Contribution from the Department of Chemistry, University of Wisconsin—Milwaukee, P.O. Box 413, Milwaukee, Wisconsin 53201, and Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Reactions of Trimethylphosphine Analogues of Auranofin with Bovine Serum Albumin

Anvarhusein A. Isab,[1a,b] C. Frank Shaw III,*[,1a] James D. Hoeschele,[1c] and James Locke[1a]

Received June 10, 1987

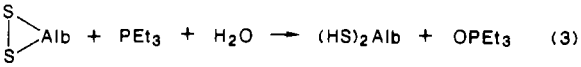
The reactions of bovine serum albumin (BSA) with (trimethylphosphine)(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)-gold(I), $Me_3PAuSAtg$, and its chloro analogue, Me_3PAuCl, were studied to develop insights into the role of the phosphine ligand in the serum chemistry of the related antiarthritic drug auranofin ((triethylphosphine)(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)gold(I)). ^{31}P NMR spectroscopy, protein modification, and gel-exclusion chromatography methods were employed. Comparison of the reactions of the methyl derivatives to the previously reported reactions of auranofin and Et_3PAuCl with BSA demonstrated that similar chemical species are formed but revealed three major differences: (1) The smaller and less basic trimethylphosphine is displaced to form the corresponding phosphine oxide (Me_3PO) more readily than is triethylphosphine. (2) (Trimethylphosphine)gold(I) chloride forms the bis((trimethylphosphine)gold(I))-μ-thiolato complex, Alb-$S(AuPMe_3)_2^+$, at cysteine-34 in the albumin crevice more readily than (triethylphosphine)gold(I) chloride forms Alb-$S(AuPEt_3)_2^+$. (3) The multiple weak binding sites for R_3PAu^+ are more easily resolved for the methyl derivative than for the ethyl derivative. Despite these differences, the results for the methyl analogues provide important confirmation for previously developed chemical models of auranofin reactions in serum. Me_3PO was not observed in reaction mixtures lacking tetraacetylthioglucose (AtgSH); this result affirms the role of AtgSH, displaced by the reaction of $Me_3PAuSAtg$ at Cys-34, in the generation of the phosphine oxide (an important metabolite in vivo). The weak binding sites on albumin react with Me_3PAuCl, but not $Me_3PAuSAtg$, demonstrating the importance of the strength and reactivity of the anionic ligand-gold bond on the reactions of auranofin analogues. The gold binding capacity of albumin is enhanced after Me_3PO is formed, consistent with the reductive cleavage of albumin disulfide bonds by trimethylphosphine.

Introduction

Auranofin [$Et_3PAuSAtg$[2] = (triethylphosphine)(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)gold(I)], was recently approved as a chrysotherapy agent. In vivo, it loses, via ligand-exchange reactions, its phosphine and thiolate ligands.[3] In cell culture model systems, the acetylthioglucose ligand (AtgSH) is lost during uptake into the cells; less phosphine than gold is taken up, and the efflux of phosphine is more rapid than that of gold.[4] In animal models, up to 90% of the phosphine is excreted as triethylphosphine oxide over 24-72 h.[3] The rapid displacement of the thiol and slower oxidation of the phosphine can be modelled by the in vitro reactions of $Et_3PAuSAtg$ with bovine serum albumin (BSA).[5-8] The in vivo processes, however, may be more complex since the phosphine is oxidized more rapidly in whole blood than in serum. The cysteine-34 residue of the mercaptalbumin (AlbSH) component of BSA displaces the thiosugar ligand immediately upon mixing.[6-9] Ligands with moderate to high affinities for gold, e.g. AtgSH, 1-thioglucose, glutathione, or cyanide, displace the phosphine,[6,10] which is oxidized to Et_3PO by the albumin disulfide bonds (reactions 2 and 3).[6-10] The

$$\text{AlbSH} + \text{Et}_3\text{PAuSAtg} \rightarrow \text{AlbSAuPEt}_3 + \text{AtgSH} \quad (1)$$

$$\text{AlbSAuPEt}_3 + \text{RSH} \rightarrow \text{AlbSAuSR} + \text{PEt}_3 + \text{H}^+ \quad (2)$$



(3)

oxygen is provided by the water of the reaction media (reaction 3).[11] Cysteine-34 of albumin is protected in a crevice environment,[12] estimated to be as deep as 10 Å.[13] It has an unusually low pK_{SH} value (ca. 5),[14] which correlates with a high affinity for gold(I)[15,16] and with the ^{31}P chemical shift of $AlbSAuPEt_3$ (δ_P = 38.8 vs $OP(OMe)_3$), which is further upfield than that of any other (triethylphosphine)gold(I) thiolate.[6,17]

Modification of ligands to alter their steric requirements and electronic properties (e.g. acidity/basicity, π-bonding ability), and hence their reactivity, is an effective strategy for learning what factors control specific chemical reactions. Similar strategies are used to determine the structure-activity relationships (SAR's) of drugs. J.D.H. has studied the SAR of auranofin,[18] using a number of analogues described in the first report of auranofin and its antiarthritic activity.[19] It was of interest, therefore, to extend these studies to the biochemical level. One such study, using an analogue with a modified thiolate ligand, demonstrated that the rate of phosphine oxidation increases as the affinity of the thiol for gold(I) increases.[6] The phosphine ligand substantially alters the properties of auranofin compared to the oligomeric gold(I)

(1) (a) University of Wisconsin—Milwaukee. b) On sabbatical leave from the King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia. (c) Warner-Lambert Co.

(2) Abbreviations: AAS = atomic absorption spectroscopy; Ac-BSA = sulfhydryl modified (acetamide) BSA; AlbSH = mercaptalbumin; AtgSH = 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose; Au_b/BSA = mole ratio of gold bound to BSA or Ac-BSA; Au_i/BSA = initial mole ratio of gold to BSA in the reaction mixture BSA = bovine serum albumin; $R_3PAuSAtg$ = auranofin (R = Et) or the trimethylphosphine analogues; SAR = structure activity relationship; TMP = $(CH_3O)_3PO$ (internal reference).

(3) Intoccia, A. P.; Flanagan, T. L.; Walz, D. T.; Gutzait, L.; Swagzdis, J. E.; Flagiello, J., Jr.; hwang, B. Y.-H.; Dewey, R. H.; Noguchi, H. *Bioinorganic Chemistry of Gold Coordination Compounds*; Sutton, B. M.; Ed.; Smith Kline & French Laboratories: Philadelphia, 1983; pp 21-33.
(4) Snyder, R. M.; Mirabelli, C. K.; Crooke, S. T. *Biochem. Pharmacol.* **1986**, *35*, 923-932.
(5) Coffer, M. T.; Shaw, C. F., III; Eidsness, M. K.; Watkins, J. W., II; Elder, R. C. *Inorg. Chem.* **1986**, *25*, 333-340.
(6) Coffer, M. T.; Shaw, C. F., III; Hormann, A. L. *J. Inorg. Biochem.* **1987**, *30*, 177-187.
(7) Malik, N. A.; Otiko, F. G.; Sadler, P. J. *J. Inorg. Biochem.* **1980**, *12*, 317-322.
(8) Razi, M. T.; Otiko, G.; Sadler, P. J. *ACS* Symp. *Ser.* **1983**, *No. 209*, 371-384.
(9) Ecker, D. J.; Hempel, J. C.; Sutton, B. M.; Kirsch, R.; Crooke, S. T. *Inorg. Chem.* **1980**, *25*, 3139-3143.
(10) Isab, A. A.; Hormann, A. L.; Coffer, M. T.; Shaw, C. F., III. *J. Am. Chem. Soc.* **1988**, *110*, 3278-3284.
(11) Isab, A. A.; Shaw, C. F., III; Locke, J. *Inorg. Chem.*, in press.
(12) Brown, J. R. *Albumin Structure Function and Uses*; Rosenor, V. M., Oratz, M., Rothschild, M. A., Eds.; Pergamon: New York, 1977; pp 27-51.
(13) Hull, H. H.; Chang, R.; Kaplan, L. J. *Biochem. Biophys. Acta* **1975**, *400*, 132-136.
(14) Lewis, S. D.; Misra, D. C.; Shafer, J. A. *Biochemistry* **1980**, *19*, 6129-6137.
(15) Isab, A. A.; Sadler, P. J. *J. Chem. Soc., Dalton Trans.* **1982**, 135-140.
(16) Boles-Bryan, D. L.; Hempel, J. C.; Mellinger, D.; Hashim, M.; Pasternack, R. *Inorg. Chem.* **1987**, *26*, 4180-4185.
(17) Shaw, C. F.; Coffer, M. T.; Mirabelli, C. K.; Klingbeil, J. *J. Am. Chem. Soc.* **1988**, *110*, 729-734.
(18) Hoeschele, J. D. *Proceedings of the International Symposium on the Synthesis and Application of Isotopically Labeled Compounds*; Duncan, W. P., Susan, A. B., Eds.; Elsevier: New York, 1983.
(19) Sutton, B. M.; McGusty, E.; Walz, D. T.; DiMartino, M. J. *J. Med. Chem.* **1972**, *15*, 1095-1098.

0020-1669/88/1327-3588$01.50/0

drugs such as myochrysine (gold sodium thiomalate) or solganol (gold thioglucose), which require parenteral administration. Therefore, we have initiated an examination of the reactions of the trimethylphosphine analogue of auranofin. Replacing the ethyl groups by methyl groups reduces the steric bulk and the basicity of the phosphine. The steric requirements of phosphine ligands can be quantitated by the cone angle (θ = 118° for Me_3P and 132° for Et_3P) described by Tolman.[20] The basicity can be ordered by the electronic factor, ν, (ν = 2064.1 for Me_3P and 2061.7 for Et_3P; ν decreases as basicity increases) also described by Tolman.[20]

Experimental Section

Reagents. Sephadex G-50 and 5,5′-dithiobis(2-nitrobenzoic acid) were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (fatty acid free, lot 10282520-49) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN); D_2O (99.8%), CH_3OD (99.5%), DMSO, and tetraacetylthioglucose (AtgSH) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Sulfhydryl modified BSA (Ac-BSA) was prepared by using iodoacetamide as previously described.[6] Auranofin was a gift from Smith Kline & French Laboratories (Philadelphia, PA).

Me_3PAuCl. Prepared as previously described.[18] Anal. Calcd for C_3H_9PAuCl: C, 11.68; H, 2.94; Cl, 11.49. Found: C, 11.59; H, 2.81; Cl, 11.56.

$Me_3PAuSAtg$. Prepared as previously described.[18] Anal. Calcd for $C_{17}H_{28}O_9SPAu$: C, 32.08; H, 4.43; Found: C, 32.36; H, 4.35.

^{31}P NMR Measurements. Spectra were obtained in deuteriated solvents on a Bruker WP-250 multinuclear NMR spectrometer operating at 101.3 MHz. Gated proton decoupling was employed to avoid denaturation of the protein. The ratio of gold initially added to protein in the NMR samples is reported as Au_i/BSA. Chemical shifts were measured and are reported relative to internal trimethylphosphate (TMP). Typically, 3000–4000 scans were accumulated for each spectrum.

Analyses and Chromatography. Gold was determined by atomic absorption spectroscopy and albumin by its ultraviolet absorbance at 278 nm (ϵ_{278} = 39 600 L/(mol cm)). Aliquots of the NMR samples were chromatographically analyzed on Sephadex G-50 (1 × 25 cm column) and eluted with 100 mM NH_4HCO_3 buffer, pH 7.9. The gold bound to the protein is reported as a mole ratio, Au_b/BSA. The sulfhydryl (mercaptalbumin = AlbSH) content of BSA was analyzed by using Ellman's reagent, DTNB, as previously described.[5]

$Me_3PAuSAtg$/BSA Reactions. BSA (200 mg.) was dissolved in 2.5 mL of 100 mM NH_4HCO_3/D_2O buffer, pH 7.9, and analyzed spectroscopically (ϵ_{278} = 39 600 L/(mol cm)) to determine the exact concentration, typically 1.26 mM. $Me_3PAuSAtg$ (250 μL of 12.5 mM in CH_3OH) was added, yielding 1.15 mM BSA and Au_i/BSA = 0.98. Of this solution, 500 μL was set aside for chromatographic analysis and the ^{31}P NMR spectrum was obtained on the remainder. Then an additional 200 μL of $Me_3PAuSAtg$ was added to 2.2 mL of the original sample, yielding Au_i/BSA = 2.1. An NMR spectrum was obtained on the initially clear sample, which developed a slight precipitate during the 1.5-h spectral accumulation.

Me_3PAuCl/BSA Reactions. BSA (4.7 mM) in 1.7 μL of NH_4HCO_3/D_2O buffer and Me_3PAuCl (53 μL of a 150 mM solution in DMSO) were mixed, yielding 4.6 mM BSA and Au_i/BSA = 1.0 A white solid precipitated but quickly redissolved upon shaking. The NMR spectrum of this solution was obtained. Two additional spectra were obtained after adding 53- and 159-μL aliquots of Me_3PAuCl to yield Au_i/BSA = 2.0 and 4.8, respectively. Finally, a supersaturated 33 mM AtgSH solution was prepared by heating solid AtgSH in 3.0 mL of NH_4HCO_3/D_2O buffer. It was quickly added to the last NMR sample. A precipitate that formed was filtered off, after which an NMR spectrum was obtained. An aliquot (600 μL) of the final sample was used for chromatographic analysis.

Me_3PAuCl/Ac-BSA Reaction. Ac-BSA (1.70 mL of 2.98 mM in NH_4HCO_3/D_2O buffer) was mixed with Me_3PAuCl (35 μL of 150 mM in DMSO, yielding 2.8 mM AcBSA and Au_i/BSA = 1.1, after which the ^{31}P NMR spectrum was measured. An additional 70-μL aliquot of Me_3PAuCl was added, yielding Au_i/BSA = 3.1, and the spectrum was remeasured. No ppt formation was observed. An aliquot (500 μL) of the final sample was chromatographed to determine Au_b/BSA.

Results

The ^{31}P NMR chemical shifts of the two gold complexes Me_3PAuCl and $Me_3PAuSAtg$ (AtgSH = 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose) were determined in several organic solvents and in the buffer system used for the protein reactions, 100 mM NH_4HCO_3, pH 7.9 (Table I). The aqueous solutions were obtained by adding small volumes of concentrated DMSO or DMF solutions of the gold complex to the buffer. The chemical shifts were unaffected by the choice of organic solvent used to dissolve the gold complex. Nor did adding DMSO to either aqueous solution affect the chemical shift. Since the sulfur in DMSO is more oxidized than the sulfur in methionine, which is itself a weak ligand for gold, it is not surprising that DMSO does not displace the chloride ligand from gold. Since Me_3PAuCl and $Me_3PAuSAtg$ dissolve more rapidly and more completely in DMSO than in DMF, DMSO was used in the subsequent studies.

The experiments described below were performed by using a single source of bovine serum albumin (BSA) with a high content of mercaptalbumin (AlbSH). The SH titer, 0.58, determined immediately before the reactions were conducted, indicated that little or no oxidation of the Cys-34 had occurred during preparation and storage of the albumin. The 0.58 mole ratio of mercaptalbumin to BSA is close to the in vivo value, 0.6–0.7.

When a concentrated solution of $Me_3PAuSAtg$ was added to serum albumin (Au_i/BSA = 0.98), three ^{31}P NMR peaks, 49.6,

Table I. ^{31}P NMR Chemical Shifts of Me_3PAuX (X = Cl^- and $AtgS^-$)[a]

solvent	chem shift, ppm X = Cl^-	chem shift, ppm X = $AtgS^-$	solvent	chem shift, ppm X = Cl^-	chem shift, ppm X = $AtgS^-$
H_2O/DMF[b]	−13.1		MeOH	−10.8	−1.7
H_2O/DMSO[c]/DMF[c]	−13.1		DMSO	−10.4	−1.7
H_2O/DMSO[d]	−13.1	−1.7	DMF	−10.1	−3.2

[a] 2–3 mM solutions in the solvent indicated. [b] 2/18 v/v DMF/100 mM NH_4HCO_3 buffer, pH 7.9. [c] 1/2/18 v/v DMSO/DMF/100 mM NH_4HCO_3 buffer, pH 7.9. [d] 1/34 v/v DMSO/100 mM NH_4HCO_3 buffer, pH 7.9.

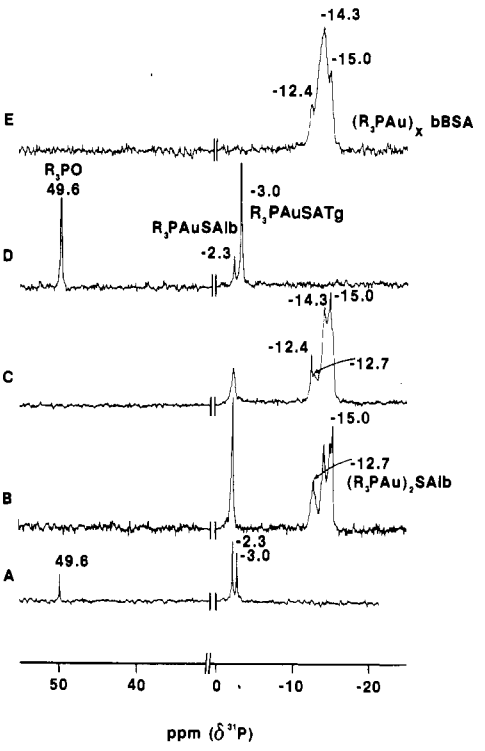


Figure 1. ^{31}P NMR spectra (101.3 MHz) in 100 mM NH_4HCO_3 buffer, pH 7.9; (a) 1.15 mM BSA + 1.13 mM $Me_3PAuSAtg$; (b) 4.65 mM BSA + 4.54 mM Me_3PAuCl; (c) 4.42 mM BSA + 8.80 mM Me_3PAuCl; (d) 1.61 mM BSA + 7.74 mM Me_3PAuCl + 20.2 mM AtgSH; (e) 2.81 mM Ac-BSA + 8.73 mM Me_3PAuCl. Sulfhydryl titers: BSA, 0.58; Ac-BSA, 0.01. Resonances are reported in ppm vs TMP and are assigned in the text.

(20) Tolman, C. A. *Chem. Rev.* **1979**, *18*, 313–345.

−2.3, and −3.0 ppm vs $(MeO)_3PO$, were observed (Figure 1a). The peak at 49.6 ppm is due to trimethylphosphine oxide, resulting from displacement and oxidation of the phosphine ligand. The peaks shifted upfield from the standard are due to the gold complex and its albumin reaction product. When additional $Me_3PAuSAtg$ was added (Au_i/BSA = 2.1; not shown), the −3.0 ppm resonance grew in intensity relative to the −2.3 ppm resonance. On that basis we assign the lower-field peak to the albumin complex, $AlbSAuPME_3$, and the higher-field peak to unreacted $Me_3PAuSAtg$. The downfield shift of the resonance when albumin displaces the tetraacetylthioglucose ligand parallels the shift that occurs in the corresponding reaction of auranofin with albumin.

To verify these assignments, the reaction of albumin with equimolar Me_3PAuCl was studied (Figure 1b). Chloride ion is a weaker ligand for gold(I) than is tetraacetylthioglucose. The resulting spectrum contained the −2.3 resonance expected for the reaction of Me_3PAuCl at Cys-34 of the albumin. The −3.0 ppm peak is absent, consistent with its assignment to $Me_3PAuSAtg$; no Me_3PO (49.6 ppm) was formed. No resonance for Me_3PAuCl (−13.1 ppm in aqueous buffer) was observed. Additional peaks, not present in the spectrum shown in Figure 1a, were observed at −12.8, −14.3, and −15.0 ppm. By analogy to previous reactions of hemoglobin and albumin with Et_3PAuCl and $Et_3PAuSAtg$,[5,17] we assign the upfield resonances to Me_3PAu^+ bound at lower affinity sites that are unreactive toward $Me_3PAuSAtg$. These sites are probably amino acid residues such as histidine and methionine, which are expected to shift the (trimethylphosphine)gold(I) resonances upfield. The formation of $AlbSAuPMe_3$ and the population of the weak binding sites can be represented as follows:

$$AlbSH + Me_3PAuCl \rightarrow AlbSAuPMe_3 + Cl^- + H^+ \quad (4)$$

$$AlbSAuPMe_3 + \text{excess } Me_3PAuCl \rightarrow (Me_3PAu)_xAlbSAuPMe_3 + Cl^- \quad (5)$$

To gain more insight into the behavior of the low affinity sites, additional Me_3PAuCl was added to the solution and the spectra were remeasured. After a second equivalent of Me_3PAuCl was added (Figure 1c), a fourth weak binding site was populated (−12.4 ppm) and the $Me_3PAuSAlb$ peak at −2.35 ppm decreased in intensity relative to the internal standard, while the resonances of the remaining weak binding sites increased. When the Au_i/BSA ratio was increased to 4.8 (not shown), the $AlbSAuPMe_3$ peak was absent and the peaks at −12.4, −14.3, and −15.0 ppm grew more intense, obscuring the −12.7 ppm resonance. The decrease in the −2.3 ppm resonance and the concomitant increase of the −12.7 ppm resonance are attributed to reaction of additional Me_3PAuCl forming a thiolate-bridged digold complex [$AlbS(AuPMe_3)_2^+$], at cys-34:

$$AlbSAuPMe_3 + Me_3PAuCl \rightarrow AlbS(AuPMe_3)_2^+ + Cl^- \quad (6)$$

Since the metal-coordinated sulfur of Cys-34 in $Me_3PAuSAlb$ more closely resembles a thioether than a thiol, the upfield shift of the new resonance, from −2.35 to −12.7 ppm, also supports the assignment. (The absence of the −2.3 and −12.7 ppm resonances from spectra obtained with sulfhydryl-modified albumin, vida infra, supports this assignment.)

Addition of tetraacetylthioglucose (AtgSH) to the sample having the 4.8 Au_i/BSA ratio dramatically changed the spectrum (Figure 1d). All of the weak binding sites (−12.4, −12.7, −14.3, and −15.0 ppm) were depopulated. A very intense resonance at −3.0 ppm indicated that $Me_3PAuSAtg$ was formed. $AlbSAuPMe_3$ (−2.3 ppm) was regenerated. Me_3PO (49.6 ppm), which was not generated in the absence of the thiol (i.e. in Figure 2b,c), appeared as a very intense resonance. The formation of $Me_3PAuSAtg$ and the regeneration of $AlbSAuPMe_3$ result from reactions of the AtgSH with the weakly bound Me_3PAu^+ moieties:

$$(Me_3PAu)_xAlbSAuPMe_3 + xAtgSH \rightarrow xMe_3PAuSAtg + AlbSAuPMe_3 + xH^+ \quad (7)$$

$$AlbS(AuPMe_3)_2^+ + AtgSH \rightarrow Me_3PAuSAtg + AlbSAuPMe_3 + H^+ \quad (8)$$

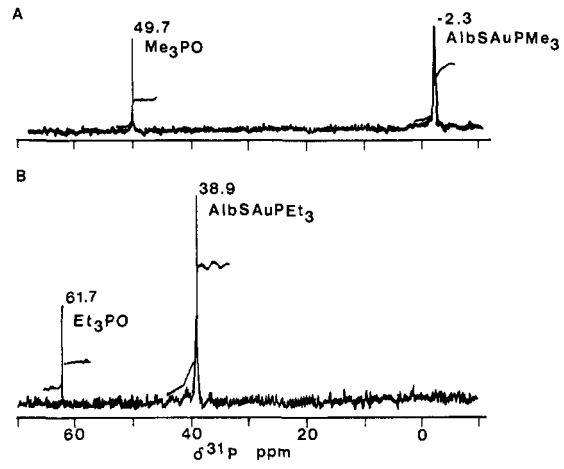


Figure 2. ^{31}P NMR spectral comparison of (a) Me_3PO and (b) Et_3PO formation. BSA (2.0 mM in 60% D_2O/40% 100 mM Tris-HCl, pH 7.9; SH titre, 0.52) was allowed to react with 1.2 mM $R_3PAuSAtg$ for 24 h. Other conditions were as in Figure 1.

The assignments of the peaks at −12.4, −14.3, and−15.0 ppm as Me_3PAu^+ weakly bound at sites other thanCys-34 were tested by reacting Me_3PAuCl with iodoacetamide-blocked albumin (Ac-BSA). The iodoacetamide converts Cys-34 of mercaptalbumin to a thioether, $AlbSCH_2CONH_2$, that is unreactive toward most gold(I) complexes. The peaks at −12.4, −14.3, and −15.0 are present, indicating that they are not generated by reactions at the free thiol group. The −2.3 and −12.7 ppm resonances, assigned to $AlbSAuPMe_3$ and $AlbS(AuPMe_3)_2^+$, respectively, are absent, confirming the role of Cys-34 in the formation of these species.

Chromatography of three of the albumin NMR samples provided additional data supporting the proposed NMR assignments. The gold to protein ratio (Au_b/BSA) of the albumin isolated from the 1/1 $Me_3PAuSAtg$/BSA mixture was 0.60, in good agreement with the sulfhydryl titer of the protein, 0.58, confirming the assignment of $AlbSAuPMe_3$ (−2.3 ppm). The iodoacetamide-modified albumin isolated after reaction with Me_3PAuCl had an Au_b/BSA ratio of 0.22, which was less than one-tenth of the initial ratio, Au_i/BSA = 3.1. This indicates that the Me_3PAu^+ bound at the weak binding sites dissociates extensively during the chromatographic separation over Sephadex G-50.

The value of 1.94 Au_b/BSA obtained for the albumin sample treated with a total of 4.8 equiv of Me_3PAuCl and then with AtgSH greatly exceeds the initial mercaptalbumin content of the protein (SH titer = 0.58). Me_3PO was formed during the reaction. The mechanism of its formation can be assumed to be similar to that for Et_3PO in the analogous auranofin reactions (eq 2 and 3): disulfide bond reduction by the phosphine.[5,6,11] The increased thiol content of the albumin allows it to bind additional gold ions and concomitantly displace the trimethylphosphine ligands. The loss and subsequent oxidation of the Me_3P ligand explains the absence of a ^{31}P NMR spectroscopic signature for these additional gold ions.

The intensity of the Me_3PO resonance in the spectrum shown in Figure 1a (and similar spectra not reproduced here) indicates the Me_3PO is generated more readily than is Et_3PO in the related reactions of auranofin. This was confirmed by comparing the extent of phosphine oxide formation with identical protein and gold ($Me_3PAuSAtg$ or $Et_3PAuSAtg$) concentrations. After 24 h, 48% of the trimethylphosphine was converted to Me_3PO, but only 28% conversion to Et_3PO was observed (Figure 2).

Discussion

A comparison of the reactions of albumin with $Me_3PAuSAtg$ and Me_3PAuCl to those of the ethyl analogues (auranofin and Et_3PAuCl) shows several major differences, even though analogous protein complexes are formed. The most notable contrast is the more rapid and more complete formation of Me_3PO compared to Et_3PO in analogous reactions. This process, as for aurano-

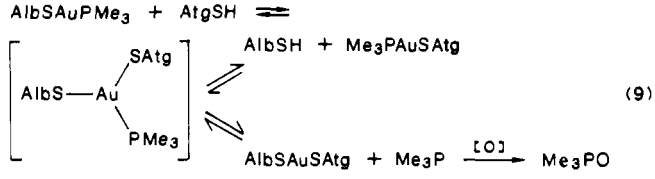
Table II. Reactions of (Trimethylphosphine)gold(I) Complexes (Me_3PAuX) with Albumin and Iodoacetamide-Modified Albumin

reactants			products	
protein (SH titer)	X	Au_i/BSA	^{31}NMR resonances[a]	Au_b/BSA[b]
BSA (0.58)	$AtgS^-$	1.0	−2.3, −3.0, +49.6	0.60
		2.1	−2.3, −3.0	
BSA (0.58)	Cl^-	1.0	−2.3, −12.8, −14.3, −15.0	
		2.0	−2.3, −12.4, −12.7, −14.3, −15.0	
		4.8	−12.4, −14.3, −14.9	
BSA (0.58) + AtgSH[c]	Cl^-	4.8	−2.3, −3.0, +49.6	1.94
Ac-BSA (<0.01)	Cl^-	3.1	−12.4, −14.3, −15.0	0.22

[a] In ppm vs TMP in 100 mM NH_4HCO_3 buffer, pH 7.9. [b] Mole ratio of gold bound to albumin determined after chromatographing an aliquot of the NMR sample. [c] 33 mM AtgSH was added to the sample listed immediately above, yiuelding 1.6 mM BSA and 10 mM AtgSH after mixing.

fin,[5,6,11] is driven by acetylthioglucose displacement of the phosphine from the albumin gold complex. Me_3PO (49.6 ppm) was generated only when AtgSH was present in solution, added either directly (Figure 1d) or as $Me_3PAuSAtg$ (Figure 1a). In the solutions prepared with only Me_3PAuCl (Figure 1b,c,e), Me_3PO is not a product.

Ligand exchange reactions of gold(I) occur via associative mechanisms.[16,18] Our previously proposed three-coordinate transition state for phosphine displacement (reaction 9),[6,21] can rationalize the greater rate of oxidation when Me_3P replaces Et_3P:



(9)

Me_3P is a weaker base than Et_3P and it has a smaller cone angle[20] indicating that it is less bulky. Because the albumin Cys-34 residue is located within a crevice,[12] estimated to be as deep as 10 Å,[13] the smaller size and steric requirements of Me_3P should favor the formation of the three-coordinate transition state. The weaker basicity favors dissociation of the phosphine from the transition state. Thus, both factors should accelerate the displacement of the Me_3P, leading to more rapid formation of Me_3PO.

In these reactions there was no accumulation of the bis(phosphine)gold(I) cation, $[(Me_3P)_2Au^+]$. Although the mechanism of $[(Et_3P)_2Au^+]$ formation in reactions of auranofin with albumin is uncertain, it is frequently observed as a minor product.[5,6]

Assigning the resonance at −12.7 ppm as a (μ-thiolato)digold adduct of albumin, $AlbS(AuPMe_3)_2^+$, is consistent with its observation only when both $AlbSAuPMe_3$ and Me_3PAuCl are present to react according to reaction 6: i.e. in the spectra shown in Figure 1b,c but not those shown in Figure 1a,d,e. The decrease in $AlbSAuPMe_3$ (δ_P = −2.3) as Me_3PAuCl is added to the $AlbSAuPMe_3$ and the regeneration of $AlbSAuPMe_3$ when AtgSH is added are well explained by the proposed reactions 6 and 8. The corresponding triethylphosphine species, $AlbS(AuPEt_3)_2^+$, was observed after chromatographing albumin adducts having both thiolate-bound and weakly bound gold, $(Et_3PAu)_xAlbSAuPEt_3$, which rearrange to form the bridged species.[5] An inorganic analogue, $[AtgS(AuPEt_3)_2^+]$, has been prepared and analyzed by various physiochemical techniques.[16,22,23] The smaller steric requirements of the Me_3P ligand will favor formation of the digold complex in the crevice environment of albumin. Electronic factors also favor this species, since the weaker basicity and trans influence of the trimethylphosphine, compared to those of Et_3P, will strengthen the bond trans to phosphine. Enhancing the strength of the bond between gold and the bridging thiol, which is a relatively poor ligand for gold compared to a terminal thiolate, should help to counteract any steric or energetic requirements imposed by the crevice environment.

Table III. Comparison of Bioavailability and Antiarthritic Activities of Orally Administered Auranofin Analogues, R_3PAuX

analogue		% antiinflammatory response[a]		bioavailability	
X (dose mg of Au/kg)	R	day 3	day 16	serum [Au]	ref
Cl^- (10)	Me	9.6	~0	1.7	19
	Et	14.4	36.6	5.4	
TgS^- (20)	Me	14.5	~0	2.3	19
	Et	27.2	44.5	6.9	
$AtgS^-$ (20)	Me	21–31	...	...	24
	Et	36–60	...	...	

[a] Reduction in hind paw volume (left/day 3 and right/day 16) compared to untreated adjuvant control.

The additional weak binding sites for Me_3PAuCl (−12.4, −14.3, −15.0 ppm) are not populated by reaction with $Me_3PAuSAtg$ because the amino acid side chains involved (presumed to be principally histidine and methionine residues) can not compete with the very high affinity AtgSH ligand. These binding sites are very well resolved in the ^{31}P NMR spectra (Figure 1b,c,e). In contrast the weak binding sites previously observed in reactions with Et_3PAuCl were characterized by broad, poorly resolved bands at ca. 25 and 28 ppm.[5] Future strategies for identifying these weak binding sites, which may serve as models for reactions of gold complexes in thiol-poor environments, can profitably exploit the greater resolution obtained by using Me_3PAu^+ as the probe.

The order of the ^{31}P chemical shifts observed here for various species generally parallels those found in the triethylphosphine system:

Me_3PO (49.6 ppm) > $AlbSAuPMe_3$ (−2.3 ppm) > $Me_3PAuSAtg$ (−3.0 ppm) > Me_3PAuCl (−13.1 ppm) ≃ $(Me_3PAu)_xAlb$ (−12 to −15 pm)

The only exception is the resonance assigned to $AlbS(AuPMe_3)_2^+$ (−12.7 ppm), which here is grouped with weak binding sites, while for the ethyl analogue it fell between the $Et_3PAuSAtg$ and Et_3PAuCl resonances.

The absorption and antiarthritic activities of triethylphosphine complexes, Et_3PAuX, are greater than those of the corresponding trimethylphosphine complexes. Table III shows serum gold levels and rat hind paw adjuvant arthritis assay data for three pairs of trimethylphosphine–triethylphosphine auranofin analogues, in which the anions are chloride, thioglucose and acetylthioglucose.[19,24] In the assay, a mycobacterium suspension injected into the left foot induces an adjuvant arthritis (secondary lesion)

(21) A reviewer questioned the validity of eq 2 and 3 and our postulated mechanism (eq 9), because the triply labelled [^{3}H,^{195}Au,^{35}S]-auranofin albumin studies of Ecker et al.[9] failed to detect any displacement of phosphine from the albumin–gold–phosphine complex. Ecker et al. allowed their reactions, with or without added cysteine or glutathione, to proceed for only 20 min; the more detailed, time-dependent study published by this laboratory[6] shows a slow but continuing evolution of Et_3PO over 24 h. Less extensive formation of Et_3PO over shorter time intervals has been reported in three previous ^{31}P NMR studies.[5,7,8] In fact, Ecker's demonstration that the $AlbSAuPEt_3$ complex, after separation from the displaced ATgSH ligand, does not form Et_3PO over 2 weeks[9] strongly supports the role of the thiol as proposed by our mechanism.

(22) Hempel, J.; Mikuriya, Y. *Bioinorganic Chemistry of Gold Coordination Compounds*; Sutton, B. M., Ed.; Smith Kline & French Laboratories, Philadelphia, 1983; pp 37–46.

(23) Cancro, M. P. M.S. Thesis, University of Wisconsin—Milwaukee, 1980.

(24) Hoeschele, J., unpublished data.

in other joints and is traditionally measured as swelling of the injected and uninjected hind feet.[19] The effectiveness of chrysotherapy agents is measured by the reduction of swelling on day 3 (injected foot) and/or day 16 (uninjected foot) compared to untreated adjuvant controls. The trimethylphosphine analogues consistently produce lower serum gold levels and are less effective in the rat hind paw adjuvant arthritis assay.[19,24]

The facile displacement and oxidation of Me_3P observed here may reduce the absorption of $Me_3PAuSAtg$ from the gastrointestinal tract. The phosphine ligands are believed to be responsible for the uptake of orally administered auranofin analogues; the oligomeric gold(I) thiolates, which lack phosphine ligands, are not orally absorbed. Oxidation of a phosphine converts a very strong, soft Lewis base into a hard, relatively weak base that does not bind gold(I) in the biological milieu. Thus, stronger phosphine bases (e.g., Et_3P), which are less easily displaced and oxidized, may be essential for oral absorption of gold drugs.

Acknowledgment. A.A.I. thanks the Chemistry Department of the King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia, for a sabbatical leave. We thank Frank Laib for his assistance in obtaining the spectra of Figure 2.

Contribution from the Department of Inorganic and Analytical Chemistry, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel, The Technion-Israel Institute of Technology, Haifa, Israel, and Department of Chemistry and Laboratory for Molecular Structure and Bonding, Texas A&M University, College Station, Texas 77843

Further Studies of the Monooxo-Capped Tritungsten Carboxylate Clusters

Avi Bino,*[1a] F. Albert Cotton,*[1b] Zvi Dori,*[1c] Miriam Shaia-Gottlieb,[1c] and Moshe Kapon[1c]

Received March 28, 1988

An improved method for the preparation of the $[W_3O(O_2CCH_3)_6(H_2O)_3]^{2+}$ ion has been found, starting with sodium tungstate, and the compound $[W_3O(O_2CCH_3)_6(H_2O)_3]ZnCl_4 \cdot 4H_2O$ (**1**) has been shown to contain the ion in a completely ordered arrangement. The structure is thus obtained without ambiguity and with more precision than before and has the following principal dimensions (Å): W-W = 2.693 (1), 2.693 (1), 2.712 (1); W-(μ_3-O)(av) = 1.98 [1]; W-OH_2(av) = 2.17 [2]; W-O(acetate)(av) = 2.078, 2.055 on the capped and uncapped sides, respectively. Compound **1** crystallizes in space group $P2_1/n$ with a = 14.324 (2) Å, b = 17.081 (2) Å, c = 13.715 (2) Å, β = 90.87 (3)°, and Z = 4. Conversion of the cation in compound **1** to the $[W_3O(O_2CCH_3)_5(OCH_3)(H_2O)_3]^{2+}$ cation has been effected, and this has been isolated and structurally characterized as the compound $[W_3O(O_2CCH_3)_5(OCH_3)(H_2O)_3]ZnCl_4 \cdot 7H_2O$ (**2**), which crystallizes in space group $P\bar{1}$ with unit cell dimensions a = 14.063 (7) Å, b = 14.216 (7) Å, c = 9.613 (5) Å, α = 103.78 (5)°, β = 100.91 (5)°, γ = 107.38 (5)°, and Z = 2. The cation in compound **2** (aqueous solution) has a proton NMR spectrum in accord with its solid-state structure, and its UV-visible spectrum shows expected similarities to that of its symmetrical parent.

Introduction

It is now well established that the elements molybdenum and tungsten have a marked tendency to form trinuclear, triangular cluster compounds in mean formal oxidation states in the range III-V. In these compounds there are bridging and capping groups that may be O, S, OR, R, Cl, and Br, and no doubt others. One of the well-characterized types of compounds has the composition $[M_3(\mu_3\text{-}X)_2(O_2CR)_6L_3]$; the essential features are an equilateral triangle of metal atoms with two capping atoms or groups (usually, but not always, the same), one above and one below, with two μ_2,η^2-O_2CR groups spanning each edge of the triangle and, finally, one monodentate ligand L (commonly H_2O) occupying a position in the M_3 plane external to each metal atom.[2]

In 1982 the surprising observation was reported that this type of cluster species could exist in an incomplete form, namely, with one capping group missing while all the other components remained roughly in the same arrangement.[3] This discovery was the result of investigating a dark blue byproduct in the preparation of the $[W_3O_2(O_2CCH_3)_6(H_2O)_3]^{2+}$ ion. It was possible to characterize this incomplete or hemicapped species, $[W_3O(O_2CCH_3)_6(H_2O)_3]^{2+}$, by an X-ray crystallographic study of its $ZnBr_4 \cdot 8H_2O$ salt. In addition, molecular orbital calculations gave a satisfactory account of the electronic structure and rationalized the deep blue color.

However, we considered this to be only the opening chapter in studying and understanding this type of system. Some of the points or questions that most obviously called for further work were as follows.

(1) Improved Preparative Procedure. The very small amounts of material, tediously accumulated from many preparations of the bicapped compound, did not provide a satisfactory basis for further chemical study.

(2) X-ray Study of an Undisordered Compound. In the bromozincate compound the cation resided on a 2-fold axis and was systematically disordered so that the upper and lower faces of the W_3 triangle were indistinguishable. Thus, the capping oxygen atom appeared as two half-oxygen atoms and it was not possible to observe whether the arrangement of the three acetate groups on the capped side was any different from the arrangement of the three on the uncapped side. Because of the way the structure was refined, it was clear that no large difference was to be expected, but nevertheless, small differences that would certainly have been anticipated were totally masked by the disorder.

(3) Could the "Missing" Cap Be Introduced? It was recognized that if the hemicapped structure could be synthesized in good yield we would then have an opportunity to obtain species with one μ_3-O and some other capping group of choice, provided we could learn to introduce the latter by design.

In this paper we report on our efforts to date to address these three points. We have been successful with the first two. With respect to the third point, we have not yet succeeded, but in the course of trying we have made an unexpected synthetic discovery that is of interest in its own right.

Experimental Section

Synthesis of $[W_3O(O_2CCH_3)_6(H_2O)_3]ZnCl_4 \cdot 4H_2O$ (1). A mixture of $Na_2WO_4 \cdot 2H_2O$ (5.0 g) and granular (mesh 20) zinc (5.0 g) in 60 mL of acetic anhydride was refluxed for 10 h. The reaction mixture was cooled to room temperature, and a green-yellow solid that separated was isolated by filtration and washed with ethanol and ether. One gram of this solid was dissolved in water and the solution filtered. The filtrate was poured on a column of Dowex 50W-X2 cation-exchange resin, whereby a yellow solution of $[W_3O_2(OCCH_3)_9]^-$ emerged from the column, while a blue ion was retained. After the column had been washed with water, the blue ion was eluted with 0.5 M aqueous HCl and

(1) (a) The Hebrew University of Jerusalem. (b) Texas A&M University. (c) The Technion-Israel Institute of Technology.
(2) See for example: Cotton, F. A.; Dori, Z.; Marler, D. O.; Schwotzer, W. *Inorg. Chem.* **1984**, *23*, 4033, 4738 and earlier references cited therein.
(3) Ardon, M.; Cotton, F. A.; Dori, Z.; Fang, A.; Kapon, M.; Reisner, G. M.; Shaia, M. *J. Am. Chem. Soc.* **1982**, *104*, 5394.

0020-1669/88/1327-3592$01.50/0