The reduction potentials of I-III (Table XII) are considerably more negative than those expected for a Rieske center analogue. Furthermore, they are even more negative than those found for the conventional 2Fe-2S ferredoxin analogues.

Direct comparisons of redox potentials between synthetic analogues and proteins are difficult to make. Potentials for the proteins are obtained in aqueous media and are reported vs the saturated hydrogen electrode (SHE). In contrast, the potentials for the synthetic analogues are obtained in organic solvents (CH_3CN, DMF, etc.) and are reported vs the saturated calomel electrode (SCE). Under the conditions described above regarding solvent and reference electrode, the redox potentials as reported for the most acceptable synthetic analogues (in DMF vs SCE) are more negative by about 1 V than those of the protein centers. Thus, the reduction of the $[Fe_2S_2(SEt)_4]^{2-}$ complex is found at -1.44 V (DMF vs SCE), while the reduction of the 2Fe-2S ferredoxins takes place at around -0.4 V (H_2O vs SHE). The midpoint redox potentials for the Rieske protein from *Thermus thermophilus*[13] and *Pseudomonas putida*[42] are found at +0.15 and -0.11 V, respectively (vs SHE). An acceptable synthetic analogue for the Rieske centers probably should undergo reduction between -0.9 and -1.1 V (in DMF vs SCE). The pH dependence of the midpoint potential in the Rieske protein from *T. thermophilus* has led[43] to the suggestion of the following equilibria:

$$[Fe_2S_2]^{2+},LH + e^- \rightarrow [Fe_2S_2]^{+},LH \text{ at pH } <8$$

$$[Fe_2S_2]^{2+},L^- + e^- + H^+ \rightarrow [Fe_2S_2]^{+},LH \text{ at pH } >8$$

and the proposal that LH is probably coordinated imidazole.

Cognizant of these results, we are currently directing our attention to the synthesis and structural characterization of Fe_2S_2 complexes that contain bidentate ligands that contain OH^- or SH^- and imidazole as functional groups.

Acknowledgment. The support of this work by a grant (GM 26671) from the National Institutes of Health is gratefully acknowledged.

Supplementary Material Available: Tables of fractional atomic coordinates and thermal parameters, hydrogen atom coordinates, anisotropic thermal parameters, and selected bond distances and angles and figures showing edge-on views of the two anions in $[Ph_4P]_2Fe_2S_2(OC_6H_4\text{-}p\text{-}CH_3)_4$ and temperature dependences of isotropic shifts (19 pages); tables of observed and calculated structure factors (35 pages). Ordering information is given on any current masthead page.

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GC-MS and ^{17}O NMR Tracer Studies of Et_3PO Formation from Auranofin and $H_2{}^{17}O$ in the Presence of Bovine Serum Albumin: An in Vitro Model for Auranofin Metabolism

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Received June 1, 1988

^{17}O NMR spectroscopy and gas chromatographic-mass spectral analysis have been used to monitor the source of oxygen in the triethylphosphine oxide formed by the reaction of the antiarthritic drug auranofin ((2,3,4,6-tetra-*O*-acetyl-β-D-1-glucopyranosato)(triethylphosphine)gold(I)) and bovine serum albumin (BSA) in the presence of reduced glutathione (GtSH). A procedure to extract Et_3PO from aqueous solutions and concentrate it for subsequent analyses was developed. When the in vitro reaction is carried out aerobically in ^{17}O-enriched water, $Et_3P^{17}O$ is generated. The chemical ionization (CH_4) mass measurement, $(m + 1)/z = 135$, and the ^{17}O NMR parameters ($\delta_O = 40.6$ and $^1J_{PO} = 156 \pm 5$ Hz) unambiguously establish its identity. The SH titer of the albumin (mole ratio of protein SH groups to BSA) increases during the reaction, confirming that albumin disulfide bonds are reduced in the reaction. Under aerobic conditions, the enriched Et_3PO accounts for at least 60% of the Et_3PO formed. The significance of these results for the in vivo formation of Et_3PO, an auranofin metabolite, is discussed.

Auranofin ((2,3,4,6-tetra-*O*-acetyl-β-D-1-glucopyranosato)-(triethylphosphine)gold(I), $Et_3PAuSAtg$),[2] a newly licensed chrysotherapy agent, undergoes ligand-exchange reactions that render its metabolism different from that of organic drugs. The products of its metabolic reactions in vivo and model reactions in vitro have been characterized by a variety of physicochemical techniques including radiotracering,[3] ^{31}P NMR,[4,5] ^1H NMR,[5] and EXAFS and XANES spectroscopies,[6] and HPLC.[7] Et_3PO is the principal, and probably exclusive, metabolite generated from the phosphine ligand after it is displaced from the gold(I).[3,5,6,8-10]

Sadler proposed that disulfide bonds in serum albumin oxidize triethylphosphine when $[Au(PEt_3)_2^+]$ is added to whole blood.[5] In packed red blood cells pretreated with auranofin, Et_3PO formation is stimulated by the addition of 2,3-dimercaptopropanol.[5] In reactions of serum albumin with auranofin analogues, auranofin generated a small amount of Et_3PO, but none was generated by

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the deacetylated analogue, $Et_3PAuSTg$ ((β-D-1-thioglucopyranosato)(triethylphosphine)gold(I)).[9] A detailed examination of the reactions of various thiols with the serum albumin metabolite, $Et_3PAuSAlb$, where AlbSH represents the mercaptalbumin form of bovine serum albumin, demonstrated that the thiols having the greatest affinity for gold(I) promote the most rapid formation of Et_3PO and that 40% or greater conversion to Et_3PO could be obtained with excess thiol present:[10]

$$\begin{array}{ccccc} \text{AlbSAuPEt}_3 + \text{RSH} & \overset{-H^+}{\rightleftharpoons} & \text{AlbSAuSR} + \text{Et}_3\text{P} & & \\ & & \downarrow [O] & & (1) \\ & & \text{Et}_3\text{PO} & & \end{array}$$

The studies cited above are consistent with the suggestion that albumin disulfides are the oxidants but do not provide direct evidence in support of their participation.[4-6,8-10] Since triethylphosphine is pyrophoric, O_2 might oxidize it after it is displaced from gold. Although reaction 1 proceeded to the same extent, and as rapidly, under argon as under aerobic conditions,[10] this constitutes only negative evidence for the role of disulfides as oxidants. Furthermore, albumin often contains an oxidation artifact (putatively a sulfenic acid, AlbSOH) formed during storage[6] and might contain spuriously oxidized methionine residues (methionine sulfoxides), which could act as oxidants. O_2, sulfenic acid, and sulfoxides contain oxygen atoms that would be transferred to the phosphine, while the disulfide linkages do not contain any transferable oxygen. Water is the most probable source of oxygen when disulfides (RSSR) drive the formation of Et_3PO (eq 2). This reaction is analogous to well-known, commercially

$$\text{RSSR} + \text{Et}_3\text{P} + \text{H}_2\text{O} \rightleftharpoons \text{Et}_3\text{PO} + 2\text{RSH} \qquad (2)$$

important reactions of disulfides with tri-*n*-butylphosphine and triphenylphosphine.[11-13] Thus, demonstrating the incorporation of labeled oxygen from the water into the Et_3PO, as it is formed by the reaction of bovine serum albumin (BSA) with auranofin and a thiol such as glutathione (GtSH), would provide strong support for the role of the disulfide bonds as oxidants. This paper describes an investigation by ^{17}O NMR and GC–MS tracer techniques of the source of the oxygen in Et_3PO. Measurement of the albumin thiol groups generated by the reaction are also reported.

Experimental Section

Materials. BSA (fatty acid free, Lot 1028252049) was obtained from Boeringer Co., $H_2{}^{17}O$ (Lot F-7959: 34% $H_2{}^{16}O$; 20% $H_2{}^{17}O$; 46% $H_2{}^{18}O$) and $H_2{}^{16}O$ (F-3580: 99.99%) were obtained from Cambridge Isotope Laboratories, glutathione (reduced) and DTNB were from Sigma Biochemicals, $CDCl_3$ and $SOCl_2$ were from Aldrich Chemical Co., $CHCl_3$ (ACS grade) was from Fisher Chemical Co., and Et_3PO was from Strem Chemical Co. Auranofin was generously provided by Smith Kline and French Laboratories.

Et_3PO Generation. In a typical reaction, BSA (240 mg; SH titer 0.60) was dissolved in 1 mL of H_2O (natural abundance, 99.9% $H_2{}^{16}O$ or 20% $H_2{}^{17}O$). The [BSA] was determined to be 3.5 mM from its UV spectrum (ϵ_{278} = 39 600 L mol^{-1} cm^{-1}). $Et_3PAuAStg$ (75 mM in CH_3OH) was added to yield a 2.5 mM auranofin concentration. Sufficient GtSH (solid) was added to yield a final 2.0 mM concentration. Et_3PO formation was allowed to proceed for 24 h with gentle stirring.

SH Titers. Aliquots of several typical reaction mixtures were fractionated over Sephadex G-75 with 100 mM NH_4HCO_3 buffer, pH 7.9, as the eluant. The albumin-containing fractions were pooled, and the gold, protein, and DTNB-reactive thiols were measured as previously described.

Et_3PO Extraction. Aqueous solutions of Et_3PO or the reaction mixtures were extracted six times with 5–10 volumes of $CHCl_3$ or $CDCl_3$. A white, gelatinous albumin precipitate formed and was kept with the aqueous phase, from which the $CHCl_3$ was decanted. The combined extracts were evaporated to ca. 1 mL on a rotary evaporator to concentrate the nonvolatile Et_3PO for spectroscopic analysis.

NMR Measurements. ^{17}O NMR spectra were obtained at 320 K on a Bruker WP 250 Multinuclear NMR spectrometer operating at 33.9 MHz. The chemical shifts were measured in $CHCl_3$ solutions and are reported relative to internal $H_2{}^{17}O$ (as 0.0 ppm). The acquisition parameters were 20-μs pulse width, 205-ms acquisition time, and 20-kHz spectral width. Typically, 10 000 acquisitions were required for enriched samples after extraction from the reaction medium. A 29-Hz line broadening was applied to enhance the signal-to-noise ratio. The spectrum of an authentic, concentrated Et_3PO sample (1/1 v/v in $CDCl_3$) was obtained at natural abundance in 4000 scans.

Under previously described conditions,[6] ^{31}P NMR spectra were obtained at 101.3 MHz on $CDCl_3$ solutions with $(MeO)_3PO$ as the internal reference.

GC–MS Analysis of Et_3PO. A Hewlett-Packard 5985 GC–MS instrument operating in the chemical ionization mode with methane gas was used. Concentrated $CHCl_3$ extracts of Et_3PO were passed through a SPB-5 capillary column (30 m; 0.25-mm i.d.; 0.25-μM film thickness) at an initial oven temperature of 100 °C and programmed to rise at 5 °C/min for 8 min. Authentic Et_3PO was used to calibrate the retention time.

MS Analysis of H_2O Enrichment. The oxygen of water is conveniently incorporated into SO_2 by using thionyl chloride in a conventional vacuum line. A 12-μL portion of water (or reaction mixture) and 12 μL of $Cl_2S{=}O$ were introduced through a septum inlet; the molar excess of water contained in equal volumes ensured that there was no spurious formation of SO_2 by reaction of unconverted thionyl chloride with moisture on the glass surfaces of the vacuum system during the subsequent fractional distillation of reactants and products. Traps at −45 °C (chlorobenzene slush), −110 °C (CS_2 slush), and −178 °C collected H_2O, SO_2, and HCl respectively. The SO_2 was transferred to a small flask fitted for the Hitachi RMU-6E inlet. Conventional electron impact analysis of the peaks at m/z 64, 66, and 68, after correction for the abundance of sulfur isotopes, provided the ratio of ^{16}O and ^{18}O incorporated into the SO_2. Triplicate analyses of the original water and the reaction media after 24 h demonstrated to significant dilution of the water.

Warning: The reaction of H_2O and $SOCl_2$ is extremely exothermic and vigorous and, therefore, potentially violent if conducted on a moderate or large scale. Using microliter quantities as described here should pose no substantial hazard.

Results

GC–MS and ^{17}O NMR spectroscopy were selected as the most sensitive tools to monitor the origin of the oxygen in triethylphosphine oxide. For the GC–MS experiments it was necessary to extract the Et_3PO from the aqueous solutions in order to remove the protein and glutathione before injection; for the ^{17}O NMR experiments it was necessary to remove it from the 10 000-fold excess of $H_2{}^{17}O$. $CHCl_3$ was chosen for the extraction because it is (1) volatile, permitting the excess to be stripped without losing the high-boiling Et_3PO (bp 243 °C), (2) sufficiently polar to extract Et_3PO, which is highly water soluble (and hydrocarbon insoluble), (3) readily available in deuteriated form for NMR measurements, and (4) nonoxygenated, thereby not interfering with subsequent ^{17}O NMR measurements. Six successive extractions, each using 5–10 volumes of $CHCl_3$, recovered sufficient Et_3PO from 5 mM aqueous solutions, with or without albumin, so that high-quality GC–MS and NMR spectra could be obtained.

Control experiments, in which authentic Et_3PO was incubated with enriched water (20% $H_2{}^{18}O$ or 20% $H_2{}^{17}O$ + 46% $H_2{}^{18}O$) then extracted and analyzed by GC–MS, established that direct H_2O/Et_3PO exchange does not occur under the conditions used for the reactions described below.

Previous research in our laboratory showed that excess glutathione drives Et_3PO formation further toward completion than occurs only in the presence of auranofin and serum albumin.[10] Albumin and auranofin react to form $Et_3PAuSAtg$, from which glutathione (GtSH) and the liberated AtgSH displace the Et_3P, thereby initiating its oxidation (eq 1). In the present study, reaction mixtures were stirred for 24 h and then extracted with chloroform for analysis of the Et_3PO.

The critical experiments were performed with 20% ^{17}O-enriched water. The ^{17}O NMR spectrum of the concentrated extract showed two resonances. The 0.0 ppm resonance, due to residual water extracted into the chloroform, provides a convenient reference signal. The 40.6 ppm resonance is that of $Et_3P^{17}O$. The

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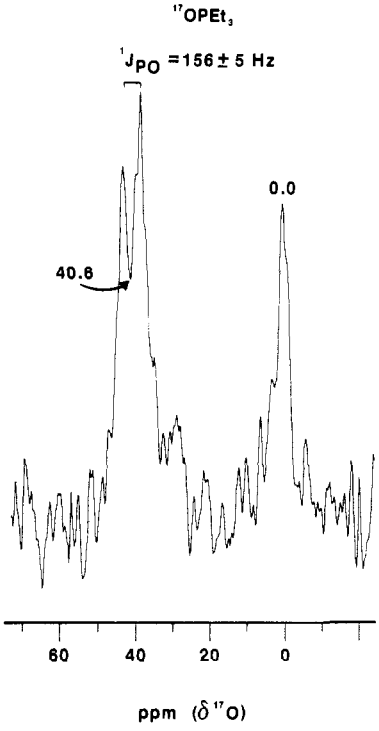

Figure 1. ^{17}O NMR spectrum of $Et_3P^{17}O$ generated by the reaction of BSA (3.5 mM), GtSH (2.0 mM), and $Et_3PAuSAtg$ (2.5 mM) in enriched water (20% $H_2{}^{17}O$) and extracted into $CHCl_3/CDCl_3$. The chemical shifts were measured and are reported relative to $H_2{}^{17}O$.

splitting, 156 ± 5 Hz, is due to one-bond phosphorus–oxygen scalar coupling, $^1J_{PO}$. The same chemical shift was observed at natural abundance after extracting authentic Et_3PO from aqueous solution, but the poorer signal-to-noise ratio obscured the expected P=O coupling (not shown).

The ^{31}P NMR spectra of the extracted Et_3PO solutions were also recorded. The chemical shift value of 49.8 ppm relative to $(MeO)_3PO$ is in excellent agreement with that for authentic Et_3PO in $CDCl_3$, 49.7 ppm. $^1J_{PO}$ was not resolved in the ^{31}P NMR of the ^{17}O-enriched sample since the ^{17}O magnetic moment is $^5/_2$.

The chloroform extracts were analyzed by GC–MS, with the use of CH_4 chemical ionization to facilitate observation of the parent ion. The $(m + 1)/z$ and $(m + 29)/z$ peaks are generated by addition of H^+ and $C_2H_5^+$, respectively, to the parent molecule. Reactions were run in depleted water (99.99% $H_2{}^{16}O$) as well as in enriched water (34% $H_2{}^{16}O$; 20% $H_2{}^{17}O$; 46% $H_2{}^{18}O$). A single parent ion +1 peak at mass 135 and the corresponding parent ion +29 peak are observed for the Et_3PO generated in depleted water (Figure 2a). The spectrum of Et_3PO generated in the enriched water (Figure 2b) contains three isotopic parent ion peaks arising from ^{16}O, ^{17}O, and ^{18}O incorporation and three corresponding parent ion +29 peaks. The observation of $Et_3P^{17}O$ by NMR spectroscopy and GC–MS and of $Et_3P^{18}O$ by GC–MS clearly establishes that water is a source of oxygen for Et_3PO formation in the presence of albumin.

The relative abundances of ^{16}O, ^{17}O, and ^{18}O (100/25/42) in Figure 2b correspond to 60, 15, and 25%, respectively. Comparing the percent of ^{16}O or the sum of ^{17}O and ^{18}O to the isotopic abundance of the water indicates that about 60–66% of the oxygen originated from the enriched water solvent. Either dilution of the water with natural-abundance water (99.8% $H_2{}^{16}O$) during the reaction or oxidation by another oxidant could cause the observed result.

To determine whether water from the BSA and glutathione diluted the isotopic enrichment of the reaction medium, it was measured before and after the reaction. For this purpose we used 20% $H_2{}^{18}O$, which is more economical than $H_2{}^{17}O$. Direct analysis of the water was precluded because our water-intolerant GC–MS instrument is equipped with a moisture removal system and our conventional mass spectrometer has a high background in the mass

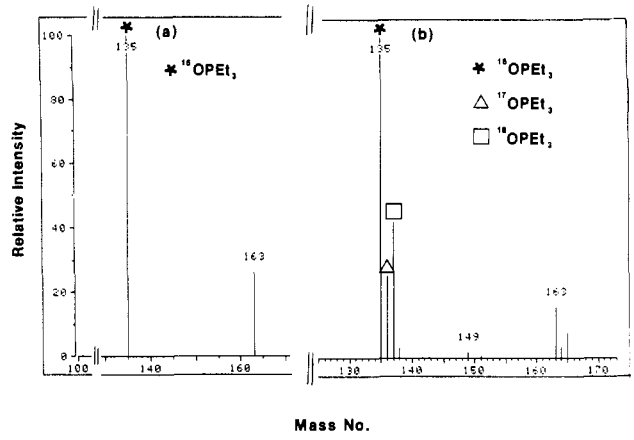

Figure 2. GC–mass spectra of (a) $Et_3P^{16}O$ generated in depleted water and (b) $Et_3P^{16}OP$ (*), $Et_3P^{17}O$ (▲), and $Et_3P^{18}O$ (■) generated in enriched water. The composition of the reaction mixture was similar to that used for Figure 1. Et_3PO was extracted into $CHCl_3$ in each case.

region of water. Therefore, we developed an indirect method using thionyl chloride to convert the water to SO_2.

$$H_2{}^*O + Cl_2S{=}O \rightarrow 2HCl + S^*O_2 \qquad (3)$$

Cl_2SO and a molar excess of water were reacted in vacuo; then the products and excess water were fractionally distilled, collecting the enriched SO_2. Electron impact mass spectroscopy was used to analyze the abundances of the ions at m/z 64, 66, and 68, which depend on the natural abundance of ^{32}S and ^{34}S, the ^{16}O and ^{18}O composition of the H_2O, and the H_2O/Cl_2SO stoichiometry. Within experimental error (three repetitions), the SO_2 generated from water and from the reaction media after 24 h had indistinguishable isotopic compositions (e.g., 0.29 ± 0.04 and 0.30 ± 0.03 $^{66}SO_2$, respectively, in excellent agreement with the value, 0.29, calculated for complete exchange of 20% $H_2{}^{18}O$ with the two sulfur oxygens).

Therefore, since the water is not isotopically diluted, another oxidant must supply the 34–40% of the Et_3PO oxygen not derived from the water. Under the aerobic conditions used, it is probably O_2, but methionine sulfoxide, cysteine sulfenic acid, or other spuriously oxidized groups on the albumin cannot be eliminated as sources. This second source of oxygen does not, however, invalidate the major conclusion that water is the *principal* source of the oxygen incorporated into Et_3PO in the presence of serum albumin.

To verify that albumin disulfide bonds are being reduced, we isolated the protein and analyzed its SH titer, which should increase if eq 2 correctly describes the oxidation–reduction chemistry. A reaction similar to those above was allowed to proceed for 24 h, after which the reaction mixture was fractionated over a gel-exclusion column to isolate the albumin component. In a typical experiment, the Au/BSA ratio was found to be 0.55 and the SH/BSA ratio, determined by using 5,5′-dithiobis(2-nitrobenzoic acid), was 0.83. Since gold bound to Cys-34 of albumin masks its reactivity with DTNB, this experimentally measured SH/BSA ratio is a lower limit for the stoichiometry of thiols generated by Et_3P oxidation. The actual value may be larger due to two factors: (1) there is reoxidation of reduced thiols under the aerobic conditions employed and (2) the newly created thiols may coordinate to gold(I) in lieu of the displaced and oxidized Et_3P, thus masking their reactivity with DTNB.

Discussion

The presence of a strong ^{17}O NMR signal (40.6 ppm) for $Et_3P^{17}O$ generated in ^{17}O-enriched H_2O and most convincingly the appearance of $Et_3P^{17}O$ and $Et_3P^{18}O$ peaks in the mass spectra demonstrate that water is the principal, but not exclusive, source of oxygen when Et_3PO is generated by serum albumin, glutathione, and auranofin under aerobic conditions. The increased SH titer of the albumin establishes that the disulfide bonds are being reduced in the course of the reaction. These observations can be

incorporated into the reaction scheme

$$\text{AlbSH} + \text{Et}_3\text{PAuSAtg} \longrightarrow \text{AlbSAuPEt}_3 + \text{AtgSH} \quad (4a)$$

$$\text{AlbSAuPEt}_3 + \text{RSH} \underset{\text{RSH = GtSH, AtgSH}}{\rightleftharpoons} \text{AlbSAuSR}^- + \text{Et}_3\text{P} + \text{H}^+ \quad (4b)$$

$$\text{PEt}_3 + \text{H}_2{}^*\text{O} + \text{BSA}(\text{S}_2) \longrightarrow \text{Et}_3\text{P}^*\text{O} + (\text{HS})_2\text{BSA} \quad (4c)$$

$$\text{PEt}_3 + \tfrac{1}{2}\text{O}_2 \longrightarrow \text{Et}_3\text{PO} \quad (4d)$$

Previous results established that the rates of Et_3PO formation are comparable under aerobic or anaerobic conditions[10] and that the rate of reaction 4a is much faster than Et_3PO formation.[6] Accordingly, step 4b, displacement of the Et_3P, must be the rate-limiting step for the overall reaction.

The incorporation of oxygen from water and the increased SH titer of the albumin provide the first direct evidence that albumin disulfides oxidize triethylphosphine in these reactions.[14,15] Albumin is rich in disulfide bonds since 34 of its 35 cysteine residues form intramolecular disulfide bonds.[16] Even the remaining cysteine (Cys-34) forms mixed disulfides with cysteine or glutathione in 30–40% of the molecules in vivo.[17] In eq 4b, the disulfide is associated with BSA, since all of the multiple albumin species, not just mercaptoalbumin (AlbSH), contain the 17 intramolecular disulfide bonds.[17] One of the disulfide bonds is postulated to be close to Cys-34,[18] which is the high-affinity binding site for Et_3PAu^+,[6] and this disulfide might be preferentially reduced.

In three related systems ((i) reactions using cyanide to displace the phosphine from $Et_3PAuSAtg$,[19] (ii) reactions of the methyl analogue of auranofin, $Me_3PAuSAtg$, with albumin,[20] and (iii) reactions of $Au(PEt_3)_2^+$ with albumin[21]), the albumin thiol titer increases concomitantly with Et_3PO formation, which is consistent in each case with disulfide oxidation of the phosphine. Hemoglobin, which contains no disulfide bonds, forms a complex, $Hb(SAuPEt_3)_2$, via the Cys-β-93 thiols.[22] It is noteworthy that, unlike $AlbSAuPEt_3$, the hemoglobin complex does not generate Et_3PO when it is treated with thiols.[22]

Although an in vivo role for albumin disulfides as oxidants has not been established yet, the rate of phosphine oxide generation is greater than auranofin is added to whole blood (i.e., in the presence of both serum and red cells) than when it is added to serum alone or red blood cells alone.[8] In addition to its role as oxidant, the albumin also labilizes the phosphine due to the trans effect of the unusual Cys-34 thiol group, which has a higher affinity for gold(I) than any other known thiol group.[10,19] While our in vitro studies clearly establish the feasibility of nonenzymatic phosphine oxidation mechanisms, there exists the possibility that reactions in vivo may involve other oxidants, for example, cytochrome P-450 monooxygenases, which are known to oxidize diphenylmethylphosphine and diphenyl(3-aminopropyl)phosphine to the corresponding phosphine oxides.[23]

The chemical shift value, $\delta_O = 40.6$, and the $^1J_{PO}$ coupling constant, 156 Hz, for $Et_3P^{17}O$ can be compared to the values of 80.9 ppm and 74 Hz, respectively, which are average values for all four oxygens in H_3PO_4.[24] The coupling constant shows better agreement with the range of values reported for phosphoryl oxygens, 120 Hz (Me_3PO) to 205 Hz (Cl_3PO).[25]

Acknowledgment. We thank Smith Kline and French Laboratories for partial funding of this research. A.A.I. thanks The King Fahd University of Petroleum and Minerals for a sabbatical leave. We also thank Dr. Christopher K. Mirabelli (Smith Kline and French Laboratories) and Mary T. Coffer (UWM) for helpful discussions. The skilled technical assistance of Dr. Suzanne Wehrli and Frank Laib is acknowledged.

Registry No. Et_3PO, 597-50-2; auranofin, 34031-32-8.

(14) A reviewer suggested that glutathione might be the oxidant. Reductions of its functional groups ($-NH_3^+$, $-CO_2^-$, $-CONH-$, and $-CH_2SH$) by phosphine are without precedent, while the reduction of disulfide bonds with various phosphines is a well-established reaction.[11-13] Furthermore, several laboratories have established previously that glutathione and other thiols undergo ligand-exchange reactions with auranofin but do not generate Et_3PO.[5,7] Nevertheless, we incubated a mixture of glutathione and auranofin at the concentrations employed here; after 24 h, the ^{31}P NMR spectrum had only a single resonance at 36.2 ppm due to the rapid exchange of thiol ligands but no resonance at 61 ppm, the chemical shift of Et_3PO in aqueous solution.

(15) Another reviewer raised the possibility that the chloroform was responsible for the oxidation of the phosphine. Our previously published studies, in which $CHCl_3$ extractions were not employed, monitored the reaction for 24 h by ^{31}P NMR spectroscopy and found conversions up to 50%;[10] only a few minutes is required to extract the Et_3PO from the reaction mixture and then strip off the chloroform, so it is not likely to be a significant oxidant.

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