Molecular Properties of Acyl Carrier Protein Derivatives*

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Charles O. Rock
From the Department of Biochemistry, St. Jude Children’s Research Hospital, Memphis, Tennessee 38101

John E. Cronan, Jr.
From the Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Ian M. Armitage
From the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510

Acyl carrier protein (ACP) functions as the acyl carrier in fatty acid biosynthesis (1) and as an acyl donor in glicerolipid synthesis (2, 3) in *Escherichia coli*. ACP is an acidic protein of molecular weight of 8847 (4), and exists in solution as an asymmetric monomer (5). Acyl moieties are bound to ACP by thioesters of the only sulphydryl group of the protein. This sulphydryl is the terminal portion of the 4'-phosphopantetheine prosthetic group attached to serine-36 of ACP via a phosphodiester linkage (4). Native ACP has a high α-helical content and is reversibly denatured by high pH (5, 6). Recent experimental findings suggest that the fatty acid moiety of native acyl-ACP interacts with the protein. The 13C NMR spectrum of 6,6-difluoro-C14:0-ACP shows two fluorines to be nonequivalent giving rise to an AB quartet indicative of a substantial degree of immobilization (7), however, the 13P NMR spectrum of 13,13-difluoro-C14:0-ACP gave a single resonance indicative of relatively unhindered rotation at carbon-13 (7). Fatty acid-protein interaction is also suggested by the finding that acyl-ACP is more stable to pH-induced denaturation than ACP (5). A model for the secondary structure of ACP has been proposed and a cluster of hydrophobic residues implicated in the formation of a fatty acid-binding domain (5).

Several investigators (8, 9) have reported that purified ACP gives rise to two bands on native gel electrophoresis. In these reports, the slower migrating band was converted to the faster migrating component by treatment with reducing agents. On the basis of this result, both laboratories concluded that the slower migrating ACP band was a dimeric species of ACP cross-linked through the single sulphydryl group of the protein. We have recently reported (5), that the dimersization of *E. coli* ACP does not readily occur in solutions stored at alkaline pH, and the separation between the two ACP species observed by Etemadi and Josse (8) seems too small to be consistent with the presence of ACP dimers. Recently, we have developed an improved method for the purification of ACP (10). Nondenaturing gel electrophoresis of ACP purified by this method in the absence of reducing agents revealed the presence of several ACP species. This result has led us to characterize the chemical modifications of ACP that give rise to these multiple forms and to explore the molecular basis for their resolution on native gels.

**EXPERIMENTAL PROCEDURES**

Materials—Sodium dodecyl sulfate, ammonium persulfate, Tris, glycine, Affi-Gel 501, N,N-methylenebisacrylamide, acrylamide, N,N,N',N'-tetramethylethylenediamine, Coomassie Blue R-250, and bromphenol blue were purchased from Bio-Rad Laboratories. Frozen *E. coli* B cells (late log) were obtained from Grain Processing Co. GSH, GSSG, CoASH, and dihydrothreitol were purchased from P-L Biochemicals. PIPES and CHES were purchased from Calbiochem and guanidine HCl (ultrapure) was obtained from Schwarz/Mann. DTNB was a Sigma product. All other chemicals were reagent grade or better.

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* The abbreviations used are: ACP, acyl carrier protein; ACP, holo-acyl carrier protein; acyl-ACP, acyl carrier protein; (ACP), acyl carrier protein dimer linked through the prosthetic group sulphydry; apo-ACP, acyl carrier protein lacking the prosthetic group; ACPSG, ACPSCoA, and ACPS-TNB, ACP mixed disulfides with glutathione, CoASH, and thionitrobenzoate (TNB), respectively; (Ac)-ACP, ACP with all five amino groups and the prosthetic group sulphydry acetylated; DTNB, 5,5'-dithio-bis(2-nitrobenzic acid); SDS, sodium dodecyl sulfate; CHES, cyclohexylaminoethanesulfonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; GSH, glutathione; R., Stokes’ radius; bis-Tris, 2,2'-bi-(hydroxyethyl)-2',2'-nitroethanol.
Native Gel Electrophoresis—The separating gel contained 20% (w/v) acrylamide, 0.5% (w/v) N,N'-methylenebisacrylamide, 0.10% (v/v) N,N',N'-tetramethylethylene diamine, 0.375% Tris-HCl, pH 9.0, and polymerization was accomplished by the addition of freshly prepared ammonium persulfate solution to a concentration of 0.03% (w/v). Using the same stock solutions for a period of several days, the separations were found to steadily deteriorate and the culprit was identified as the separating gel buffer. The pH of the buffer does not change, and we do not understand the exact nature of the problem. Consistent results were obtained when the separating gel buffer was freshly prepared. A stacking gel was poured over the separating gel and contained the same components except the acrylamide concentration was 5% and the Tris HC1 buffer was 0.125 M, pH 6.8. The running buffer consisted of 3.1 g of Tris and 14.4 g of glycine/liter. Samples contained glycerol and bromphenol blue and the pH adjusted to between 6.0 and 7.5. Gels were run at 25 mA until the tracking dye reached the gel bottom. Staining gels overnight in 50% methanol, 10% acetic acid, 0.1% Coomassie Blue R-250, and destaining in 10% methanol, 10% acetic acid. ACP is an acidic protein and stains poorly using most protocols. Several combinations of methanol, acetic acid, and different stains were tried and the above procedure was determined empirically to be the most satisfactory.

Preparation of ACP Derivatives—ACP was purified from E. coli B cells in the absence of reducing agents using the 2-propanol method described previously (10). ACPSH mixed disulfides with GSH and CoASH were prepared under similar conditions. The ACPSH sample was reduced with dithiothreitol and the reducing agent removed using a Sephadex G-25 column. Reaction mixtures contained ACPSH (1 mM), sodium borate, pH 8.5 (50 mM), and either GSSG or CoASSCoA (50 mM) in a final volume of 1 ml. The reaction mixture was incubated overnight at room temperature, the low molecular weight components were resolved from the protein on a G-25 column. ACPS-TNB was prepared by reacting a reduced solution of ACPSH (1 mM/ml) with 2 volumes of 10 mM DTNB in 50 mM Tris-HCl, pH 8.5, for 1 h at room temperature. (Ac)-ACP-Sac was prepared by mixing 0.1 ml of reduced ACPSH (0.1 mM) in 10 mM Tris-HCl, pH 8.5, with 0.1 ml of tetrahydrofuran. Acetic anhydride (10 μl) was added, the mixture allowed to stand at room temperature for 30 min and another 10 μl of acetic anhydride was added and the incubation continued for another 30 min. (Ac)-ACP-Sac was precipitated by the addition of 0.4 ml of water and the derivatized protein collected by centrifugation. The apo-ACP sample was a gift from Dr. Mary Polacco and was prepared by treating ACPSH with hydrofluoric acid (11). When necessary, ACP derivatives were concentrated by loading the sample onto a 0.1-ml DEAE-cellulose column (DE-52, Whatman) and elution of the ACP with 0.5 M LiCl in the desired buffer. Acyl-ACP was prepared by a slight modification of the previously published procedure (12). The modification was to substitute 20 mM bis-Tris-HCl, pH 6.0, for the 20 mM Tris-HCl, pH 7.4, buffer in all the chromatographic steps. This alteration was employed to reduce the formation of (ACP), by the reaction of ACPSH with acyl-ACP (see “Results”). (ACP), formation and thus lower acyl-ACP yields by comparison with the system without the modification were observed in those previous reactions (12). [14C]ACPSH and [3H]ACPSH labeled in the prosthetic group was prepared biosynthetically as described previously (5).

31P NMR—31P NMR spectra were recorded on an extensively modified Bruker HFX-90 single-coil pulsed Fourier transform spectrometer operating at 36.4 MHz. D.O present in a 3-mm co-axial capillary insert was used for the field frequency lock, and all spectra were obtained under conditions of proton noise decoupling. Chemical shifts are reported in parts per million relative to an external standard of 85% phosphoric acid run coincidently with the sample. Sample volumes were 1.0 ml and ACP concentrations were 1 μM.

RESULTS

Identification of ACPSSh—When ACP, isolated in the absence of reducing agents from E. coli B cells, was subjected to native gel electrophoresis, the major protein band was found to migrate slower than the ACPSH standard (Fig. 1). This new ACP species was found to be converted to a species migrating with standard ACPSH when the sample was reduced with dithiothreitol prior to loading the gel. This result suggested that the new ACP species was a mixed disulfide with another molecule. The 31P NMR spectrum of ACP purified from E. coli B also revealed the presence of a new ACP species as shown by a resonance shifted 0.3 ppm upfield from standard ACPSH (Fig. 2). During the course of these studies, the 31P NMR spectra were obtained on four different preparations of ACP from 0.5 kg of E. coli B cells. The spectrum shown in Fig. 2A contained the most ACPSH found in the four purifications. In the other three instances, only the resonance corresponding to the new ACP species was observed and the ACPSH peak occurred only as an unresolved shoulder on the main peak. The spectrum in Fig. 2A was chosen to illustrate the chemical shift difference between the two peaks. Corresponding with the results in the electrophoresis experiments, this resonance was converted to the standard ACPSH position by dithiothreitol. SDS and SDS-urea gel electrophoresis (5) of the new ACP form exhibited a single band both in the presence and absence of dithiothreitol that migrated with the ACPSH standard. These data show that the molecular weight of the compound linked to ACPSH was small compared to the molecular weight of ACP. This compound was identified by dissolving 1 μmol of ACP mixed disulfide in 1 ml of 20 mM Tris-HCl, pH 8.0, followed by reduction with 5 μmol of dithiothreitol. After 30 min at room temperature, the solution was titrated to pH 3.9 with acetic acid and the precipitate allowed to flocculate. The ACP pellet was removed by centrifugation and an aliquot of the supernatant chromatographed on an amino acid analyzer. A single component eluting at the same position as the GSH standard (9 min) was detected. To further support the identity of this species as ACPSHG, synthetic ACPSHG was prepared as described under “Experimental Procedures.” Authentic ACPSHG was found to have the same migration on gel electrophoresis (Fig. 1) and an identical 31P NMR chemical shift (Fig. 2B) as the unknown ACP species extracted from the cells.

Characterization of (ACPSH)—We have observed that homogeneous acyl-ACP (12) slowly decomposes (t1/2 = 2 years for C16:0) when stored in 50 mM Tris-HCl, pH 7.4, at -20°C. Long chain-saturated (C14:0 and C16:0) acyl-ACP have been found to be quite stable upon storage, whereas unsatu-
rated acyl-ACPs and shorter chain lengths were found to be considerably less stable. Surprisingly, the cleavage of the fatty acid did not result in the formation of ACPSH, but rather in a protein that has a higher molecular weight. The properties of this ACP species were investigated by gel filtration chromatography (Fig. 3). The breakdown product of acyl-ACP was found to possess an R, appropriate for a dimeric species of ACP (Fig. 3). Addition of dithiothreitol to the sample resulted in an elution volume identical with that shown in spectrum in C. SDS and SDS-urea gel electrophoresis of (ACPSS) samples showed a band having a molecular weight corresponding to twice the size of ACPSH in the absence of reducing agents and this band was converted to ACPSH by addition of dithiothreitol to the sample prior to electrophoresis. Similar results were obtained when the mobility of (ACPSS) was examined in the native gel electrophoresis system (Fig. 1). Definitive evidence for the existence of dimeric ACP was provided by sedimentation equilibrium experiments (Fig. 4). The absence of dithiothreitol, (ACPSS) was found to possess a molecular weight twice that found in the presence of dithiothreitol (Fig. 4). (ACPSS) was also detected in acyl-ACP samples by virtue of its characteristic $^{31}$P NMR chemical-shift (Fig. 5). As found in our other experiments, (ACPSS) was converted to ACPSH by the addition of dithiothreitol to the sample.

The degradation of acyl-ACP in the presence of ACPSH is surprising in light of the low reactivity of acyl-ACP to simple thiols (14). At 37°C and pH 8.0, the reaction had an appreciable rate (Fig. 6) and is dependent on the concentration of ACPSH (Fig. 6). Thin-layer chromatography on Silica Gel G layers developed in hexane:diethyl ether:acetic acid (80:20:1, v/v), of samples from experiments as in Fig. 6 and [14C]acyl-ACP samples that have significantly degraded on storage revealed the presence of free fatty acids but no fatty aldehydes were detected. We do not understand the molecular details of this reaction, but a direct attack of ACPSH on acyl-ACP may be expected to produce a fatty aldehyde as a product. Since free fatty acid was the only product observed, it appears that hydrolysis of acyl-ACP precedes dimerization, however, (ACPSS) is not found when ACPSH is treated similarly to a mixture of acyl-ACP and ACPSH. This observation is of practical significance in storing acyl-ACP since acyl-ACP preparations that are initially homogeneous have much longer half-lives than if the acyl-ACP is contaminated with ACPSH.

Although (ACPSS) was not readily formed in ACPSH solutions (5), significant quantities of (ACPSS) were formed when ACPSH was added to a mixed disulfide of ACP and another thiol. For example, when ACPSH was mixed with ACPSTNB in 50 mM Tris-Cl, pH 8.0 (ACPSS) was readily formed and could be assayed spectrophotometrically by observing the release of the TNB anion. (ACPSS), prepared by this method was found to have the same $^{31}$P NMR spectrum as the (ACPSS) isolated from the acyl-ACP preparations (Fig. 5). (ACPSS) was also formed when ACPSH was chromatographed on thiol affinity resins. When ACPSH was passed through Bio-Gel P-100 columns (47 x 0.3 cm) of Bio-Gel P-100 (18 ml bed volume) was equilibrated with 0.25 M LiCl, and developed at a flow rate of 1.1 ml/h. The sample size in all cases was 100 μl and 325-μl fractions were collected.

**Fig. 2.** $^{31}$P NMR spectra of ACPSH and ACPSSG. A, $^{31}$P NMR spectrum of an ACP sample purified from E. coli B cells in the absence of reducing agents; B, $^{31}$P NMR spectrum of synthetic ACPSSG; C, $^{31}$P NMR spectrum of ACPSH. Addition of dithiothreitol to the samples in A and B resulted in spectra having a chemical shift identical with that shown in spectrum in C.

**Fig. 3.** Resolution of ACP monomers and dimers by gel filtration chromatography. A column (47 x 0.3 cm) of Bio-Gel P-100 (18 ml bed volume) was equilibrated with 0.25 M LiCl, and developed at a flow rate of 1.1 ml/h. The sample size in all cases was 100 μl and 325-μl fractions were collected. Panel A, a sample containing $^{[14C]}$(ACPSS) and acyl-$^{[14C]}$ACP. ACPSH and acyl-ACP have previously been shown in a similar solvent (5). Panel B, a sample containing purified $^{[14C]}$(ACPSS). Panel C, a sample containing purified $^{[14C]}$(ACPSS) that had been reduced with dithiothreitol prior to application to the column. In all cases, recovery of applied radioactivity was >95%.
through an organomercurial agarose column (Affi-Gel 501), two fractions were obtained. One ACP fraction did not absorb to the column and the other was eluted with dithiothreitol. Examination of these two fractions in the native gel electrophoresis system revealed that the fraction that did not bind to the column was (ACPS)$_2$, and the fraction eluted with dithiothreitol was ACPSH. Rigorous exclusion of oxygen was not attempted and the oxidation of ACPSH to (ACPS)$_2$ may have been accomplished by the presence of metal ions other than mercury or the support. 

31P NMR Investigations—ACPSH has been shown to be reversibly denatured by high pH and temperature (5, 6) and Ca$^{2+}$ has been shown to stabilize the protein to these effects (6, 15). Therefore, we have investigated the 31P NMR spectra of ACPSH under these conditions to determine whether the phosphorus chemical shift is sensitive to the conformational state of ACPSH. The 31P chemical shift of ACPSH was not altered by changing the pH or by the presence or absence of dithiothreitol (Table I). Denaturation of ACP by heat was accompanied by a small but consistently observed downfield chemical shift (Table I). The addition of Ca$^{2+}$ to ACPSH resulted in an upfield shift in the 31P resonance (Table I). The small range of 31P chemical shifts observed in these studies suggests that the conformation of the phosphorus attached to serine-36 was not drastically altered by major structural changes in ACPSH.

We have also investigated the 31P NMR spectra of ACPSH having a variety of sulfhydryl group modifications (Table II). All ACP disulfides examined exhibited a downfield chemical shift as compared to ACPSH (Table II). In all cases, addition of dithiothreitol to the sample resulted in the disappearance of the ACP disulfide resonance and the appearance of the ACPSH resonance at $-0.01$ ppm. ACPSH-SCoA exhibited two additional resonances at $+3.56$ and $-11.37$ ppm arising from the phosphates of the CoA moiety. These chemical shifts were those of CoASH and were not affected by the addition of dithiothreitol to the ACPSH-SCoA sample. All acyl-ACP chain lengths examined possessed the same chemical shift (Table II). In contrast to the ACP disulfides, the acyl-ACP chemical shift was upfield from ACPSH and not affected by the addition of dithiothreitol.

Structural Consequences of Sulphydryl Group and Protein

![Graph](image)

**Fig. 4. Sedimentation equilibrium of (ACPS)$_2$.** The sedimentation experiment was performed by a modification (5) of the method of Bothwell et al. (13). The values plotted are the log of the fraction of protein remaining in the top 40% of the tube after centrifugation at about 68,000 rpm (14 p.s.i.) for 20 h versus the molecular weight normalized to a partial specific volume of 0.730 ml/g. The buffer used was 0.02 M LiCl in bis-Tris-HCl, pH 6.5. All samples contained 10 mg/ml of Dextran T-10 to stabilize against convection. Dithiothreitol (DTT) (when added) was present at 20 mM. The [$^{13}$C](ACPS)$_2$ sample was the same as that shown in Fig. 3, B, and the distributions of the radiolabeled ACP species were determined by scintillation counting. The distributions of the standards were determined spectrophotometrically at 240 nm or 418 nm (cytochrome c). The F values in the (ACP) experiments were obtained in the presence of an internal standard of cytochrome c (4.5 mg/ml). Essentially identical results were obtained without the internal standard. Standard proteins were ['$^{13}$C]ACPS (8,850; $\delta = 0.02$), sperm whale myoglobin (16,900; $\delta = 0.728$), and chromotryptinogen (25,700; $\delta = 0.734$).

![Graph](image)

**Fig. 5. 31P NMR spectra of acyl-ACP and (ACPS)$_2$.** A. 31P spectrum of (ACP)$_2$-ACP + AC containing (ACPS)$_2$, contamination; B, 31P spectrum of (ACP)$_2$, prepared by the DTNB method; C, 31P NMR spectrum of pure C$_{16}$-ACP. Addition of dithiothreitol to the (ACP)$_2$ samples resulted in a spectrum possessing the same chemical shift as ACPSH, but did not affect the position of acyl ACP.

![Graph](image)

**Fig. 6. Deacylation of acyl-ACP catalyzed by ACPSH.** Incubations contained 0.1 mM Tris-HCl, pH 8.0, 5 mM dithiothreitol, 1.0 $\mu$M [1-'$^{14}$C]Cyto-ACP (specific activity 50 Ci/mmol) with no additions, 0.4 mg/ml of ACPSH, or 1.6 mg/ml of ACPSH. The capped Eppendorf tubes were incubated at 37°C and 5-ml samples were withdrawn after the tubes were blended on a Vortex mixer and centrifuged for 2 min on an Eppendorf microcentrifuge to sediment any water droplets in the sides of the tubes. The amount of [1-'$^{14}$C]acyl-ACP remaining was measured using the filter disc assay (18).
**Properties of ACP Derivatives**

**TABLE I**

| Temperature | pH<sup>a</sup> | Additions<sup>a</sup> | Chemical shift<sup>b</sup> |
|-------------|----------------|----------------------|--------------------------|
| 7°C         | 3.4            | 8 m guanidine HCl    | -0.18                    |
| 28          | 5.1            | None                 | -0.01                    |
| 60          | 5.1            | None                 | +0.27                    |
| 28          | 5.0            | 15 mM Ca<sup>2+</sup> | -0.17                    |
| 28          | 7.3            | None                 | -0.01                    |
| 28          | 9.4            | None                 | -0.01                    |
| 41          | 9.4            | None                 | +0.22                    |
| 28          | 9.4            | 15 mM Ca<sup>2+</sup> | -0.17                    |
| 41          | 9.4            | 15 mM Ca<sup>2+</sup> | -0.11                    |
| 49          | 9.4            | 15 mM Ca<sup>2+</sup> | +0.09                    |
| 28          | 9.4            | 30 mM Ca<sup>2+</sup> | -0.28                    |

<sup>a</sup> Buffers were: 25 mM sodium acetate, pH 5.1, 25 mM Tris-HCl, pH 7.3, 25 mM CHES, pH 9.4.

<sup>b</sup> All spectra were first collected in the absence of dithiothreitol. Two other resonances were observed at +3.96 and -11.37 ppm corresponding to the +3P resonances from the CoA moiety.

**TABLE II**

| Sample<sup>a</sup> | Chemical shift<sup>b</sup> |
|---------------------|--------------------------|
| ACP SH              | -0.01                    |
| (ACPS)<sub>2</sub>   | +0.24                    |
| ACPS S S G           | +0.30                    |
| ACPS S S C<sub>O</sub>A | +0.18                  |
| ACPS T N B           | +0.07                    |
| Acyl-ACP            | -0.58                    |

<sup>a</sup> All samples were dissolved in 20 mM PIPES, pH 6.8, 0.2 M LiCl.

**DISCUSSION**

ACP has been shown to undergo pH-induced denaturation manifested by a decrease in the α-helical content as measured by optical methods (6) and a dramatic increase in the R, of the protein (5). Native gel electrophoresis at pH values above the same structure of the transition region that ACP is known to have is the effect of sulfhydryl and protein modification on the stabilization of the native ACP conformation. ACP is a very acidic protein and carries 21 negative charges and only 6 positive charges per mole at the pH of the separating gel (4). This preponderance of negatively charged residues means that modifications of the protein that add or subtract 1 or 2 negative charges/mole will have little effect on the velocity of migration during electrophoresis. Therefore, the molecular dimensions of the ACP species is the primary variable that determines the migration of the ACP derivative in this gel system (Fig. 1). Modifications that stabilize the native ACP structure migrate faster in these gels than the ACPSH standard and modifications that destabilize ACP migrate more slowly than ACPSH. The separations described in this report were not observed when the native separating gel also contained urea (8 m), SDS or both, further supporting the conclusion that conformational isomers are being resolved. We have previously shown that the presence of an acyl group of ACP stabilizes the protein to pH-induced denaturation and that acyl-ACP has a smaller R, than ACPSH at elevated pH (5). Accordingly, acyl-ACP was found to migrate faster on native gels than ACPSH (Fig. 1). All acyl-ACP chain lengths examined (C<sub>10</sub> to C<sub>13</sub>) had an identical mobility in the native gel electrophoresis system indicating that they all possessed the minimum structural features conferring maximum stability to the protein moiety. All of the ACP-mixed disulfides prepared in this report were found to destabilize the structure of ACP (Fig. 1). A model of ACP secondary structure has been proposed (5), and the stabilization induced by the acyl group has been attributed to the interaction of the acyl moiety with hydrophobic amino acid residues present in adjacent helical segments of the protein (5). If hydrophobic interactions are primarily responsible for the stabilization of acyl-ACP, then the introduction of polar groups into this segment of the molecule would be expected to destabilize the structure. Therefore, the destabilization of ACP structure by charged ligands bearing little resemblance to a fatty acid chain is consistent with this model.

The effects of protein modifications on the stability of ACPSH was also examined. Fully acetylated ACP has been shown by spectral methods to be abnormally sensitive to pH-induced denaturation (6). (Ac)<sub>2</sub>-ACPSh migrates in the native gel system just below (ACPSH)<sub>2</sub> (Fig. 1), demonstrating that (Ac)<sub>2</sub>-ACPSh has a large molecular radius consistent with this ACP derivative being in a random coil configuration. Removal of the prosthetic group caused a slight destabilization of ACP structure.

Previous investigators have observed that ACP preparations give rise to multiple bands on native gel electrophoresis and that the slower migrating bands were converted to the faster migrating band by addition of dithiothreitol to the sample prior to electrophoresis (8, 9). Since CoASH readily dimerizes in solution and the prosthetic group of ACP has the same structure as CoASH, the notion that ACPSH also dimerizes in solution has been generally accepted (1). Therefore, these workers (8, 9) concluded that the slower migrating band was (ACPSH)<sub>2</sub> and no further characterization of these ACP species was reported. Since our present data (Fig. 1) demonstrate that a variety of sulfhydryl modifications of ACPSH migrate more slowly than ACPSH in a native gel electrophoresis system similar to that used by previous workers (8, 9), the identification of these bands as (ACPSH)<sub>2</sub> must...
be considered suspect. We have observed that ACP purified from *E. coli* B cells is primarily ACPSSG, and further that this ACP species migrates more slowly in native gel electrophoresis than ACPSH and is converted to ACPSH by reducing agents. A comparison of our gels to those shown by Etemadi and Josse (8) suggests that ACPSSG was present in their samples. Since GSH is the major low molecular weight sulfhydryl compound in *E. coli* (16), ACPSSG could arise by spontaneous nonenzymatic oxidation of ACPSH with GSSG. However, CoASH has been shown to be converted to CoASSG under anaerobic conditions in stationary phase *E. coli* (17), and this result suggests that ACPSSG may be formed under similar conditions to protect ACPSH from spurious oxidations. We are currently investigating the formation of ACPSSG in *E. coli* cultures under a variety of growth conditions to ascertain its role in ACP metabolism.

The high nucleophilicity of the sulfhydryl of ACPSH makes the formation of (ACPS)$_2$ rapid under certain conditions. When ACPSH is reacted with ACPS-TNB, (ACPS)$_2$ is formed and can be monitored by release of TNB anion. Most surprisingly, acyl-ACP also appears to react with ACPSH to form (ACPS)$_2$ (Fig. 5). This rate is much slower than the above reactions and in this case, a specific protein-protein interaction may be involved since acyl-ACP is much less reactive to simple thios (Fig. 6; Refs. 12, 14). This observation is of practical significance in the preparation and storage of acyl-ACP, and has resulted in the modification of the previous preparative method (see “Experimental Procedures”) by lowering the pH in the chromatography steps to 5.9 where acyl-ACP hydrolysis and thus (ACPS)$_2$ formation is reduced.

Although the $^{31}$P chemical shifts reported in this paper (Tables I and II) are significant for phosphate resonances, the exact nature of the structural alterations in the phosphate group responsible for the shifts is not known. However, it can be concluded that the environment of the phosphate group is altered as a consequence of sulfhydryl and protein modifications. Each electrophoretically distinct form of ACP was also found to possess a characteristic $^{31}$P chemical shift. In all cases, protein modifications that resulted in a less stable ACP structure were found to have chemical shifts displaced downfield from ACPSH and modifications resulting in stabilization of ACP were found to have chemical shifts upfield from ACPSH.

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