Acyl Carrier Protein from *Escherichia coli*. Structural Characterization of Short-Chain Acylated Acyl Carrier Proteins by NMR†

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ABSTRACT: Acylated acyl carrier proteins (ACPs) with acyl chain lengths of 2, 4, 6, 8, and 10 carbons were investigated by NMR and nuclear Overhauser methods at 500 MHz. Chemical shift changes of downfield aromatic and upfield, ring-current-shifted, isoleucine proton resonances monotonically vary as a function of acyl chain length with the most prominent shifts occurring with chain lengths between four and six carbons. Chemical shifts are largest for one of the two phenylalanines; however, substantial shifts do exist for Tyr-71, His-75, and two isoleucines. Since these residues are distributed throughout the molecule, their associated resonance chemical shifts are most probably explained by an induced conformational change. Comparative NOE measurements on reduced ACP (ACP-SH) and ACP-S-C8 suggest, however, that these induced conformational changes are small except for around one of the phenylalanines. A tertiary structural model for acyl-ACP consistent with our previous model for ACP-SH [Mayo, K. H., Tyrell, P. M., & Prestegard, J. H. (1983) *Biochemistry 22*, 4485–4493] is presented.

Acyl carrier protein (ACP)[1] functions as a coenzyme in the synthesis and subsequent metabolism of fatty acids in bacteria and plants (Prescott & Vagelos, 1972; Stumpf, 1977). In this function, ACP can exist as a free sulfhydryl, as a disulfide aduct, or as an acylated protein where the acyl moiety can be from 2 to 18 carbon atoms long. The various acyl derivatives exhibiting differences in activity as substrates for some enzymes in fatty acid metabolism are enhanced over differences among functional analogues such as acyl-CoAs (Thompson, 1981). This suggests that the conformation of ACP and acyl-ACPs may play a role in the regulation of fatty acid synthesis. Investigation of the conformation of ACP and various acyl-ACPs is, therefore, of utmost importance in resolving mechanistic questions.

Among all known ACPs, ACP from *Escherichia coli* is perhaps the best characterized (Prescott & Vagelos, 1972; Schultz et al., 1969). A small protein of 8847 daltons, ACP from *E. coli* contains 77 amino acid residues, a large proportion being acidic (~29%) and a small proportion (~8%), clustered at the NH terminus, being positively charged. Although the sequence of ACP has been known for some time (Vanaman et al., 1968a,b), no X-ray crystal structure yet exists. Structural models have thus been divised by combining a variety of physical observations. From an interpretation of optical rotary dispersion (Takagi & Tanford, 1968; Prescott et al., 1969) and circular dichroism studies (Schultz, 1975), ACP seems to possess a high α-helix content. The predictive algorithm of Chou & Fasman (1974a,b, 1978a,b) has been applied to ACP (Rock & Cronan, 1979), and a secondary structure consistent with high α-helix content has been predicted. Four α-helical regions are predicted to exist between residues 3–21, 26–32, 37–53, and 58–69, with β-turns interrupting the α-helical segments, yielding a rough, first approximation to the secondary structure. In our previous paper (Mayo et al., 1983) we have studied aspects of the solution conformation of *E. coli* ACP-SH primarily by using nuclear Overhauser (NOE) methods; NOEs among His-75, Tyr-71, Phe-I, and three Ile's suggested a folding pattern of the four α-helices and allowed a tertiary structural model for ACP-SH to be proposed (Mayo et al., 1983).

Primarily on the basis of hydrophobic binding studies of acyl-ACPs to octyl-Sepharose, Cronan (1983) has suggested the presence of an acyl-chain binding site able to shield from six to eight carbons of a fatty acid chain from interaction with hydrophobic chromatography resins. This hypothesis of an acyl chain binding site has also been suggested by ^{19}F NMR studies on ^{19}F-labeled fatty acids bound to ACP (Galley et al., 1978). The tertiary structural model of ACP-SH (Mayo et al., 1983) is consistent with the proposal of a specific hydrophobic binding site since a hydrophobic cleft formed by the arrangement of the four α-helices is present in the model. The placement of an acyl chain in perturbations of this proposed structure will be investigated in this present study by using 500-MHz proton NMR.

Materials and Methods

Native acyl carrier protein (ACP) was purified from *Escherichia coli* B cells (Grain Processing) by using the method of Rock & Cronan (1980) and reduced to free sulfhydryl by the method of Cronan & Klages (1981). Purity was checked by proton NMR, amino acid analysis, and native acrylamide gel electrophoresis (Ray & Cronan, 1976). Ellman's test for sulfhydryl reactivity (Ellman, 1959) verified the existence of an active, free sulfhydryl on the prosthetic group prior to acylation. Acyl-ACPs were synthesized from ACP-SH and the corresponding *N*-acylimidazole as described by Cronan & Klages (1981). The acyl-ACPs were precipitated from the reaction mixture by lowering the pH and were examined by NMR without further purification. Yields ranged from 90% for acetyl- and octanoyl-ACPs, to over 70% for butanoyl- and hexanoyl-ACPs, to approximately 30% for decanoyl-ACP.

Samples for ^1H NMR measurements were passed through a Chelex column, freeze-dried, and redissolved in D_2O several

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[1] Abbreviations: ACP, acyl carrier protein; ACP-SH, reduced acyl carrier protein; CoA, coenzyme A; DSS, sodium 4,4-dimethyl-4-silapentanesulfonate; NOE, nuclear Overhauser effect.

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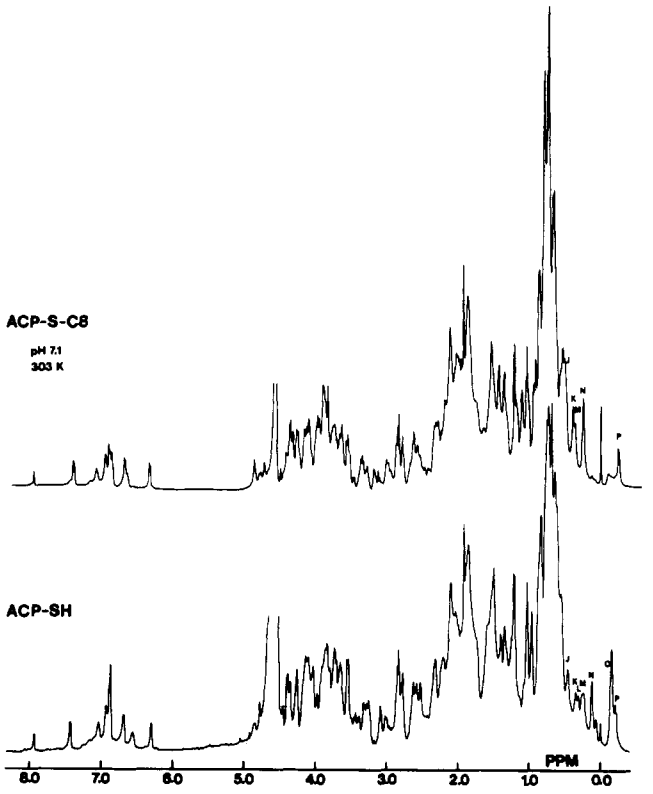

FIGURE 1: 500-MHz NMR spectra of ACP-SH and ACP-S-C8. The spectrum represents (512) accumulations over a period of ~12 min by using a 90° pulse on a 0.5-mL sample at concentrations of 3 mM ACP, pH 7.1, at 303 K. The large, central HDO resonance has been omitted for clarity.

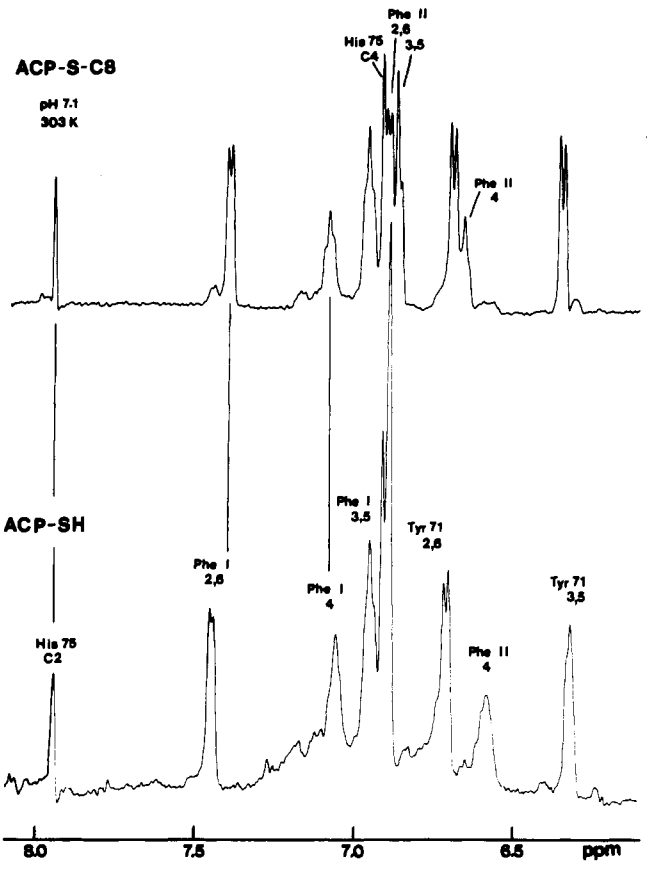

FIGURE 2: Downfield aromatic region. Conditions are identical with those in Figure 1. The data have been processed with a Lorentzian to Gaussian transformation to improve resolution.

hours before the experiment. The final protein concentration was approximately 3 mM in 20 mM potassium phosphate buffer at the pH indicated in the text.

^{1}H NMR spectra were recorded in the Fourier mode on a Bruker WM-500 spectrometer at 303 K. The solvent deuterium signal was used as the field-frequency lock. All chemical shifts are quoted in parts per million (ppm) downfield from sodium 4,4-dimethyl-4-silapentanesulfonate (DSS). NOEs were generated by irradiating the desired peak for 0.4 s at a power level sufficient to null z magnetization of the irradiated peak in 0.05 s. A 2-ms delay was introduced before the observation pulse and accumulation to reduce transient effects, and a time of 1.5 s was allowed between accumulations (accumulation time of 1.48 s) to allow for recovery of z magnetization. The FIDs resulting from several hundred transients were multiplied by an exponential which produced 10-Hz line broadening prior to Fourier transformation. Difference spectra were obtained by subtracting the free induction decay (FID) of the irradiated peak spectrum from the FID of a control spectrum that had been irradiated in a region where no resonances occur under the same experimental conditions. NOEs were determined by dividing areas of peaks in the difference spectrum by the area of the irradiated peak and correcting for differences in the number of protons.

RESULTS

Effect of Acylation and Fatty Acid Chain Length on Chemical Shift. Chemical shift changes on acylation are most clearly seen by comparison of spectra of ACP-SH and octanoyl-ACP (i.e., ACP-S-C8). A 500-MHz NMR spectrum of reduced ACP (i.e., ACP-SH) is shown in Figure 1. Chemical addition of an octanoyl fatty acid chain to the free sulfhydryl of the ACP prosthetic group (i.e., ACP-S-C8) produces an only slightly modified NMR spectrum.

Most resonances in these spectra suffer from extensive overlap, and small changes cannot be easily analyzed. Two regions, however, appear to be much less convoluted; these are the upfield ring-current-shifted hydrophobic methyl proton resonances (i.e., upfield of 0.5 ppm) and the downfield aromatic proton resonances (i.e., 6.0–8.0 ppm). The downfield aromatic resonances are more clearly shown in Figure 2 and have been labeled in accord with our previous work on ACP-SH (Mayo et al., 1983) as shown. The most notable changes are in the resonances of Phe-II. On addition of an octanoyl side chain to ACP-SH the Phe-II four proton resonance, for example, has been downfield shifted (~0.1 ppm). The ACP-S-C8 sample contained some unmodified ACP-SH; therefore, ACP-SH resonances in the ACP-S-C8 spectrum can also be observed (~10%).

A more complete dependence of chemical shift changes as a function of acyl chain length at two pH values (i.e., pH 5.8 and 9.4) is given in Figure 3 for well-resolved downfield aromatic and upfield ring-current-shifted resonances. As a function of acyl chain length, the chemical shifts are seen to vary monotonically with the greatest shifts occurring on elongation from four to six carbons. At pH 5.8 these shifts occur in both upfield and downfield directions and are largest for Phe-II and Ile I. More moderate changes are observed for Tyr-71 and His-75. Not included in this figure are resonances L and O assigned to methyl groups on aliphatic hydrophobic residues near Phe-II (Mayo et al., 1983). These shift markedly downfield (>0.1 ppm) on chain elongation and, in fact, merge with the complex methyl region in octanoyl-ACP where they cannot be discerned.

ACP is believed to undergo a conformational change above pH 7.5; so the above experiments were repeated at pH 9.4. Effects of acylation of comparable magnitudes are seen both

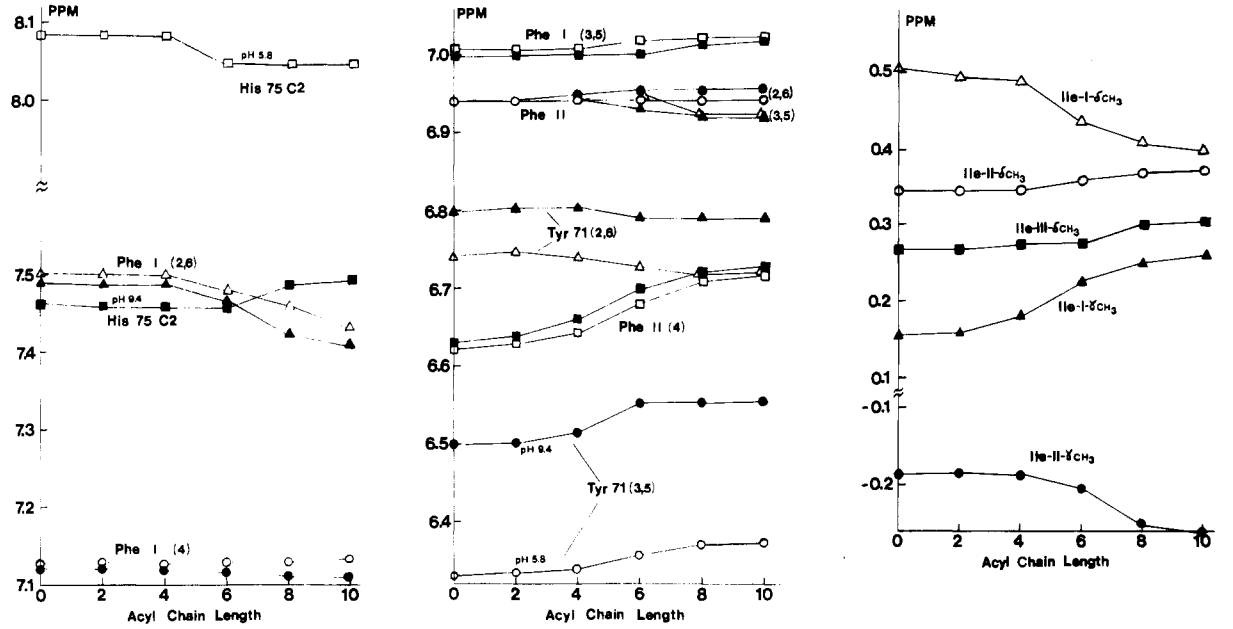

FIGURE 3: Effect of acyl-chain length on chemical shifts. Chemical shift data are plotted as a function of acyl-chain length for downfield aromatic and upfield, ring-current-shifted methyl proton resonances. The filled-in symbols are for data at pH 9.4, and the open symbols are for data at pH 5.8. Symbols are merely connected by solid lines.

above and below pH 7.5. For several residues, shifts on elongation of acyl chains change direction between pH 5.8 and pH 9.4. These include His-75, Ile I, and Ile II. His-75 exhibits a $pK_a \simeq 8.0$ (unpublished results), and differences may be connected with this for that residue; the origin of the effects on Ile I and II is not understood.

Nuclear Overhauser Measurements. Chemical shift changes on varying the acyl chain length can be due either to direct acyl-chain-residue interactions or to indirect effects resulting from induced conformational changes. The former may be useful in localizing an acyl-chain binding site, while the latter are of more general interest. In order to differentiate these effects, nuclear Overhauser (NOE) experiments similar to those previously performed on ACP-SH (Mayo et al., 1983) were undertaken on ACP-S-C8. Since NOE difference spectra for ACP-S-C8 are similar to those already published for ACP-SH, these spectra are not shown here; rather, these NOE results on ACP-S-C8 are summarized in Table I along with data for ACP-SH.

For a system of dipolar coupled spins, the magnitude of the nuclear Overhauser effect on a given spin following saturation of another spin for a fixed length of time is a complex function of the motional properties of the spin system, the resonance frequency of the spectrometer, and the inverse sixth power of the distances separating all pairs of spins (Dobson et al., 1980). The inverse sixth power dependence is an inherent source of information about structural properties of macromolecules (Sykes et al., 1974; Oldfield et al., 1975; Chapman et al., 1978; Krishna et al., 1978; Gordon & Wüthrich, 1978; Poulsen et al., 1980), but it must first be separated from motional factors. In simple cases where there is an interaction at a known distance and it can be assumed that all interactions are modulated by the same motion, motional dependence of nuclear Overhauser enhancements can be largely removed by dividing all enhancements by that arising from a well-defined standard. This procedure is followed in Table I. In the case at hand, our standard enhancement arises from the aromatic (2,6)–(3,5) proton interaction; the distance between proton pairs is known from X-ray measurements to be 2.5 Å. All other enhancements (η_j) arising from interaction with a given aromatic proton have therefore been scaled by the (2,6)–(3,5) ring proton enhancement to give a ratio, R. This procedure is a good first approximation although it assumes that a single correlation time characterizes the cross-relaxation process. The use of a more general model for the molecular dynamics might affect the results somewhat, especially where methyl groups are involved; since we are dealing more with a semiquantative comparison between two similar proteins for which the NOE results, in most cases, do not change, the need for a more complex model is probably unnecessary at this level of investigation. The data in Table I, in some cases, do show very small changes in these ratios (<40%) on comparing data on ACP-SH and ACP-S-C8. The major exception is Phe-II, where, for example, ratios change by factors of 2–3 for interaction with F2 and F5 resonances from proximate residues.

Discussion

The nuclear Overhauser (NOE) data presented here suggest that structural changes in ACP which occur following acylation with fatty acids of eight carbon atoms or less are generally small. Although this conclusion is based on data concerning only aromatic amino acid residues in ACP, these residues have been suggested to lie in a hydrophobic cleft positioned between four α-helix segments in ACP (Mayo et al., 1983) and should, therefore, be well situated to reflect perturbations on acylation. Of these aromatic residues, only Phe-II demonstrates significant internuclear distance changes. If we convert the ratios in Table I to distances as was done in our previous work, a change on the order of ~0.2–1.0 Å in the case of the Phe-II β-CH_2 ring distances and interresidue distance changes on the order of ~1.0 Å for unassigned proton resonances F2 and F5 are found.

Chemical shift changes as a function of acyl-chain length observed here are also largest for Phe-II proton resonances. Two upfield, ring-current-shifted resonances, L and O, assigned to γ- or δ-methyl protons of an aliphatic hydrophobic residue (i.e. Leu, Val, or Ile) proximal to Phe-II (Mayo et al., 1983), are downfield shifted on chain elongation so much so that observing their shift dependencies is made virtually impossible. Other resonances given in Figure 3 demonstrating acyl-chain-induced shift dependencies are more attenuated. Under the same solvent and temperature conditions chemical shift

Table I: Nuclear Overhauser Effects (NOEs) at pH 7.7

		ACP-SH		ACP-S-C8		
resonance irradiated	resonance showing NOE	η_j^a (t = 0.4 s)	R_{SH}^b	η_j^a (t = 0.4 s)	R_{C8}^b	ΔR %^{c}
His-75 C(2)	Tyr-71 (2,6)	0.01		0.01		
	Tyr-71 (3,5)	0.01		0.005		
Phe-I (2,6)	Phe-I (3,5)	0.2	1.0	0.2	1.0	0
	B1 (Phe-I αCH)	0.2	1.0	0.2	1.0	0
	B2 (proximate residue	0.04	0.2	0.03	0.2	0
	B3 (Phe-I βCH)	0.17	0.9	0.16	0.8	−12
	B4 (Phe-I βCH)	0.15	0.8	0.17	0.9	+12
	K (Ile II δCH_3)	0.02	0.1	0.02	0.1	0
	P (Ile II γCH_3)	0.02	0.1	0.02	0.1	0
Tyr-71 (2,6)	Tyr-71 (3,5)	0.15	1.0	0.17	1.0	0
	His-75 C(2)	0.01	0.1	0.01	0.1	0
	G1 (Tyr-71 αCH)	0.13	0.9	0.12	0.7	+22
	G2 (proximate residue)	0.02	0.1			
	G3 (Tyr-71 βCH)	0.08	0.5	0.12	0.7	+40
	G4 (Tyr-71 βCH)	0.10	0.7	0.09	0.5	−40
	K (Ile II δCH_3)	0.02	0.1	0.02	0.1	0
	M (Ile III δCH_3)	0.02	0.1			
	N (Ile I γCH_3)	0.01	0.1			
	P (Ile II γCH_3)	0.01	0.1			
Tyr-71 (3,5)	Tyr-71 (2,6)	0.27	1.0	0.23	1.0	0
	His-75 C(2)	0.03	0.1	0.01	0.1	0
	K (Ile II δCH_3)			0.02	0.1	
	J (Ile I δCH_3)	0.02	0.1	0.01	0.1	0
	N (Ile I γCH_3)	0.02	0.1	0.02	0.1	0
Phe-II (2,6; 3,5)	Phe-II (4)	0.29	1.0	0.24	1.0	0
	F1 (Phe-II αCH)	0.14	0.5	0.13	0.5	0
	F2 (proximate residue)	0.04	0.1	<0.01	<0.04	<−150
	F3 (Phe-II βCH)	0.07	0.2	0.08	0.3	+50
	F4 (Phe-II βCH)	0.07	0.2	0.16	0.7	+250
	F5 (proximate residue δ- or γCH_3)	0.06	0.2	0.01	0.1	−100
	L (Leu-δCH_3 or	0.02	0.1	?^{d}		?^{d}
	O Val-γCH_3)	0.02	0.1	?^{d}		?^{d}

^{a}NOE = 1 + η; t is the mixing time; the error in these measurements is ±0.01. ^{b}R is the ratio of η_j at a given resonance to η_j arising from the 2,6 to 3,5 protons of the aromatic rings. ^{c}ΔR % is the percent change in the average internuclear distance on going from ACP-SH to ACP-SC8. A minus sign denotes a greater distance apart while a plus sign denotes a lesser distance apart. ^{d}Chemically shifted?

differences of various proton resonances can only be due to direct acyl-chain residue interactions or due to indirect effects resulting from induced conformational changes. Since conformational changes involving Phe-II are known to occur, at least part of the chemical shift differences are therefore due to indirect effects.

A working model of the ACP-SH tertiary structure has been proposed (Mayo et al., 1983). According to this model, Phe-I and Phe-II have been tentatively assigned to Phe-28 and Phe-50, respectively. Some understanding of the possible conformational perturbations can be achieved by adding an acyl chain to this model (Figure 4). If we assume no gross conformational change in ACP-SH upon acylation as the comparative NOE data strongly suggest, then we may place the acylated form of the prosthetic group in this model beginning at the point of its covalent linkage to Ser-36. Doing this would place the free sulfhydryl of the prosthetic group near the top of α-helix segment 1, while acylation places the fatty acid chain between α-helices 3 and 4 near Phe-50. As the chain is elongated from four to six carbons it may be necessary to move Phe-50 aside to accommodate the chain. Largest NOE changes are noted for Phe-II, and its chemical shift changes are greatest between butanoyl-ACPs and octanoyl-ACPs.

The Tyr-71, His-75, Phe-28, and Ile I, II, and III residues are located at the opposite end of the molecule, removed from any direct contact with the fatty acid chain. Changes in the chemical shifts of these amino acid residues as a function of acyl chain length, therefore, can probably be ascribed to small induced conformational changes either in the protein or in the prosthetic group which in the molecule lies near these amino acids.

The existence of a hydrophobic binding site for the acyl chains in ACP has been proposed primarily on the basis of pH stabilization data and hydrophobic binding data of acyl-ACPs to octyl-Sepharose (Rock & Cronan, 1979; Rock & Garwin, 1979); these data have been interpreted as indicating the presence of an acyl-chain binding site that binds the first six to eight carbon atoms of the acyl chain. Chains of 8–10 or more carbon atoms would then extend into the solvent and be more accessible to binding a hydrophobic column matrix. The model in Figure 4 supports this proposal.

Precedent for structural changes occurring in ACP as a consequence of fatty acid chain elongation and changes in pH has already been set (Schultz, 1975; Rock & Cronan, 1979; Rock et al., 1981). On the basis of electrophoretic mobilities, for example, the Stokes radius of ACP-SH is suggested to increase from 19.6 Å at pH 7.2 to 37 Å at pH 9.4, an increase of almost 2-fold (Rock & Cronan, 1979). Furthermore, the addition of fatty acyl chains to ACP-SH decreases the amount of expansion; at pH 9.4 the Stokes radius of long-chain (≥C8) acyl-ACPs is only 30.5 Å, compared to 37 Å for the non-acylated species (Rock & Cronan, 1979). The changes of ~20% in radius, if distributed uniformly over the amino acids of a globular protein, would translate to increases of approximately 3 Å in average interresidue distances. It is clear that we do not see changes of this magnitude in the regions around the aromatic residues at pH 7.7. However, these estimates of the Stokes radii based on electrophoretic mobilities must be viewed with caution. First of all, ACP is assumed by the Stokes model to be spherical, although it is, in fact, likely to be very asymmetric. Furthermore, ACP is a highly, negatively charged molecule, and the effects on both charge and asymmetry on hydrodynamic behavior are not well understood. Our

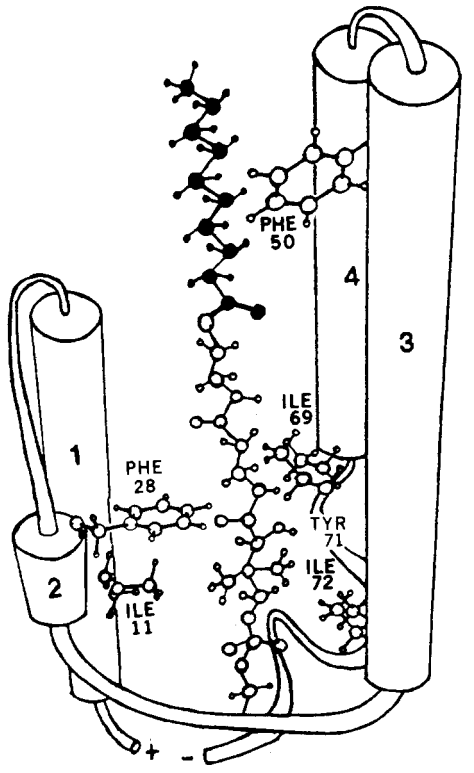

FIGURE 4: Proposed tertiary structure of ACP-P-C10. The proposed gross conformation of ACP-S-C10 is shown. α-Helix regions (1–4) are depicted as cylinders, and β-turns and random coils are shown as solid lines. The only amino acid residues shown are those that have been discussed in the text. The prosthetic group is shown as open circles between the four helices, and the decanoyl acyl chain is shown as filled-in circles attached to the prosthetic group.

data are consistent with radii changes in that some conformation change does exist. Changes in induced chemical shift patterns at pH 5.6 and 9.4 are also observed. Our data, however, suggest a smaller and possibly more localized alteration in structure.

ACKNOWLEDGMENTS

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