The Reactions of Gold(0) with Amino Acids and the Significance of these Reactions in the Biochemistry of Gold

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Gold(0) dissolves in a wide range of aqueous amino acid solutions exposed to dioxygen. In particular, the thiol-containing molecules cysteine, penicillamine and glutathione give solutions of gold complexes which can be identified by circular dichroism. Animal experiments suggest that gold complexes can be absorbed readily through skin. Gold concentration in human skin which had been in prolonged contact with gold and gold concentrations from the skin of patients treated with Myocrisin, a gold(I) compound, are reported. The possible significance of these results in terms of erosion of jewelry and skin-irritant reactions is discussed.

Introduction

Gold can become involved in human biochemistry through four main paths,

(a) by normal ingestion through food and drink
(b) by the wearing of jewelry
(c) from dental fillings
(d) as a drug in the treatment of rheumatoid arthritis [1] (chrysotherapy)

Many sources of water contain gold, albeit at very low concentrations and, consequently, some absorption by plant and animal life is possible. It is only in a limited number of cases [2], however, that plants containing measurable quantities of gold (up to 1 ppm) have been found – generally in areas where there is a significant amount of gold in the soil. Thus, ingested gold (a) is very low in concentration and usually beneath detectable limits. Little is known of the effect of the wearing of jewelry (b) or of the effect of dental fillings (c) on body gold concentrations, but there are suggestions that gold jewelry can produce an irritant reaction in some subjects.

Patients who have received a course of gold injections (d) may retain gold for many years after the injections have been stopped [3]. Gold from this source has been found widely dispersed in the body, in serum, organs and skin [4]. Whether it is stored as insoluble gold(I) complexes or as gold(0) is, as yet, unknown. Both are possible, in that there are present *in vivo* many thiols in membranes and proteins capable of stabilizing gold(I) as gold(I)–thiol complexes. On the other hand, if there is no easily accessible thiol, reduction to gold(0) will readily take place.

Gold has three common oxidation states – (0), (I) and (III). The chemistry of gold is dominated by the ease with which the equilibrium between these oxidation states can be altered. In general, gold(III) is stabilized by 'hard' [5] ligands such as oxygen or nitrogen donors or halide ions. Gold(I) prefers 'softer' [4] sulphur of phosphorus-donor ligands. In the presence of reducing agents and in the absence of ligands capable of stabilizing gold(I) complexes, both gold(III) and gold(I) are easily reduced to gold(0).

Recent work has suggested that the dissolution and uptake of gold(0) in plants occurs through ligands secreted from the roots – in particular cyanide and amino acids [6]. In this paper we have examined the solubility of gold *in vitro* in solutions of different amino acids. Further, animal experiments involving the diffusion of gold complexes through skin are reported as well as gold concentrations in human skin samples from below gold rings and of skin samples from patients undergoing chrysotherapy. The purpose of this study was to assess the ability

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TABLE I. The Effect of pH on the Solubility of 24 ct Gold in 10^{-1} *M* Solution of Cysteine, Glutathione. Penicillamine, Alanine and Histidine Solutions. Solutions were Analysed after Standing in Contact with the Gold for one Month.

	pH	gold (μg/ml)	appearance
L-cysteine	1.2	0.3	black precipitate
	7.2	2.0	white/black precipitate
	9.5	22.1	white precipitate
glutathione	1.6	0.6	black precipitate
	7.2	8.0	black precipitate
	9.5	33.1	black precipitate
D-penicillamine	1.6	0.4	black precipitate
	7.2	10.2	black precipitate
	9.5	18.5	black precipitate
L-alanine	1.6	0.2	clear solution
	7.2	0.1	clear solution
	9.5	0.2	clear solution
L-histidine	1.6	1.2	faint black precipitate
	7.2	2.1	faint black precipitate
	9.5	1.8	faint black precipitate

of amino acids to oxidatively dissolve gold and to consider whether gold might be dissolved by sweat and hence could be a possible source of allergic reactions in skin.

Experimental

A piece of gold foil of known purity weighting approximately 30 mg was placed in 20 ml of 10^{-1} *M* aqueous solution of the appropriate amino acid, and the pH adjusted as required with dilute hydrochloride acid or sodium hydroxide. 5 ml of 10^{-1} *M* hydrogen peroxide was added in some cases. The details of these experiments are specified in the Results section. Gold colloid was prepared by the addition of excess stannous chloride in hydrochloric acid to sodium tetrachloroaurate in aqueous solution. An estimated five-fold excess of amino acid was added and the ciruclar dichroism spectra recorded after one week. Gold [7] and copper concentrations were determined by atomic absorption spectrometry using a PE72 Spectrophotometer and a PE HGA72 carbon furnace atomiser.

Wistar AG strain hairless rats were used in groups of five. They were treated daily for four days with the test compounds, which were applied in ethanolic vehicle to a 2 × 2 cm area of anterior dorsal skin and allowed to evaporate. On day five, the animals were killed under ether and bled out by the pharyngeal route. The gold solutions used were 20 mg/day in 0.2 ml ethanol of (a) Myocrisin (sodium gold(I) thiomalate) and (b) Auranofin (2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-5-triethylphosphine gold(I)). Serum separated from each blood sample was analysed for gold as before [7]. Skin samples were obtained from patients and the samples dried and analysed as previously described [9].

Results

The gold concentrations in μg/ml obtained from 10^{-1} *M* amino acid solutions after fourteen days in contact with colloidal gold were as follows. glycine 0.496, leucine 0.104, alanine 0.150, asparagine 0.252, histidine 2.160, tryptophan 0.841, cysteine 22.02, penicillamine 18.35. CD spectra were obtained from these solutions with cysteine, penicillamine, glutathione and alanine. The major peaks were,

L-cysteine solution (mμ)	300(+)	370(–)	
glutathione solution	310(+)	360(–)	
L-alanine solution	315(+)	388(–)	
D-penicillamine solution	400(–)	460(+)	510(+)

Similar spectra but with lower signal-to-noise ratios were obtained when the same amino acid solutions were left in contact with massive 24 ct gold for the same time.

Different gold concentrations were obtained by varying the pH of the test solutions (Table I). Raising the pH increased the solubility of gold in solutions of thiol-containing ligands. With the non-thiol-containing amino acids, changes in pH had little effect on solubility. Precipitates were observed in a number

TABLE II. Gold and Copper Concentrations (μg/ml) from 9, 18, 22 ct. Gold in Histidine and Glycine (10^{-1} *M*) Solutions ± Hydrogen (10^{-1} *M*) Peroxide.

		gold (no hydrogen peroxide)	copper (no hydrogen peroxide)	gold (with hydrogen peroxide)	copper (with hydrogen peroxide)
histidine	9ct	4.7	1.2	11.1	21.8
	18	1.9	0.8	7.1	1.3
	22	1.2	0.6	6.8	0.6
glycine	9	0.6	0.3	4.6	1.0
	18	0.3	0.3	3.6	0.5
	22	0.2	0.3	3.2	0.4

TABLE III. Gold in Skin Analyses (μg of dry weight).

(a) Skin samples below gold rings on fingers 0.09, 0.07 μg/g.
(b) Skin samples from patients with rheumatoid arthritis receiving chrysotherapy and showing a generalised skin rash.

Unaffected	Skin from rash
(μg/g)	(μg/g)
11.8	4.9
0.9	1.1
1.2	0.3
30.2	26.4

of bases. These were removed and, in each case, found to contain gold. The black precipitate obtained from cysteine at pH 1.6 was almost pure gold (96.1% Au), whereas the white precipitate at pH 9.5 contained 9.6% Au and appeared to be mainly cystine (from CH and N analysis and IR spectra).

Table II lists the results obtained for *9, 19 and 22 ct gold* in massive form in the absence and presence of hydrogen peroxide – the latter added to provide a stronger oxidant than dioxygen. Apart from gold, the other major constituent in these alloys is copper and the analytical figures for copper and gold were:

9ct – 58.9%, Au, 40.6% Cu
18 ct – 89.5% Au, 9.1% Cu
22 ct – 95.6% Au, 6.1% Cu

Animal experiments were carried out to see if gold could be absorbed across skin into blood – the results suggested that this is surprisingly easy. The serum analyses for gold gave values of 3.07 ± 0.35 ppm and 0.82 ± 0.15 ppm for Auranofin and Myocrysin, respectively. Comparative data on the skin absorption of gold compounds into blood in humans is not readily obtainable. However, skin samples taken from below ring fingers showed measurable amounts of gold, indicating that dissolution of gold and absorption of the complexes formed can occur (Table IIIa). For comparison, the skins of a number of patients receiving gold injections and showing skin rashes were also analysed (Table IIIb). These showed a wide range of results. In cases where a biopsy included an inflamed and normal area of skin, the gold concentration in the inflamed area was often less than in the non-inflamed area.

Discussion

The results show that gold(0) in air is soluble in a wide range of amino acids, with cysteine, penicillamine and histidine being the most effective. Using colloidal gold, more concentrated solutions (~10^{-4} *M*) could be obtained and the CD spectra were better defined. The cysteine–gold CD spectrum is fairly typical of a gold(I)–cysteine complex (also gold(I)–glutathione) and the penicillamine spectra typical of a gold(III)–(penicillamine)$_2$ complex [10]. The alanine complex is probably also gold(I) but, since the complex is unstable and repeated attempts to isolate it failed, the oxidation state of the gold in the complex is unknown.

The effect of pH on the complexing of gold with cysteine, glutathione, penicillamine, alanine or histidine is shown in Table II. With the thiol ligands, there was an increased concentration at higher pH values. However, in all but the alanine solutions, precipitates occurred, making comparisons rather difficult. These precipitates presumably arise because the gold complexes formed either are insoluble and precipitate or are unstable and produce a precipitate of gold(0).

The effect of using different-purity gold (7, 18, 22 ct) is shown in Table II. Histidine and glycine were used as potential ligands. The addition of hydrogen peroxide to a comparable series of solutions to examine the effects of adding an oxidising agent facilitated the oxidation of gold(0). The thiol ligands were not used in this series of experiments, as they are easily oxidised to the disulphide by hydrogen peroxide. The results suggest that (a) the higher the copper content, the more gold and copper dissolves and (b) the presence of hydrogen peroxide facilitates the oxidative dissolution of the metal. Since copper is known to dissolve easily in amino acid solutions [11], the enhanced solubility of gold could be due to the increased surface area exposed by the dissolving copper.

These results suggest that gold metal is soluble in a range of aqueous amino acid solutions. Thus gold jewelery, if in close contact with skin, could be slowly soluble in sweat and therefore the thinning of gold rings with time, thought to be due to abrasion, could also be due to dissolution.

Some of this dissolved gold complex could then be adsorbed onto and perhaps through the skin. Animal experiments show that diffusion of gold complexes through the skin of rats into sera occurs. However, in humans the evidence is less direct. In two cases examined, analyses of samples of skin from below ring fingers did show a measurable amount of gold, although no gold was detected in the sera. There is also a history [12] of patients who, on starting chrysotherapy, produce rashes specifically in those areas of this skin which has been in regular contact with gold jewelry. These rashes are different, both in speed of appearance and specificity of site, from the fairly frequent skin rashes associated with gold injections. It is tempting to suggest that these specific rashes are examples of localised gold irritancy resulting from injected gold reaching a part of the skin already sensitised by absorbed gold. The figures in Table III for the gold concentrations in skin samples show that the values found below ring fingers are only a little below some of the values found from the skin of patients with more generalised Myocrisin-induced rashes. The wide range of values found in the latter group suggests that absolute concentrations are not important, and differences between inflamed and non-inflamed areas are probably due to increased blood circulation and increased exfoliation in the inflamed area. There are also examples of people who cannot wear gold jewelry and who prefer silver or platinum mountings because of irritant skin reactions. However, further work is necessary to confirm that gold alone is the cause of these reactions and that copper or other metals are not also involved in this form of irritancy.

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