axial ligands increases, the one-electron reduction potentials of **1**-X (E_{red}, V vs. SCE) are more negatively shifted in the order of **1**-$NCCH_3$, 0.39 V > **1**-$OOCCF_3$, 0.13 V > **1**-N_3, −0.05 V. Rate constants of electron transfer from ferrocene derivatives to **1**-X were analyzed in light of the Marcus theory of electron transfer to determine reorganization energies (λ) of electron transfer [1]. The effect of the axial ligands is the deceleration of the electron-transfer rate in the order of **1**-$NCCH_3$ > **1**-$OOCCF_3$ > **1**-N_3. In sharp contrast to this, the rates of the proton-coupled electron-transfer reduction of **1**-X are markedly accelerated in the presence of acid in the opposite order such as **1**-$NCCH_3$ < **1**-$OOCCF_3$ < **1**-N_3. Such contrasting effects of the axial ligands on the electron-transfer and proton-coupled electron-transfer reactions of non-heme oxoiron(IV) complexes are discussed in light of the counterintuitive reactivity patterns observed in the oxo-transfer and hydrogen-atom abstraction reactions by non-heme oxoiron(IV) complexes.

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P037

Photoinduced hydrogen production with artificial photosynthesis based on chlorophyll–carotenoid conjugated system

Yutaka Amao, Yuko Maki, Yoshiko Fuchino

Department of Applied Chemistry, Oita University, Oita 870-1192, Japan. amao@cc.oita-u.ac.jp

Light-harvesting site in photosynthesis protein consists of Mg chlorophyll-*a*, *b* (MgChl-*a*, *b*) and carotenoid dye such as β-carotene. MgChl-*a* and *b* play an important role in photosynthesis such as light harvesting, the photoinduced electron transfer and so on. On the other hand, carotenoid dyes also have important functions such as the absorption of UV light, photo-protection of MgChl-*a* and *b*, and photosynthesis protein. In photosynthesis protein, MgChl-*a*, *b* and carotenoid dyes are assembled via the hydrogen bond, hydrophobic interaction and coordination bond, not covalently. Thus, MgChl-*a*, *b* and carotenoid dyes can be assembled using hydrophobic interaction of surfactant micellar as photosynthesis protein mimics. In this work, artificial photosynthesis system, anionic water-soluble carotenoid dye crocetin (λ_{max} =536 nm) electrostatically immobilised onto the surface of cationic surfactant cetyltrimethylammonium bromide (CTAB) micellar including MgChl-*a* and *b* (Cro/MgChl), is prepared and applied to the photoinduced hydrogen production system with platinum nano-particle catalyst.

The fluorescence at 680 nm due to MgChl-*a* and *b* is observed. However, the fluorescence at 572 nm due to crocetin is disappeared. In contrast, the fluorescence also is observed at 680 nm with excitation to absorption band of MgChl-*a* and *b* (660 nm). Moreover, the weak fluorescence at 680 nm is observed with 536 nm excitation in the CATB micellar including MgChl-*a* and *b* without crocetin (MgChl). These results indicate that the photoinduced energy transfer from the photoexcited state of crocetin to MgChl-*a* and *b* occurs. When the sample solution containing Cro/MgChl, methylviologen, NADPH and colloidal platinum was irradiated, the hydrogen production was observed.

P038

Electronic structure description of tris(dithiolene) complexes of molybdenum and tungsten

Stephen Sproules[1], Eckhard Bill[1], Serena DeBeer-George[2], Karl Wieghardt[1]

[1]Max-Planck-Institut für Bioanorganische Chemie, Mülheim an der Ruhr, Germany.

[2]Stanford Synchrotron Radiation Lightsource, Stanford, USA. sproules@mpi-muelheim.mpg.de

Pterin-containing molybdenum and tungsten enzymes catalyze a variety of two-electron oxygen redox reactions vital to the global cycling of carbon, nitrogen and sulfur. The metal is coordinated by a bidentate dithiolene ligand that links it to the pyranopterin cofactor, and thus the protein. Molybdenum and tungsten tris(dithiolene) complexes have been utilized to model the active sites of these enzymes in order to investigate the role of the dithiolene as a redox active ligand in the absence of an oxo moiety. There has been recent controversy over the true electronic structure of these compounds, with the neutral complex being described as possessing either a Mo(V) [1] or Mo(IV) [2] center, with one or two oxidized ligands, respectively. We present here a systematic study of the three-membered electron transfer series $[M(dithiolene)_3]^{0/1-/2-}$ (M = Mo, W), and the use of electron paramagnetic resonance (EPR) and X-ray absorption spectroscopic (XAS) studies in concert with theoretical calculations to assign oxidation levels to this electron transfer series.

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P039

Cytochrome P450s involved in oxidative coupling reactions: intermediates involved in generation of the chromopyrrolic acid (CPA) scaffold of rebeccamycin by joint action of RebO and RebD

Tatyana Spolitak, David P. Ballou

Department of Biochemistry, University of Michigan, Ann Arbor, MI 48109, USA. dballou@umich.edu

The cytochromes P450 participate in a wide variety both biosynthetic and biodegradative processes, usually by catalyzing monooxygenation. In addition to oxygenations, P450s also catalyze atypical P450 chemistry such as reductions, isomerizations, and oxidative couplings. This work presents experimental evidence for catalytic intermediates involved in the rebeccamycin biosynthetic pathway, which includes two P450-dependent reactions (RebD and RebP) that effect the carbon–carbon coupling of two modified tryptophans to form the rebeccamycin scaffold. Our results suggest that these oxidative coupling reactions involve the oxo-ferryl species (Cpd I) of RebD. We show that RebD can carry out peroxidase chemistry, implying that it can form Cpd I, thought to be a key oxidant in the coupling reaction of RebD [1]. In the presence of RebO to produce the modified tryptophan substrate (B), Cpd II was observed, suggesting that a nascent Cpd I had oxidized the substrate to form a tryptophan radical. The radical is suggested to form C, which in subsequent steps is coupled to form the rebeccamycin agycone.

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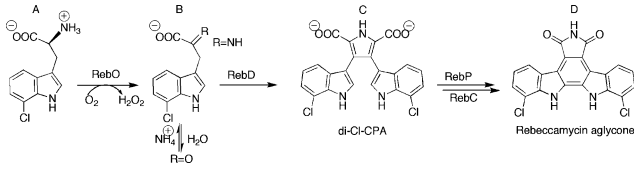

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P040

Towards the understanding of His411–Fe^{IV}=O spectroscopic properties in ferryl intermediate of cytochrome *c* oxidase + O_2 reaction: a theoretical QM/MM, MD approach

Vangelis Daskalakis[1], Stavros C. Farantos[1,2], Victor Guallar[3], Constantinos Varotsis[2,*]

[1]Institute of Electronic Structure and Laser, Foundation for Research and Technology, Hellas, P.O. Box 1527, 711 10 Heraklion, Greece.

[2]Department of Chemistry, University of Crete, P.O. Box 2208, 71003 Voutes, Heraklion, Greece.

[3]Barcelona Supercomputing Center, Centro Nacional de Supercomputación and Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain. vdas@her.forthnet.gr; varotsis@edu.uoc.gr

Cytochrome *c* oxidase (CcO), found in the inner mitochondrial membranes or in many bacteria, catalyzes the four electron reduction of molecular oxygen to water. Four protons are pumped across the inner mitochondrial membrane, by C*c*O, attributing to the electrochemical gradient [1] needed by ATP-synthase. QM/MM and MD calculations are used to probe the spectroscopic characteristics of the ferryl intermediates in aa_3 cytochrome c oxidase (C*c*O) from *P. denitrificans*. We link proton pump activity in C*c*O enzyme to $\nu(Fe^{IV}=O)$ stretching vibrational frequency in a higher level of theory, than previously applied [2], and to the interesting δ(His411–Fe^{IV}=O) bending vibration. We find that the His411–Fe^{IV}=O moiety vibrations become highly coupled depending on the protonation state of the heme a_3 ring A propionate/Asp399 pair and we propose a mechanism of the resonance Raman enhancement of the δ(His411–Fe^{IV}=O) bending vibration due to an 1:2 resonance phenomenon involving Cu_B motion towards heme a_3. Implications of this Cu_B motion for the C*c*O mechanism of action will also be discussed. Proposed experiments can probe only the δ(His411–Fe^{IV}=O) vibration in the 350–360 cm^{-1} lower frequency region of the spectrum, as MD calculations show that by exciting the Fe^{IV}=O bond we can chose to enhance either both ν(Fe=O) and δ(His411–Fe=O) or only the latter.

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P041

Directional electron transfer of four heme cytochrome c_3 investigated by EQCM measurement

Noriyuki Asakura, Hiromu Matsumoto, Takumi Tezuka, Ichiro Okura

[1]Department of Bioenginerring, Tokyo Institute of Technology.
nasakura@bio.titech.ac.jp

A simultaneous measurement of electrochemistry and quartz crystal microbalance (EQCM) applied for monitoring of the cytochrome c_3 and hydrogenase interaction. Cytochrome c_3 has four hemes in one molecule and is the substrate for hydrogenase which catalyses both hydrogen evolution and uptake. Reduced cytochrome c_3 donates electrons to hydrogenase and hydrogen evolution occurs. While oxidized cytochrome c_3 accepts electrons from hydrogenase in the case of hydrogen uptake. The role of each heme in the electron transfer of cytochrome c_3 is not still clarified.

In this study, an electron-donating heme (electron exit) and an electron-accepting heme (electron entrance) were investigated by EQCM measurement.

Cytochrome c_3 was immobilized on a quartz crystal gold electrode and the immobilized cytochrome c_3 redox was controlled by electrode potential. When the immobilized cytochrome c_3 is reduced, hydrogen evolution complex is formed, and otherwise hydrogen uptake evolution is formed when the cytochrome c_3 is oxidized. The complex interchange driven by cytochrome c_3 redox was monitored by EQCM measurement. In order to investigate the electron entrance and exit in cytochrome c_3, two types of cytochrome c_3 immobilized electrode were prepared. One is that the heme I is faced toward electrode, and the other is that the heme IV is faced toward electrode. EQCM measurement for the two electrodes was carried out. The result shows that heme I is the electron entrance and that heme IV is the electron exit.