^{31}P NMR Studies of the Formation of a (Cysteine-34)(μ-thiolato)bis(gold(I) triethylphosphine) Species of Bovine Serum Albumin and a Related Model Titration

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Systematic spectroscopic titrations of bovine serum albumin (62% mercaptalbumin, AlbSH, content) with Et_3PAuCl demonstrate evidence for the formation of a cysteine-34 μ-thiolato species, $AlbS(AuPEt_3)_2^+$, characterized by a ^{31}P NMR chemical shift of 35.6 ppm vs trimethyl phosphate (TMP). When iodoacetate-modified albumin ($AlbSCH_2COO^-$) is similarly titrated, this species does not form, verifying its association with Cys-34. The μ-thiolato species forms only when Et_3PAuCl reacts at the saturated strong binding site $AlbSAuPEt_3$ concomitantly with its reaction at the unpopulated weak binding sites (primarily histidines). When 2,3,4,6-tetraacetylthioglucose (ATgSH) is used to titrate an albumin sample treated with excess Et_3PAuCl, only one Et_3PAu^+ is removed from $AlbS(AuPEt_3)_2^+$ simultaneously with its removal from the histidines. A model system, the reaction of Et_3PAuCl with auranofin ($Et_3PAuS(ATg)$) which forms $ATgS(AuPEt_3)_2^+$, an analogue of the protein species, was also studied by ^{31}P NMR. $ATgS(AuPEt_3)_2^+$ contains diastereotopic (and hence nonequivalent) Et_3PAu^+ moieties characterized by distinct chemical shifts (δ_p = 34.71 and 34.97 ppm vs TMP) which can be resolved at a 1:1 ratio of Et_3PAuCl: $Et_3PAuS(ATg)$. At higher and lower ratios, rapid chemical exchange of the excess gold complex (Et_3PAuCl or $Et_3PAuS(ATg)$) and the μ-thiolate species was observed.

Introduction

The mechanisms of chrysotherapy, the treatment of rheumatoid arthritis with various gold(I) compounds, are uncertain, although these treatments have been used for many years. The metabolites formed from the gold(I) drugs are yet poorly understood.[1,2] Since the gold(I) species from those drugs were found to bind predominately to albumin in the bloodstream in vivo,[3] the binding sites and ligation of gold(I) have been topics of interest.[1,3-5] In vitro studies have demonstrated that the strong binding site is the free thiol residue Cys-34[4,6,7] of albumin. The nature of various weak binding sites that have been described depends on the particular gold complexes.[6-8] Auranofin, the second-generation gold drug [$ATgSAuPEt_3$ = (triethylphosphine)(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)gold(I)] binds to albumin exclusively through the displacement of its thioglucose ligand by Cys-34:[4,6]

$$\mathrm{AlbSH + ATgSAuPEt_3 \rightleftharpoons AlbSAuPEt_3 + ATgSH} \quad (1)$$

The strong and weak albumin binding sites for Et_3PAuCl (an analogue of auranofin with a weaker anionic ligand) have been characterized by ^{31}P NMR[4,6,8] and EXAFS spectroscopies.[6] These data clearly support the initial binding of a single Et_3PAu^+ at Cys-34 to form $AlbSAuPEt_3$, characterized by a chemical shift of 38.8 ppm, and subsequent formation of multiple, weakly-bound complexes, attributed to reaction at histidine and possibly methionine and characterized by chemical shifts of 23–29 ppm.

In addition, a poorly characterized species with a chemical shift of 35.9 ppm has sometimes been noted.[6,9] A possible explanation for this resonance is the formation of a (μ-thiolato)-digold species. Two model complexes of this type have been described previously: [$ATgS(AuPEt_3)_2^+$][10-14] and [$PhCH_2S(AuPPh_3)_2^+$].[15] We undertook systematic ^{31}P NMR titrations of bovine serum albumin (BSA, which is strongly homologous to human serum albumin) with Et_3PAuCl to examine the nature of the putative μ-thiolato species. Model titrations using auranofin in lieu of albumin in the reaction with Et_3PAuCl are also reported.

Experimental Section

Materials. Auranofin and Et_3PAuCl were obtained from Smith Kline & French Laboratories; BSA (fatty acid free, Lot No. 107400823) was obtained from Boehringer Mannhein Biochemicals; ATgSH, DTNB (5,5′-dithiobis(2-nitrobenzoic acid)) and Sephadex G-100 were obtained from Sigma Chemical Co.; ICH_2COONa, D_2O, CH_3OH, and TMP (trimethyl phosphate) were obtained from Aldrich Chemical Co.

Sulfhydryl-Modified BSA (Ac-BSA).[16] Iodoacetate-blocked BSA was prepared by adding a 100-fold excess of solid ICH_2COONa to 3 mL of 2 mM BSA (pH 7.9, 100 mM NH_4HCO_3 buffer). The solid dissolved quickly, and after an incubation of 10 min, the mixture was separated chromatographically on a Sephadex G-100 column eluted with 100 mM NH_4HCO_3. The BSA-containing fractions were pooled and concentrated to ca. 2 mM on an Amicon ultrafiltration cell. Albumin then was

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quantitated by its UV absorption at 278 nm (ϵ_{278} = 39 600 M^{-1} cm^{-1}), and the SH titre of albumin was measured using the DTNB method.[17]

^{31}P NMR Titration. Before each NMR measurement, an aliquot of 20 (or 25) μL of methanolic solution of 162 (or 105) mM Et_3PAuCl was added sequentially to 2 mL of 2.56 mM BSA (SH titre 0.62) in 100 mM NH_4HCO_3 buffer, pH 7.9, or to 2.5 mL of 1.12 mM Ac–BSA (SH titre 0.01) in 100 mM NH_4HCO_3 buffer, pH 7.9, respectively. ^{31}P NMR spectra were obtained on a Bruker-250 spectrometer at 101.258 MHz with broad-band proton decoupling. The 10 000 scans were accumulated with a 30° pulse width, 0.5-s relaxation delay, 16K data points, and 10-H decoupling power. The spectral widths were 20 000 Hz. The temperature was 293 K, and D_2O was used as an internal lock. The NMR chemical shifts are reported relative to TMP as 0 ppm. The integrated intensities of resonances at 35.6 and 38.8 ppm were obtained with use of the same acquisition parameters, since they exhibit similar longitudinal relaxation times. The longitudinal relaxation time of the species characterized at 35.6 ppm was measured by the progressive saturation method[18] after titrating to the point where the resonance at 38.8 ppm disappeared completely.

The model reaction between Et_3PAuCl and $Et_3PAuS(ATg)$ was examined by ^{31}P NMR titrations using the same acquisition parameters on the Bruker and at 202.5 MHz on a General Electric GN-500 spectrometer. Aliquots of Et_3PAuCl and $Et_3PAuS(ATg)$ in D_2O–MeOH (60:40) solutions were mixed in appropriate ratios.

Results and Discussion

Because serum albumin is microheterogeneous, consisting in vivo of a mixture of mercaptalbumin (AlbSH, in which Cys-34 is fully reduced) and endogenous mixed disulfides of Cys-34 with glutathione and cysteine (AlbSS(Gt) and AlbSS(Cy), respectively),[19] the SH titre must be measured and incorporated into the experimental design. The value of 0.62 obtained for the bovine albumin used in this study is within the in vivo range of 0.60–0.70.

Proton-decoupled ^{31}P NMR spectroscopy was chosen as the method to examine the formation of the species giving rise to the 35.6 ppm resonance because of its sensitivity and its high resolution compared to other techniques such as EXAFS and Mossbauer spectroscopy. Figure 1 shows the results of an NMR titration of albumin with Et_3PAuCl under conditions that lead to the formation of the desired species. The first addition (0.64 Au/BSA, ~1.0 Au/AlbSH) produced $AlbSAuPEt_3$, δ_P = 38.8 ppm, as the only major species (with possibly a trace of weakly bound gold, 23–29 ppm). The next addition of 1 equiv of gold to the mercaptalbumin (1.27 Au/BSA total, Figure 1B) substantially populated the weak binding sites (23–29 ppm), which are attributed to the histidine residues.[6,8] The next two additions further populated these sites and also gave rise to the previously observed 35.6 ppm resonance (Figure 1C,D). Further additions caused the growth of the 35.6 ppm resonance and concomitant loss of $AlbSAuPEt_3$ accompanied by growth of the 23–29 ppm resonances. The $AlbSAuPEt_3$ resonance is very weak after adding 8.2 equiv of Et_3PAuCl per AlbSH and disappears completely after adding 10.2 equiv of gold per AlbSH (6.33 Au/BSA), Figure 1H.

The 35.6 ppm chemical shift is too large for nitrogen or thioether bases, and is within the range observed for thiolate adducts.[8] On the basis of the known ability of thiols to form digold μ-thiolate species,[10–15] we propose that this species is a (μ-thiolato)bis[(triethylphosphine)gold(I)] adduct formed by further reaction of Et_3PAuCl at Cys-34:

$$AlbSAuPEt_3 + Et_3PAuCl \rightarrow AlbS(AuPEt_3)_2^+ + Cl^- \quad (2)$$

The upfield shift (38.8 to 35.6 ppm) indicates that the bonds

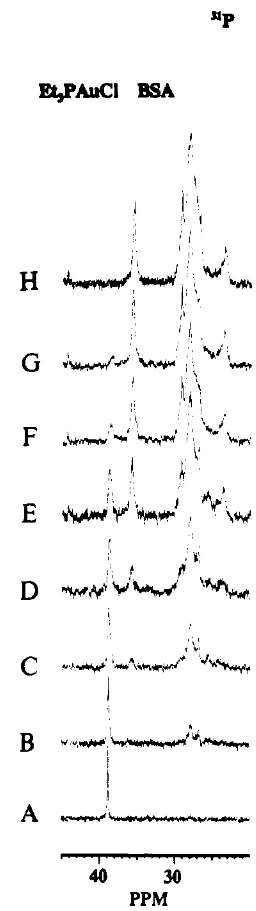

Figure 1. {^1H}^{31}P NMR (101.3 MHz) spectra of BSA (2.56 mM, SH/BSA = 0.62) titrated with Et_3PAuCl in ratios of Et_3PAuCl to BSA of (A) 0.64, (B) 1.27, (C) 1.90, (D) 2.53, (E) 3.16, (F) 3.80, (G) 4.43, and (H) 6.33. The resonances at 38.8, 35.6, and 23–29 ppm are assigned to $AlbSAuPEt_3$, $AlbS(AuPEt_3)_2^+$, and $(Et_3PAu\text{–}His)_n$AlbSH, respectively.

between the bridging thiolate and gold are weaker than for the terminal thiolate, as would be expected.[20]

Integrations of the 35.6 and 38.8 ppm resonances provides further evidence that the species giving rise to them are interconverted during the titration. The T_1 value of $AlbS(AuPEt_3)_2^+$, measured by the progressive saturation method, was found to be 1.4 ± 0.1 s, which is experimently equal to the value of 1.3 ± 0.1[6] measured previously for $AlbSAuPEt_3$. Thus, the intensities of two resonances can be compared directly. A typical plot of the intensities (arbitrary units) vs the moles of gold added is shown in Figure 2. The decreasing intensity of the 38.8 ppm resonance corresponds to the increase in the intensity at 35.6 ppm, consistent with eq 2 above. The elongated sigmoidal curves reflect the competing reactions at other weak binding sites (δ_P = 23–29 ppm). The quantitative ratios of the intensity increases at 35.6 ppm to the decreases at 38.8 ppm, measured after each addition, average 1.95 ± 0.03, which is very close to the ideal ratio of 2.00 predicted by eq 2. Thus, the correlated sigmoidal changes in the intensities of the resonances and the ratio of the changes provide strong support for assigning the 35.6 ppm resonance to $AlbS(AuPEt_3)_2^+$.

To further confirm that the reaction was occurring at Cys-34, a sulfhydryl-modified albumin, Ac–BSA, was employed. The carboxymethylation procedure generated $AlbSCH_2COO^-$ and

(20) A reviewer suggested that bond angle changes might account for the chemical shift change. We have compared the chemical shifts and bond angles (∠CPC, ∠CPAu, and ∠PAuX) for the following compounds: $Et_3PAuS(ATg)$ (38.1 ppm; 105.8 ± 2.9, 112.9 ± 2.5, and 173.6),[25] $(AuSCH_2CH_2PEt_2)_2$ (105.2 ± 4.1, 113.4 ± 5.1, and 173.5),[27] Et_3PAuCN (36.3 ppm; 105.0 ± 1.9, 113.6 ± 0.9, and 176.6),[21] and $Et_3PAu(ptm)$ (29.6 ppm; 104.7 ± 9.3, 113.4 ± 1.2, and 179.8),[28] and find no significant correlation of δ_P with the bond angles about phosphorus or gold.

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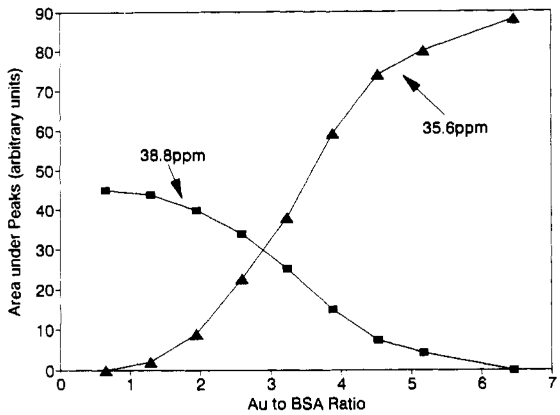

Figure 2. Integrated areas under the peaks at 38.8 and 35.6 ppm (see Figure 1) vs the Au:BSA ratio. The integrations were performed in the absolute intensity mode. The ratios $(A_i - A_0)_{35.6ppm}/(A_0 - A_i)_{38.8ppm}$ of the changes in the integrated intensities (A_i) after the ith incremental addition of gold have an average value of 1.95 ● 0.03 (theoretical value 2).

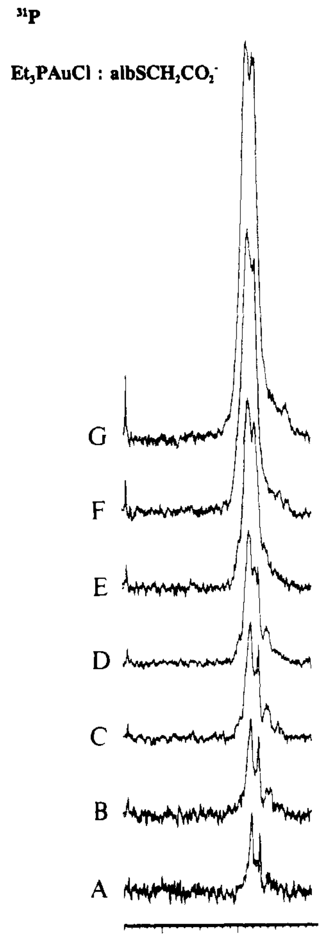

Figure 3. $\{^1H\}^{31}P$ NMR (101.3 MHz) spectra of Ac–BSA (1.12 mM, SH:Ac-BSA = 0.01) titrated with Et_3PAuCl in ratios of Et_3PAuCl to Ac–BSA of (A) 0.94, (B) 1.87, (C) 2.80, (D) 3.75, (E) 6.55, (F) 11.27, and (G) 17.07. Beyond the ratio of 17, the Et_3PAuCl precipitated and remained suspended in the solution; at lower ratios, fleeting precipitates formed and quickly dissolved. Note the absence of the 38.8 and 35.6 ppm resonances when Cys-34 is modified by acetylation.

reduced the SH titre to 0.01 SH/BSA. A titration, analogous to that of Figure 1, in which a total of 17 equiv of Et_3PAuCl was added to the Ac–BSA (Figure 3) was carried out. The 23–29 ppm resonances of the weak binding sites increased throughout the titration, but neither the $AlbSAuPEt_3$ nor the $AlbS(AuPEt_3)_2^+$ resonances were detected, confirming that the 35.6 ppm resonance is associated with Cys-34.

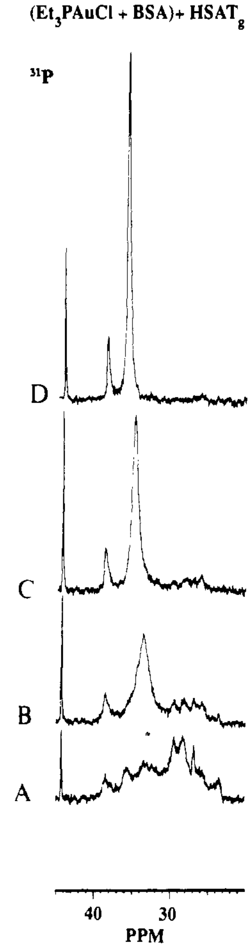

Figure 4. $\{^1H\}^{31}P$ NMR (101.3 MHz) spectra of Et_3PAuCl-treated BSA (1.12 mM; SH titre 0.55; Au:BSA = 3.9, the point where the 38.8 ppm resonance disappears) titrated by HS(ATg) in ratios of Et_3PAuCl:BSA: HS(ATg) of (A) 3.9:1:0.7, (B) 3.9:1:1.4, (C) 3.9:1:2.1, and (D) 3.9:1:2.8. The 44.1 ppm resonance is assigned to $(Et_3P)_2Au^+$. The broad resonance upfield from 38.8 ppm (spectra A–C) represents the averaged chemical shifts of auranofin (36.4 ppm), $ATgS^+(AuPEt_3)_2$ (34.97 and 34.71 ppm), $AlbS(AuPEt_3)_2^+$ (35.6 ppm), and $(Et_3PAu\text{–}His)_nAlbSH$ (23–29 ppm). It shifted to 36.4 ppm (auranofin) when sufficient HS(ATg) was added. At the ratio of 3.9:1:3.5 (not shown), the 44.1 ppm resonance disappeared and a 61.7 ppm (Et_3PO) resonance appeared.

A trace quantity of $(Et_3P)_2Au^+$ (44.1 ppm) is observed in Figure 3B and increases with successive additions of Et_3PAuCl. This forms via a ligand scrambling of various Et_3PAuX species present (X = Cl, Cys-34, histidine). This phenomenon has been observed previously in aqueous solutions of Et_3PAuCN.[21]

Beyond the 17 equiv shown, further additions of Et_3PAuCl, which is added to the aqueous albumin as a methanolic solution, resulted in the precipitation of Et_3PAuCl. Interestingly, bovine serum albumin contains 17 histidine residues, and the insolubility may result after they are saturated with weakly-bound Et_3PAu^+.

To verify that the $AlbS(AuPEt_3)_2^+$ forms reversibly and to assess its stability, Et_3PAuCl-treated albumin was titrated with ATgSH, which is a high-affinity ligand for gold(I). The BSA (SH titre 0.55) was first titrated with Et_3PAuCl until the 38.8 ppm resonance disappeared (such as in Figure 1H). After 2.8 equiv of ATgSH was added (Figure 4D), resonances for $Et_3PAuS(ATg)$ (36.4 ppm), $Au(PEt_3)_2^+$ (a sharp band at 44.1 ppm), and $AlbSAuPEt_3$ (38.8 ppm) indicated the species present. Thus, the net reactions generating auranofin and regenerating $AlbSAuPEt_3$ are

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$$AlbS(AuPEt_3)_2^+ + ATgSH \rightarrow AlbSAuPEt_3 + Et_3PAuS(ATg) + H^+ \quad (3)$$

$$(Et_3PAu\text{–}His)_n AlbSX + ATgSH \rightarrow (Et_3PAu\text{–}His)_{n-1} AlbSX + Et_3PAuS(ATg) \quad (4)$$

The intermediate spectra (Figure 4A–C, obtained after incremental additions of 0.7, 1.4, and 2.1 equiv of ATgSH, respectively) show the progressive losses of the $AlbS(AuPEt_3)_2^+$ and histidine-bound Et_3PAu^+, the regeneration of $AlbSAuPEt_3$, and the systematic narrowing and shifting of the band initially at 33.5 ppm and finally at 36.4 ppm. The gradual shift of this band indicates that Et_3PAu^+ undergoes chemical exchange among the newly formed $Et_3PAuS(ATg)$, some of the weakly bound gold species, and perhaps $ATgS(AuPEt_3)_2^+$ which may form by transfer of two weakly-bound Et_3PAu^+ to ATgSH. The formation of $(Et_3P)_2Au^+$, which is present throughout the titration, apparently results when Et_3P is displaced from gold and then extracts a second Et_3PAu^+ from another site.

Cysteine-34 is clearly the favored binding site for Et_3PAu^+, even though less than 1 equiv is present compared to the 17 histidine residues. After one Et_3PAu^+ binds to form $AlbSAuPEt_3$, steric and electronic factors reduce the affinity of Cys-34 for the second gold moiety. Cys-34 is located in a crevice estimated by EPR spin-labeling to be 1000 pm (10 Å) deep,[22] and the first (phosphine)gold moiety can be expected to retard access of the second. Terminal thiolates are stronger ligands for gold(I) than are bridging thiolates.[23] Thus, the presence of one gold bound to Cys-34 will reduce its affinity for the second gold(I) which requires the formation of a μ-thiolato bridge. As a result of these electronic and steric factors, the (μ-thiolato)bis[(triethylphosphine)gold(I)] species, conclusively identified from the chemical behavior illustrated in Figures 1–4, forms simultaneously with the population of the histidine sites. Conversely one of the two Et_3PAu^+ moieties is removed from Cys-34 as the histidine-bound gold is displaced by ATgSH, and the other is retained (as $AlbSAuPEt_3$) because Cys-34 is a higher affinity thiol than ATgSH.[5,16]

The complex [μ-$ATgS(AuPEt_3)_2^+$] contains a thiolato ligand bridging two Et_3PAu^+ moieties.[10–14] We undertook a ^{31}P NMR examination of the reaction between Et_3PAuCl and $Et_3PAuS(ATg)$,[10] which leads to $ATgS(AuPEt_3)_2^+$, as a model for the formation of $AlbS(AuPEt_3)_2^+$. A typical set of spectra are shown in Figure 5. When either Et_3PAuCl or $Et_3PAuS(ATg)$ is present in excess ($x > 0.52$ or $x < 0.48$; $x = [Et_3PAuCl]/([Et_3PAuCl] + [EtPAuS(ATg)])$), only a single peak is observed, due to rapid ligand exchange among the Et_3PAu^+ species present. These regions of the titration are discussed below.

Very close to the equivalence point ($0.48 < x < 0.52$; $[Et_3PAuCl] \approx [Et_3PAuS(ATg)]$), two nonexchanging peaks are observed at 34.97 and 34.71 ppm shown in Figure 5D ($x = 0.51$). Exact control of the stoichiometry was essential; with even a slight excess of Et_3PAuCl or $Et_3PAuS(ATg)$, these resonances were broadened and overlapped one another. Three independent titrations at 101.3 MHz and one at 202.5 MHz were conducted to verify that this observation is not an artifact. The chemical shift difference between the two resonances was the same at both field strengths, demonstrating that they are, indeed, due to two nuclei in different environments and not due to a scalar coupling phenomenon. These resonances at 34.97 and 34.71 ppm are assigned to two diastereotopically nonequivalent[24] Et_3PAu^+ moieties bound to the sulfur of ATgSH. ATgSH is an asymmetric ligand with five chiral centers, including C_1 to which the thiol group is bonded. Newman projections (Figure 6) from S to C_1 are shown for auranofin and its μ-thiolato species. The auranofin rotamer is that found in the crystal structure.[25] The orientation of the two golds and the lone pair on the sulfur with respect to the C_1 substituents of the μ-thiolato species is arbitrary among the three possible rotamers. Regardless of the relative rotamer populations, the two phosphines cannot become equivalent in the absence of a dissociative mechanism[24] or inversion at sulfur.[26] Clearly, under the conditions used here, neither mechanism operates rapidly enough to make the two phosphorus nuclei equivalent on the ^{31}P NMR time scale.

A plot of δ_P, the average chemical shift, vs mole fraction of gold as Et_3PAuCl (Figure 7) demonstrates that the exchange

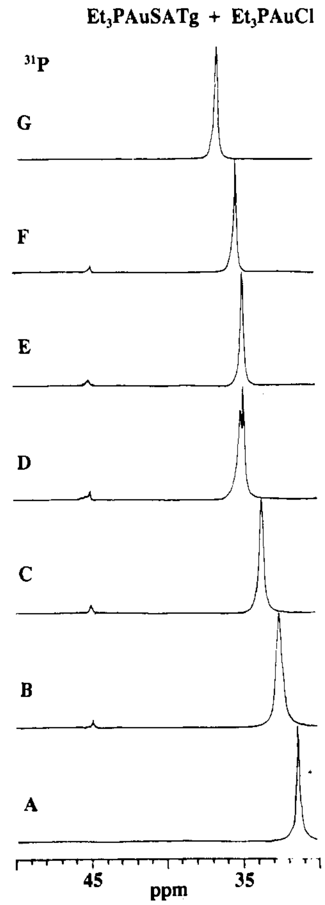

Figure 5. $\{^1H\}^{31}P$ NMR (101.3 MHz) spectra of Et_3PAuCl (25.86 mM), $Et_3PAuS(ATg)$ (10.77 mM) and their mixtures in 60:40 D_2O–MeOH solutions. The mole fraction ratios of $Et_3PAuCl:Et_3PAuS(ATg)$ (total gold from 10.77 to 25.86 mM) are (A) 1:0, (B) 0.69:0.31, (C) 0.55:0.45, (D) 0.51:0.49, (E) 0.42:0.58, (F) 0.20:0.80, and (G) 0:1. TMP was the internal standard.

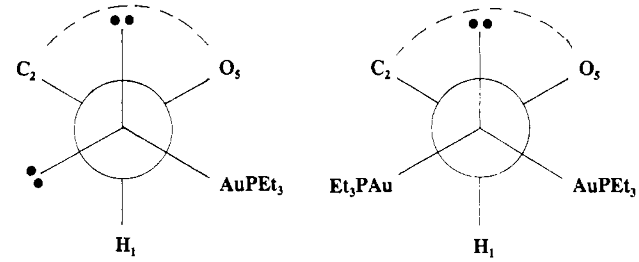

Figure 6. Newman projections (S–C_1) of crystalline $Et_3PAuS(ATg)$[25] (left) and for one of three possible $(Et_3PAu)_2S(ATg)^+$ rotamers (right).

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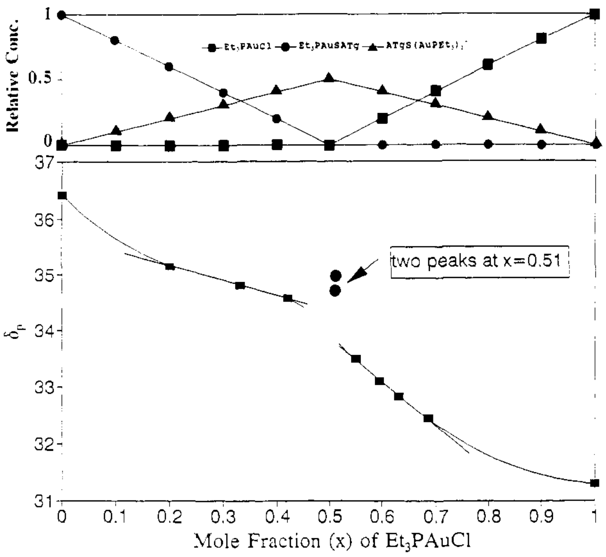

Figure 7. ^{31}P NMR chemical shifts (ppm) of $Et_3PAuSATg/Et_3PAuCl$ mixtures during the titration shown in Figure 5 (bottom) and relative concentrations of Et_3PAuCl, $Et_3PAuS(ATg)$, and $(Et_3PAu)_2S(ATg)^+$ (top) vs the initial mole fractions of Et_3PAuCl (x = $[Et_3PAuCl]/\{[Et_3PAuCl] + [Et_3PAuS(ATg)]\}$). Notice that, at the equivalence point ($[Et_3PAuCl] = [Et_3PAuS(ATg)]$; $x = 0.5$), $(Et_3PAu)_2S(ATg)^+$ is the only species present (top) and that no exchange takes place (bottom).

reactions occurring when $x < 0.48$ or > 0.52 are not just simple interchanges of anions between Et_3PAuCl and $Et_3PAuS(ATg)$. If that were so, the data would define a single straight line between the limiting values for pure $Et_3PAuS(ATg)$ ($x = 0$, $\delta_P = 36.4$ ppm) and Et_3PAuCl ($x = 1$, $\delta_P = 31.3$ ppm). Rather, the presence of two distinct straight line regions from $x = 0.25$ to $x = 0.45$ and $x = 0.55$ to $x = 0.75$ with a discontinuity at $x = 0.5$ (the equivalence point) indicates that $(Et_3PAu)_2S(ATg)^+$ is exchanging with the excess complex present in each region. Between $x = 0.25$ and $x = 0.45$, auranofin is in excess and an exchange between it and the μ-thiolato species is most likely, although equilibration to form a trinuclear gold species, $(Et_3PAu)_3(S(ATg))_2^+$, could also explain the linear shifts. In the region from $x = 0.55$ to $x = 0.75$, excess Et_3PAuCl most likely undergoes exchange with $(Et_3PAu)_2S(ATg)^+$, although its equilibration with the chloride to form a triply bridging thiolate, $(Et_3PAu)_3S(ATg)^{2+}$ could also explain the data. Thus, the graph (Figure 7) reveals the complexity of the exchange processes in a way that is rarely evident from simply comparing sequential spectra (e.g., Figure 5), although neither treatment reveals the species undergoing exchange.

$[(Et_3PAu)_2S(ATg)]^+$ has been reported to form by the reactions of $Et_3PAu^+NO_3^-$ with $Et_3PAuS(ATg)$[10-12] and Et_3PAuCl with $Et_3PAuS(ATg)$,[11] and upon treatment of auranofin with HCl,[11,13] Hill et al.[14] obtained a crystal structure of $[(Et_3PAu)_2S(ATg)^+][NO_3^-]$, in which two $(R_3PAu)_2SR^+$ moieties were associated via gold–gold interactions to form a dimer, $[(R_3PAu)_2SR^+]_2$, but disorder about the plane of the gold atoms precluded a high-resolution structure determination. Recently Fackler et al.[15] reported the structure of $[(Ph_3PAu)_2SCH_2Ph^+][NO_3^-]$ which provided a second and well-defined model for the albumin μ-thiolato species. This also has a dimeric structure with strong gold–gold interactions. These examples provide evidence that (μ-thiolato)digold species are indeed stable gold complexes.

The two (μ-thiolato)digold species studied here behave quite differently. The inorganic model system $ATgS(AuPEt_3)_2^+$ undergoes rapid exchange with an excess of either Et_3PAuCl or $Et_3PAuS(ATg)$ (Figure 5). The protein digold adduct $Alb\text{-}S(AuPEt_3)_2^+$ does not exchange on the NMR time scale with $AlbSAuPEt_3$ or excess Et_3PAuCl (Figure 1). The protein environment of Cys-34 apparently modifies the kinetic properties of the gold adducts, slowing exchange rates, although Cys-34 has an unusually high affinity for gold(I).[16] Sadler and Isab previously found that, for low-molecular weight thiols, there is a correlation of affinity for gold(I) and the exchange rates, both increasing as pK_{SH} decreases.[23] The barrier to exchange by the albumin adduct, despite the high affinity of Cys-34 for gold, must be a steric consequence of the bulky protein ligand and, in particular, the crevice environment of Cys-34.[22]

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