Contribution from the Departments of Chemistry, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201, and University of Cincinnati, Cincinnati, Ohio 45221

Reactions of Auranofin and Et_3PAuCl with Bovine Serum Albumin

Mary T. Coffer,[†] C. Frank Shaw III,*[†] M. K. Eidsness,[‡] J. W. Watkins II,[‡] and R. C. Elder[‡]

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The stoichiometries and products of reactions of microheterogeneous bovine serum albumin (BSA) with the new gold-based antiarthritic drug $Et_3PAuSATg$ (-SATg = 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato) and Et_3PAuCl have been studied by ^{31}P NMR, XANES, and EXAFS spectroscopy and chromatography. For $Et_3PAuSATg$, there is a single mode of binding. The ratio of bound gold to BSA, the integrated ^{31}P NMR intensities, and the loss of gold binding upon protein thiol modification are consistent with the formation of $AlbSAuPEt_3$ ($\delta(^{31}P)$ = 38.8 vs. $OP(OCH_3)_3$) and displacement of $ATgS^-$ by the reduced thiol (principally cys-34) of mercaptalbumin (AlbSH). XANES and EXAFS spectroscopies have confirmed the formation of AlbS-$AuPEt_3$ and were used to calculate Au-P and Au-S bond distances (d_{Au-P} = 229 pm and d_{Au-S} = 227 pm). Et_3PAuCl also reacts at the free thiol to yield $AlbSAuPEt_3$ ($\delta(^{31}P)$ = 38.8), and when excess Et_3PAuCl is present, additional weaker binding sites are populated ($\delta(^{31}P)$ = 27 and 28). EXAFS analysis of the gold bound at the low-affinity sites is consistent with coordination via phosphorus and a low-atomic-weight donor atom, most likely nitrogen (d_{Au-P} = 226 pm and d_{Au-N} = 206 pm). The thiol content of BSA gradually decreases during storage although the ability to react with $Et_3PAuSATg$ is not diminished. Evidence consistent with the formation of a sulfenic acid derivative of AlbSH and its reduction by $Et_3PAuSATg$ and azide is given.

Introduction

Chrysotherapy has been widely accepted for many years, although the mechanism of action of the gold(I) drugs is unknown. The degradation of joint tissues can be suppressed by administration of aurothioglucose, (AuSTg),[1] aurothiomalate (AuSTm), and (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato)(triethylphosphine)gold(I) ($Et_3PAuSATg$). This suppression may be due to inhibition of thiol-containing enzymes, such as acid phosphatase and β-glucuronidase, involved in the inflammatory process.[2]

In vivo studies have demonstrated that Au in the bloodstream is predominantly albumin-bound.[3] A principal physiological role of albumin is the transport and modulation of organic drugs, endogenous metabolites, and metal ions. Many divalent metal ions (Cu^{2+}, Zn^{2+}, Pb^{2+}, Ca^{2+}, Mg^{2+}, and Ni^{2+}) react at specific sites (e.g. Cu^{2+} at the N-terminus). Thus, the reactivity of gold drugs toward albumin may effect their distributions in vivo.

Bovine serum albumin (BSA) is strongly homologous to human serum albumin (HSA) and is frequently substituted for HSA since it exhibits less microheterogeneity. The chemistry of BSA is nonetheless complicated by microheterogeneity. Mercaptalbumin (AlbSH), in which cys-34 is reduced, represents 60% of the albumin in vivo. Non-thiol forms of the protein (e.g. mixed disulfides formed with endogenous cysteine or glutathione) account for the remaining 40% of the BSA.[4] Following isolation and storage, the thiol titer (SH/BSA) diminishes even further.

Gerber demonstrated that the binding of AuSTm to BSA inhibits the free thiol (predominantly cys-34) reactivity.[5] Recent spectroscopic, radiotracer, and physiochemical studies of the interaction of AuSTm with albumin confirmed that both strong and weak binding occur.[6] EXAFS (extended X-ray absorption fine structure) spectroscopy proved extremely useful for structural elucidation. Strong binding occurs at the free thiol with retention of thiomalate; weak binding results from ligation of AuSTm oligomers to the sulfur of the tightly bound AuSTm.[6]

This study focuses on the interaction of the recently approved (FDA) drug auranofin with BSA. The phosphine ligand differentiates $Et_3PAuSATg$ from AuSTm and AuSTg and alters its pharmacological properties, enabling it to be administered orally and increasing its lipid solubility. In addition, $Et_3PAuSATg$ is monomeric while gold(I) thiolates exist as oligomers, $(AuSR)_n$ (n has been estimated for AuSTm to be between 7 and 8 at pH 7.3[7]). Displacement of the potential reducing agents, Et_3P or ATgSH, from auranofin upon protein binding may cause complicating side reactions. However, the Et_3P ligand also enables the chemical reactions of this complex in biological systems to be studied by ^{31}P NMR spectroscopy.[8,9]

Reactions of BSA with Et_3PAuCl, a model compound and synthetic precursor of $Et_3PAuSATg$, were also studied to determine whether the anionic ligand significantly alters the binding properties of Et_3PAuX complexes. The strength of the Au-X bond may determine the extent of Et_3PAu^+ binding to proteins, including thiol-dependent enzymes, and may be related to the serum Au levels observed in vivo. Thus, a careful examination of the extent of $Et_3PAuSATg$ and Et_3PAuCl binding to BSA and its relationship to thiol content was undertaken using EXAFS and XANES (X-ray absorption near edge spectroscopy) and ^{31}P NMR analysis to determine the coordination environment of the albumin-bound Au and the fate of the phosphine ligand.

Experimental Section

Isolation of Albumin-Gold Complexes and Other Products. Ethanolic solutions (37 mM) of $Et_3PAuSATg$ or Et_3PAuCl were added to 2.0 mL of 1.8 mM BSA or AlbSSCy solutions in aqueous 0.10 M NH_4HCO_3 buffer, pH 7.9, to yield solutions of various Au/BSA ratios (0.32-1.20).[10] Ethanol in the final mixture did not exceed 10%. After incubation at 4 °C for 0-48 h, the samples were separated on a 1.5 × 40 cm Sephadex G-100 column at 2-4 °C with an elution rate of 15-25 mL/h (0.10 M NH_4HCO_3 eluent). Fractions were collected and analyzed for protein and Au content by absorbance measurement at 278 nm (ϵ_{278} = 39 600 L/(mol cm)) and by atomic absorption spectroscopy, respectively. High- and low-molecular-weight fractions were pooled, lyophilized, and stored at -20 °C and then redissolved for ^{31}P NMR studies.

Reactions with excess $Et_3PAuSATg$ were carried out by adding 1.00 mL of 19.2 mM ethanolic $Et_3PAuSATg$ (19.2 μmol) to 10.0 mL of a

(1) Abbreviations: HSA = heterogeneous human serum albumin; BSA = heterogeneous bovine serum albumin; AlbSH = mercaptalbumin component of BSA; AlbSSCy = disulfide derivative of mercaptalbumin; AuSTg = (1-thio-β-D-glucopyranosato)gold(I); AuSATg = (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato)gold(I); AuSTm = (thiomalato)gold(I), sodium salt; GtSH = glutathione; DTNB = 5,5′-dithiobis(2-nitrobenzoic acid).

(2) Ennis, R. S.; Granda, J. L.; Posner, A. S. *Arthritis Rheum.* **1968**, *11*, 756-764.
(3) Kamel, H.; Brown, D. H.; Ottaway, J. M.; Smith, W. *Analyst (London)* **1977**, *102*, 645-647.
(4) Simpson, R. B.; Saroff, H. A. *J. Am. Chem. Soc.* **1958**, *80*, 2129-2131.
(5) Gerber, D. A. *J. Pharmacol. Exp. Ther.* **1964**, *143*, 137-140.
(6) Shaw, C. F., III; Schaeffer, N. A.; Elder, R. C.; Eidsness, M. K.; Trooster, J. M.; Calis, G. H. *J. Am. Chem. Soc.* **1984**, *106*, 3511-3521.
(7) Isab, A. A.; Sadler, P. J. *J. Chem. Soc., Dalton Trans.* **1981**, 1657-1663.
(8) Malik, N. A.; Sadler, P. J. *Biochem. Soc. Trans.* **1979**, *7*, 731-732.
(9) Malik, N. A.; Otiko, G.; Sadler, P. J. *J. Inorg. Biochem.* **1980**, *12*, 317-322.

(10) A reviewer questioned the choice of ammonium bicarbonate buffer since NH_3 might compete for Et_3PAu^+. When NH_4HCO_3 is used, an additional desalting step is omitted from spectroscopic sample preparation. Furthermore, the chromatographic results demonstrate that Et_3PAuCl and $Et_3PAuSATg$ bind to albumin even in the presence of a 100-fold excess of NH_3 over protein. This is not surprising since Et_3PAu^+ is a soft acid and NH_3 is a hard base.

* To whom correspondence should be addressed.
[†] University of Wisconsin—Milwaukee.
[‡] University of Cincinnati.

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Table I. ^{31}P NMR and Stoichiometric Data for Chromatographically Isolated Albumin–Gold Complexes

Reaction	$\left(\frac{Au}{BSA}\right)$ mixture	Incubation time (hrs)	AlbSH BSA	$\left(\frac{Au}{BSA}\right)_{HMW}$	$\left(\frac{Au}{AlbSH}\right)_{HMW}$	$\delta^{31}P$ hmw fractions	$\delta^{31}P$ lmw fractions
$Et_3PAuSATg/BSA$	1:1	0	0.57	0.50	0.88	38.7	37.0
		1	0.57	0.61	1.07	—	—
		24	0.57	0.57	1.00	38.8	37.0, 57.2
		48	0.57	0.62	1.09	—	—
	5:1	0	0.35	0.39	1.11	39.0	37.0
		24	0.58	0.58	1.00	39.1	37.0
$Et_3PAuSATg/AlbSSCy$	1.5:1	0	0.00	0.04	—	—	—
$Et_3PAuSATg/BSA$ dimer	1.07:1	0	0.26	0.25	0.99	—	—
Et_3PAuCl/BSA	1.12:1	0	0.39	1.11	2.85	44.0, 38.9, 35.9, 28, 27	—
Et_3PAuCl/BSA	0.41:1	0	0.39	0.44	1.13	38.8	—
$Et_3PAuCl/AlbSSCy$	1.45:1	0	0.00	1.07	—	44.1, 28.4, 27.8, 24.9	—

0.37 mM BSA solution (3.7 μmol). After 0 or 24 h, the samples were separated on a 2.5 × 40 cm G-100 Sephadex column and 6.42-mL fractions were collected. Protein fractions were pooled, lyophilized, and redissolved in D_2O/NH_4HCO_3 buffer and Millipore filtered to yield 1–1.5 mM BSA (or AlbSSCy) solutions for ^{31}P NMR. Low-molecular-weight fractions were redissolved in CH_3OD and filtered to yield 0.5–7.5 mM Au solutions.

Direct Analysis of Reaction Mixtures. Several $Et_3PAuSATg/BSA$ (Au/BSA = 1.03, [BSA] = 2.8 mM) and Et_3PAuCl/BSA (Au/BSA = 1.04, [BSA] = 3.2 mM) reaction mixtures were monitored over time via ^{31}P NMR spectroscopy and without chromatography. The Et_3PAuX complexes, in ethanolic solution, were added dropwise to the protein solutions in D_2O/NH_4HCO_3 buffer (the EtOH content did not exceed 10%) 15–20 min prior to acquisition of the initial spectra. Subsequent spectra were obtained at 1.2–3.5-h intervals.

^{31}P NMR Spectroscopy. Broad-band proton-decoupled ^{31}P NMR spectra (101.3 MHz) were obtained at 295 K on a Bruker WM-250 spectrometer in D_2O/NH_4HCO_3 or CH_3OD. The spectral range examined was from −40 to +120 ppm.

Solutions (ca. 2 mL) were placed in 10 mm o.d. NMR tubes along with 1–3 μL of $OP(OCH_3)_3$ as reference ($\delta(^{31}P)$ = 2.74 vs. 85% H_3PO_4). Spectra were accumulated in ca. 100 000 pulses (8 μs, ca. 45°) and 0.541-s acquisition time. Spin–lattice relaxation times were evaluated with use of the progressive saturation technique.[11]

The integrated intensities of an equimolar $Et_3PAuSATg/BSA$ sample (5.0 mM BSA, 10% methanol) were obtained with use of the typical acquisition parameters and also under fully relaxed conditions (90° pulse, 15-s delay between pulses). The reaction mixture was incubated approximately 3 weeks at 4 °C to assure completeness of $OPEt_3$ generation before spectrum acquisition.

Analyses. The mercaptalbumin content of the BSA preparations was determined approximately twice a month with use of 5,5′-dithiobis(2-nitrobenzoic acid), DTNB.[12] Au concentrations were determined by atomic absorption on an Instrumental Laboratories 357 spectrometer, calibrated with serial dilutions of a Spex $KAu(CN)_2$ standard (171.4 μg/mL).

Azide Treatment of BSA. After addition of 1.0 mL of a 3.1 mM NaN_3 solution (NH_4HCO_3 buffer, pH 7.9) to 10.0 mL of a 28.7 μM BSA solution (SH titer 0.43), the sulfhydryl titer of the resulting solution (N_3^-/BSA = 10.8) was monitored over a 24-h period. As a control, 0.28 mM NaN_3 (NH_4HCO_3 buffer) solution lacking BSA was similarly analyzed.

Isolation and Reaction of Albumin Dimers. Dimeric albumin was partially separated from monomeric albumin by gel filtration of 11.8 μmol of protein on a 2.5 × 40 cm Sephadex G-100 column in NH_4HCO_3 buffer (pH 7.9), lyophilized, and redissolved in 2.6 mL of NH_4HCO_3 buffer (78% of the albumin in these fractions was dimer). After Millipore filtering the albumin (1.23 μmol) and addition of $Et_3PAuSATg$ (0.25 mL of 4.96 mM solution), the sample was immediately chromatographed and analyzed as above.

Preparations of EXAFS Samples. Protein was isolated from the $Et_3PAuSATg/BSA$ and $Et_3PAuCl/AlbSSCy$ reaction mixtures, as described above. $Et_3PAuSATg/BSA$ mixtures were incubated for 0, 1, 24, and 48 h in order to determine whether changes in the oxidation state or coordination sphere occurred subsequent to the initial Au binding. Typically, 150-mg samples were prepared.

Preparation of AlbSAuSATg for EXAFS. $(AuSATg)_n$ solution (0.2 mL, 9.19 mM in 1/1 EtOH/butanol) was added to 14.6 mL of a 0.506 mM BSA solution (12.75 mL of 0.10 M NH_4HCO_3 buffer, 1.10 mL of butanol, and 0.75 mL of ethanol) and then filtered to yield a solution with 1:4 AuSATg/BSA stoichiometry. After 45 min, the reaction mixture was chromatographed as above and analyzed. The protein fractions were pooled, lyophilized, and stored frozen at −20 °C until the EXAFS analysis was performed.

EXAFS Measurements and Analyses. The X-ray absorption spectra were measured at the Stanford Synchrotron Radiation Laboratory, on beam line IV-1. Fluorescence and absorbance measurements were simultaneously collected. The lyophilized protein samples were packed into sample cells with 5 mm path length. The spectra of the model compounds $Na_3[Au(S_2O_3)_2]\cdot 2H_2O$, $[(etu)_2Au]Cl\cdot H_2O$ (etu = ethylenethiourea), $[Au(NH_3)_4](NO_3)_3$, and $[(Ph_2MeP)_2Au]PF_6$ were recorded on powdered solid samples diluted with Li_2CO_3 packed into cells of path length 1 mm. Simultaneous wavelength calibration was obtained with gold foil placed between the second and third ionization chambers.

The EXAFS information was extracted from the absorption spectrum by following the technique of Hodgson[13–15] using programs modified for compatibility with an Amdahl 470/V7A computer and TSO IBM software in use at the University of Cincinnati. Other details of the data collection and analysis have been published previously.[6,16]

Reagents. The following reagents were obtained from Sigma Chemicals (St. Louis): DTNB, bovine serum albumin (fatty acid free, A7030, lots 93F-0044 and 62F-0510; fatty acid free, crystallized and lyophilized, A-7511, lots 22F-9340, 10F-9371, and 90F-9315), and Sephadex G-100.

D_2O (99.8% D), CH_3OD (99.5% D), and $OP(OCH_3)_3$ were obtained from Aldrich Chemical Co. (Milwaukee). Sulfhydryl-modified albumin (lot 11M) was ordered from Miles Biochemicals. Auranofin ((2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato)(triethylphosphine)gold(I)) and chloro(triethylphosphine)gold(I) were the gifts of Smith Kline and French Laboratories.

Results

$Et_3PAuSATg/BSA$ Reaction. After chromatography of the $Et_3PAuSATg/BSA$ reaction mixtures, the molar ratio of gold bound per BSA was consistently less than 1 (Table I). The unbound gold eluted as a single low-molecular-weight species. Recoveries of BSA and Au ranged from 94 to 105% and 92 to 110%, respectively.

Initial experiments showed strong agreement between sulfhydryl titer (moles of mercaptalbumin, AlbSH, per mole of BSA) and the molar ratio of gold bound per BSA (Au/BSA) as shown in Table I. The average ratio of gold to mercaptalbumin (Au/AlbSH) was 1.05 ± 0.05. Samples chromatographed immediately after mixing had Au/AlbSH ratios that ranged from 0.75 to 0.90,

(11) Freeman, H.; Hill, H. D. W.; Kaptein, K. *J. Magn. Reson.* **1972**, *7*, 82–98.
(12) Ellman, G. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77.
(13) Cramer, S. P.; Hodgson, K. O. *Prog. Inorg. Chem.* **1979**, *25*, 1–39.
(14) Eccles, T. K. Ph.D. Thesis, Stanford University, 1979.
(15) Cramer, S. P. Ph.D. Thesis, Stanford University, 1979.
(16) Elder, R. C.; Eidsness, M. K.; Heeg, M. J.; Tepperman, K. G.; Shaw, C. F., III; Schaeffer, N. A. *ACS Symp. Ser.* **1983**, *No. 209*, 385–400.

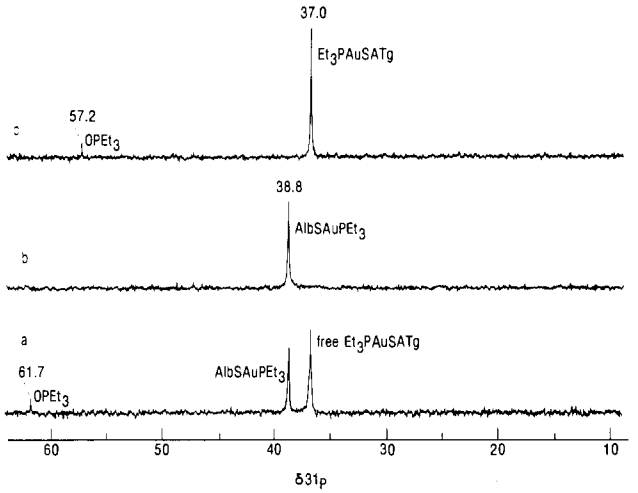


Figure 1. Broad-band proton-decoupled ^{31}P NMR spectra (101.3 MHz, 0.54-s pulse delay) of (a) an equimolar $Et_3PAuSATg$/BSA mixture (2.8 mM BSA) incubated for 24 h, (b) the hmw fractions (gold–albumin complex), and (c) the lmw fractions (unreacted auranofin) isolated by chromatography, as in Table I, of a similar reaction mixture (incubated 24 h). (b) and (c) were concentrated by lyophilizing and redissolving in D_2O/NH_4HCO_3 buffer and CH_3OD, respectively. Chemical shifts are reported relative to $OP(OCH_3)_3$ ($\delta(^{31}P)$ = 2.74 relative to H_3PO_4).

indicating that the reaction is not instantaneous. Incubating the samples for 1, 24, or 48 h prior to gel filtration or adding a fivefold excess of $Et_3PAuSATg$ did not increase the Au/AlbSH ratio beyond unity. These data suggest that the reaction is complete within 1 h and that there exists a single gold binding site, the thiol form of cys-34.

To confirm that cys-34 of AlbSH is the sole binding site, commercially obtained sulfhydryl-blocked albumin (AlbSSCy, SH/BSA = 0.003) was reacted with $Et_3PAuSATg$. Immediate fractionation of the sample ($Et_3PAuSATg$/AlbSSCy = 1) and subsequent analysis showed that the Au/BSA ratio was 0.037. The decreased gold-binding capability of the modified BSA provides strong evidence for the role of cys-34 as the sole $Et_3PAuSATg$ binding site.

ATgSH displaced from the albumin-bound gold was detected in the low-molecular-weight (lmw) chromatographic fractions with DTNB. After correction for the partial reaction of $Et_3PAuSATg$ with DTNB (0.5 DTNB reduced per $Et_3PAuSATg$), 53% of the expected ATgSH was recovered. Control experiments demonstrated that ATgSH is partially oxidized during gel filtration.

The albumin peak often exhibited a front-running shoulder, due to the presence of dimeric BSA,[17] which binds less gold than the major protein peak. Analysis of the equimolar reaction of $Et_3PAuSATg$ with a dimer-enriched BSA sample (78% dimer) showed that the bound Au/BSA ratio was 0.25. The correspondence between this value and the SH titer of the dimer (0.26) suggests that the only reaction of $Et_3PAuSATg$ with dimeric BSA occurs at free thiol groups.

Unchromatographed reaction mixtures of $Et_3PAuSATg$ and BSA ($Et_3PAuSATg$/BSA = 1.03) were also monitored over 24 h by ^{31}P NMR spectroscopy. The spectra contained peaks at 61.6, 38.6, and 36.6 ppm (Figure 1a) and did not change significantly between 1 and 24 h. The resonances were assigned to $OPEt_3$ (verified by determination of the $OPEt_3$ chemical shift in D_2O), protein-bound phosphine, and free $Et_3PAuSATg$, respectively. Malik and Sadler[8] also observed three resonances at 68.3 (5%), 45.3 (30%), and 42.9 (65%) ppm (relative to 85% H_3PO_4) in agreement with the results reported here.

To verify the assignments, the protein-bound and free gold–phosphine species were separated by gel exclusion chromatography, lyophilized, and redissolved for ^{31}P NMR analysis. The single peak at 38.8 ppm in the protein sample (Figure 1b) is indicative of $AlbSAuPEt_3$ formation.

(17) Andersson, L. O. *Biochim. Biophys. Acta* **1966**, *117*, 115–133.

Table II. Spin–Lattice Relaxation Times[a]

compd (n)[b]	T_1, s[c]
$Et_3PAuSATg$ (3)	1.7 ± 0.2
$AlbSAuPEt_3$ (4)	1.3 ± 0.1
$OPEt_3$ (5)	3.8 ± 0.6

[a] Measured by progressive saturation in 3–5 mM albumin solutions. [b] Number of independent determinations. [c] Mean values and standard deviations.

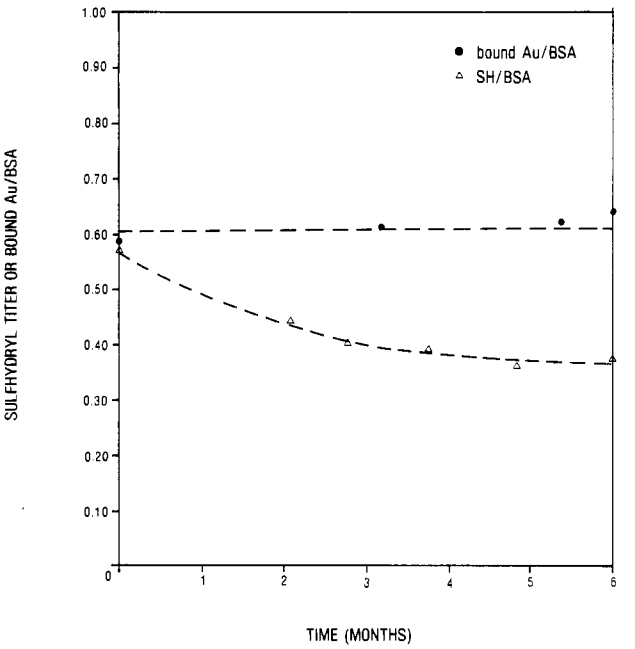


Figure 2. Variation of the Au binding capacity (●) and sulfhydryl titer (△) of a single BSA preparation over a 6-month period. Au/BSA ratios were determined by chromatographing $Et_3PAuSATg$/BSA samples immediately after mixing and analyzing the protein-containing fractions.

The lmw fractions, redissolved in CH_3OD, contained unreacted $Et_3PAuSATg$ (37.0 ppm). In spectra with good signal/noise ratios, a second peak of low intensity at 57.2 ppm (Figure 1c) was assigned to $OPEt_3$ ($\delta(^{31}P)$ = 61.6 in aqueous solution and 57.2 in CH_3OD).

Spin–lattice relaxation times (Table II) for $Et_3PAuSATg$, $OPEt_3$, and $AlbSAuPEt_3$ were measured in solutions of 3–5 mM albumin by the progressive saturation technique.[11] Use of concentrated protein solutions was necessary since the correlation times (τ_c) of these species vary with solution viscosity and affect the T_1 values. Under these conditions the T_1 values are short compared to those obtained for organic phosphines and their complexes (typically 8–20 s) in less viscous media.[18]

Table III shows the distribution of products determined by integration of several NMR spectra. Two 5.0 mM protein solutions were compared under fully relaxed conditions (pulse delay based on T_1 measurements) and with the 0.54-s pulse delay routinely utilized in this work. The similarity of the distributions demonstrates that approximate data can be obtained with the shorter pulse delays, since their use underrepresents the $OPEt_3$ intensity only slightly. From the effect of delay times on the integrated intensities, it can be estimated that less than 10% of the Et_3P is displaced from gold and oxidized. At the 5 mM concentrations more conversion to $OPEt_3$ was observed with the samples, indicating that formation of the oxide is concentration-dependent. In addition, the bound Au/BSA ratios determined after isolation of the protein and the value based on NMR integration were in excellent agreement. For the sample that had incubated 24 h prior to NMR analysis (Table III, sample a) the protein-bound phosphine (38.6 ppm) represented 41% of the phosphorus-containing products and the free auranofin peak ac-

(18) Deihl, P.; Fluck, E.; Kosfeld, R. "NMR Basic Principles and Progress"; Springer-Verlag: Berlin, 1979; Vol. 16, pp 90–91.

Table III. Product Distributions as a Function of NMR Acquisition Parameters and Protein Concentrations

	concn of reactants, mM		acquis params		product mole ratios[a]			
sample	Au	BSA (SH/BSA)	delay, s	pulse, deg	$OPEt_3$	AF	$AlbSAuPR_3$	Au/BSA[b]
a	2.9	2.8 (0.40)	0.54	45	0.03	0.56	0.41	0.38
b	2.1	1.9 (0.35)	10.0	90	0.07	0.27	0.66	0.68
c	5.0	5.0 (0.34)	0.54	45	0.11	0.24	0.65	...
			15.0	90	0.15	0.21	0.64	...
	5.0	5.0 (0.35)	8.00	45	0.20	0.28	0.52	...
			0.54	45	0.17	0.25	0.58	...

[a] Total Phosphorus 1.00. [b] Determined after chromatographic isolation.

counted for approximately 56%. The agreement between the percentage of protein-bound phosphine and the protein SH titer, 0.40, implies that the reaction is limited only by the AlbSH content when $Et_3PAuSATg$ is present in excess.

Oxidation of Cys-34 during Storage. Although the bound Au/AlbSH ratio determined from the initial $Et_3PAuSATg$/BSA experiments was approximately unity (Table I), subsequent experiments, which utilized an "aged" BSA source (SH titer 0.3–0.4), revealed that the decrease in thiol content was not accompanied by a decrease in Au binding (Figure 2). Over a 6-month period, the protein SH titer dropped from 0.57 to 0.37 while the Au/BSA ratio remained constant at 0.61 ± 0.01.

Assuming that the thiol titer is reduced by oxidation, reduction by PEt_3 or ATgSH could regenerate AlbSH, maintaining the Au/BSA binding ratio at 60%. If PEt_3 were the reducing agent, the amount of $OPEt_3$ generated would correspond to the difference between the protein SH titer and its gold-binding capacity. In fact, $OPEt_3$ represented at most 10% of the total phosphine (Figure 1a) for the reaction of $Et_3PAuSATg$ with 5-month-old protein. ATgSH regenerated AlbSH when incubated with "aged" albumin. Thus, it is likely that ATgSH released from the reaction of $Et_3PAuSATg$ with AlbSH is the primary reducing agent.

Formation of a stable sulfenic acid derivative of AlbSH would explain these observations. Sulfenic acid derivatives of several proteins can be reduced to the mercapto form by various nucleophilic reagents (e.g. N_3^-, $S_2O_3^{2-}$, DTT, β-mercaptoethanol).[19] Upon incubation of "aged" BSA with azide (N_3^-/BSA = 10.8) the SH titer increased from 0.43 to 0.57 after 24 h. A control demonstrated that N_3^- did not interfere with the DTNB assay.

Et_3PAuCl/BSA Reactions. Gel filtration chromatograms of equimolar Et_3PAuCl/BSA reaction mixtures showed most of the Au bound to the protein but also exhibited trailing of gold from the high-molecular-weight (hmw) fractions into the eluting buffer. The dramatic increase in the ratio of bound Au/BSA over the SH titer of the BSA and the trailing of Au (which was not observed in the $Et_3PAuSATg$/BSA reaction) suggest that weak-binding sites exist for Et_3PAuCl.

In contrast, a stable protein–gold complex was isolated without loss of gold from the equimolar reaction of Et_3PAuCl with AlbSH (Et_3PAuCl/AlbSH = 1) and analyzed by ^{31}P NMR (Figure 3a). The appearance of a single peak at 38.8 ppm, identical with that obtained in the $Et_3PAuSATg$/BSA studies, indicates that both $Et_3PAuSATg$ and Et_3PAuCl react at cys-34 with retention of PEt_3 and displacement of the anionic ligand to form $AlbSAuPEt_3$.

The Et_3PAuCl/BSA reaction mixture (Et_3PAuCl/BSA = 1.06) was also analyzed by ^{31}P NMR spectroscopy. In addition to the 38.8 ppm peak ($AlbSAuPEt_3$), broad peaks at 27 and 28 ppm and a sharp peak at 44.2 ppm were detected (Figure 3d). The spectrum of the BSA isolated from this mixture (Figure 3b) contained all the peaks originally detected in the unchromatographed sample, indicating that all the resonances represent protein-bound phosphine. The 27 and 28 ppm resonances, however, were diminished almost beyond detection, and the 38.8 ppm peak was reduced in intensity. In addition, a new, sharp peak at 35.9 ppm was present.

To simplify the ^{31}P spectrum and restrict binding to the low-affinity sites, the reaction of the gold complex with AlbSSCy was investigated. The ^{31}P NMR spectrum of the protein isolated from

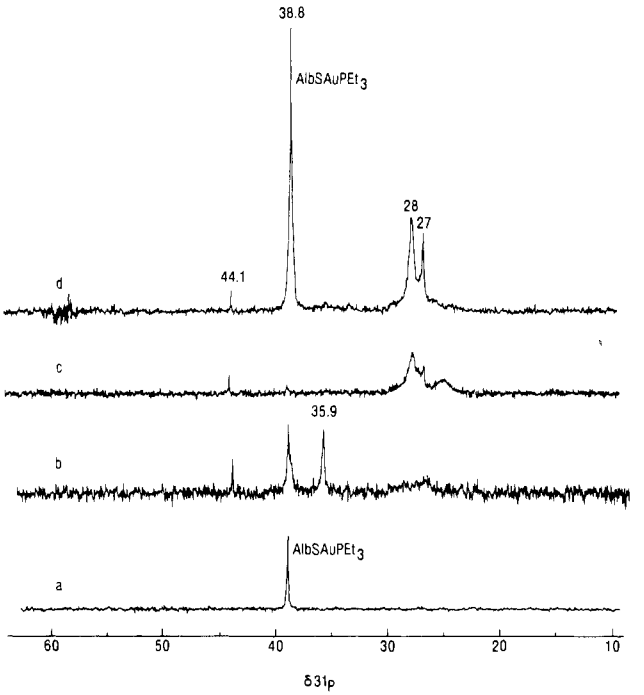


Figure 3. Broad-band proton-decoupled ^{31}P NMR spectra (101.3 MHz, 0.54-s pulse delay) of Et_3PAu^+ bound at (a) the tight-binding site, (b) and (d) the tight- and weak-binding sites, and (c) only the weak-binding sites. The concentrated protein fractions in (a) were obtained from an equimolar Et_3PAuCl/AlbSH reaction (1.89 mM BSA; SH titer 0.39). The protein fractions in (b) were obtained from a 2.86/1.00 Et_3PAuCl/AlbSH mixture (1.87 mM BSA; SH titer 0.39). The protein fractions in (c) were obtained from a 1.45/1.00 Et_3PAuCl/AlbSSCy reaction (1.62 mM BSA). The solution in (d) is a 2.86/1.00 Et_3PAuCl/AlbSH reaction mixture (without chromatography, 3.2 mM BSA; SH titer 0.37). The signal at 44.2 ppm may be due to $[(Et_3P)_2Au^+]$ formed in situ. Samples were chromatographed as in Figure 1. Chemical shifts are reported relative to $OP(OCH_3)_3$ ($\delta(^{31}P)$ = 2.74 relative to H_3PO_4).

the Et_3PAuCl/AlbSSCy mixture (Figure 3c) contained peaks at 27, 28, and 44.1 ppm and a slight peak at 25 ppm. Thus, the absent 38.8 and 35.9 ppm resonances must be associated with cys-34 while the remaining resonances result from binding at other sites or species formed by dissociation of weakly bound Et_3PAu^+ during chromatography.

A comparison of the Et_3PAuCl/BSA spectra (Figure 3b–d) revealed that the appearance of the 35.9 ppm resonance was accompanied by a decrease in the intensity of the peaks attributed to $AlbSAuPEt_3$ (38.8 ppm) and the weakly bound Et_3PAu^+ (27 and 28 ppm). Also, the intensity of the 44.1 ppm peak varied inversely with the intensities of the 27 and 28 ppm resonances. These observations and the fact that the 44.1 ppm resonance was detected only when the weak-binding sites were populated (Et_3PAuCl/AlbSH > 1) imply that these species (35.9 and 44.1 ppm peaks) are formed via loss of Et_3PAu^+ and/or PEt_3 from the low-affinity sites.

EXAFS/XANES Spectroscopy. To confirm the binding of the $AuPEt_3$ moiety to the cys-34 thiol and to explore the ligands involved in the weak-binding sites, the following complexes were prepared for EXAFS analysis: $AlbSAuPEt_3$,

(19) Allison, W. S. *Acc. Chem. Res.* **1976**, *9*, 293–299.

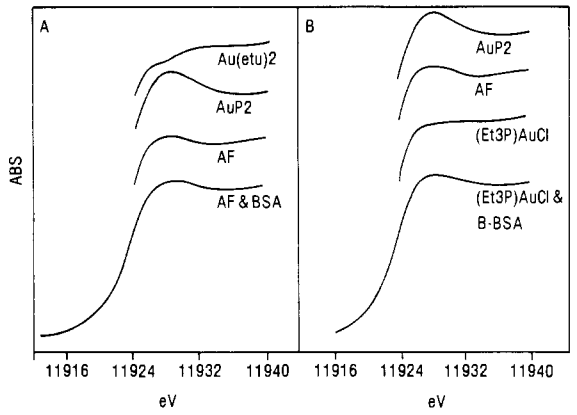


Figure 4. (A) XANES spectra of the gold L_{III} edge region: for bis(ethylenethiourea)gold(I), $Au(etu)_2$, gold(I) coordinated by two sulfur atoms; for bis(diphenylmethylphosphine)gold(I), AuP_2, gold coordinated by two phosphorus atoms showing the pronounced peak in the edge structure characteristic of Au–P coordination; for auranofin, AF, showing the somewhat reduced peak characteristic of a single Au–P interaction; for the product of auranofin incubation of BSA, AF, and BSA, with an Au–P peak indicative of retention of the Au–P bond in the product complex. (B) XANES spectra of the gold L_{III} edge region: for bis(diphenylmethylphosphine)gold(I), AuP_2; for auranofin, AF, showing the peak characteristic of Au–P bonding; for (triethylphosphine)gold(I) chloride, Et_3PAuCl, which with Cl trans to P lacks the Au–P peak; for the product of (triethylphosphine)gold(I) chloride incubation with blocked BSA, Et_3PAuCl, and B-BSA (B-BSA = AlbSSCy), showing the characteristic Au–P peak.

$(Et_3PAu)_{0.78}$AlbSSCy, and $AlbS(AuSATg)_{0.45}$. Auranofin was used to prepared the $AlbSAuPEt_3$, and Et_3PAuCl was reacted with AlbSSCy to prepare the weakly bound Et_3PAu^+ species. The third sample, $AlbS(AuSATg)_{0.45}$, which represents a product that might form if Et_3P were displaced from $Et_3PAuSATg$, was prepared by reaction of $(AuSATg)_n$ with BSA.

The L_{III} XANES spectra of gold(III) complexes feature a prominent transition at 11.923 keV, due to a bound state transition of an electron from a 2p orbital into a vacant 5d orbital. Gold(0) in the colloidal or bulk forms has characteristic bands at 11.945 and 11.967 keV.[16] The absence of these bands, as observed here, confirms that the gold remains in the +1 oxidation state.

A band at 11.930 keV has been identified in the XANES spectra of numerous gold–phosphine species.[16] In the spectra of $(Et_3PAu)_{0.78}$AlbSSCy and $AlbSAuPEt_3$, the presence of the expected band at 11.930 keV confirmed that the Et_3P is ligated to gold (Figure 4). Its presence in the $(Et_3PAu)_{0.78}$AlbSSCy spectrum (Figure 4b) provides evidence that Cl^- has been displaced, since the starting material, Et_3PAuCl, is exceptional in that it lacks this band.

Analysis of the EXAFS portion of the spectra, using the Fourier transforms and fitting the Fourier-filtered EXAFS spectra with transferable constants from model compounds with known structures, yielded the results of Table IV. The EXAFS data of $AlbSAuPEt_3$ could be fit fairly well either by S alone, P alone or a combination of the two. In all three models the coordination number was approximately 2 and the bond distances were reasonable for Au–S and Au–P bonds.

The data for $(Et_3PAu)_{0.78}$AlbSSCy could be fit well by invoking a two-coordinate model involving P and N (or O) ligation, as indicated by the remarkable improvement in the goodness of fit (Table IV). Other models involving some combination of P, S, and Cl ligands produced unreasonable coordination numbers or bond lengths and poorer fits of the Fourier-filtered EXAFS data. The calculated Au–P and Au–N bond distances, 225 and 206 pm respectively, agree well with crystallographically determined distances for P and N complexes of gold(I). The coordination numbers for N, 1.1, and P, 1.1, also indicate the displacement of Cl^- from the complex, thereby maintaining the two-coordinate environment of gold. EXAFS cannot distinguish between N or O coordination, but the chemistry of gold(I) suggests that oxygen coordination is unlikely.[20]

Table IV. EXAFS Results for Albumin–Gold Complexes

complex[a]	Au/BSA[b]	donor atom(s)	coord no.	Au–X dist, pm	GOF[c]
$AlbS(AuPEt_3)_{1.0}$	0.58	S	2.1	227	0.24
		P	2.3	230	0.20
		S[d]	0.6	227	0.19
		P[d]	1.7	229	
$(Et_3PAu)_{0.78}$AlbSSCy	0.78	P	1.6	224	0.23
		N	3.0	207	0.47
		P[d]	1.1	225	0.06
		N[d]	1.1	206	
$AlbS(AuSATg)_{0.45}$	0.20	S	2.3	228	0.11
$AlbS(AuSTm)_{0.76}$[e]	0.44	S	2.1	228	0.25
$AlbS(AuSTm)_{7.4}$[e]	2.65	S	2.0	230	0.24

[a] These formulas give the stoichiometries between Au and AlbSH in the samples. [b] This column lists the ratios of Au to total albumin. [c] Goodness of fit computed as $GOF = \{[\sum k^6(\text{data} - X)^2]/N\}^{1/2}$, in which data are the N filtered data points and X the fit values. [d] These are two-shell fits. [e] Reference 6.

The EXAFS/XANES results for $AlbS(AuSATg)_{0.45}$ (Table IV) are identical with those previously obtained for $AlbS(AuSTm)_{0.76}$.[6] As expected, the XANES band at 11.930 keV is absent, since the starting gold complex is a (thiolato)gold(I) species. The coordination number of 2.3 is consistent with the formation of a complex having the sulfurs of ATgSH and AlbSH as donor atoms. The bond length, 228 pm, is the same as that of $AlbS(AuSTm)_{0.76}$. The differences in the coordination spheres determined for this complex, where only sulfur ligation was expected, and those of the Et_3PAu^+ complexes demonstrate the importance of EXAFS spectroscopy for structural analysis of gold–protein species.

Discussion

The agreement between the SH titer of the albumin and the bound Au/BSA ratios, the similarity of the chemical shift of the phosphine–gold–albumin adduct, 38.8 ppm, to those of other thiolate adducts, Et_3PAuSR, the presence of the 38.8 ppm species in the protein fractions after gel filtration, and the presence of ATgSH in the lmw fractions strongly support the conclusion that auranofin reacts at and binds to the free SH residue of albumin, cys-34, with displacement of ATgSH. The coordination sphere determined by EXAFS/XANES is also consistent with this model. The inability of EXAFS to distinguish AuS_2, AuP_2, and SAuP coordination is expected and borne out by the similarity in the goodness of fit for all three models.[16] Phosphorus and sulfur roles are most likely interchanged in model 3; namely, the Au–S bond is likely to be longer than the Au–P bond. This interchange is found in EXAFS fits of auranofin as well. On the basis of XANES, PEt_3 is ligated to gold; on the basis of NMR, only one PEt_3 is ligated to gold; on the basis of EXAFS, the second ligand must be sulfur. Thus, the complex formed between AlbSH and auranofin must be $AlbSAuPEt_3$ arising from reaction 1.

$$Et_3PAuSATg + AlbSH \rightarrow AlbSAuPEt_3 + ATgSH \quad (1)$$

As shown by Sadler and co-workers, ^{31}P NMR is a powerful tool for probing the reactions of phosphine–gold(I) complexes with biological entities.[8,9] Some care must be exercised since products formed by the loss of PEt_3 from gold would be transparent to this technique. In the present case, however, the agreement of the amount of $AlbSAuPEt_3$ formed as determined by NMR integration (41% of the total phosphine in the equimolar $Et_3PAuSATg$/BSA reaction; Table III, sample a) and by isolation of the complex (bound Au/BSA = 0.38) with the SH titer of BSA (0.40) demonstrates that it is the only major protein-bound gold species.

The equimolar reaction of Et_3PAuCl with AlbSH generated only the 38.8 ppm resonance, indicating that cys-34 is the high-affinity binding site for it (Figure 3a). All of the gold was protein-bound upon gel filtration, and the trailing of gold observed

(20) Puddephatt, R. J. "The Chemistry of Gold"; Elsevier: Amsterdam, 1978; p 58.

upon population of the weak-binding sites was not evident. Thus, cys-34 is the preferred site of reaction:

$$Et_3PAuCl + AlbSH \rightarrow AlbSAuPEt_3 + Cl^- + H^+ \quad (2)$$

The existence of additional weaker binding sites for Et_3PAu^+ explains the trailing of gold from the protein into the eluting buffer during chromatography. The broadenss of the ^{31}P NMR peaks at 27 and 28 ppm arising from weakly bound Et_3PAu^+ may indicate rapid exchange among these sites. Because the spectra of the protein fractions from the Et_3PAuCl/AlbSSCy reaction also contained these broad resonances, the weak-binding sites must be topologically remote from cys-34.

Recent ^{31}P NMR studies of model compounds by Kinsch and Stephan suggest that these chemical shifts may be indicative of coordination of Et_3PAu^+ to imidazole, lysine, carboxylate, or thioether donor groups of albumin.[21] When methanolic solutions of Et_3PAuCl are added dropwise to aqueous solutions of histidine or methionine at gold/ligand ratios between 0.5 and 2.0, a single, broadened resonance is observed for the Et_3PAuX species formed by ligand exchange.[22] The broadening is indicative of rapid Et_3PAu^+ exchange between chloride and histidine or methionine and closely resembles the behavior of the Et_3PAu–protein resonances at 27 and 28 ppm. The EXAFS spectrum of $(Et_3PAu)_x$AlbSSCy demonstrates that the weakly bound Et_3PAu^+ is coordinated to a ligand of low atomic number. Since oxygen is a poor ligand for gold, binding to a nitrogen donor, e.g. histidine, lysine, or the N-terminal amino group, is most likely.

The resonance at 35.9 ppm is observed only in the reaction of AlbSH with excess Et_3PAuCl and after chromatography was not detected in the Et_3PAuCl/AlbSSCy reaction. Thus, it is reasonable to postulate that this resonance represents a thiolate-bridged dimeric gold species, $[(\mu\text{-AlbS})(AuPEt_3)_2^+]$:

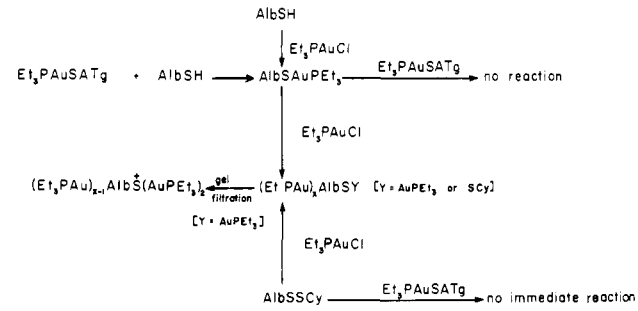
$$(Et_3PAu)_xAlbSAuPEt_3 \xrightarrow{\text{gel filtration}} (Et_3PAu)_{x-1}(AlbS^+)(AuPEt_3)_2 \quad (3)$$

The equilibrium constants for a similar reaction between Et_3PAuCl and $Et_3PAuSATg$ to form $[(Et_3PAu)_2SATg^+]$ are 2×10^3 in aqueous solution and 8.3×10^1 in 95% methanol.[23] The chemical shift of $[(Et_3PAu)_2SATg^+]$, 36.0 ppm, is approximately 1 ppm upfield from that of auranofin. The 35.9 ppm shift of the postulated albumin-bridged digold species is similar in value and also upfield from the shift of the parent complex, $AlbSAuPEt_3$. The formation of $[ATgS(AuPEt_3)_2^+]$ is hindered by high chloride concentrations and/or organic solvent. Therefore, the detection of $[AlbS(AuPEt_3)_2^+]$ only after removal of Cl^- and ethanol from the reaction mixture is reasonable. Although the thermodynamic and kinetic factors favoring the transfer of Et_3PAu^+ are unclear, the localization of cys-34 in a crevice of the protein may favor the binding of the second Et_3PAu^+ here over more exposed locations on the protein surface.

The resonance at 44.1 ppm is observed in the reaction of BSA or AlbSSCy with Et_3PAuCl, but not with $Et_3PAuSATg$. Sadler et al.[9] assigned as $[(Et_3P)_2Au^+]$ a similar resonance (50.3 ppm vs. 85% H_3PO_4) observed in the reaction of Et_3PAuCl with glutathione, GtSH, which generates the oligomeric gold(I) thiolate, $[AuSGt]_n$. In the present case, an oligomer of albumin, $[AlbS\text{-}Au]_n$, analogous to the $[AuSGt]_n$ polymer postulated by Sadler could not form, and displacement of PEt_3 by a second ligand of BSA would be required.

The decrease in the albumin SH titer during storage without a decrease in its ability to react with $Et_3PAuSATg$ (Figure 2) was unexpected. Either the product generated via oxidation of the SH group retains the ability to bind Et_3PAu^+ or the oxidation is chemically reversed during the reaction with auranofin. The first possibility is eliminated by the ^{31}P NMR data, in which only one class of binding sites is observed. Thus, the oxidation is most likely reversed by one of the reaction components. PEt_3 is an unlikely reductant because the $OPEt_3$ (at most 10%) generated was insufficient to account for the additional gold bound. Thus, ATgSH released by reaction of auranofin with AlbSH (eq 1) appears to be the reducing agent.

Scheme I



The two most probable mechanisms of thiol oxidation are formation of intermolecular disulfide-linked dimers or oxidation of cys-34 to a sulfenic or sulfinic acid group. An alternative, nonoxidative mechanism is formation of a thiouronium ring with an adjacent arginine, previously postulated for albumin.[24] Because dimeric BSA was not reduced by auranofin, it is unlikely that dimer formation is responsible for the decrease in SH titer during storage.

Direct oxidation of cys-34 is the most plausible alternative mechanism. Although sulfenic acids are labile and are often transient intermediates, bulky aliphatic thiols do form isolable sulfenic acids, e.g. Me_3CSOH.[25] Stable sulfenic acids of cysteine in glyceraldehyde-3-phosphate dehydrogenase, papain, and creatine kinase have been detected by their reduction with azide:[19,26]

$$\text{protein-SOH} + 2N_3^- + 2H^+ \rightarrow \text{protein-SH} + 3N_2 + H_2O \quad (4)$$

Formation of sulfur oxidation states higher than disulfides (i.e., RSO_xH, x = 1, 2, or 3) in albumin have been reported.[27] Treatment of the "aged" BSA samples with azide increased their SH titers, consistent with the postulated formation of sulfenic acid. The simplest reaction that can account for the ability of $Et_3PAuSATg$ to reduce the sulfenic acid and form $AlbSAuPEt_3$ is

$$AlbSOH + ATgSH + Et_3PAuSATg \rightarrow AlbSAuPEt_3 + ATgSSATg + H_2O \quad (5)$$

The ATgSH is generated by reaction of auranofin with AlbSH present in the BSA sample (eq 1). Summing eq 1 and 5 gives

$$2Et_3PAuSATg + AlbSOH + AlbSH \rightarrow 2AlbSAuPEt_3 + ATgSSATg + H_2O \quad (6)$$

Reaction 6 predicts that the maximum Au/AlbSH ratio should be 2.00 and the maximum Au/BSA ratio should be 1.00. The mean Au/AlbSH ratio for approximately 25 reactions was 1.8. Consistent with this equation, very little ATgSH was detected in the reactions where the Au/AlbSH ratio greatly exceeded unity. Thus, all the present observations can be explained by the formation of AlbSOH during storage and its reduction by ATgSH displaced from auranofin. In contrast, the reaction of AuSTm with albumin does not generate free thiols and the bound Au/AlbSH ratio should be approximately unity, as observed by Schaeffer et al.[6]

Scheme I summarizes the reactions of Et_3PAuCl and $Et_3PAuSATg$ with AlbSH and AlbSSCy. $(Et_3PAu)_xAlb$ represents weakly bound Et_3PAu^+. The dramatic differences between the reactivities of auranofin and Et_3PAuCl toward BSA demonstrate the sensitivity of the chemistry of Et_3PAuX species to

(21) Kinsch, E. M.; Stephan, D. W. *Inorg. Chim. Acta* **1984**, *91*, 263–267.
(22) Coffer, M. T.; Shaw, C. F., III, unpublished observations.
(23) Hempel, J., personal communication.
(24) Brush, J. S.; Jensen, E. V.; Jacobsen, H. I. *Biochim. Biophys. Acta* **1962**, *74*, 688–697.
(25) Shelton, J. R.; Davis, K. E. *J. Am. Chem. Soc.* **1967**, *89*, 718–719.
(26) You, K.; Benitez, L. V.; McConachie, W. A.; Allison, W. S. *Biochim. Biophys. Acta* **1975**, *384*, 317–330.
(27) Noel, J. K. F.; Hunter, M. J. *J. Biol. Chem.* **1972**, *247*, 7391–7406.

variation of the anionic ligand. The additional binding modes of the chloro complex reflect the relative weakness of the Au–Cl bond compared to the Au–SR bond. Thus, Cl^- is displaced by cys-34 ($AlbSAuPEt_3$ formation) and also by other ligands on albumin which cannot displace $ATgS^-$.

Under physiological conditions during chrysotherapy, albumin concentrations in serum far exceed the levels of gold obtained with auranofin. Thus $AlbSAuPEt_3$ will be the dominant circulating form of gold until the oxidation of the phosphine to Et_3PO is completed over approximately 24 h. Et_3PAuCl has antiarthritic activity but causes severe gastrointestinal irritation.[28] Reaction at additional binding sites not available to auranofin may in part explain this difference in biological activities, if the Et_3PAuCl concentration exceeds the availability of membrane sulfhydryl sites in the stomach or gut.

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(28) Walz, D. T.; DiMartino, M. J.; Charkin, L. W.; Sutton, B. M.; Mishu, A. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 142-152.

Contribution from the Department of Inorganic Chemistry, The University, Newcastle upon Tyne NE1 7RU, England

Kinetics and Mechanism of the Reduction of Rhodium(III) Complexes $Rh(H_2O)_5X^{2+}$ by Cr^{2+}

Elaine F. Hills, Monika Moszner, and A. Geoffrey Sykes*

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Reduction of the yellow rhodium(III) ions $[Rh(H_2O)_6]^{3+}$, $[Rh(H_2O)_5Cl]^{2+}$, and $[Rh(H_2O)_5Br]^{2+}$ by Cr^{2+} in aqueous $HClO_4$ solution to give the green rhodium(II) aqua dimer Rh_2^{4+} has been studied by the stopped-flow method at 25 °C, I = 2.0 M ($LiClO_4$). An $[H^+]^{-1}$ term is dominant in the reduction of $[Rh(H_2O)_6]^{3+}$ (pK_a = 3.4), and rate constants (M^{-1} s^{-1}) for the different oxidants are $[Rh(H_2O)_5OH]^{2+}$ (2.3×10^5), $[Rh(H_2O)_5Cl]^{2+}$ (2.2×10^3), $[Rh(H_2O)_5Br]^{2+}$ ($>8 \times 10^3$). The products $[Cr(H_2O)_5Cl]^{2+}$ and $[Cr(H_2O)_5Br]^{2+}$ have been identified, and reactions are of the inner-sphere type. Addition of Cr^{3+} (0.03 M) and Co^{2+} (0.03 M) as possible reactants with monomeric Rh^{2+} had no effect on the rate of formation of Rh_2^{4+}. Free halide ions react with Rh_2^{4+} and induce Rh metal formation. Reduction of $[Rh(H_2O)_6]^{3+}$ with V^{2+} also gives Rh metal, probably as a result of outer-sphere generation of monomeric Rh^{2+}.

Introduction

The aquarhodium(II) ion was first prepared by Maspero and Taube.[1] Its chromatographic behavior compared to that of the 3+ Rh(III) and Cr(III) aqua ions has indicated a dimeric 4+ structure, which has only slight paramagnetism from proton NMR measurements. The dimer has a single metal–metal bond and is believed to have the structure $[Rh_2(H_2O)_{10}]^{4+}$. Other studies have enlarged on these properties,[2-4] but on the whole the ion has not been extensively studied. In addition to investigating the manner of its formation we intended studying substitution reactions with simple monodentate 1- anions. These attempts lead to formation of Rh metal, which in the case of the halide ions is believed to be of some relevance to the Rh(III) to Rh(II) redox interconversions described.

Experimental Section

Hexaaquarhodium(III) was prepared by the method of Ayres and Forrester[5] with some modifications. The reaction of $RhCl_3 \cdot xH_2O$ (Johnson Matthey Chemicals) and concentrated (72%) perchloric acid (BDH, AnalaR) was carried out in a three-necked round-bottomed flask complete with thermometer and condenser, which was connected to a water pump to reduce the pressure and hence help concentrate the solution. The reaction mixture obtained was diluted and loaded onto a Dowex 50W-X8 column (1.6 cm in diameter, 10 cm long) washed with 0.5 M and then 1.0 M $HClO_4$, which resulted in the removal of chloro complexes of charge <2.0. The major fraction of $[Rh(H_2O)_6]^{3+}$ was eluted as a bright yellow band with 2 M $HClO_4$. Solutions were standardized spectrophotometrically by using the known spectrum of $[Rh(H_2O)_6]^{3+}$, with peaks at 311 (ϵ = 67.4 M^{-1} cm^{-1}) and 396 nm (ϵ = 62 M^{-1} cm^{-1}).[6] The two absorbance peaks are well-defined and are of nearly equivalent intensity, not as indicated in ref 7.

Solutions of the green aqua Rh(II) dimer were prepared according to the method of Ziolkowski and Taube,[2] which involved the addition of stoichiometric amounts of Cr^{2+} to a 0.03 M solution of $[Rh(H_2O)_6]^{3+}$ in 2 M $HClO_4$. After separation of the different bands on a Dowex 50W-X2 column, the Rh_2^{4+} ion was eluted with 3 M $HClO_4$ and gave the literature spectrum with peaks at 402 (ϵ = 63 M^{-1} cm^{-1} per Rh) and 648 nm (ϵ = 46.5 M^{-1} cm^{-1}).[4] Solutions stored under N_2 at 0 °C were stable for ~3 weeks. Subsequently some disproportionation to Rh metal and Rh(III) was noted.

To prepare solutions of $[Rh(H_2O)_5Cl]^{2+}$ and $[Rh(H_2O)_5Br]^{2+}$ a freshly generated (unpurified) solution of Rh_2^{4+} was treated with an excess of Cl_2 (gas) or Br_2 in solution (eq 1). The 2+ ions were purified

$$Rh_2^{4+} + X_2 \rightarrow [Rh(H_2O)_5X]^{2+} \quad (1)$$

chromatographically by elution from a Dowex 50W-X8 resin with 0.5 M $HClO_4$ and concentrated by reloading onto a short column and eluting with 2 M $HClO_4$ to give >0.1 M solutions. Peak positions (and ratios) were consistent with literature spectra: for $[Rh(H_2O)_5Cl]^{2+}$, 335 (ϵ = 50 M^{-1} cm^{-1}) and 426 nm (ϵ = 50.4 M^{-1} cm^{-1});[6] for $[Rh(H_2O)_5Br]^{2+}$, 342 (ϵ = 70.6 M^{-1} cm^{-1}) and 441 nm (ϵ = 70.6 M^{-1} cm^{-1}).[2] Essential features of the latter were confirmed in a quantitative experiment in

(1) Maspero, F.; Taube, H. *J. Am. Chem. Soc.* **1968**, *90*, 7361.
(2) Ziolkowski, J. J.; Taube, H. *Bull. Acad. Pol. Sci. Ser. Sci. Chim.* **1973**, *21*, 113; *Chem. Abstr.* **1973**, *78*, 143 334*h*.
(3) Wilson, C. R.; Taube, H. *Inorg. Chem.* **1975**, *14*, 405.
(4) Wilson, C. R.; Taube, H. *Inorg. Chem.* **1975**, *14*, 2276.
(5) Ayres, G. H.; Forrester, J. S. *J. Inorg. Nucl. Chem.* **1957**, *3*, 365.
(6) Wolsey, W. C.; Reynolds, C. A.; Kleinberg, J. *Inorg. Chem.* **1963**, *2*, 463.
(7) Swaminathan, K.; Harris, G. M. *J. Am. Chem. Soc.* **1966**, *88*, 4411.

0020-1669/86/1325-0339$01.50/0