Supporting Information

The Mode of Action of Anticancer Gold-Based Drugs:a Structural Perspective

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Materials

Hen egg white lysozyme (HEWL- L7651), sodium chloride, sodium acetate, acetic acid were purchased from Sigma. Au_2phen[1a], Auoxo6[1b,c], Aubipy[c] [1d-f] and AuSac2[1g] were synthesised and characterised as described previously (see the respective references). Purity was in all cases > 99% (metal basis). Auranofin (EI-206-0100) was purchased from Vinci-Biochem.

Methods

UV-vis spectrophotometric studies

To assess the compound interactions with HEWL, spectrophotometric studies were performed by a Varian Cary 50 Bio UV-vis spectrophotometer. 10 μl of freshly prepared concentrated solutions (10^{-2}M) of the individual complex in a proper solvent (DMSO for Auoxo6 and Au_2phen; ethanol for Auranofin, and acetone for AuSac2) were added to a solution of HEWL (10^{-5}M) in 50 mM acetate buffer and 1M NaCl at pH 4.5. The concentration of each gold compound in the final sample was 10^{-4} M. Electronic spectra of HEWL were recorded before and after the addition of each gold compound at a stoichiometric ratio of 10:1 (metal to protein). Each samples was monitored by collection of the electronic spectra for 24 hours at room temperature.

Crystallization and X-ray diffraction data collection

Crystals of HEWL-gold compound adducts were obtained by both co-crystallization and soaking procedures. Ligand-free HEWL crystals were grown at 293 K using the hanging drop vapour diffusion method, 1.0-1.4 M NaCl as a precipitating agent in 0.050 M acetate buffer at pH 4.5 and protein concentration 15 mg/mL. Crystals of 0.3-0.4 mm size appeared within 24 hours. After two days, crystals were soaked in a solution formed by gold based drugs dissolved in a proper solvent (DMSO for Aubipyc, Auoxo6 and Au_2phen; ethanol for Auranofin, and acetone for AuSac2) and 1.2 M NaCl in the same buffer. At the final, concentrations of metallodrugs and protein were in at least in 1:1 ratio. Crystals of HEWL in the presence of the gold-based drugs have been also obtained by co-crystallization using complex in a 1:1 ratio. These crystals were obtained at 293 K using 0.6-1.0 M NaCl as a precipitating agent in 0.050 M acetate buffer at pH 4.5. All the crystals were fished with nylon loops and flash-frozen at 100 K in a supercooled nitrogen gas produced by an Oxford Cryosystem (and maintained at 100 K during the data collection) without cryoprotectant, using a procedure recently developed [2]. This procedure partly dehydrate the crystals, enhancing in some cases the resolution of X-ray diffraction data [3]. Complete data sets were collected at the CNR Institute of Biostructure and Bioimages, Naples, Italy using a Saturn944 CCD detector equipped with CuKα X-ray radiation from a Rigaku Micromax 007 HF generator. Data were processed and scaled using HKL2000 [4]. Details of data collection statistics of the crystals where gold adducts are formed in significant amounts are reported in Table S1.

Structure resolution and refinement: The structures were solved with Fourier difference method, using the PDB file 4J1A [5], without water molecules and ligands, as the starting model. The refinement was carried out with CNS [6] or REFMAC [7], model building and map inspections were performed using O [8] or COOT [9]. Statistics on the refinements are reported in Table S1. Structure validation has been carried out using Procheck [10]. Coordinates and structure factors have been deposited in the Protein Data Bank (PDB codes 4LFP, 4LGK, 4LFX, for HEWL-AuSac2, HEWL-Au_2phen, HEWL-Auoxo6 complexes, respectively).

Table S1.Data collection and refinement statistics for the analyzed HEWL-gold compound adducts

	HEWL-AuSac$_2$	HEWL-Au$_2$phen	HEWL-Auoxo6
PDB code	4LFP	4LGK	4LFX
Crystal colour	pink	yellow	yellow
Note	Crystal obtained by co-crystallization	Crystal obtained by co-crystallization	Crystal obtained by soaking
Data-collection temperature (K)	100	100	100
Data reduction			
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$
Unit-cell parameters			
a = b = (Å)	78.04	77.43	78.27
c = (Å)	37.32	37.19	37.21
Molecules per asymmetric unit	1	1	1
Observed reflections	86345	22175	35615
Unique reflections	12749	7204	6133
Resolution (Å)	50.0-1.72 (1.75-1.72)	50.0-1.95 (1.98-1.95)	50.0-2.10 (2.14-2.10)
Completeness (%)	99.3 (99.8)	84.3 (98.5)	86.2 (68.2)
Rmerge†	7.5 (54.8)	11.3 (45.1)	13.9 (20.1)
I/σ(I)	36.7 (2.2)	6.1 (3.8)	5.8 (3.5)
Multiplicity	6.8 (3.8)	3.1 (3.1)	5.8 (2.0)
Refinement			
Resolution (Å)	50.0-1.72	50.0-1.96	50.0-2.10
number of reflections in working set	10435	6194	5407
number of reflections in test set	576	325	302
R factor/Rfree (%)	0.180/0.236	0.211/0.300	0.204/0.281
Number of protein atoms	1029	1023	1035
Occupancy of Au	0.6	0.4	0.5
Number of water molecules/other ions (Na^+, Cl^-)	135/4	167/3	108/4
B-factor (Au atom, Å^2)	41.7	49.3	42.5
Ramachandran values (%)			

*Crystals of HEWL-Auoxo6 adduct obtained by co-crystallization diffract to 2.8 Å. Electron density maps show, also in this case, the binding of one Au(I) ion close to His15. Au ion refines with 0.5 occupancy and a B-factor value of 53.9 Å

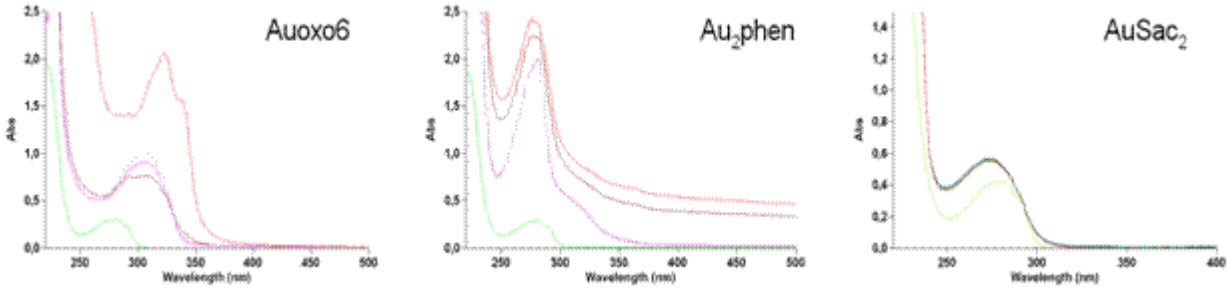

Figure S1. Time course UV-vis spectra of each gold compound in the presence of HEWL (green line) dissolved in 50 mM acetate buffer/ 1M NaCl pH 4.5 over 24 h incubation. Concentration of the complex is 10^{-5} M (with a metallodrug-protein molar ratio of 10:1). The figure shows spectra recorded after 0 h (red line), 1 h (brown line), 12h (pink line) and 24 h (grey line). Auoxo6 and Au_2phen manifest large spectral changes when incubated in the reference buffer, implying that they are undergoing relevant chemical transformations and/or degradation (most likely gold(III) reduction and consequent ligand detachment). Reduction attenuates the interaction of gold center to its ligands and facilitates gold transfer to other ligands and ultimately to the protein. This means that probably, in the case of Auoxo6 and Au_2phen, gold is first reduced and released from its orginal ligands; then it binds the protein, though the two latter processes - ligand release and protein binding- may be somewhat overlapped. The case of the gold(I) complex Ausac2 features a different situation where ligand exchange is the main molecular mechanism accounting for adduct formation. In our case, it is evident that upon interactions with the protein both saccharinate ligands are displaced. Moreover, the presence of high concentrations of chloride in the medium may play a role in the overall reaction process.

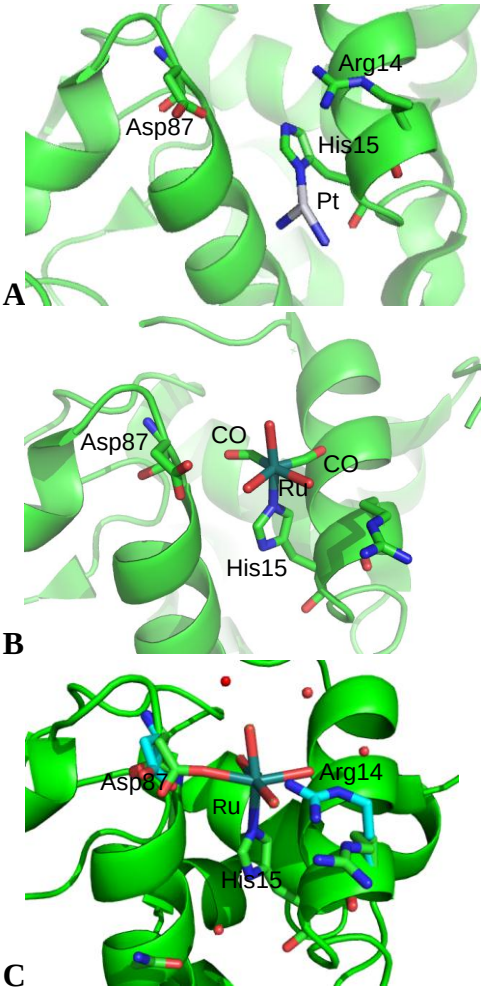

Figure S2. Interactions between HEWL and metal (Pt and Ru) containing drugs. In all cases, metal binding preferentially occurs at His 15. A) complex with cisplatin, pdb code 2I6Z [11]; Pt(II) has occupancy equal to 0.3. It is bound to the the ND1 of His 15 and to the nitrogens of two ammonia molecules in cisplatin. The fourth ligand is not detected. C) complex with [fac-Ru(CO)(3)Cl(κ(2)-H(2)NCH(2)CO(2))], a Ru(III) containing CO releasing molecule, pdb code 2XJW; D) complex with AziRu, a Ru(III) containing complex analogous to NAMI-A, pdb code 4J1A [5]. In the last two cases, Ru(III) is coordinated to NE2 atom of His15. Covalent bonds are shown in solid stick. Arg14 and Asp87 side chains are also shown

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