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Protein Recognition of Gold-Based Drugs: 3D Structure of the Complex Formed When Lysozyme Reacts with Aubipy^c

Luigi Messori,[†] Maria Agostina Cinellu,[‡] and Antonello Merlino*[,§,∥]

[†]Department of Chemistry, University of Florence, Via della Lastruccia, 3-13, Florence I-50059, Italy

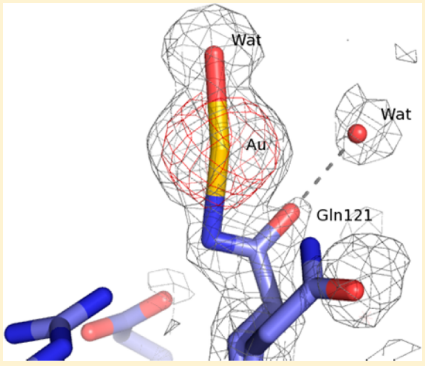
[‡]Department of Chemistry and Pharmacy, University of Sassari, Via Vienna 2, Sassari I-07100, Italy

[§]Department of Chemical Sciences, University of Naples Federico II, via Cintia, Napoli I-80126, Italy

[∥]CNR Institute of Biostructure and Bioimages, via Mezzocannone 16, Napoli I-80100, Italy

Supporting Information

ABSTRACT: The structure of the adduct formed in the reaction between Aubipy^c, a cytotoxic organogold(III) compound, and the model protein hen egg white lysozyme (HEWL) has been solved by X-ray crystallography. It emerges that Aubipy^c, after interaction with HEWL, undergoes reduction of the gold(III) center followed by detaching of the cyclometalated ligand; the resulting naked gold(I) ion is found bound to the protein at Gln121. A direct comparison between the present structure and those previously solved for the lysozyme adducts with other gold(III) compounds demonstrates that coordinated ligands play a key role in the protein−metallodrug recognition process. Structural data support the view that gold(III)-based antitumor prodrugs are activated through metal reduction.



KEYWORDS: *Gold-based drugs, metallodrugs, protein−ligand interactions*

Knowledge of the interactions of medicinal gold compounds with proteins is crucial to define their mechanism of action; indeed these compounds are believed to be activated upon protein interaction[1] and then to exert their biological actions by hitting and selectively damaging a few protein targets. Despite numerous papers reporting on the interactions of gold compounds with proteins, only few structural studies have appeared so far, and very few Au binding sites have been characterized in proteins.[2−5]

By using hen egg white lysozyme (HEWL) and bovine pancreatic ribonuclease (RNase A) as model proteins, we previously solved the X-ray structures of a few protein adducts with gold compounds of medicinal interest.[6,7] These two proteins have often been used to study protein modifications induced by metal-based bioactive agents.[6−15] We found that the reaction of three structurally different medicinal gold compounds, i.e., AuSac2, Auoxo6, and Au2phen (Figure 1), with HEWL invariantly leads to the formation of the same type of metal−protein adduct bearing a gold(I) ion tightly anchored to His15 with Cl^- as the second ligand.[6] In the same work, we tried to crystallize the complex between HEWL and Aubipy^c (Figure 1), i.e., [(bipy^{dmb}-H)Au(OH)][PF_6] (where bipy^{dmb}-H = deprotonated 6-(1,1-dimethylbenzyl)-2,2′-bipyridine), a cyclometalated gold(III) complex exhibiting remarkable anticancer properties in vitro,[16] but attempts to obtain a stable adduct in the solution used to grow HEWL−Aubipy^c crystals (1.0 M NaCl and 0.050 M sodium acetate, pH 4.0−4.4) failed.[6]

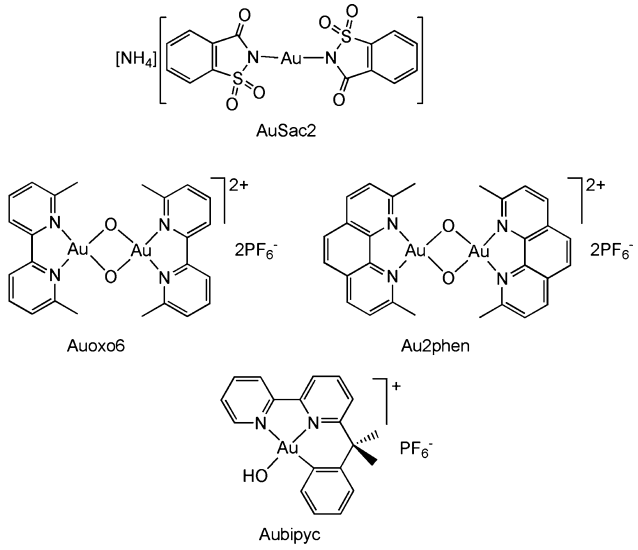


Figure 1. Structure of AuSac2, Auoxo6, Au2phen, and Aubipy^c.

Notably, Au(I) ions bind to His residues even in the complex formed between RNase A and Auoxo6.[7]

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Here we report the crystal structure of the HEWL−gold(I) adduct, solved using X-ray diffraction data collected on crystals of HEWL obtained in the presence of Aubipy^c (1:10 protein to metal ratio) via the hanging drop method. These crystals have been grown using a reservoir solution of 0.6 M $NaNO_3$, 0.1 M sodium acetate pH 4.4, and 20% ethylene glycol.

We have also obtained crystals of HEWL in the presence of Au2phen and Auoxo6 exactly under the same experimental conditions, but inspection of electron density map did not reveal the formation of protein−gold adducts (data not shown).

Crystals have been flash-cooled in a cold gaseous nitrogen stream, without any cryoprotectants, after mother liquor removal (Figure 2). This methodology can yield improved low-temperature crystalline order,[17] higher resolution of X-ray diffraction,[18] and reduced mosaicity.

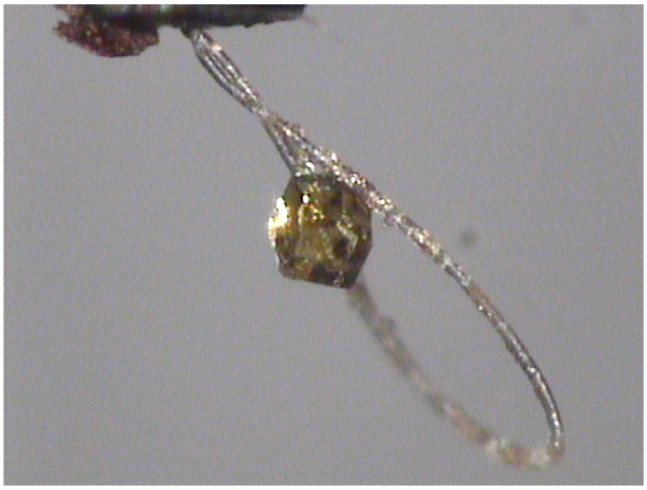

Figure 2. Crystal of HEWL−Aubipy^c mounted in a nylon loop and flash-cooled in the absence of mother liquid and cryoprotectants.

A data set from crystals of HEWL−Au(I) has been collected at 1.80 Å resolution at Institute of Biostructures and Bioimages, Naples, Italy. Each diffraction image was taken by oscillating 1.0°; the integrated intensities were merged and scaled using HKL2000.[19] The results of the data collection are summarized in Table S1, Supporting Information. The structure was solved by difference Fourier method using PDB 4J1A[20] without water and ligands as starting model and subjected to several cycle of restrained refinement using Refmac5.7[21] and rebuilding in Coot.[22]

The final model, which includes 1235 non-hydrogen atoms, refines to an R factor of 0.181 (R_{free} = 0.244). The average B factor for all atoms of the structure is 31.2 Å^2. A full list of the refinement statistics is reported in Table S2, Supporting Information. This model has been deposited in the Protein Data Bank under the accession code 4OOT.

The overall conformation of the protein in the complex (Figure 3) is very similar to that of the metal-free protein (CA root-mean-square deviation from the HEWL structure (PDB code 2VB1)[23] is 0.79 Å) implying that the structural effects of gold coordination are highly localized. The gold atom (occupancy 0.6) is clearly detected by inspection of the electron density maps (Figure 4).

At variance with our previous studies,[6] here the gold atom is bound to Gln121 side chain. The coordination geometry of the metal is completed by a water molecule. The geometry is nearly

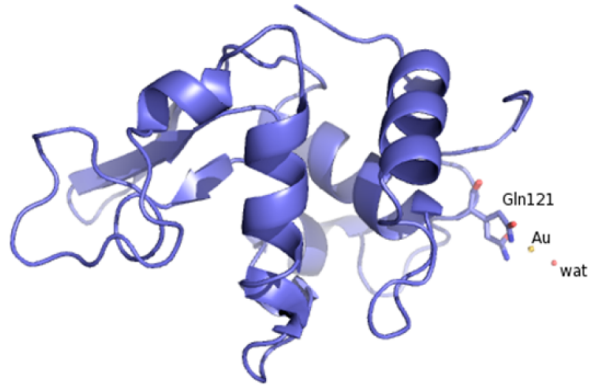

Figure 3. Ribbon diagram of HEWL with gold ion bound to Gln121.

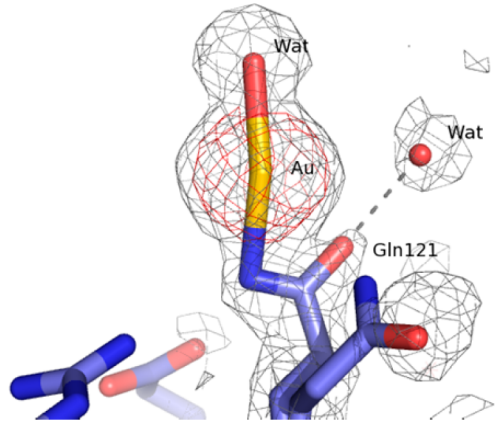

Figure 4. Structural details of the gold center in HEWL−Aubipy^c. $2F_o - F_c$ electron density map is contoured at 2σ (red) and 0.5σ (gray). Gln121 adopts two distinct conformations.

linear, strongly indicating that the gold atom is in the oxidation state +1 (Figures 3 and 4).

Since gold(I) has a preference for soft ligands such as sulfur and phosphorus,[4] secondary Au sites were searched close to Cys or Met residues. Yet, there is no evidence of gold atoms bound to these residues. Similarly, no evidence is obtained for the formation of colloidal gold.

Reduction of gold(III) to gold(I) appears to be a common feature in the behavior of medicinal gold(III) complexes, as already observed in many cases,[6,7] although not a general rule. Indeed, in some cases, gold(III) complexes, particularly of polydentate ligands, have been reported to maintain their oxidation state, provided the availability in the protein of coordination environments that favor a higher oxidation state.[24] Aubipy^c was shown to manifest a reasonable stability of its gold(III) chromophore in aqueous solutions (at physiological pH)[16] and to display a conspicuous resistance toward reduction.[25] It was also found that Aubipy^c retains its cyclometalated ligand upon interaction with HEWL, at least in ammonium acetate solutions.[26] In line with these previous observations, we propose that Aubipy^c could react with the Gln121 amidic side chain to give the gold(III) adduct $[Au(bipy^{dmb}\text{-}H)\{HN(CO)R\}]^+$ (R = protein). This hypothesis is strongly supported by the recent isolation of the acetamido complex $[Au(bipy^{dmb}\text{-}H)\{NH(CO)CH_3\}](PF_6)$, obtained from reaction of Aubipy^c with $CH_3(CO)NH_2$.[27] Upon binding to Gln121, the gold ion may then be reduced by ethylene glycol, thus allowing the detaching of the terdentate ligand. In

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fact, it is known that this latter solvent is capable of providing reducing species, without the need for a separate reducing agent.[28]

X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) studies carried out on AubipyOH ([Au(2,2′-bipyridine)(OH)2](PF_6)), in complex with bovine serum albumin,[29,30] showed that the protein-bound gold center manifests a lower coordination number than in AubipyOH alone, strongly suggesting that gold(III) to gold(I) reduction has occurred upon protein interaction.[29] Data are also in agreement with the fact that gold(I) centers are anchored to the protein mainly through nitrogen donors.[30] Aubipy^c reduction and gold(I) binding to proteins is consistent with the observation that this molecule is able to inhibit mitochondrial thioredoxin reductase, producing apoptotic cell death.[31]

In a more general framework, these data reveal that the formed HEWL−gold adducts are distinct when different gold-based bioactive agents react with the same protein (compare data reported in ref 6 and the present work), although the metallodrug−protein reactions invariantly lead to formation of Au(I) ions.

The higher redox stability of Aubipy^c, imparted by the cyclometalated ligand, with respect to Auoxo6 and Au2phen, may account for the different coordination site.

In this respect, it is worth reminding that the interactions of ruthenium complexes with proteins critically depend on the studied model protein (lysozyme or cyt c): significant changes in reactivity were indeed highlighted for different ruthenium compounds and for different proteins.[14,20,32] The same may hold for gold complexes.

The understanding of the factors that drive formation of protein adducts with gold compounds is important for the biopharmaceutical development of this class of metal-based agents. To this end, we have been investigating the reactions of a variety of gold compounds with the model protein hen egg white lysozyme to examine the effects of the carrier ligand on the formation of gold−protein complexes.

Upon analyzing the structure of the HEWL−gold adduct arising from Aubipy^c and comparing this with those previously solved,[6,7] we may conclude the following:

(a) In spite of its higher redox stability, Aubipy^c undergoes reduction of the gold(III) center after reaction with HEWL, losing its original ligands. These observations indicate that, similar to other cytotoxic gold(III) agents, Aubipy^c likely behaves as a redox activated species.

(b) The resulting gold(I) ions are bound to Gln121. Remarkably, although lysozyme has been very often used to study the interaction between metal-based agents and proteins,[6,8−15] the binding site close to Gln121 has never been reported before for a metal compound.

(c) In the conditions used to grow lysozyme−Aubipy^c crystals, the reaction between Aubipy^c and HEWL produces an adduct that is distinct from that produced in the reaction between the same protein and other cytotoxic Au(III) and Au(I) compounds.[6] This result suggests that the nature of the final protein−gold adduct is strongly dependent on the nature of the starting gold compound and that the carrying ligand plays a major role in determining the type of protein−gold adduct.

As a final comment, we note that cytotoxic gold(III) compounds generally react with the model protein lysozyme upon metal center reduction. Although the in vivo relevance of our data is necessarily limited by the inevitable use of the chosen crystallization conditions (i.e., the chemicals and the pH), these findings suggest that the likely activation mechanism of these prodrugs is based on metal reduction. Gold(III) compounds may undergo redox reactions with proteins rich in sulfur containing residues, but they also interact with His or Gln. Further work on protein−gold(III) compound interactions will help to improve our understanding of the chemistry and reactivity of these candidate drugs.

■ ASSOCIATED CONTENT

Supporting Information

Data collection and processing; structure solution and refinement. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(A.M.) E-mail: antonello.merlino@unina.it.

Author Contributions

The manuscript was written through contributions of all authors.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

HEWL, hen egg white lysozyme; Aubipy^c, [(bipydmb-H)Au-(OH)][PF_6]

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