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Cytotoxicity and DNA binding properties of a chloro glycylhistidinate gold(III) complex (GHAu)

Stefania Carotti [b], Giordana Marcon [a], Maja Marussich [a], Teresita Mazzei [b], Luigi Messori [a], Enrico Mini [b], Pierluigi Orioli [a,*]

[a] *Department of Chemistry, University of Florence, via Gino Capponi 7, 50121 Florence, Italy*
[b] *Department of Pharmacology, University of Florence, via Gino Capponi 7, 50121 Florence, Italy*

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Abstract

The chloro glycylhistidinate gold(III) complex (GHAu) is shown to be fairly cytotoxic towards the established A2780 ovarian carcinoma human cell line either sensitive or resistant to cisplatin. Remarkably, GHAu is far more cytotoxic than the corresponding zinc(II), palladium(II), platinum(II) and cobalt(II) complexes implying that cytotoxicity is essentially to be ascribed to the presence of a gold(III) center. Circular dichroism (CD) spectra, atomic absorption measurements and DNA melting profiles suggest that GHAu in vitro is able to bind DNA, the presumed target for several antitumor metal complexes, and to modify its conformation, even if the observed changes are generally small. Implications of these findings for the mechanism of action of cytotoxic gold(III) complexes are discussed.

Keywords: Gold(III) complexes; DNA; Cytotoxicity; Cancer

* Corresponding author. Tel.: +39-55-2757554; fax: +39-55-2757555.
E-mail address: orioli@risc1.cerm.unifi.it (P. Orioli)

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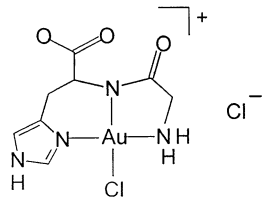
1. Introduction

In the last two decades several studies dealt with the evaluation of gold compounds as potential antitumor agents, much of the work focusing on gold(I) complexes [1–3]. Also, a few gold(III) complexes were screened in vitro and shown to display interesting cytotoxic properties [2]. This is not a surprise since the Au(III) ion is isoelectronic with Pt(II) and both give rise to square planar geometries [4]. The strict relationship to platinum(II) compounds makes gold(III) complexes good candidates for development and testing as potential anticancer drugs but the relatively high kinetic lability and the usually high redox potentials significantly hindered such studies [5,6]. To minimise these effects and render gold(III) complexes suitable for biomedical applications, opportune ligands must be selected; we thought that the GlyHis dipeptide (GH hereafter) might be appropriate for this goal. The synthesis and crystal structure of the GlyHisAuCl complex (GHAu hereafter) were previously described by Lippert et al. [7]. The solution properties, the cytotoxicity and the DNA binding properties of this complex are reported on here. In view of the promising results obtained in the preliminary biological assays, additional pharmacological studies of GHAu and similar compounds, both in vitro and in vivo, are presently being carried out.

2. Materials and methods

2.1. Synthesis of the compounds

The gold(III) complex GlyHisAuCl (Fig. 1) was prepared according to Ref. [7]. The purity of the obtained material was checked through elemental analysis and ^{1}H NMR spectroscopy. The other metal glycylhistidinate complexes were prepared according to the reported procedures [8–10] and checked by the same methods. All other reagents were of analytical grade.



MW=506.09

Fig. 1. Schematic drawing of the chloro glycylhistidinate gold(III) complex.

2.2. Solution studies

GHAu solutions were prepared by dissolving the compound in a 0.05 M phosphate buffer, pH 7.4, containing either 100 mM or 4 mM NaCl. GHAu concentrations were usually $1–2 \times 10^{-3}$ M for the ^{1}H NMR studies and 1×10^{-3} M for the circular dichroism (CD) studies. Calf thymus DNA was purchased from Sigma (St. Louis, MO). The ^{1}H NMR spectra were recorded on an Avance 600 Bruker Instrument. Electronic spectra were carried out on a Cary 5 spectrophotometer; CD spectra on a Jasco J500 dichrograph. Data analysis was performed using the respective software packages. Thermal denaturation studies of DNA were carried out on a Cary 5 instrument equipped with thermostated cuvettes, applying a rate of temperature increase of 0.5°C/min. Data were analysed according to Ref. [11].

2.3. Cytotoxicity studies

For cytotoxicity studies the representative cisplatin-sensitive ovarian carcinoma A2780/S human cell line was used. The platinum resistant A2780/R cell line was produced by repeated 1 h weekly exposure to 50 μM cisplatin of the sensitive parental cell line [12]. Cell lines were maintained in RPMI 1640 medium supplemented with fetal bovine serum (FBS) and antibiotics at 37°C in a 5% CO_2 atmosphere and subcultured twice weekly. Experiments were conducted on exponentially growing cells. Inhibition of cell growth by the various complexes was monitored through the Sulforhodamine B (SRB) assay. The SRB assay was conducted in 96-well plates using RPMI 1640 medium + 5% FBS, according to the procedure described by Skehan et al. [13]. Incubation times of 72 h were used in standard experiments; in some selected experiments, exposure times were reduced to either 4 or 1 h. In all cases results were read after 72 h.

2.4. Atomic absorption measurements

Atomic absorption measurements were carried out with a Varian AA475 instrument. Calibration curves were built up for GHAu. Samples for atomic absorption spectroscopy (AAS) were prepared as follows: aliquots of GHAu solutions were added to buffered calf thymus DNA solutions in such a way to obtain r_i values (added metal to basepair molar ratios) of ≈ 0.1. Samples were left to stand overnight at 25°C. DNA was then precipitated by addition of 70% ethanol, and the pellet washed three times and then resuspended in the buffer. Gold content was measured both in the supernatant and in the pellet through AAS.

3. Results

3.1. Behavior of GHAu in solution

The behavior of GHAu within a physiological environment was followed

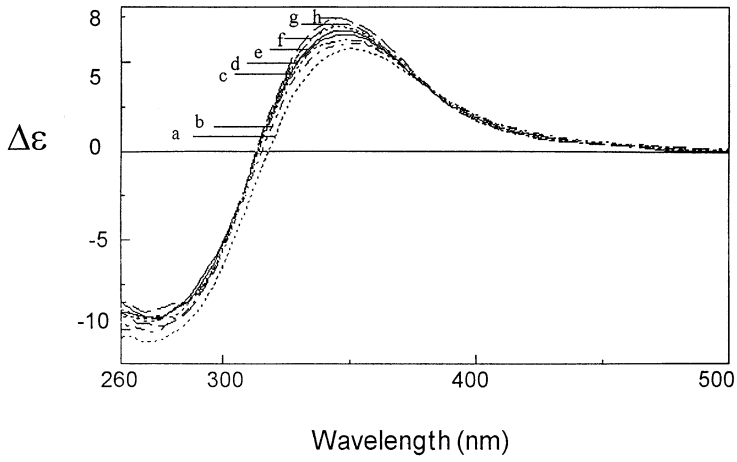


Fig. 2. Circular dichroism (CD) spectra of chloro glycylhistidinate gold(III) complex (GHAu) after dissolution in the physiological buffer. Spectra were recorded at the following times: (a) 30, (b) 60, (c) 120, (d) 180, (e) 240, (f) 300, (g) 360 and (h) 420 min. Conditions: phosphate buffer 50 mM, NaCl 4 mM, pH 7.4, GHAu concentration 1×10^{-3} M, $T = 25$°C.

through CD and ^{1}H NMR spectroscopies. Upon dissolution in a buffer containing 0.05 M sodium phosphate and 0.004 M NaCl, at pH 7.4, GHAu exhibits a characteristic CD spectrum with a positive band at 360 nm and a negative one at 290 nm. By comparison with the CD spectrum of the free ligand these bands are assigned as ligand-to-metal charge transfer bands. The CD spectrum of GHAu, after dissolution in the buffer, undergoes slow changes with time (i.e. a significant increase in the positive band at 360 nm) that reach completion within ca. 7 h (at 25°C); afterwards the complex is stable in the buffer for days (Fig. 2).

Hydrolysis of GHAu under the same conditions was analysed by high resolution ^{1}H NMR spectroscopy. Inspection of the aromatic region of the NMR spectrum revealed, beyond the characteristic 2H and 4H imidazole signals of GHAu (respectively located at 8.71 and 7.45 ppm), the appearance, within a short time, of a number of new species that very likely correspond to hydrolysis products of GHAu — indeed several signals are observed within the imidazole 2H and 4H region. The new signals, whose relative intensities varied significantly with time, may be assigned either to the monomeric aquated species or to various polymeric species, slowly exchanging on the NMR time scale; simultaneously the signals of the initial species progressively decreased in intensity and eventually disappeared. After about 6 h only two predominant species remained in solution, one of which corresponds to the tetrameric species previously described by Lippert (Fig. 3) [7]; afterwards, the ^{1}H NMR spectrum did not change for days. Nonetheless, during all these hydrolysis and/or oligomerisation processes, gold remained in the $3+$ oxidation state as

demonstrated by the electronic and CD spectra, pointing out that dipeptide coordination prevents gold(III) reduction to gold(I) or to metallic gold. Only after very long standing of the complex in the physiological buffer was formation of some metallic gold observed.

3.2. Cytotoxicity of GHAu

The cytotoxicity of GHAu was evaluated towards the human ovarian carcinoma A2780 cell line either sensitive (A2780/S) or resistant (A2780/R) to cisplatin; for comparison purposes the cytotoxicity of cisplatin (CDDP) on the same cell lines was measured. Cytotoxicity profiles are shown in Fig. 4 and Table 1. It is apparent that GHAu exhibits a relevant cytotoxic activity on both cell lines: IC_{50} values fall in the micromolar range and are comparable with those of CDDP. Remarkably, GHAu retains a high cytotoxicity towards the cisplatin resistant cell line: the resistance ratio (i.e. IC_{50} of CDDP on resistant cells/IC_{50} of CDDP on sensitive parental cells) is in fact equal to 1.63 and 10 for GHAu and CDDP, respectively. To obtain more detailed information on the cytotoxic properties of GHAu, comparative studies were carried out with the free ligand and with a number of metal glycylhistidinate complexes — namely GHZn(II), GHCo(II), GHPd(II) and

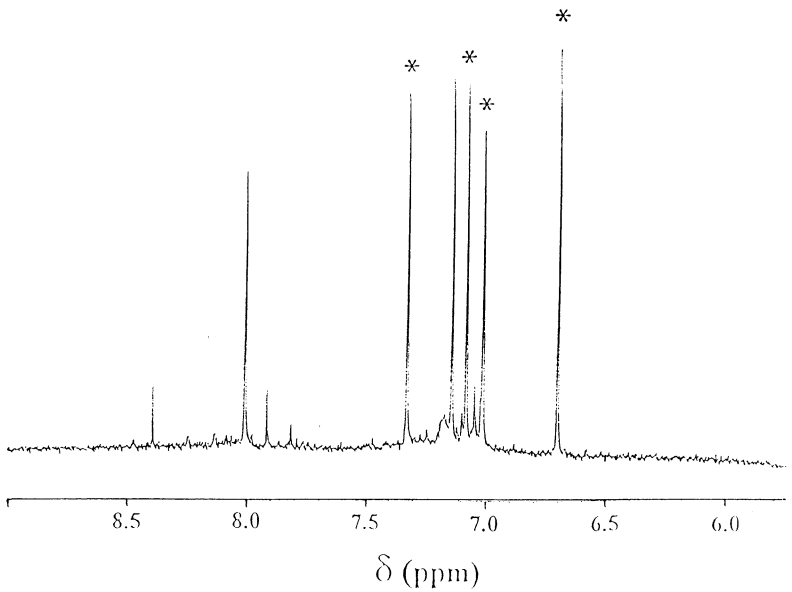


Fig. 3. A total of 600 MHz ^{1}H NMR spectrum of chloro glycylhistidinate gold(III) complex (GHAu) after dissolution in a deuterated buffer containing phosphate 50 mM and NaCl 100 mM, pH 7.4. The spectrum was taken 6 h after dissolution in the buffer, when hydrolysis/oligomerisation reactions reached completion. Resonances labeled with an asterisk belong to the tetrameric species (see Ref. [7]).

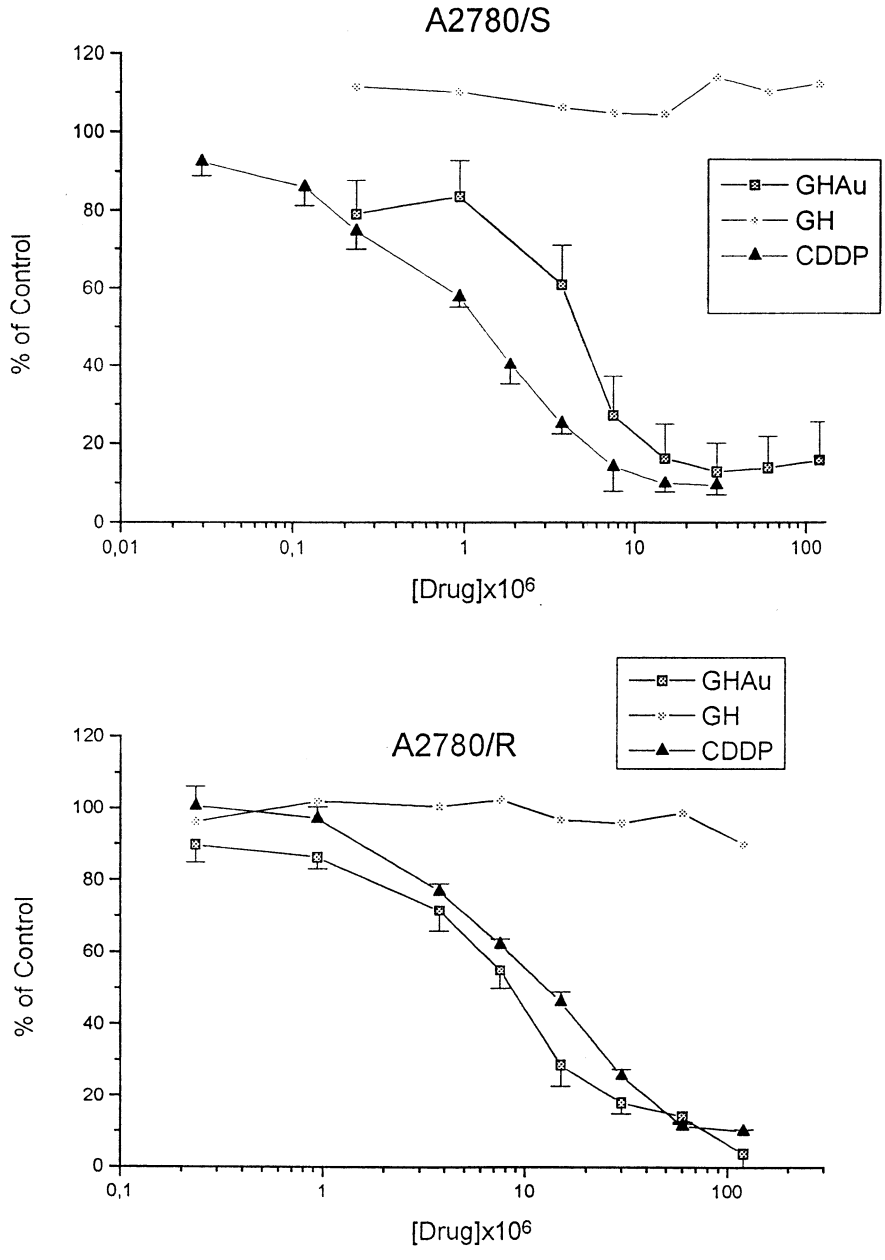


Fig. 4. Drug sensitivity profiles of cisplatin-sensitive and -resistant human tumor cell lines [A2780/S and A2780/R of ovarian carcinoma] towards chloro glycylhistidinate gold(III) complex (GHAu). Graphs (with error bars) show the percentage of growth compared to control upon incubation with increasing amounts of the gold(III) complex. For comparison purposes the curves obtained with cisplatin (CDDP) are reported. The values reported in these graphs are the average of two experiments for GHAu and of five experiments for CDDP.

Table 1
IC_{50} values (μM) of various metal-glycylhistidinate complexes against the human ovarian carcinoma A2780 cell lines sensitive or resistant to cisplatin expressed as mean ± S.E. of at least three determinations or mean of two determinations

	A2780/S	Exp. no.	A2780/R	Exp. no.	Resistance ratio
GHAu	5.2 ± 1.63	3	8.5 ± 2.3	3	1.63
GHZn	165.0 ± 37.75	3	157.5	2	0.93
GHCo	200.0 ± 41.63	3	258.3 ± 111.96	3	1.29
GHPt	117.3 ± 1.45	3	282.5	2	2.06
GHPd	223.3 ± 55.48	3	260.0	2	1.16
CDDP	1.6 ± 0.58	5	16.1 ± 3.85	5	10.0

GHPt(II). The results shown in Table 1 clearly demonstrate that GHAu is by far the most active compound, while the other metal glycylhistidinate complexes are poorly cytotoxic (in all cases the measured IC_{50} values are higher than 100 μM). The GH ligand, as expected, is completely devoid of toxicity. Analysis of the present results unequivocally points out that the important cytotoxicity observed for GHAu must be specifically ascribed to the presence of the gold(III) center.

Additional experiments were carried out in order to establish the dependence of the cytotoxic effects on the exposure time. It was found that cytotoxicity of GHAu strictly depends on the incubation times; by reducing the incubation time with GHAu from 72 h to either 4 or 1 h the measured IC_{50} values on A2780/S cells increased from 5.2 to 30 and > 120 μM, respectively.

3.3. DNA binding properties

The mechanisms responsible for the cytotoxic activity of gold(III) complexes are still largely unknown; however, by analogy with the case of the isoelectronic and isostructural platinum(II) compounds, some authors hypothesised that DNA may represent the primary target also for this class of compounds. Preliminary experimental data support this view [14,15].

To shed new light on this issue, the reaction of GHAu with calf thymus DNA was followed in vitro through CD spectroscopy and analysis of the temperature dependence of the DNA helix-to-coil transition. CD spectra of calf thymus DNA in the UV region were measured upon addition of increasing amounts of GHAu (Fig. 5). It is evident that addition of GHAu brings about minor changes of the CD spectrum of calf thymus DNA that are suggestive of the occurrence of small DNA conformational distortions. More extensive CD spectral changes are observed at higher metal to basepair ratios. An additional experiment was carried out to monitor possible alterations of the gold(III) chromophore following DNA addition: an excess of calf thymus DNA was added to a solution containing GHAu in the reference buffer (in such a way to reach a final metal to bp ratio of 0.1) while recording the visible CD spectrum of GHAu. The CD spectrum of the gold(III) chromophore is scarcely affected by DNA addition, implying that gold remains in

the 3+ state and that the overall conformation of the complex is scarcely affected as well.

In any case, AAS measurements showed that a large amount of added GHAu (about 50%) is associated to DNA after overnight incubation at room temperature, precipitation with ethanol and extensive washing of the pellet ($r = 0.1$), providing evidence for important binding of gold to DNA.

The effects of GHAu on the thermal profile of the helix-to-coil transition were also monitored: it is found that addition of GHAu to calf thymus DNA to a final r value of 0.1, in 50 mM phosphate buffer, pH 7.4, 4 mM NaCl, slightly destabilises the double helix (T_m decreases by ≈ 1.5°C from 82.7 to 81.2°C).

4. Discussion

In the search for new metal-based anticancer drugs a series of gold(III) complexes, with different donors and chemical structures, have been considered [16,17]. The need for ligands capable of stabilising the gold(III) center against hydrolysis/reduction reactions taking place within a physiological environment clearly emerged. Among the synthesised and tested compounds, GHAu, previously characterised by Lippert et al. [7], seems particularly promising.

Notably ^{1}H NMR and CD studies in solution under physiological conditions demonstrate that the presence of the glycylhistidinate ligand prevents reduction and/or precipitation of gold(III) ions even if chloride hydrolysis and oligomerisation reactions are shown to occur during the first hours after mixing. In the authors' opinion the fact that the gold(III) center does not undergo reduction renders GHAu suitable for further biological studies.

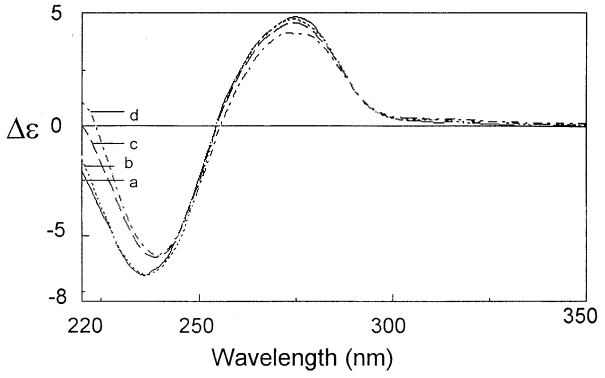


Fig. 5. Circular dichroism (CD) spectra of calf thymus DNA upon addition of increasing amounts of chloro glycylhistidinate gold(III) complex (GHAu). Spectra were recorded at the following r values: (a) 0, (b) 0.1, (c) 0.4 and (d) 0.8 (from top to bottom at 260 nm). Conditions: phosphate buffer 50 mM, NaCl 4 mM, pH 7.4, calf thymus DNA 8×10^{-5} M (basepair concentration).

The in vitro pharmacological evaluation of GHAu toward the reference tumor cell line A2780 demonstrates that GHAu is endowed with relevant cytotoxic effects, only slightly smaller than those produced by equimolar amounts of cisplatin on the same cell line. Notably, resistance to cisplatin does not affect significantly sensitivity to GHAu; indeed the resistance factor determined for GHAu is only 1.6 to be compared with the value of 10 found for cisplatin. The observed cytotoxic properties of GHAu may be specifically ascribed to the gold(III) center: in fact analogous complexes of the glycylhistidinate ligand with bipositive metal ions — namely zinc(II), cobalt(II), palladium(II) and platinum(II) — are virtually inactive when tested on the same cell lines; as expected, the glycylhistidinate ligand is not cytotoxic.

In the assumption that the observed cytotoxicity is a consequence of a direct interaction with DNA, the DNA binding properties of GHAu in vitro toward calf thymus DNA were evaluated. AAS results indicated that GHAu binds calf thymus DNA to a significant extent, whereas CD spectra showed that only minor conformational distortions of the solution structure of DNA are induced. CD spectra of GHAu in the visible show that the gold(III) chromophore is poorly affected by DNA addition and that its oxidation state is not modified. DNA melting studies showed that GHAu addition very slightly destabilises the double helix structure. If one considers that chloride is a good leaving group and that the overall gold(III) chromophore is not largely modified upon reaction with DNA, it is reasonable to hypothesise that the interaction of GHAu with DNA predominantly occurs through monodentate binding to nucleobases.

In conclusion, the present study points out that GHAu holds promise as a potential antitumor compound and that DNA represents a possible target for its action. More extensive studies are now needed to test the effects of GHAu and parent compounds in vivo on representative tumor models.

Acknowledgements

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