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The Protein Chemistry of Antiarthritic Gold(I) Thiolates and Related Complexes

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Our laboratory has extensively examined the inorganic biochemistry of antiarthritic gold drugs and selected analogues, focusing on their protein chemistry. The reactions of oligomeric gold(I) drugs and the monomeric oral drug, auranofin, with serum albumin, hemoglobin and metallothionein and the nature of the gold binding sites on these proteins are described in this Comment. A possible mechanism for oxidation of triethylphosphine in serum and additional insights into the pharmacology of gold drugs are discussed.

INTRODUCTION

Chrysotherapy, the treatment of rheumatoid arthritis (RA) with gold complexes, is probably the longest-established of any currently used inorganic therapeutic regimen. The name chrysotherapy is derived from *chrysos*, the Greek word for gold. Forestier, a French physician, reported in 1929 that gold complexes apparently had greater efficacy on RA than on tuberculosis for

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which they were then prescribed.[1] Subsequent multi-center double blind studies confirmed that chrysotherapy can actually induce a remission of the disease state. The early use of chrysotherapy was marred by serious toxicity and occasional deaths and cortisone temporarily replaced it as the preferred treatment. Disenchantment with the side effects of cortisone and the availability of Atomic Absoption Spectroscopy for monitoring serum gold levels of patients led to a resurgence of gold therapy beginning in the early 1970s.

The introduction of Auranofin, a second-generation chrysotherapeutic agent, has stimulated interest in the biochemistry of gold. Three major reviews appeared in the period 1976–1980.[2,3,4] Two subsequent symposium volumes have collected further information on the subject.[5,6] At present about a dozen inorganic, biochemistry and pharmacology laboratories are pursuing various fundamental questions related to the mechanisms of chrysotherapy. Although these labs are many fewer than the multitude studying platinum anti-tumor agents, the progress in gold biochemistry during the past decade has been remarkable.

Myochrysine (AuSTm) and solganol (AuSTg), used clinically in the United States, are oligomers of gold(I) (Fig. 1). AuSTm was shown by WAXS to be an open chain structure in which the gold ions are linked by bridging thiomalate ligands.[7] Both AuSTm and AuSTg are more complex than indicated by the simple formulas. AuSTm contains an excess of the thiolate, consistent with the open chain structure and AuSTg, presumed to be a cyclic structure contains an oxidized form of the ligand. Auranofin, in contrast, is a well defined monomeric complex with triethylphosphine and 2,3,4,6-tetra-O-acetyl-β-1-D-thioglucose ligands (Fig. 1). The coordination about gold is essentially linear ($\angle$SAuP = 173.6°) with conventional Au–S and Au–P bond lengths of 231 pm and 227 pm, respectively.[8]

Gold is spectroscopically reticent. One might even describe it as spectroscopically incompetent. The NMR signals of the 100% abundant isotope gold 197, for which $I = \frac{3}{2}$, has never proven to be chemically useful. The principal oxidation states Au(I) and Au(III) are d^{10} and spin-paired d^8 configurations, precluding ESR studies. The UV-visible spectra have been cleverly exploited in some cases,[9] but are often swamped by the ligand absorbances, especially in the case of protein complexes.

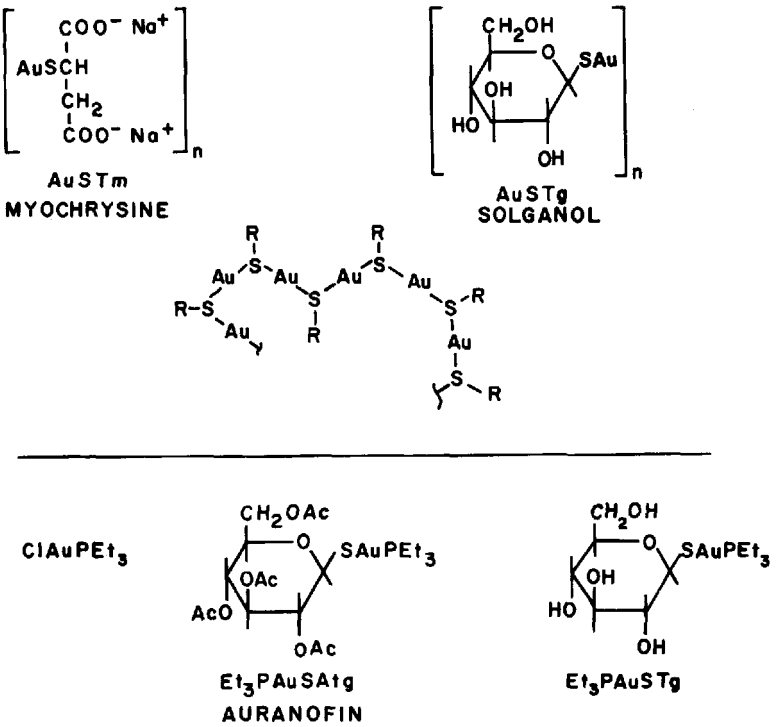


FIGURE 1 Structures of (above) oligomeric gold(I) thiolates AuSTg and AuSTm and (below) Et_3PAuCl, auranofin, and deacetylated auranofin.

Two somewhat esoteric techniques, EXAFS/XANES and Mössbauer spectroscopy, can provide useful information. Extended X-ray Absorption Fine Structure Spectroscopy and X-ray Absorption Near Edge Spectroscopy can provide information about the coordination numbers, oxidation state and ligating atoms of gold complexes.[10,11] Mössbauer Spectroscopy provides information about the coordination number and geometry, oxidation state and to a lesser degree, the type of ligands bound to gold.[12] Sadler[13,14] and Stephan[15] demonstrated the utility of ^{31}P NMR as a probe for reactions of auranofin and its analogues with proteins and we have exploited the power of this technique to probe many systems. It is clear from the literature, however, that the brute force combination of chromatographic separations with atomic absorption

spectroscopy have contributed much to our current understanding of gold(I) biochemistry.

Inorganic chemists have now prepared complexes of gold in five oxidation states, −I, I, II, III, and V, in addition to the elemental state, gold(O). The −I, II, and V states occur only under special conditions of ligation, solvent, etc. and there is at present no reason to believe that they are significant in biological systems. The injectable and oral gold drugs presently in clinical use are all gold(I) complexes. *A priori*, it is possible that oxidation to gold(III) or reduction to gold(O) may occur after their administration. Indeed, deposits of gold, called aurosomes, found in various tissues and readily identified because they are electron dense, were often suggested to be colloidal gold. Collaborative EXAFS/XANES studies between our laboratory and Prof. Elder at the University of Cincinnati demonstrated that the deposits are predominantly gold(I) coordinated to two sulfur ligands.[11] $AuCl_4^-$ can be reduced by free sulfhydryl groups,[2,3,4] by methionine residues,[16] and even by the disulfide bonds of cysteine, oxidized glutathione, or insulin.[17,18] The latter result was demonstrated by cleavage of the insulin α- and β-chains by $AuCl_4^-$ (Fig. 2). Thus, there exist at least three mechanisms by which gold(III) can be reduced *in vivo*. Conversely, Smith, Brown and co-workers[19] have identified conditions under which oxidation to gold(III) can be effected by molecular oxygen.

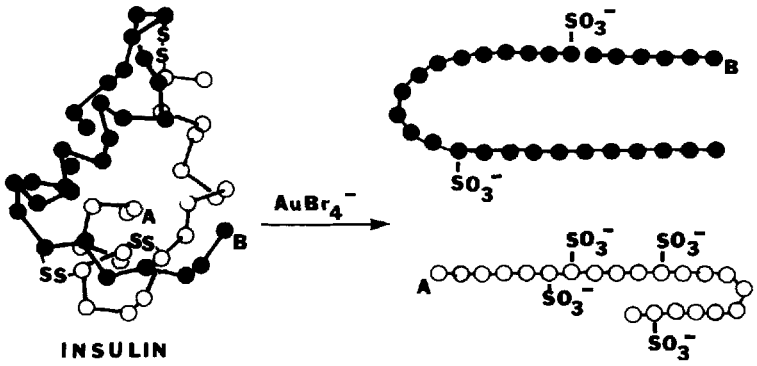


FIGURE 2 The cleavage of insulin into its oxidized A and B chains demonstrates that protein disulfide bonds are active reductants of gold(III) complexes.

While there may be specific macromolecules or cells which stabilize metallic gold or gold(III), none have been identified to date. Thus, it is reasonble to assume that the bulk of gold in circulation and in tissues will be gold(I). Our studies with isolated proteins, described below, support this conclusion.

Our recent contributions to the field have focused on the chemical reactions of the gold drugs and selected analogues with proteins that are potential ligands or binding sites *in vivo*. In this Comment, our results with serum albumin, hemoglobin, and metallothionein will be reviewed and compared to those from other laboratories. The emphasis will be on the inorganic biochemistry of the reactions. For each protein a short introduction will describe its relevant properties.

I. HEMOGLOBIN

Hemoglobin (Hb) is well known to inorganic chemists as the prototypical oxygen carrier. The reversible binding of dioxygen to the four ferrous ions of the heme groups and the subtleties of cooperativity among the four subunits ($\alpha_2\beta_2$) still engage legions of inorganic biochemists. Less well known, but important as precursors to our gold work, are the extensive studies of hemoglobin reactions with mercury compounds, prompted by their use as thiol reagents. Human Hb contains six thiol groups, one on each α chain (α-104) and two on each β chain (β-112 and β-93) (Fig. 3). When the protein is in its native state, only the β-93 cysteines are reactive toward thiol reagents. The α-104 and β-112 thiols are buried in the interior of the protein and are accessible only after denaturation. Gold has been observed to accumulate in red blood cells after administration of auranofin to patients and laboratory animals and after administration of gold(I) thiolates to tobacco smoking patients. Thus, gold might bind to hemoglobin during therapy, provided that other, higher-affinity rbc binding sites do not outcompete the hemoglobin.

Auranofin and Analogues. Sadler and co-workers were the first to report reactions of hemoglobin with gold complexes.[13,14] When red blood cells were incubated with Et_3PAuCl (8 mM), the binding of

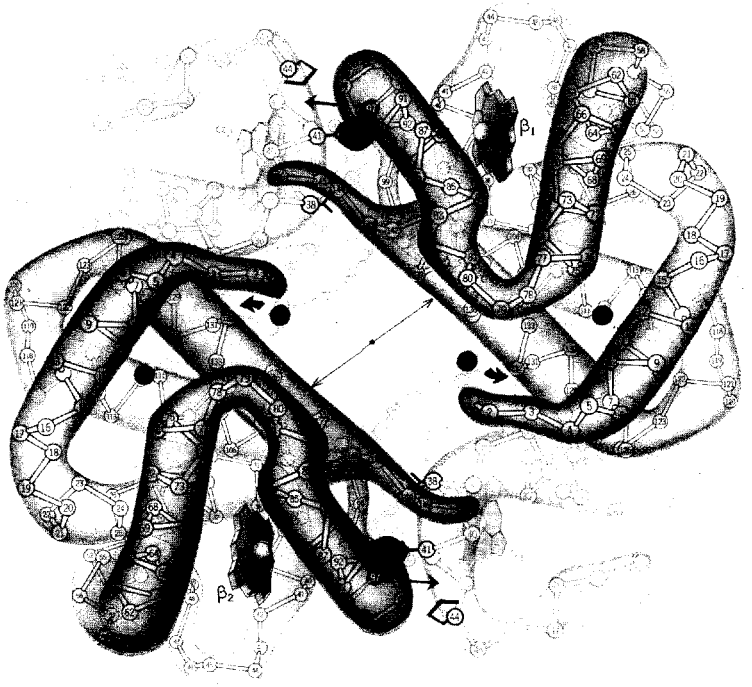


FIGURE 3 The structure of hemoglobin showing the α- and β-chains with the cysteine residues. Cys-β-93 residues (●) are near the surface and accessible to sulfhydryl reagents. Cys-α-104 and Cys-β-112 residues (•) are in the interior and accessible only under denaturing conditions.

Et_3PAu^+ to two thiolate groups was detected by ^{31}P NMR. These were identified as hemoglobin and glutathione.[14] Subsequent studies in our laboratory confirmed that the Cys-β-93 residues were the principal binding site of gold.[20] When auranofin and two analogues, Et_3PAuCl and $Et_3PAuSTg$, were compared, it was found that auranofin reacted only slightly, $Et_3PAuSTg$ to a somewhat greater extent, and Et_3PAuCl completely at this site. Thus the reaction can be formulated as an equilibrium ligand displacement which occurs at gold(I) and is sensitive to the displaced anion[20]:

$$nEt_3PAuX + Hb(SH)_2 \underset{X^- = Cl^- >\!\!> TgS^- \; > ATgS^-}{\rightleftharpoons} Hb(SAuPEt_3)_n(SH)_{2-n} + nX^- \quad (1)$$

Covalent modification of the Cys-β-93 sites by a reagent such as iodoacetamide eliminates their reaction with gold:

$$Et_3PAuX + Hb(SCH_2CONH_2)_2 \longrightarrow \text{no reaction} \qquad (2)$$

Excess Et_3PAuCl is ble to populate additional weak binding sites, whose ^{31}P NMR signature (25, 28 ppm) is consistent with binding to histidine or possibly methionine residues.[20] This reaction occurs for the sulfhydryl-modified protein and after saturating the Cys-β-93 sites of the unmodified protein. $Et_3PAuSAtg$ does not react at these sites, and $Et_3PAuSTg$ reacts only weakly. These weak binding sites are primarily of academic interest, because they are populated only when the gold concentration is more than twice that of hemoglobin, a situation not likely to be achieved *in vivo*.

Another interesting reaction which occurs at high concentrations of Et_3PAuCl is denaturation of the hemoglobin, leading to the formation of a deep green colored met-Hb which contains 20 to 30 equivalents of bound gold and a high spin ferric ion.[21] The kinetics of formation of the met-Hb are biphasic and complex. Superoxide ion release may be involved in the reaction.[21] The Cys-β-93 strong binding sites and the weak binding sites that we observed at lower Et_3PAuCl/Hb ratios[20] are certainly populated as a prelude to the oxidation. The formation of the cystienyl gold complex is not a prerequisite, however, since NEM-modified hemoglobin also reacts in this manner.[21] The oxidation, like the population of the weak binding sites observed by ^{31}P NMR, occurs at gold/HB ratios far larger than those achieved during therapy.

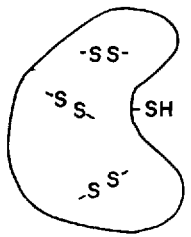
AuSTm Reactions. The binding of AuSTm to hemoglobin has been less extensively studied. Up to 2.0 equivalents of gold bind to Hb, even when excess gold is used.[20] Loss of the gold binding ability when the protein is modified by iodoacetamide confirms that the Cys-β-93 sites are the principal binding sites, as suggested by the 2/1 Au/Hb stoichiometry. The rate of binding was measured under pseudo-first-order conditions where both Cys-β-93 groups would be populated. The reaction is rapid and complete within 5 minutes when 1 mM AuSTm and 100 μM HbO_2 were used. Only a single reaction phase could be detected over the course of the reaction, ruling out an anti-cooperative effect due to electrostatic repulsion of one AuSTm unit for the second.

The extent of the AuSTm and auranofin reactions were compared using similar concentrations of protein and gold. AuSTm reacts more completely than does auranofin.[20] This order of gold binding affinities stands in striking contrast to the propensity of red blood cells to accumulate gold in non-smoking patients: auranofin administration leads to gold accumulation, but myochrysine administration does not. Thus, the relative affinities of Hb for these compounds, established by direct reaction, rules out any role for hemoglobin as the driving force for RBC gold accumulation *in vivo*.[20]

II. SERUM ALBUMIN

Albumin is the most abundant protein and the principal source of sulfhydryl groups in serum. The chemistry of the albumin thiol and disulfide groups is, like much of albumin chemistry, complex and difficult to probe directly.[23a] Thirty-four of its 35 cysteine residues are present as 17 disulfied bonds (Fig. 4). The remaining cysteine, Cys-34, occurs in the reduced form and as "mixed" disulfide bonds to glutathione and free cysteine:

Mercaptalbumin	AlbSH	~60–70%
Cysteine Disulfide	AlbSSCy	~30–40%
Glutathione Disulfide	AlbSSGt	



MICROHETEROGENEOUS
Alb SH ~ 0.7 in vivo
AlbSSCy
AlbSSGt ~ 0.3 in vivo
Dimers - isolation artifact
SH "loss" - storage artifact

35 Half Cystines
"1" Cysteine
17 Disulfides

Crevice ~ 1000 pm

FIGURE 4 A schematic representation of serum albumin including cysteine-34 in its crevice environment and some of the 17 internal disulfide bonds.

In addition to these species, which are present in circulating blood, dimerized artifacts form during isolation, and an oxidized artifact forms during prolonged storage.[23a] Our work on auranofin–albumin reactions (*vide infra*) led to evidence favoring a cysteinyl-34 sulfenic acid, AlbSOH, as the product of the oxidation.[22] Commercial bovine serum albumin (BSA) generally has a higher mercaptalbumin content (SH titre) than human serum albumin (HSA) preparations and has been used extensively in our research. Since Cys-34 is the principle binding site of the gold(I) complexes, knowledge of the albumin SH titre (the ratio of AlbSH to total BSA) is essential for correct interpretation of reaction stoichiometries.

Two additional features of albumin are noteworthy. First, the pK_{SH} value of Cys-34 (-5)[23] is unusually low compared to those of cysteine (8.5) or glutathione (8.9). According to the inverse correlation between pK_{SH} and gold affinity noted by Sadler and Isab,[24] the low pK_{SH} value suggests that the albumin should be a high affinity binding site. Second, Cys-34 is located in a crevice estimated to be 1000 pm (10 Å deep).[25] This aspect of its structure is symbolically represented by the kidney-bean shape of the albumin structure in Figs. 4–9. The crevice environment may restrict the access of gold to cys-34 and may affect ligand exchange reactions after it does bind. Unfortunately, the crystal structure of albumin has not progressed beyond the identificaton of the space group[26] and detailed information about the Cys-34 environment is not available. A large body of indirectly obtained chemical and structural information has been accumulated and summarized.[23a] The binding of other metals has been studied extensively and was recently reviewed.[27]

AuSTm Binding Sites. In the absence of any ESR or NMR signals, we undertook collaborative EXAFS/XANES and Mössbauer studies to obtain structural information about the complexes formed by serum albumin and AuSTm. A complex containing approximately two golds per mercaptalbumin was prepared for the Mössbauer experiment.[28] A single broad quadrupole doublet was resolved by computer fitting into two doublets (IS_1 = 1.88 mm/s, QS_1 = 6.68 mm/s; and IS_2 = 1.70 mm/s, QS_2 = 6.50 mm/s), results which are consistent with two slightly different $Au(I)S_2$ environments in the complex. Two complexes were prepared for the EXAFS studies.[28] The first was prepared under conditions

where gold would bind to Cys-34, but not to the additional weak binding sites populated when excess AuSTm is present. The data obtained confirmed the $Au(I)S_2$ coordination environment and provided a gold–sulfur bond length of 228 pm. Tracer studies utilizing ^{34}S-AuSTm showed that the ratios of thiomalate and gold to BSA in the complex approach the SH titre and that the rate of thiomalate binding was similar to that of gold.[28] Taken in conjunction with the structural data, this provides good evidence that an albumin–gold–thiomalate complex forms at Cys-34 and that the gold remains two-coordinate (Eq. 3 and Fig. 5):

$$[\text{AuSTm}]_n + \text{AlbSH} \longrightarrow \text{AlbSAuSTm} \qquad (3)$$

A related thiolate complex AlbSAuSATg, prepared from $[\text{AuSATg}]_n$, was also shown by EXAFS to have $Au(I)S_2$ coordination.[22]

Thermodynamic studies provide evidence for additional weak-binding sites for gold(I).[29–34] We found that incubating albumin with a large excess of AuSTm then chromatographing it over a gel-exclusion resin allowed isolation of a complex with gold to

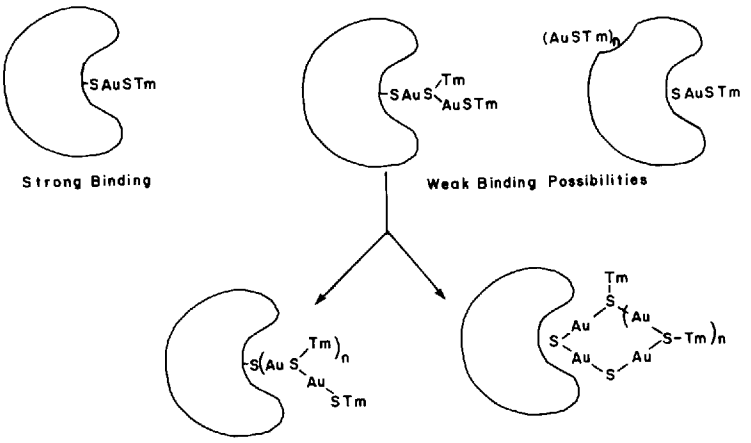


FIGURE 5 The strong (Cys-34) biding site and possible weak binding sites for AuSTm and albumin. The oligomeric structures generated from Cys-34 seem more likely for the weak binding sites, but binding of intact oligomers at a remote site can not be unequivocally ruled out.

albumin ratios approaching 3.[28] Analysis of a complex with a stoichiometry of 2.65 golds per BSA (7.4 Au/AlbSH) showed that the golds retained the AuS_2 coodination environment and remained gold(I). Since AuSTm does not undergo ligand exchange reactions with disulfide bonds or methionine, the most reasonable interpretation is that oligomers of gold(I) form at Cys-34 using the tightly bound gold as the initiation point (Fig. 5). Competition studies using flourescent probes[28] determined that intact gold(I) oligomers were not binding at remote drug-binding sites known as Anion sites I and II, but do not unambiguously eliminate the possibility of other sites remote from Cys-34 (Fig. 5). The possibility that AuSTm binds at histidine or other nitrogen sites, as does Et_3PAu^+, can be eliminated by the EXAFS data for the weakly bound gold. Thus we prefer to interpret the reaction as oligomer formation at Cys-34 and to report the gold stoichiometry relative to the mercaptalbumin content of the protein (Eq. 4):

$$\text{AlbSH} + \text{xs AuSTm}_n \longrightarrow \text{AlbS(AuSTm)}_{7.4} \qquad (4)$$

Two possible structural models consistent with the oligomeric structure of AuSTm[7] are shown in Fig. 5. The data presently available can not distinguish the linear and cyclic models.[28]

Auranofin and Analogues. Trialkylphoshine complexes of gold(I) with various anions, R_3PAuX, react with albumin in a complex series of reactions. These complexes are analogues of auranofin and, by varying X and R, one obtains insights into the nature of auranofin–albumin reaction. Auranofin, deacetylated auranofin ($Et_3PAuSTg$, a probable metabolite formed in the gastrointestinal system)[35] and Et_3PAuCl react stoichiometrically to form a single protein complex, $AlbSAuPEt_3$, readily identified by its ^{31}P NMR signature, 38.9 ppm:

$$\text{AlbSH} + Et_3PAuX \xrightarrow[X^- = Cl^-, TgS^-, AtgS]{} \text{AlbSAuPEt}_3 + X^- \qquad (5)$$

For all three complexes, the reactions are complete under the conditions used to obtain the NMR spectra (1–5 mM albumin and gold). The completeness of these reactions, compared to the equi-

librium reactions of hemoglobin with the same complexes, confirms the high affinity of Cys-34 for gold(I), as predicted by the low pK_{SH} value and the large chemical shift of $AlbSAuPEt_3$. The PAuS coordination geometry of gold in the protein complex was confirmed by EXAFS/XANES spectroscopy, which determined the gold to be gold(I) and the Au–S and Au–P bond lengths to be 227 pm and 229 pm, respectively. An elegant study by Ecker *et al.*, using triply labeled [^{3}H, ^{195}Au, ^{35}S]-auranofin, demonstrated a 0.6:0.6:1.0 gold:phosphine:albumin ratio in the resulting complex,[36] confirming the validity of Eq. (5).

Et_3PAuCl also reacts at additional weak binding sites. They can be populated in reactions of albumin with a modified cysteine-34 residue (e.g., AlbSSCy or carboxymethylated albumin), or after saturating the Cys-34 site when excess Et_3PAuCl reacts with albumin.

$$Et_3PAuCl + AlbSY \xrightarrow[Y = -CH_2CO_2^-, AuPEt_3, SCy]{} (Et_3PAu)_xAlbSY \quad (6)$$

Using the cysteine disulfide of albumin, AlbSSCy, an adduct having only weakly bound Et_3PAu^+ was prepared and studied by ^{31}P NMR and EXAFS/XANES spectroscopy. The results are consistent with binding at nitrogen donor ligands (Au–N and Au–P bond distances of 208 pm and 225 pm, respectively). Histidines are the most probable candidates, but other nitrogen donors such as the terminal amino group and lysines can not be unambiguously eliminated by the data presently available.

An infrequently observed form of binding involves two equivalents of Et_3PAu^+ bound at Cys-34. It was observed after chromatographing a sample of albumin with Et_3PAu^+ saturating Cys-34 and also bound at the weak binding sites and is characterized by a resonance at 36 ppm[22]:

$$(Et_3PAu)AlbSAuPEt_3 \longrightarrow AlbS(AuPEt_3)_2 \quad (7)$$

These Et_3PAu^+ binding sites, $AlbSAuPEt_3$, $(Et_3PAu)_xBSA$, and $AlbS(AuPEt_3)_2$, represented schematically in Fig. 6, can also be populated using other phosphine analogues of auranofin including those of Me_3P, iPr_3P, and Φ_3P.[37,38] Table I lists the NMR chemical

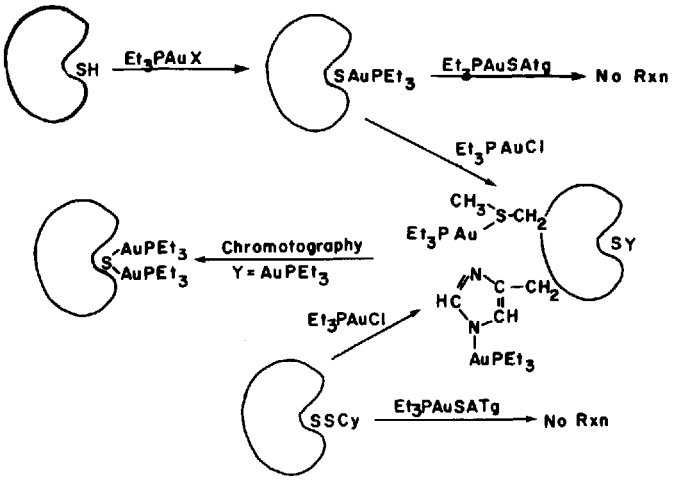


FIGURE 6 A schematic representation of the reactions of auranofin and its analogues with serum albumin.

shifts for the methyl, ethyl and isopropyl analogues of the various albumin–gold–phosphine complexes that have been identified to date. The Me_3P ligand is displaced from gold and oxidized more readily than Et_3P,[37] while iPr_3P is not displaced at all by thiols, but can be displaced by cyanide, which has a very great affinity for gold(I).[38] Thus, the ease of displacement of the phosphine is

$$Me_3P > Et_3P > iPr_3P$$

Since the basicities and the cone angles of the phosphines both increase to the right, it is not possible to distinguish the contribution of either effect. Φ_3P, which is less basic than Me_3P and has a larger cone angle than iPr_3P, should distinguish the relative importance of the two factors, but the insolubility of many of its gold complexes in aqueous media precluded meaningful comparisons.

The Albumin Oxidation Artifact. An anomaly in the gold binding stoichiometries of the albumin–gold complexes prepared from auranofin led to an unusual retrospective study of the reaction. (Retrospective—or backward looking—studies of data obtained be-

TABLE I

NMR chemical shifts for trialkylphosphinegold(I) complexes[a]

	R = Me[b]	R = Et[c]	R = iPr[d]	R = Φ[d]
$AlbSAuPR_3$	−2.3	38.8	68.5	36.4
$AlbS(AuPR_3)_2$	−12.7	36		
$(R_3PAuN)_xAlb$	−12→−15	27, 28	58.4, 58.8, 59.8	−28
R_3P			18.0	−8.4[e]
R_3PAuCl	−13.1	31.5	60.5	31[e]
$R_3PAuSAtg$	−3.0	36.6	66.5	35.9[f]
$[Au(PR_3)_2]^+$		44.1	74.7	33.7
R_3PO	49.6	61.6	64.3	

[a]Chemical shifts (ppm relative to $(MeO)_3PO$), measured in buffered aqueous solution, pH 7.9, except where otherwise noted.
[b]Ref. 37.
[c]Ref. 22.
[d]Ref. 38.
[e]1:1 MeOH:buffer.
[f]MeOH.

fore the research question or project was formulated are common in clinical sciences, but unusual in physical laboratory sciences, except perhaps in high energy physics.[39]) Mary Carlock, a masters student in my laboratory, noticed that the extent of gold binding, nominally according to Eq. (2), sometimes exceeded the mercaptalbumin content of the sample, but produced only Cys-34 bound Et_3PAu^+. Because she worked meticulously and kept marvelously detailed notebook records, she was able to plot the course of gold binding and SH titre for a single batch of albumin over six months.

The commercially obtained BSA, stored in a freezer between the occasions when she used it, declined in SH titre, while the gold binding capacity remained constant (Fig. 7). Consideration of the literature on albumin sulfhydryl groups[40] led to the hypothesis that Cys-34 was being oxidized to a sulfenic acid, AlbSOH, Eq. (8), during storage. The reversal of the oxidation can be attributed to the ability of the acetylthioglucose ligands displaced from auranofin, Eq. (8a), to reduce it back to the sulfhydryl form, Eq. (8b), which reacts further according to Eq. (8a):

$$AlbSH \xrightarrow{[O]} AlbSOH \qquad (8)$$

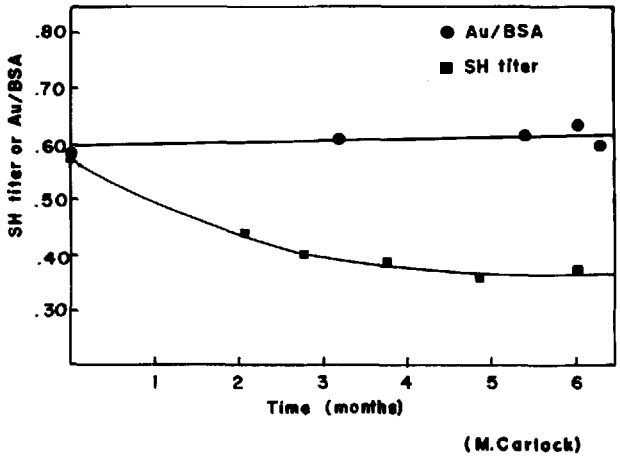


FIGURE 7 The oxidation artifact of albumin leads to a decreased SH titre over many months of storage at −4° C, but does not decrease the reaction with auranofin. The postulated formation of AlbSOH, a sulfenic acid of albumin, which can be reduced by the AtGSH displaced from auranofin, provides an explanation of this phenomenon.

$$\text{AlbSH} + \text{Et}_3\text{PAuSAtg} \longrightarrow \text{AlbSAuPEt}_3 + \text{AtgSH} \quad (8a)$$

$$\text{AlbSOH} + 2\text{AtgSH} \longrightarrow \text{AlbSH} + \text{H}_2\text{O} + \text{AtgSSAtg} \quad (8b)$$

While there is no direct spectroscopic method to verify the formation of AlbSOH, two chemical reagents, azide and mercaptoethanol, known to reduce sulfenic acids to the sulfhydryl form, increase the SH titre, a result consistent with the hypothesis. Sulfenic acids are known to form in several enzyme systems,[41] and previous workers have proposed that AlbSO_xH, x = 1, 2 or 3 can form.[40]

Albumin Oxidation of Triethylphosphine. When auranofin reacts with albumin, a small amount of Et_3PO is often observed in the NMR spectrum.[13,14,22] The finding that it is not observed when Et_3PAuCl or deacteylated auranofin is substituted suggested that the anionic ligand might be central to the mechanism of its formation.[42] This could occur as follows: first, auranofin undergoes

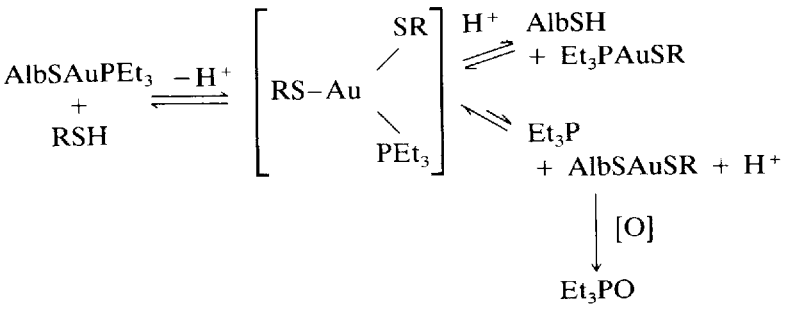
a ligand displacement reaction, liberating AtgSH (Eq. (9a), identical to Eq. (5)); second, an equilibrium displacement of the phosphine by the acetylthioglucose, Eq. (9b); thid, although the equilibrium of Eq. (5b) lies far to the left, irreversible oxidation of the phosphine (Eq. (5c)) slowly shifts it toward completion.

$$\text{AlbSH} + \text{Et}_3\text{PAuSAtg} \longrightarrow \text{AlbSAuPEt}_3 + \text{AtgSH} \quad (9a)$$

$$\text{AtgSH} + \text{AlbSAuPEt}_3 \rightleftharpoons \text{AlbSAuSAtg} + \text{Et}_3\text{P} + \text{H}^+ \quad (9b)$$

$$\text{Et}_3\text{P} \xrightarrow{[\text{O}]} \text{OPEt}_3 \quad (9c)$$

Indirect evidence from the reaction between isolated and purified albumin–gold–triethylphosphine and various thiols is consistent with a second-order process involving a three-coordinate intermediate for gold(I):



(10)

The formation of small amounts of Et_3PAuSR in equilibrium with the albumin–gold complex and slower formation of Et_3PO (40–60% over 25 h) can be explained by the intermediate shown.[42] Three thiols, tetracetylthioglucose, thioglucose and glutathione, used in comparative studies, produced relative rates of Et_3PO formation, AtgSH > TgSH > GtSH, which parallel their affinities for gold(I). The thiol dependence of the reaction is consistent with an associative mechanism and inconsistent with rate-limiting dissociation of the phosphine. The proposed mechanism is consistent with the known chemistry of gold(I). All of the ligand exchange

reactions of gold(I) reported to date, except one reaction of an organometallic species, are associative processes. The availability of the empty $6p_x$ and $6p_y$ orbitals, orthogonal to the bond axis of the linear complex, makes the three-coodinate intermediate energetically accessible.

Chromatographically isolated [^{3}H, ^{195}Au]-AlbSAuPEt$_3$ is stable to phosphine loss over a 2 week period,[36] confirming the role of the thiol, which is removed during the chromatography. The failure of Ecker *et al.*[36] to observe displacement and oxidation of the phosphine during 20 min incubations with added thiols is consistent with the very slow reaction we observed.

Reactions with Hydrogen Cyanide. The model for the phosphine oxidation predicts that a ligand with an affinity for gold(I) greater than that of thiols should accelerate the formation of Et_3PO. Cyanide has an unusually high affinity for gold(I) and its reactions with albumin–protein metabolites are of further interest because HCN absorbed into the blood of tobacco smokers may alter the metabolism of auranofin. HCN is known to alter the metabolism of AuSTm causing it to enter red blood cells, which non-cyanide metabolites can not do. Since auranofin metabolites enter cells readily, the effects of HCN on its metabolism will be more subtle.

Exactly the predicted result, very rapid Et_3PO formation, was obtained using cyanide[43] (Fig. 8). The reaction was complete in less than an hour, compared to 40–50% phosphine oxidation after 24 hours using thiols. One interesting feature of the cyanide reaction is that Et_3PAuCN can be isolated as a transient product. Conversely, if Et_3PAuCN and AlbSH are the reactants, AlbSAuPEt$_3$ is formed transiently, and the same set of final products form. Although one might expect to isolate "AlbSAuCN," the cyanide analogue of AlbSAuPEt$_3$ and AlbSAuSTm, etc., studies using H^{13}CN and H^{14}CN failed to detect any evidence for it. "AlbSAuCN" appears to undergo ligand scrambling to form $Au(CN)_2^-$, AlbSH and $\overline{\text{AlbSAuX}}$, where X is an as yet unidentified ligand, probably a protein side chain of the albumin. Since there is no reason to believe that AlbSAuCN should be less stable than the phosphine and thiomalate analogues, the driving force for the reaction may be the formation of $Au(CN)_2^-$ ($\beta_2 = 10$)[44]

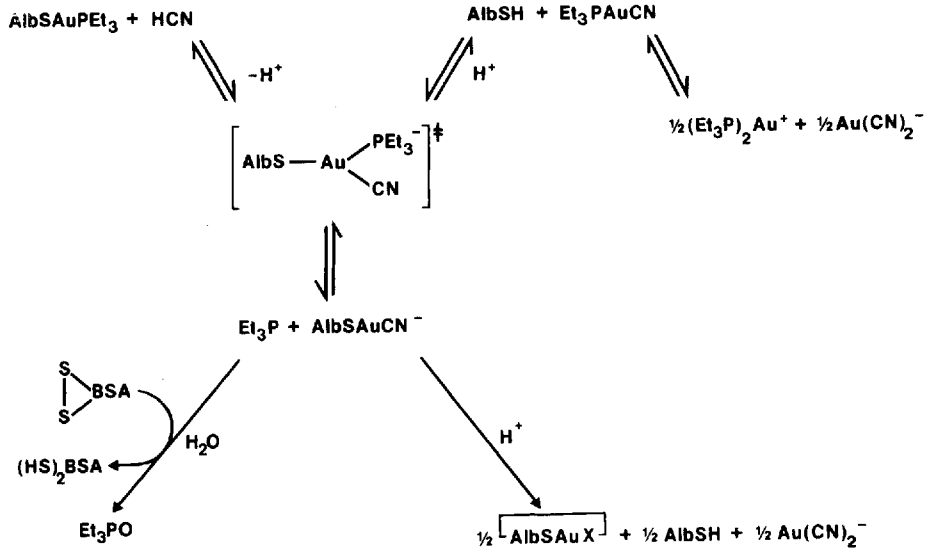


FIGURE 8 A scheme for the reactions of $AlbSAuPEt_3$ with HCN and AlbSH with Et_3PAuCN. Each leads to the same set of products which are driven by the irreversible formation of Et_3PO and AlbSAuX and $Au(CN)_2^-$.

or a high-affinity, but not yet identified, binding site where the protein chelates gold(I). Disproportionation reactions of related model complexes including R_3PAuCN (R = Et, Me, iPr and Ph)[45,46] and R'SAuCN (RS^- = CyS^-, GtS^-, TmS^-)[47,48] have been studied.

The Role of Albumin Disulfide Bonds. In order to relate the *in vitro* phosphine oxidation chemistry to physiological situations, it is important to know the oxidant for the phosphine. Sadler had proposed that the disulfide bonds of albumin are reduced when the protein is treated with $[(Et_3P)_2Au]Cl$,[13,14] and a similar reaction could occur here. Indeed, Φ_3P is used as a reductant in the commercial analysis of the disulfide content of wool. Our preliminary finding that the rate of oxidation was not accelerated by adding glutathione disulfide nor retarded by using an anaerobic atmosphere is consistent with the role of protein disulfide bonds as the oxidants.[22]

More convincing evidence is provided by ^{17}O tracer studies.[49] When a disulfide is reduced by phosphine, the mechanism requires water as the oxygen donor:

$$RSSR + H_2^*O + Et_3P \longrightarrow {}^*OPEt_3 + 2RSH \qquad (11)$$

When $OPEt_3$ generated by reacting $AlbSAuPEt_3$ and glutathione for 24 h in enriched water was extracted into $CHCl_3$ and analyzed by ^{17}O NMR, a signal for Et_3PO (δ_O = 40.6 ppm vs. H_2O; $^2J_{PO}$ = 156 Hz) was observed. Quantitative analysis by Gas Chromotography–Mass Spectroscopy confirmed the incorporation of enriched oxygen, but showed that about a third of the Et_3PO generated from an unlabeled source of oxygen, presumably O_2 since aerobic conditions were used.

$$AlbSAuPEt_3 \xrightarrow[AlbSAuSR]{RSH} Et_3P \begin{cases} \xrightarrow[H_2O]{RSSR} {}^*OPEt_3 \\ \xrightarrow{O_2} OPEt_3 \end{cases} \qquad (12)$$

Another testable consequence of Eq. (11) is that the SH titre of albumin should increase as phosphine oxide forms. This result

was observed in the reactions in $H_2^{17}O$, in the reactions with cyanide discussed above and in reactions between albumin and $[(Et_3P)_2Au]Cl$.[43,49,50] The latter study also demonstrated that both the internal and external disulfides of albumin are reduced as Et_3P is oxidized[50]:

$$\begin{matrix} S \\ | \\ S \end{matrix}\!\!\diagdown\!\!\diagup\!\!\text{BSA} + Et_3P \longrightarrow Et_3PO + (HS)_2BSA \qquad (13)$$

$$AlbSSR + Et_3P \longrightarrow Et_3PO + AlbSH + RSH \qquad (14)$$

Hemoglobin lacks disulfide bonds. All of its cysteines occur in reduced form. During our studies of hemoglobin–auranofin reactions, no Et_3PO was observed, even in the presence of excess thiols.[20] This finding is consistent with a role for the albumin disulfide bonds as oxidants, since the absence of the oxidant from hemoglobin should preclude Et_3PO formation, as observed.

New Thiol Binding Sites. During our studies on the reactions of Et_3PAuCN[43] and $[(Et_3P)_2Au^+]$[50] with albumin we obtained ^{31}P NMR evidence for a third class of Et_3PAu^+ binding sites, created by the reduction of disulfide bonds according to Eq. (13). When gold is present in excess of Cys-34 and phosphine is displaced from auranofin or an analogue, a broad resonance is observed in the region 35–36 ppm. This is in the range of chemical shifts spanned by thiol complexes and must involve reaction of Et_3PAu^+ at one or more of the new sulfhydryl groups generated by phosphine reduction of the internal disulfide bonds:

$$(HS)_2AlbSY + Et_3PAuCl \longrightarrow (Et_3PAuS)_x(HS)_{2-x}AlbSY \qquad (15)$$

The differentiation of the Cys-34 site (δ_P = 38.8 ppm) and the new sites (δ_P = 35–36 ppm) by ^{31}P NMR using Et_3PAu^+ as a probe may be an important new tool for studying the sulfur chemistry of albumin.

III. ALBUMIN–GOLD EQUILIBRIUM BINDING CONSTANTS

Few equilibrium studies of gold binding have been reported in the literature. This results in no small part from the absence of useful UV-Vis chromophores to facilitate such studies. The alternatives are cumbersome chromatographic or membrane dialysis methods which are much slower and less reproducible.

For AuSTm, several groups have reported binding constants and the number of associated binding sites on the protein.[29–31,51] Table II lists the results of four independent studies. The values for the strong binding site span a range of just over two orders of magnitude, from 6.6×10^3 to 1.5×10^6, and the weak binding sites are typically 1 to 2 orders of magnitude smaller. In each case, the studies were of fairly limited scope. The role of excess thiomalate was not considered and only the study by Schaeffer[51] compared the results to the SH titre of the protein.

Pedersen in an important series of papers[32–34] has examined the concentration, ionic strength, and pH dependence of the reactions of aurothiosulfate, $Au(S_2O_3)_2^{-3}$, with human albumin. This crystalline and presumably well-defined drug lacks the excess ligand which complicates studies using AuSTm and is not known to have any impurities present. The results suggest a different course of reaction for $Au(S_2O_3)_2^{-3}$ at the strong binding site, binding as Au^+ without retention of the thiosulfate ligands:

$$Au(S_2O_3)_2^{-3} + AlbSH \longrightarrow AlbSAu\text{-}? + 2S_2O_3^{-2} + H^+ \quad (16)$$

Since gold(I) is not stable as an aquated ion, some other group must function as the second ligand. This structure may be analogous to the species designated $\overbrace{AlbSAuX}$ which is formed in the HCN/AlbSAuPEt$_3$ reactions discussed above.

The data for the weaker binding sites were interpreted as binding of intact $Au(S_2O_3)_2^{-3}$ at other independent sites. The data seem (to this author) equally compatible with an alternative possibility, namely, that $[AuS_2O_3^-]_n$ oligomers form at the tight binding site with displacement of one ligand. Since $AuS_2O_3^-$ or $Au(S_2O_3)_2^{-3}$

TABLE II

Equilibrium binding constants for serum albumin–gold complexes[a]

Complex	Albumin	K_s	n_s	K_w	n_w	Ref.
AuSTm	HSA	1.5×10^6	0.07	1.1×10^5	0.3	29
	HSA	3.1×10^5	0.04	1.4×10^3	1.8	30
	HSA	6.1×10^3	1.0	2×10^2	6.6	31
	BSA	6.6×10^4	0.5	3×10^3	0.75	32
$Au(S_2O_3)_2^{-3}$	HSA[a]	2.2×10^5	–	–	–	33
	HSA[b]	3.9×10^4	–	4.4×10^2	–	33

[a]s = strong; w = weak; measurements were made in serum or 0.15M KCl at pH 7.4.
[b]66 μM albumin.
[c]530 μM albumin.

are both negatively charged, the ionic strength dependence of each should be qualitatively similar and indistinguishable.

Unfortunately equilibrium binding studies, such as these, provide no structural information and our structural studies including the use of radiotracers were carried out with different complexes. The discrepancies prompted us to attempt chromatographic resolution of the products of albumin–AuSTm reactions.[52] Figure 9 compares HPLC ion-exchange chromatograms of bovine serum albumin and its AuSTm reaction product(s). The chromatogram of albumin alone shows three main peaks, attributed to AlbSH (the major peak) and two attributed to AlbSSCy and AlbSSGt (the smaller, later-eluting peaks). After reaction with AuSTm, the major albumin–gold species elutes at higher ionic strength, consistent with the increased charge of AlbSAuSTm (three units more negative than AlbSH) and with the radiotracer and spectroscopic data discussed above. Unexpected from our previous work, but consistent with Pedersen's results, is a minor gold species eluting at the same ionic strength as the AlbSH. This may result from the displacement of thiomalate by a second ligand group present on albumin:

$$\text{AlbSAuSTm}(\text{—X}) + \text{TmSH} \overset{?}{\rightleftharpoons} \text{AlbSAuX} + \text{TmSH} \quad (17)$$

It is not clear, however, why binding according to Eq. (16) or (17) should have a higher affinity than binding with retention of ligand, Eq. (3), yet remain the minor form. The apparent discrepancies between equilibrium binding and structural studies of albumin–AuSTm complexes are profound and may have their origin in as yet undeciphered complexities of serum albumin. A detailed crystallographic study of the protein would clarify our understanding of its role as a gold-transport mechanism during therapy.

IV. METALLOTHIONEIN

Unique metal-thiolate clusters are found in the metal binding protein, metallothionein (MT). It uses twenty cysteine residues to form M_4S_{11} and M_3S_9 clusters, which are located at the ends of

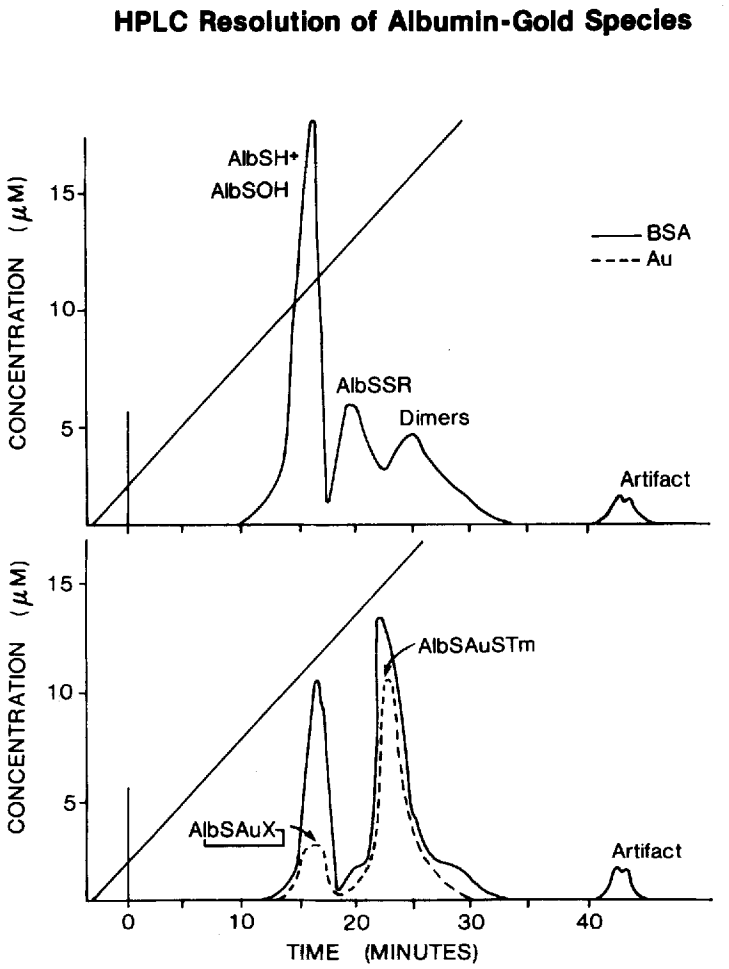


FIGURE 9 HPLC ion exchange chromatogram for the products of an albumin–AuSTm reaction. The large peak is monomeric forms of albumin, followed by smaller peaks due to disulfide forms and dimer artifacts that form during isolation. After reacting with AuSTm, much of the monomeric albumin elutes at higher ionic strengths, consistent with formation of AlbSAuSTm, which is three units more negative charge than AlbSH. An unshifted gold–albumin species may represent gold bound without ligand, as predicted by the equilibrium bindings studies of Pedersen (Refs. 32–35) and observed in the HCN/AlbSAuPEt$_3$ reactions.

the protein chain.[53,54] The amino acid sequence and the metal clusters are shown in Fig. 10. Under normal physiological conditions the protein may contain Zn^{+2} and/or Cu(I) ions, and it also binds environmentally accumulated Cd^{+2}, which stimulates the biosynthesis of additional metallothionein. Gold complexes given to laboratory animals or added to cell cultures stimulate the formation of mixed-metal gold-containing thioneins, called aurothioneins.

Since MTs are rich in thiols, one expects them to have an appreciable affinity for gold(I). On the other hand Zn^{+2} and Cd^{+2} bind tightly to the clusters and only ligands with greater thermodynamic affinities for the ions can extract them. Thus, it is not clear, *a priori*, whether or not gold complexes should have suffi-

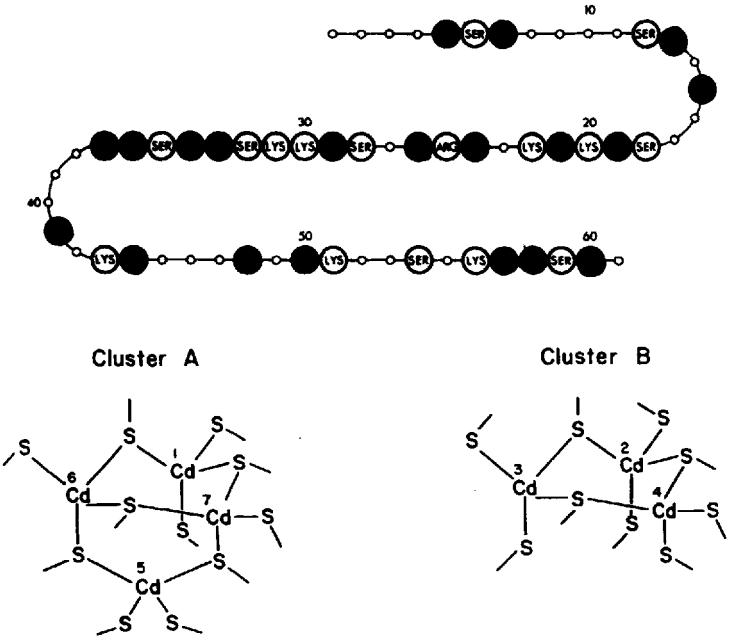


FIGURE 10 The amino acid sequence of metallothionein, representing the 20 cysteines as dark circles (●) and other amino acids as open circles (○, ○). The 3-metal (β) cluster and the 4-metal (α) cluster form at the N-terminal and C-terminal ends of the protein (figures adapted from Refs. 64 and 65). Each Cd^{+2} and Zn^{+2} in the two clusters occupies a tetrahedral 4-coordinate MS_4 site.

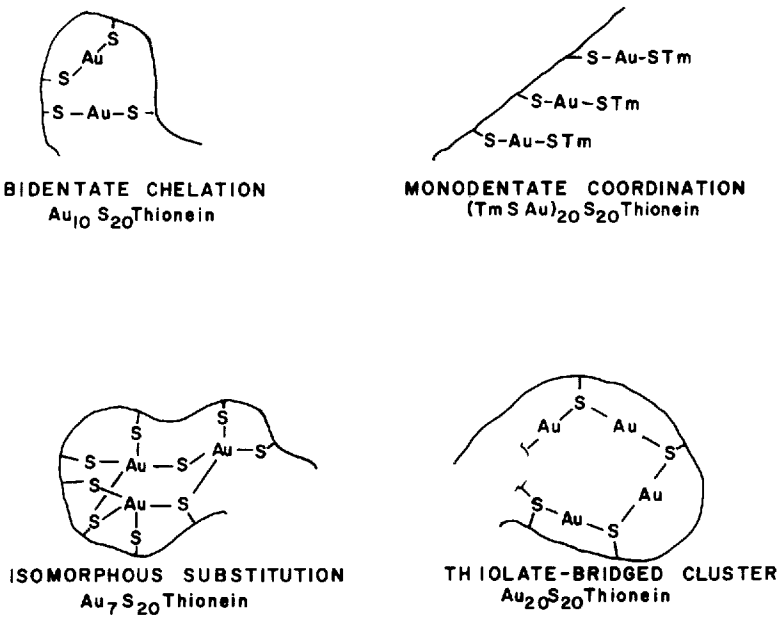


FIGURE 11 Possible structures and limiting stoichiometries for aurothioneins. Three possibilities invoke two-coordination, the usual structure of gold(I): bidentate coordination (two terminal MT cysteine ligands), monodenate coordination (one MT cysteine; one free ligand); thiolate-bridged cluster (bridging cysteines, analagous to AuSR in Fig. 1). Isomorphous substitution requires the Au^{+} to occupy the MS_4 tetrahedral structures of native MT. Good evidence exists for the existence of aurothioneins with monodentate and bidentate coordination under appropriate conditions.

cient affinities for the thiolates to be able to displace Zn^{+2} or Cd^{+2}. If not, the gold-containing thioneins formed by administering gold to animals must form by reactions with apo-thionein—that is, metal-free metalothionein. A related question is whether gold(I) will enter the four-coordinate, tetrahedral Cd^{+2} and Zn^{+2} sites or distort the structure of MT to create the two-coordinate linear binding geometry that it generally adopts with anionic ligands. Four possible binding modes[55] and the limiting stoichiometric for all-gold thioneins are shown in Fig. 11.

Experimental results *in vitro* demonstrate that the reactivity of gold(I) toward metallothionein is modulated by the ligands. AuSTm,

which has a low affinity ligand, reacts with Zn_7Th, Cd_7Th and various mixed-metal thioneins to displace the M^{+2} ions.[55] When the protein is in excess, partial displacement of Zn^{+2} and/or Cd^{+2} leads to mixed-metal thioneins. [^{35}S]-thiomalate tracer studies showed that the ligand is lost in these cases.

$$\text{AuSTm} + \text{Zn,Cd–Th} \longrightarrow \text{Au,Cd–Th} + \text{TmSH} + \text{Zn}^{+2} \qquad (18)$$

Zn^{+2} is displaced in preference to Cd^{+2}, as would be expected from their relative binding constants. EXAFS results ($Au(I)S_2$ coordination geometry) and the stoichiometry of bound gold and displaced metals were consistent with the formation of linear gold(I) coordination geometries involving two thionein cysteine residues, which implies that there is some disruption of the cluster structures.

When excess AuSTm is employed, the protein-bound Zn^{+2} and Cd^{+2} are completely displaced.[55] The ratio of gold bound to metal displaced approaches a limiting value of $20Au/7(Zn^{+2} + Cd^{+2})$ and thiomalate is retained in the complex. This is consistent with the model in which one AuSTm monomer is bound at each thiol of the protein:

$$\text{xs AuSTm} + \text{Zn,Cd–Th} \longrightarrow (\text{TmSAu})_{20}\text{Th} + 7\ \text{Zn}^{+2},\text{Cd}^{+2} \qquad (19)$$

The loss of the cluster structures was confirmed by a decrease in the K_D value for the elution of the aurothionein from a gel-exclusion resin, behavior consistent with the formation of a linear, unfolded structure.[55]

We observed that auranofin does not react with Zn,Cd–Th, nor does it displace cadmium or zinc.[56] This was confirmed by Ecker *et al.*[36] using triply labelled auranofin. Its analogue, Et_3PAuCl, which contains the more readily displaced chloride ligand, reacts with Zn,Cd–Th, displacing some zinc.[56] From the ratio of metals displaced to gold bound, it was concluded that gold was binding with and without loss of the phosphine ligand, i.e., monodentate coordination as Et_3PAuS- and bidentate coordination as AuS_2 where the sulfur donor atoms are the cysteine side chains. Similar con-

clusions can be drawn for the reaction of apo-thionein (metal free) with triply labelled auranofin, in which partial loss of phospine from the bound gold was observed.[36] Removal of the metal ions enhances the activity of the sulfhydryl groups in the apo-protein, so they are able to displace the AtgSH and phosphine ligands of auranofin.

In summary, AuSTm and Et_3PAuCl react with MT displacing the bound metal ions. Auranofin does not displace metals from or bind to MT, but does react with the apo-thionein, the metal-free protein.

V. ^{31}P-NMR CHEMICAL SHIFT–pK_{SH}–GOLD AFFINITY CORRELATIONS

The NMR studies described above for reactions of various auranofin analogues with albumin, hemoglobin and low-molecular-weight thiols led to our observation of a correlation between the ^{31}P chemical shifts of Et_3PAu^+ coordinated to various thiols and the affinity of the thiol for gold(I).[20,42] Values for several thiols are listed in Table III, along with the thiol pK_{SH} values, which are inversely correlated with affinity for gold(I), as previously reported by Isab and Sadler.[24]

Quantum mechanical studies of third row transition elements such as gold, for which relativistic corrections are required, are in their infancy. Certainly they are unable to deal with subtle substituent effects several bonds removed from gold which affect the changes in affinity observed here. Yet, it is possible to offer a simple rationalization of the correlation in terms of the durable and useful generalization, *Hard and Soft Acids and Bases*.[57] pK_{SH} values measure proton–thiolate affinities. The proton is the ultimate hard acid, and large pK_{SH} values correspond to a high affinity for H^+. Gold(I), on the other hand, is among the softest of acids. It disproportionates in water to gold(III) and metallic gold unless it is stabilized by soft ligands such as thiols, phosphines or cyanide. The high affinity for gold(I) on the part of thiols with low pK_{SH} values suggests that these are the softer thiols. Thus, there is a continuum from thiolates with soft base character, high affinity for gold(I) and low pK_{SH} values to the thiolates with somewhat harder base character, lower affinities for gold(I) and high pK_{SH} values.

TABLE III

Correlation of δ_P for Et_3PAuSR complexes with the thiol pK^a_{SH}

Complex	δ^a_P/ppm	Ref.	pK_{SH}	Ref.
$AlbSAuPEt_3$	38.8	22	<5	23
$ATgSAuPEt_3$	36.7	22	6.4	61
$TgSAuPEt_3$	36.3	42	7.6	62
$GluSAuPEt_3$	35.7	42	8.7	63
$Hb(SAuPEt_3)_x$	34	20	–	–

[a]Chemical shifts relative to $OP(OCH_3)_3$ measured in aqueous NH_4HCO_3 buffer, pH 7.9.

Ultimately, of course, one wishes to have a more fundamental explanation, but that awaits advances in theory and computational techniques.

VI. INTERPROTEIN GOLD TRANSFER

One of our earliest studies of gold in the biological milieu was an examination of the reactions of several gold complexes with kidney cytosol.[58] The similarity of the gold distribution when it was administered to rats and when it was added to the cytosol *in vitro* was explained by proposing that gold(I) equilibrates among protein and non-protein sulfhydryl groups.[58] For the gold distribution to be a thermodynamically, rather than kinetically, controlled phenomenon, there must be mechanisms for facile exchange of gold among various binding sites, allowing it to come to equilibrium. Subsequently, a related model for auranofin pharmacology, the sulfhydryl shuttle model, was proposed to explain the facile movement of Et_3PAu^+ in and out of cells.[59]

A prediction based on the chemical shift–affinity correlation stimulated us to examine gold transfer between hemoglobin and serum albumin.[20] From the chemical shifts of $Hb(SAuPEt_3)_x$ (34 ppm) and $AlbSAuPEt_3$ (38.8 ppm), one expects gold to transfer from the hemoglobin Cys-β-93 groups to the albumin Cys-34 residue. The reaction, in fact, occurs rapidly and completely:

$$xAlbSH + Hb(SH)_{2-x}(SAuPEt_3)_x \longrightarrow Hb(SH)_2 + xAlbSAuPEt_3 \quad (20)$$

An NMR spectrum accumulated for 1 hour immediately after adding the albumin to the sample showed no detectable signal due to $Hb(SH)_{2-x}(SAuPEt_3)_x$, from which we estimate that $t_{1/2}$ for the reaction at the millimolar concentrations we employed is less than 10 minutes.

The rapid transfer of gold to the higher affinity site in the absence of any added low-molecular-weight ligands to facilitate the reaction provides strong support for the (1) the chemical shift–affinity correlation, (2) the protein–sulfhydryl equilibration model,[58] and (3) the sulfhydryl shuttle model for transport of gold across membranes.[59]

VII. BIOLOGICAL SIGNIFICANCE

Clearly, an understanding of the structures, kinetics and thermodynamics of gold–protein reactions will facilitate an understanding of gold pharmacology and metabolism. Many of the studies discussed here precede *in vivo* studies that might lead to the same information. Some specific conclusions include the following:

1. Sulfhydryl groups are the high affinity binding sites for gold(I) on serum albumin (Cys-34) and hemoglobin (Cys-β-93). Binding at other weaker sites occur only under conditions of concentration and gold ligation that are not likely to occur during chrysotherapy.
2. Et_3PO can be generated by displacing Et_3P from the likely auranofin–albumin metabolite, $AlbSAuPEt_3$. Its formation is driven by uncatalyzed reactions involving low-molecular-weight thiols as the displacing agents and protein disulfide bonds as oxidants.
3. HCN accelerates the formation of Et_3PO *in vitro* and may subtly alter the *in vivo* metabolism of auranofin in patients who smoke.
4. Direct, unfacilitated transfer of gold between large protein structures can be rapid, as shown by the $Hb(SAuPEt_3)_x$/AlbSH reaction.
5. The protein environment of cysteine residues dramatically alters their affinities for gold.
6. Since hemoglobin has a low affinity for gold(I) and albumin has a high affinity, the binding of gold(I) to hemoglobin, if it occurs at all *in vivo*, does not drive the accumulation of gold by blood cells.

7. The reactivity of gold(I) complexes with metallothionein depends on the ligation of gold. When reactions occur, the binding geometry is apparently dictated by the preference of gold(I) to adapt linear, two-coordinate structures. These structural perturbations may explain the findings of Butt *et al.* that aurothioneins are rapidly degraded after they form in cells.[60]

Three additional implications of this research merit brief discussion. The first involves the difference in gold metabolism between auranofin and the injectable drugs. Patients treated with auranofin have higher levels of gold associated with their circulating immunoglobins than do patients treated with injectable drugs, which lack the phosphine ligand. Immunoglobins are rich in disulfide bonds, and poor in reduced cysteine. Therefore, it is reasonable to speculate that displacement of phosphine from AlbSAuPEt$_3$ or other phosphine-containing metabolites may lead to reduction of the immunoglobin disulfide bonds and the creation of new binding sites.

Second, the lability of the ligand exchange reactions of gold(I) with protein and non-protein thiols, coupled with *in vivo* studies demonstrating loss of the ligands from myochrysine, solgonal and auranofin, suggest that the circulating metabolites of gold will be similar for the injectable and oral gold drugs. The following metabolic scheme for gold and albumin shows that this proposal is consistent with the body of chemistry described in this Comment:

$$\text{AuSTm} + \text{AlbSH} \longrightarrow \text{AlbSAuSTm} \xrightarrow[\text{TmSH}]{\text{RSH}} \text{AlbSAuSR} \qquad (21)$$

$$\begin{array}{l} \text{Et}_3\text{PAuSATg} \\ \quad\Big\downarrow \text{ stomach \& gut} \\ \text{Et}_3\text{PAuSTg} + \text{AlbSH} \xrightarrow[\text{TgSH}]{} \text{AlbSAuPEt}_3 \xrightarrow[\text{Et}_3\text{PO}]{\text{RSH} \quad [\text{O}]} \text{AlbSAuSR} \end{array} \qquad (22)$$

Third, accepting the premise that the metabolites of the oral

and injectable gold drugs converge to common species, the pharmacological differences between auranofin and the injectable drugs must be determined in the interval between absorption into the blood and the oxidation of the phosphine ligand. This suggests that in seeking the third generation of chrysotherapy agents attention should be focused on ligands with high affinities for gold. If the ligands are less readily displaced, greater modulation of the pharmacology may be possible.

ABBREVIATIONS

AlbSH, mercaptalbumin;
AlbSSCy and AlbSSGt, mixed disulfides of albumin with cysteine and glutathione;
AlbSOH, the oxidation artifact of albumin, putatively, a sulfonic acid;
AtgSH, tetra-2,3,4,6-O-acetyl β-1-D-thioglucose;
BSA, microheterogeneous bovine serum albumin;
CySH, cysteine;
DTNB, 5,5′-dithiobis(2-nitrobenzoic acid);
EXAFS, extended X-ray absorption fine structure;
GtSH and GtSSGt, reduced and oxidized glutathione;
Hb, $Hb(SH)_2$–hemoglobin;
HSA, human serum albumin;
MT, metallothionein;
TgSH, β-1-D-thioglucose;
TmSH, thiomalate;
Th, thionein, the protein chain of metallothionein;
XANES, X-ray absorption near edge spectroscopy

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