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Purification and Characterization of *Rhodobacter sphaeroides* Acyl Carrier Protein†

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ABSTRACT: Acyl carrier protein (ACP) has been purified from the facultative phototrophic bacterium *Rhodobacter sphaeroides*. The ACP preparation was >95% homogeneous as determined by native and disodium dodecyl sulfate (Na_2DodSO_4)–polyacrylamide gel electrophoreses and N-terminal amino acid analysis. Amino acid compositional analysis revealed that the protein contains approximately 75 amino acids, has a calculated minimum molecular weight of 8700, and lacks the amino acids tyrosine and tryptophan. The presence of the characteristic 4′-phosphopantetheine prosthetic group was indicated by the occurrence of equimolar quantities of β-alanine and taurine in amino acid hydrolysates and was confirmed by independent chemical analysis. The protein displayed a p*I* of 3.8 and had a calculated partial specific volume of 0.732 mL/g. The primary structure of the protein has been determined for the first 46 amino acid residues from the N terminus of the molecule, and the region of the molecule encompassing the amino acids from residues 31 to 44 was found to have 100% homology with the identical residues in *Escherichia coli* ACP. In contrast to *E. coli* ACP, *R. sphaeroides* ACP migrated according to its molecular weight during Na_2DodSO_4 gel electrophoresis, was resistant to pH-induced denaturation, and comigrated with the *cis*-vaccenoyl-ACP derivative during native gel electrophoresis. It is proposed that the basis for these properties is the enhanced hydrophobic character of the protein.

Organisms possessing a type II (nonaggregated) fatty acid synthetase complex (bacteria and plants) utilize a freely dissociable acyl carrier protein (ACP)[1] to bind the intermediates and products of fatty acid synthesis (Vagelos, 1971; Prescott & Vagelos, 1972; Bloch & Vance, 1977). In addition to their role in bacterial fatty acid synthesis, acyl-ACP derivatives are known to directly serve as acyl donors for de novo phospholipid biosynthesis in bacteria (Ailhaud & Vagelos, 1966; van den Bosch & Vagelos, 1970; Goldfine et al., 1967; Goldfine & Ailhaud, 1971; Lueking & Goldfine, 1975a; Cronan, 1978; Rock & Cronan, 1982) and, most recently, have been shown to participate as acyl donor substrates for the production of lipid A (Anderson et al., 1985) and for the reacylation of lysophosphatidylethanolamine in cells of *Escherichia coli* (Rock, 1984). The central role played by ACP in bacterial lipid metabolism was recently highlighted by the results of Rock and Jackowski (1982), who showed, via a series of in vivo studies, that the size and composition of the intracellular acyl-ACP pool responded to the metabolic status of cellular phospholipid biosynthesis.

The non-sulfur purple facultative phototrophic bacterium *Rhodobacter sphaeroides* provides an attractive system for investigations of the regulation of bacterial fatty acid and phospholipid biosynthesis (Kaplan, 1978; Lueking et al., 1978; Niederman et al., 1978; Takemoto, 1974; Donohue & Kaplan, 1985). In contrast to *E. coli*, *R. sphaeroides* displays an obligate requirement for acyl-ACP substrates as acyl donors for de novo phospholipid synthesis (Lueking & Goldfine, 1975b; Cooper & Lueking, 1984), and both temporal and light-mediated controls of phospholipid synthesis have been demonstrated in this organism (Lueking et al., 1978; Fraley et al., 1978; Campbell & Lueking, 1983). In view of the central importance of ACP and acyl-ACP in the lipid metabolism of *R. sphaeroides* (Boyce & Lueking, 1984; Cooper & Lueking, 1984), we have purified and examined the physical

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[1] Abbreviations: ACP, acyl carrier protein (reduced form); Na_2DodSO_4, disodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DEAE, diethylaminoethyl; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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properties of ACP obtained from phototrophically grown cells of this organism.

Experimental Procedures

Chemicals. [^{3}H(N)]-β-Alanine (108 Ci/mmol), [9,10-^{3}H]palmitic acid (11 Ci/mmol), and [1-^{14}C]palmitic acid (52.5 mCi/mmol) were purchased from New England Nuclear Corp. [^{3}H]Acetic acid (26 Ci/mmol) and [^{3}H]acetic anhydride (50 mCi/mmol) were purchased from ICN Pharmaceuticals, Inc. [^{3}H]Vaccenic acid (1 mCi/mmol) was prepared as described by Cooper and Lueking (1984). [^{3}H]ACP was isolated from *R. sphaeroides* grown on [^{3}H(N)]-β-alanine-supplemented medium (3 μCi/mL; 108 Ci/mmol). Sephadex G-100, Sephadex G-75, octyl-Sepharose, *E. coli* alkaline phosphatase, DEAE-cellulose, DEAE-Sephadex A25, soybean trypsin inhibitor, bovine trypsin inhibitor, bovine serum albumin, ovalbumin, glucagon, and pepsin were purchased from Sigma Chemical Co. *E. coli* B was purchased from Grain Processing Co. Ready-Solv NA and Ready-Solv EP scintillation cocktails were from Beckman. DE-52 used in the recovery of acyl-ACP's was obtained from Whatman. Lysozyme and casamino acids were obtained from Difco Products. Polyacrylamide gel electrophoresis (PAGE) materials were purchased from Bio-Rad Laboratories, as were the molecular weight markers used in disodium dodecyl sulfate (Na_2DodSO_4)–PAGE. Precoated silica gel H uniplates (20 × 20 cm) were purchased from Analtech, Inc. All other chemicals and solvents were of reagent grade or better and were obtained from Sigma Chemical Co., Fisher Scientific, or Eastman Kodak.

Organism, Medium, and Growth Conditions. R. sphaeroides strain M29-5 (Leu^-, Met^-), derived from wild-type strain 2.4.7, was obtained from Samuel Kaplan, University of Illinois, Urbana, IL. Growth was conducted in a succinic acid minimal medium (Sistrom, 1962), as modified by Lueking et al. (1978), supplemented with 0.2% (w/v) casamino acids. Incubations were conducted photoheterotrophically at 32 °C in either completely filled screw-cap culture tubes or Roux bottles or under an atmosphere of N_2/CO_2 (95%/5%) in 5–20-L vessels. Continuous illumination was provided by a bank of four Sylvania Lumiline lamps. For growth in 5–20-L vessels, lamp banks were placed on two sides of the vessel and the vessel was continuously stirred and intermittently sparged with the N_2/CO_2 gas mixture. Culture growth was monitored turbidimetrically by using a Klett-Summerson colorimeter equipped with a no. 66 red filter. Cells were harvested in the stationary phase of growth, and yields were typically 2 g wet weight of cells/L of culture. *R. sphaeroides* stock cultures were maintained at −20 °C in succinate minimal media supplemented with casamino acids and 30% (w/v) glycerol.

Purification of ACP. R. sphaeroides ACP was purified by a modification of the procedure described by Majerus et al. (1969). Frozen cells (200 g wet weight) of *R. sphaeroides* M29-5 were allowed to thaw overnight in 200 mL of 20 mM triethanolamine hydrochloride (pH 7.5) containing 10 mM 2-mercaptoethanol and were then disrupted by two passages through an Aminco French pressure cell (16000 lb/in.^{2}). The crude cell lysate was clarified by centrifugation at 10000*g* for 20 min, and the resulting supernatant was centrifuged for 1 h at 106000*g*. The soluble fraction obtained was treated with streptomycin sulfate and ammonium sulfate, and the ACP was collected by acid precipitation as described by Majerus et al. (1969). The precipitate was resolubilized in buffer (pH 6.0), and the solution was clarified by centrifugation (10000*g*, 10 min). The preparation was then chromatographed twice over a column of DEAE-cellulose and once over a column of DEAE-Sephadex A25. ACP eluted from DEAE-cellulose at 14.4 mmho (4 °C) and from DEAE-Sephadex at 18 mmho. ACP was quantitated in column fractions by monitoring the ability of fraction aliquots to stimulate the activity of *E. coli* acyl-ACP synthetase. Acyl-ACP synthetase assays utilized enzyme preparations purified from *E. coli* B (Rock & Cronan, 1981a) through the heat supernatant step, and incubation mixtures (40 μL) contained 60 μM [^{14}C]palmitic acid (52.5 μCi/μmol), 19 μL of the column fraction, 13.25 μg of acyl-ACP synthetase, 75 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM ATP, 0.4 M LiCl, 0.5% Triton X-100, and 2.0 mM dithiothreitol. Reactions were initiated by the addition of enzyme and were terminated at 15 or 60 min by transferring 35-μL samples of Whatman filter paper disks. The filter disks were then air-dried, washed 3 times in chloroform/methanol (8:2) to remove unincorporated [^{14}C]palmitate (Green et al., 1981), dried, and counted in nonaqueous scintillation fluid.

Synthesis of Acyl-ACP. Acyl thiol ester derivatives of *E. coli* and *R. sphaeroides* ACP's were prepared with *E. coli* acyl-ACP synthetase. *E. coli* ACP was purified as described by Rock and Cronan (1981b). [^{14}C]Palmitoyl-ACP was prepared by batch synthesis as described by Rock et al. (1981b). Tritiated acyl-ACP derivatives were prepared as described by Cooper and Lueking (1984). The purity of acyl-ACP substrates was routinely monitored by native PAGE (Rock et al., 1981a), and quantitation was accomplished by monitoring the amount of radioactive fatty acid released following treatment of the acyl-ACP substrate with neutral hydroxylamine (Lueking & Goldfine, 1975a).

Compositional and Primary Structure Analysis. ACP purified by preparative elution electrophoresis was hydrolyzed (in duplicate) in 6 N HCl at 105 °C in vacuo for 24 and 48 h, with 11 nmol of protein used for each hydrolysis. The amount of cysteine and 2-mercaptoethylamine was determined following oxidation of 2 nmol of ACP in 6 N HCl containing 1.5% dimethyl sulfoxide (Spencer & Wold, 1969), and cysteine was quantitated as cysteic acid. The 2-mercaptoethylamine of the prosthetic group was detected as taurine. Tryptophan was monitored in a methanesulfonic acid hydrolysate (Liu & Chang, 1971) of 4.4 nmol of ACP and was not detected. A Beckman amino acid analyzer (Model 121MB) was employed for the separation of amino acid residues, which were then detected with the dimethyl sulfoxide–ninhydrin reagent of Moore (1968). Peak areas were integrated by employing a Spectra-Physics integrator.

Primary structure analysis was performed on the intact protein by using automated Edman degradation (Laursen, 1972) and a Beckman Model 89C sequencer (spinning cup) equipped with an Altex 345 ternary high-performance liquid chromatograph. Amino acid residues were identified as their phenylthiohydantoin derivatives (Edman & Begg, 1967). The compositional and primary sequence analyses were conducted at the Protein Sequencing Center, University of Texas, Austin, TX.

Prosthetic Group Analysis. [^{3}H(N)]-β-Alanine-labeled ACP was incubated for 22 h at 37 °C in 0.1 M Tris-HCl, pH 12.0, containing 0.1 M dithiothreitol. Upon completion of the incubation, the pH of the solution was adjusted to 6.5, and the sample was chromatographed on a column of Sephadex G-25 equilibrated in 1.0 mM KH_2PO_4 buffer (pH 6.5) to resolve the released tritiated 4′-phosphopantetheine from the residual holo-ACP and the resulting apo-ACP. Column fractions were monitored for radioactivity, and the fractions comprising the peak containing the low molecular weight labeled material were pooled, concentrated, and examined by

thin-layer chromatography as described by Jackowski and Rock (1981). The preparation was found to contain a radioactive compound that comigrated with authentic 4′-phosphopantetheine which, upon treatment with alkaline phosphatase, was converted to a compound that comigrated with authentic pantetheine.

Gel Chromatography. Sephadex G-100 or Sephadex G-75 (1.5 × 60 cm) was equilibrated in 20 mM KH_2PO_4, pH 7.0, and calibrated with standards of known molecular weights and Stoke's radii. Each standard was chromatographed separately, and 1- or 2-mL fractions were collected. The erfc^{-1} (K_{av}) was calculated by the method of Ackers (1967) as described by Mann and Fish (1972) to generate a linear calibration of Stoke's radius against four standards: bovine serum albumin, 36.5 Å; ovalbumin, 28.1 Å; soybean trypsin inhibitor, 22.6 Å; and lysozyme, 19.1 Å.

Polyacrylamide Gel Electrophoresis. Na_2DodSO_4–PAGE was performed as described by Laemmli and Favre (1973) on 20% polyacrylamide/0.5% bis(acrylamide) in a 0.75 mm thick resolving gel with a 2-cm stacking gel composed of 4% acrylamide/0.1% bis(acrylamide). The Bio-Rad low molecular weight standards were supplemented with bovine trypsin inhibitor (M_r 6510). Conformationally sensitive native PAGE was conducted on a 20% polyacrylamide/0.5% bis(acrylamide) resolving gel (pH 9.0) with a 4% polyacrylamide/0.1% bis(acrylamide) stacking gel (pH 6.8) (Rock et al., 1981a). Samples were run at 30 mA until the tracking dye reached the bottom of the gel.

Isoelectric Focusing. Isoelectric focusing of ACP was performed on a Parmacia flat-bed apparatus (FBE 300) according to the manufacturer's instructions. The gel was composed of 4.85% acrylamide/0.15% bis(acrylamide), and the carrier ampholytes were Pharmalyte pH 3–10. Reference p*I* standards were from Pharmacia and consisted of 11 proteins with a p*I* range of 3.5–9.3. Samples were applied by soaking into filter paper strips (5 × 10 mm) and laying the paper strip directly onto the gel surface. The gel was run at 30 W, and the p*I*'s of *R. sphaeroides* and *E. coli* ACP's were determined by reference to the p*I* values and migration distances of known standards.

Results

Purification of R. sphaeroides ACP. ACP was purified from phototrophically grown cells of *R. sphaeroides* by a modification of the procedure described by Majerus et al. (1969). As was suggested by Powell et al. (1973), DEAE-Sephadex A25 was substituted for DEAE-Sephadex A50 in the final step of the purification. ACP was assayed by monitoring the ability of protein fractions to stimulate the activity of a preparation of *E. coli* acyl-ACP synthetase (Rock & Cronan, 1981a). As is shown in Figure 1, ACP eluted as a symmetrical peak from a column of DEAE-Sephadex A25 developed with a linear gradient of LiCl (0.20–0.50 M) in potassium phosphate buffer, and column fractions containing ACP displayed a relatively constant specific activity when monitored with acyl-ACP synthetase (Figure 1). However, upon further analysis of the pooled ACP fractions by native gel electrophoresis and N-terminal amino acid analysis, it was found that the material obtained from the chromatography procedure described in Figure 1 was only 85% pure. In addition to the monomeric (ACPSH) and dimeric ($ACPS_2$) forms of ACP (inset of Figure 1), two slowly migrating bands were observed. However, following treatment of the sample with dithiothreitol and further purification by preparative elution electrophoresis (Experimental Procedures), a dimer-free preparation of ACP was obtained that was shown to be greater

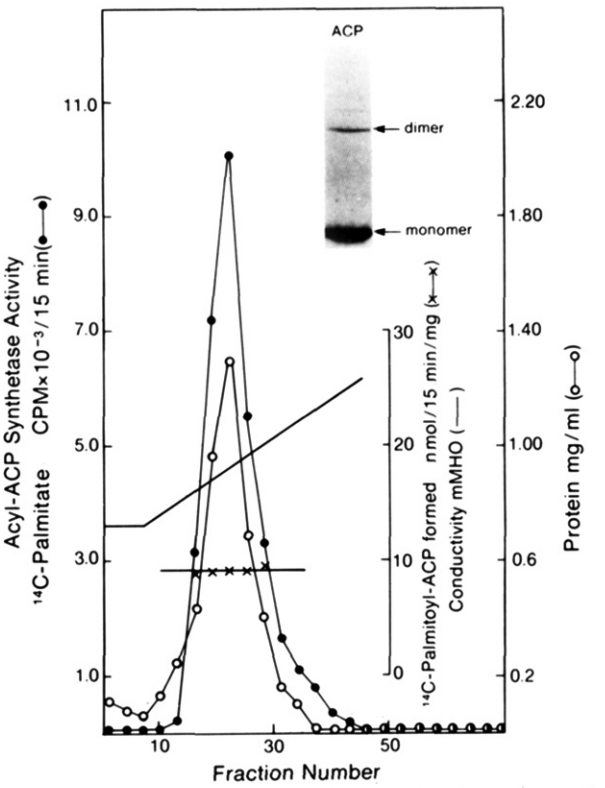


FIGURE 1: DEAE-Sephadex A25 chromatography of *R. sphaeroides* ACP. Pooled fractions obtained following DEAE-cellulose chromatography (Majerus et al., 1969) were applied to a column of DEAE-Sephadex A25 equilibrated in 20 mM KH_2PO_4 buffer (pH 6.2) containing 10 mM 2-mercaptoethanol and 0.3 M LiCl. The column was eluted with a linear (0.3–0.7 M) gradient of LiCl in phosphate buffer. Fractions containing ACP were identified by monitoring their ability to stimulate the activity of the *E. coli* acyl-ACP synthetase (●) (Experimental Procedures). ACP specific activity (×) is the value for nmol of [^{14}C]palmitoyl-ACP formed (15 min)$^{-1}$ (mg of fraction protein)$^{-1}$ (o).

than 95% homogeneous by native PAGE and N-terminal amino acid analysis. The yield of ACP was 50 mg/kg wet weight of phototrophically grown cells, which is comparable to the yield of ACP obtained from cells of *E. coli* (Majerus et al., 1969).

Properties of R. sphaeroides ACP. The results of an amino acid compositional analysis of *R. sphaeroides* ACP are presented in Table I. The protein consists of approximately 75 amino acid residues and has a calculated minimum molecular weight, including the 4′-phosphopantetheine prosthetic group, of 8700. This value is identical with the value (8700) determined by Na_2DodSO_4 gel electrophoresis (not shown) and is comparable to the values for molecular weight reported for the ACP's from other procaryotic organisms (Vanaman et al., 1968; Simoni et al., 1967; Ailhaud et al., 1967). The protein lacked the amino acids tryptophan and tyrosine (Table I) and, like *E. coli* ACP, possessed a predominance (31%) of acidic amino acid residues (p*I* 3.8). Assuming the the Asx and Glx residues are aspartic and glutamic acids, respectively, and that these residues are completely ionized at pH 7.0, a value for the maximum specific hydration of the protein of 0.489 g of H_2O/g of protein can be calculated from the data presented in Table I (Kuntz & Kauzman, 1974). The partial specific volume of the protein was calculated to be 0.733 mL/g by using the values specified by Cohn and Edsall (1943).

The data presented in Table II show the results of a comparison of amino acid compositions of eight ACP's, using the index of relatedness $S\Delta Q$ described by Marchalonis and Weltman (1971) and interpreted as described by Cornish-

Table I: Acyl Carrier Protein Amino Acid Compositions

amino acid	*R. sphaeroides*	*E. coli*[a]	*M. smegmatis*[b]	*Spinacia oleracea*[c]
Cys	(1)	0	0	0
Asx	10	9	11	12
Thr	4	6	5	6
Ser	3	3	5	4
Glx	14	18	20	16
Pro	1	1	4	2
Gly	4	4	6	4
Ala	9	7	13	9
Val	8	7	8	7
Met	1	1	1	1
Ile	7	7	6	5
Leu	4	5	8	7
Tyr	0	1	2	0
Phe	5	2	2	2
His	1	1	0	1
Lys	3	4	5	9
Arg	1	1	3	0
Trp	0	0	0	0
β-Ala	1	1	1	1
taurine	1	1	1	1
calcd M_r	8699	8847	10640	9087

[a] Vanaman et al., 1968. [b] Matsumura et al., 1970. [c] Simoni et al., 1967; Ailhaud et al., 1967.

Table II: Relatedness of *R. sphaeroides* ACP to Other ACP's

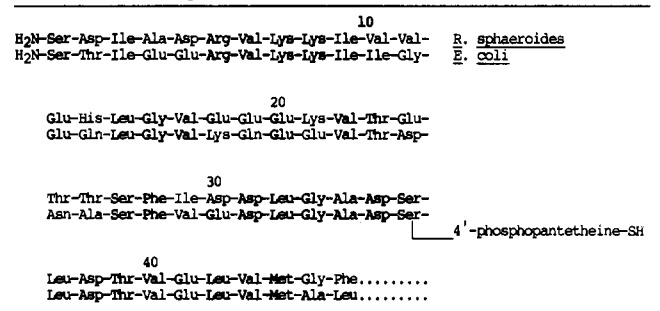
	N[a]	$S\Delta Q$[b]	test[c]	reference[d]
procaryotic ACP				
E. coli	77	62	strong	Vanaman et al., 1968
M. smegmatis	99	74	strong	Matsumura et al., 1970
Arthrobacter viscosus	78	112	weak/strong	Simoni et al., 1967
Clostridium butyricum	75	113	strong/weak	Ailhaud et al., 1967
eucaryotic ACP				
Spinacia oleracea	85	97	strong/weak	Simoni et al., 1967
Hordeum vulgare	87	112	weak	Hoj & Svendsen, 1983
Persea americana	114	115	weak	Simoni et al., 1967
E. gracilis	90	202	weak/none	Dinello & Ernst-Fonberg, 1973

[a] N is the number of amino acid residues of the apoprotein. [b] $S\Delta Q$ is the relatedness index of Marchalonis and Weltman (1971) and was calculated from published amino acid compositions. [c] Interpretation of the relatedness test was conducted as described by Cornish-Bowden (1983). [d] References refer to those for the amino acid composition.

Bowden (1983). *R. sphaeroides* ACP was found to be strongly related to the ACP's from *E. coli* and *Mycobacterium smegmatis* as well as to the ACP from spinach chloroplasts. However, *R. sphaeroides* ACP displayed little or no relatedness to the ACP from *Euglena gracilis* (see Discussion).

Prosthetic Group Identification. *R. sphaeroides* ACP was found to possess equimolar quantities of β-alanine and taurine, indicating the presence of the characteristic 4′-phosphopantetheine prosthetic group (Table I), and studies were conducted to directly identify this prosthetic group. [^{3}H]ACP was prepared by growing cells of *R. sphaeroides* in medium supplemented with [^{3}H]-β-alanine and purifying the ACP as described above. Upon incubation of this [^{3}H]ACP at pH 12 to promote the base-catalyzed release of the prosthetic group, 70% of the protein-associated radioactivity was converted to an acid-soluble form. Following treatment of this acid-soluble fraction with alkaline phosphatase, the radioactive compounds were identified as 4′-phosphopantetheine and pantetheine by thin-layer chromatography (Experimental Procedures). Interestingly, repeated attempts, under both denaturing (0.1% Na_2DodSO_4, 5.4 M guanidine hydrochloride) and nondenaturing conditions, to demonstrate the free sulfhydryl contributed by the 4′-phosphopantetheine group on the holo-ACP using sulfhydryl reactive reagents (Ellman, 1959; Grassetti & Murray, 1967) were not successful. The molecular basis for this result is unknown.

Table III: Comparison of the Primary Structures of Acyl Carrier Proteins[a] from *R. sphaeroides* and *E. coli*[b]



[a] Regions of homology are shown in boldface type. [b] Vagelos, 1971.

Table IV: Physical and Biochemical Properties of *R. sphaeroides* and *E. coli* Acyl Carrier Proteins

property	*R. sphaeroides*	*E. coli*
molecular weight		
primary structure	8699	8847[a]
gel chromatography	19000	20000[b]
SDS–PAGE	8700	20400[b]
sedimentation	7500	9000[b]
Stokes radius (Å), gel chromatography	19.8	19.6[b]
amino acid composition		
total residues	75	77[a]
acidic (%)	31	28
basic (%)	8	8
nonpolar (%)	46	39
extinction coeff, E_{280} (mM^{-1})	4.1	1.8[c]
isoelectric point, focusing	3.8	4.1[b]
hydration (mL of H_2O/g of apo-ACP), pH 6.0	0.489	0.42[b]
partial specific vol, $\bar{v}$ (mL/g)	0.733	0.731[b]

[a] Vanaman et al., 1968. [b] Rock & Cronan, 1979. [c] Rock & Cronan, 1980.

The primary structure of *R. sphaeroides* ACP has been determined for the first 46 amino acid residues from the N terminus of the molecule, and comparison of this partial sequence with the known primary structure of *E. coli* ACP revealed a 65% sequence homology. As is shown in Table III, the ACP's from both organisms possess a 14 amino acid sequence (residues 31–44) that displays 100% homology, and of the 16 unmatched amino acids present in the remaining 32 residues, 8 represent substitutions that are chemically equivalent. In *E. coli* ACP, the attachment site for the 4′-phosphopantetheine prosthetic group is Ser-36 (Vanaman et al., 1968). Although the position for attachment of the 4′-phosphopantetheine group of *R. sphaeroides* ACP was not confirmed, it is presumed to be attached in the region of the molecule that exhibits sequence homology with *E. coli* ACP.

Implications of ACP Acylation. The ACP's from *R. sphaeroides* and *E. coli* are low molecular weight, highly charged acidic proteins whose primary cellular function is to serve as a carrier of hydrophobic moieties. Both proteins exhibit anomalously high values for Stoke's radii when examined by gel chromatography (Table IV), although sedimentation analysis indicated molecular weight values of 7500 and 9000 (Table IV) for *R. sphaeroides* ACP and *E. coli* ACP, respectively. These data have been interpreted by Rock and Cronan (1979) as indicating that *E. coli* ACP possesses

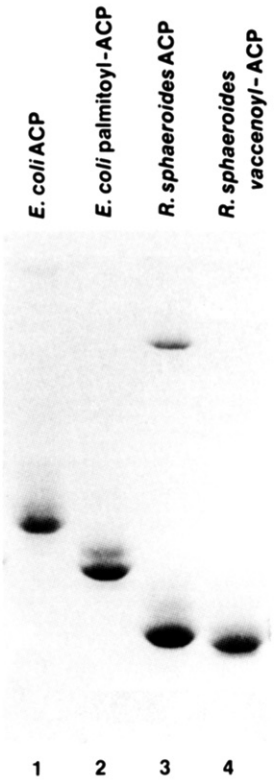


FIGURE 2: Native gel electrophoresis of *R. sphaeroides* and *E. coli* ACP and acyl-ACP. ACP was purified from cells of *R. sphaeroides* and *E. coli* as described under Experimental Procedures. Acyl-ACP derivatives were prepared with *E. coli* acyl-ACP synthetase (Experimental Procedures). Conformationally sensitive (native) gel electrophoresis was conducted as described by Rock et al. (1981a). Lanes: (1) *E. coli* ACP (monomer and dimer), (2) *E. coli* palmitoyl-ACP, (3) *R. sphaeroides* ACP (monomer and dimer), and (4) *R. sphaeroides cis*-vaccenoyl-ACP. Each lane contained 4–9 μg of protein.

an asymmetric shape and that structural asymmetry may be a general property of acidic proteins. Further, the acidic nature of *E. coli* ACP has been invoked by Rock and Cronan (1979) to explain its anomalous migration during gel electrophoresis under denaturing conditions (Table IV). However, *R. sphaeroides* ACP does not display this anomaly and, instead, was found to migrate predictably when analyzed by Na_2DodSO_4 gel electrophoresis (Table IV). In addition, *R. sphaeroides* ACP does not display the pH-induced denaturation and hydrodynamic expansion that is known to occur with *E. coli* ACP (Rock & Cronan, 1979). As is shown in Figure 2, *R. sphaeroides* ACP and *cis*-vaccenoyl-ACP migrated at identical rates when subjected to conformationally sensitive (native) gel electrophoresis, and this rate was significantly faster than the rates observed for either *E. coli* ACP or acyl-ACP. Whereas *E. coli* ACP acquired a more compact, conformationally stable form following its acylation (Figure 2), the acylation of *R. sphaeroides* ACP had no measurable effect upon the protein's conformation. However, this was only true for the *cis*-vaccenoyl thiol ester derivative of *R. sphaeroides* ACP. Analysis of *R. sphaeroides* palmitoyl-ACP by gel filtration (Figure 3) and native gel electrophoresis (Figure 4) indicated that the acylation of *R. sphaeroides* ACP with palmitic acid results in a significant destabilization of the protein's conformation. This is in contrast to *R. sphaeroides* ACP and *cis*-vaccenoyl-ACP, which display an R_s value of 19.8 Å when chromatographed on a column of Sephadex G-100 (pH 7.0). The R_s value for *R. sphaeroides* palmitoyl-ACP was found to be 15.2 Å (Figure 3). *E. coli* ACP and palmitoyl-ACP comigrated during gel chromatography at pH 7.0

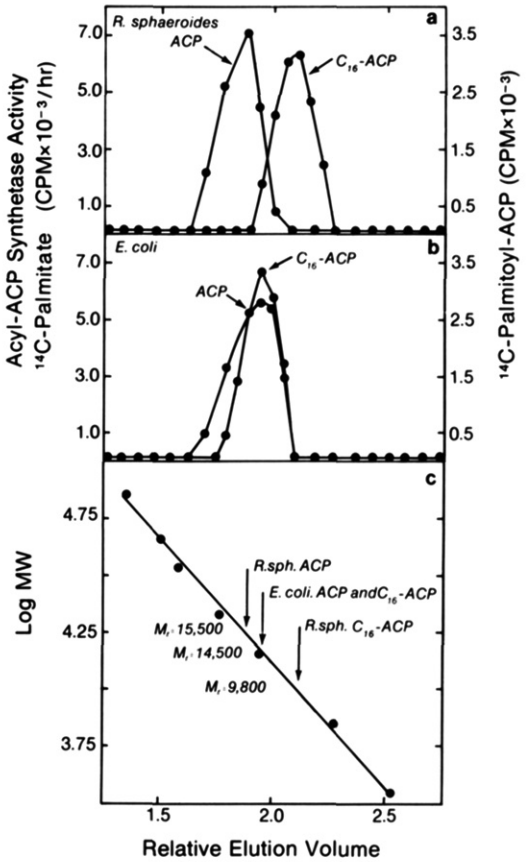


FIGURE 3: Gel chromatography of *R. sphaeroides* and *E. coli* ACP and acyl-ACP. ACP was purified from cells of *R. sphaeroides* and *E. coli* as described under Experimental Procedures. [^{14}C]Palmitoyl thiol ester derivatives of ACP ([^{14}C]palmitoyl-ACP; 52.5 μCi/μmol) were prepared enzymatically with *E. coli* acyl-ACP synthetase (Experimental Procedures). Unesterified ACP was monitored by determining the ability of fraction aliquots to stimulate acyl-ACP synthetase activity. Chromatography was conducted on a 1.5 × 60 cm column of Sephadex G-100 equilibrated with 20 mM KH_2PO_4 buffer (pH 7.0). The column flow rate was 13 mL/h, and 1–2 mL fractions were collected. (a) *R. sphaeroides* ACP (0.83 mg) and [^{14}C]palmitoyl-ACP (3.4 μg). (b) *E. coli* ACP (2.0 mg) and [^{14}C]palmitoyl-ACP (3.6 μg). (c) Column calibration: bovine serum albumin (67 000), ovalbumin (45 000), pepsin (34 700), soybean trypsin inhibitor (21 500), lysozyme (14 400), bovine trypsin inhibitor (6510), and glucagon (3500).

and displayed an R_s value of 18.5 Å, which compares favorably with the R_s value of 19.6 Å previously determined for these molecules under comparable conditions (Rock & Cronan, 1979). Thus, the resolution of *R. sphaeroides* ACP and palmitoyl-ACP by gel chromatography does not require that the elution be conducted under alkaline conditions. However, the resolution of *R. sphaeroides* ACP and palmitoyl-ACP also occurred during native gel electrophoreses at pH 9.0 (Figure 4), which are the conditions where *R. sphaeroides* ACP and *cis*-vaccenoyl-ACP were shown to comigrate.

Discussion

This paper describes the purification of ACP from phototrophically grown cells of *R. sphaeroides*. ACP was obtained in yields of 50 mg/kg wet weight of cells and was >95% homogeneous as determined by native gel electrophoresis and N-terminal amino acid analysis. Amino acid compositional analysis revealed that the protein consists of approximately 75 amino acid residues, lacks the amino acids tryosine and tryptophan, possesses a predominance of acidic amino acid residues (p*I* 3.8), and has a calculated minimum molecular weight of 8700. *R. sphaeroides* ACP was shown to possess

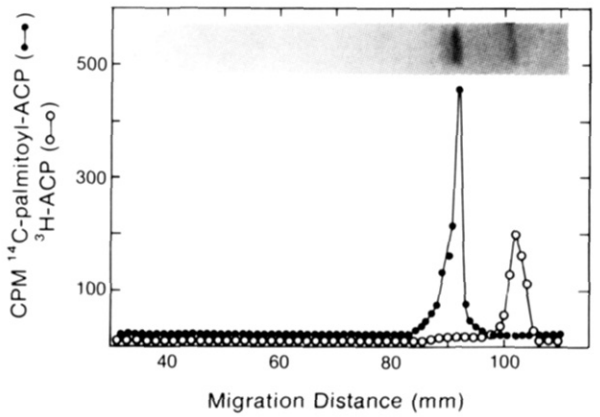


FIGURE 4: Native gel electrophoresis of *R. sphaeroides* ACP and palmitoyl-ACP. *R. sphaeroides* [^{3}H]ACP was purified from cells grown in medium supplemented with [^{3}H]-β-alanine, and [^{14}C]palmitoyl-ACP was prepared enzymatically as described under Experimental Procedures. Conformationally sensitive native gel electrophoresis was conducted with a mixture of *R. sphaeroides* ACP and palmitoyl-ACP. The lane containing the radioactive sample was frozen and cut into 1-mm sections, and the sections were dissolved and processed for scintillation counting as described by Fraley et al. (1978). Sample visualization was conducted separately with a mixture of *R. sphaeroides* ACP and palmitoyl-ACP.

the characteristic 4′-phosphopantetheine prosthetic group and displayed a high degree of sequence homology with *E. coli* ACP. This homology was especially evident in the region of the protein (residues 31–44) that, in *E. coli* ACP, is known to possess the prosthetic group attachment site. Further, structural similarity to *E. coli* ACP is indicated by the ability of *E. coli* ACP to serve as acyl donor for the in vitro assay of the *R. sphaeroides sn*-glycerol-3-phosphate acyltransferase (Cooper & Lueking, 1984; Lueking & Goldfine, 1975), by the ability of both proteins to comparably stimulate the *E. coli* acyl-ACP synthetase, and by the cross-reactivity of the proteins in immunological assays with the respective anti-ACP antibodies (unpublished observations). Comparison of the index of relatedness $S\Delta Q$ of the two proteins revealed phylogenetic relatedness between *R. sphaeroides* ACP and the ACP's from *M. smegmatis* and spinach; however, only a weak relationship of *R. sphaeroides* ACP to the ACP's from algal and other plant sources was indicated (Table II).

Importantly, *R. sphaeroides* ACP displayed some properties that were distinctly different from those of *E. coli* ACP. In contrast to *E. coli* ACP, *R. sphaeroides* ACP migrates to its predicted location during Na_2DodSO_4 gel electrophoresis (Table IV). *E. coli* ACP migrates anomalously during gel electrophoresis in the presence of anionic detergents, and Rock and Cronan (1979) have attributed this behavior to the highly acidic nature of the protein. However, in view of the results obtained with *R. sphaeroides* ACP, it seems unlikely that the apparent abnormal binding of Na_2DodSO_4 by *E. coli* ACP can be attributed solely to its acidic properties. Furthermore, *R. sphaeroides* ACP migrates considerably faster than either *E. coli* ACP or acyl-ACP during conformationally sensitive (native) gel electrophoresis (Figure 2). Since the ACP's from these two organisms are of similar size and charge, this result suggests that *R. sphaeroides* ACP exists as a highly compact molecule. In fact, the observed comigration of *R. sphaeroides* ACP and vaccenoyl-ACP during native gel electrophoresis indicates that *R. sphaeroides* ACP normally exists at a near-limiting state of compactness. With *E. coli* ACP, acylation of the protein with a long-chain saturated, or unsaturated, fatty acid results in a stabilization of the protein's structure. Acyl-ACP's are resistant to pH-induced denaturation and are easily separable from native ACP during gel electrophoresis (Figure 2).

Although the precise molecular basis for the differences in physical properties exhibited by the ACP's from *R. sphaeroides* and *E. coli* is unknown, it seems reasonable to assume that many of these differences may be directly related to the differences in the hydrophobic properties of the proteins. The percentage of nonpolar amino acid residues possessed by *R. sphaeroides* ACP is significantly higher than that present in *E. coli* ACP (46% vs. 39%), with the majority of this increase being attributable to an increased number of phenylalanine residues (Table I). Further, in contrast to *E. coli* ACP, which binds only weakly if at all to octyl-Sepharose, *R. sphaeroides* ACP binds tenaciously to this resin and is only eluted under solvent conditions that result in the simultaneous elution of its long-chain acyl thiol ester derivatives. Thus, assuming that considerable intramolecular hydrophobic interactions occur in *R. sphaeroides* ACP, this would explain its apparent compact conformation, its resistance to pH-induced denaturation, and the absence of measurable changes in its conformation upon acylation with *cis*-vaccenic acid. In this instance, the apparent destabilization of *R. sphaeroides* ACP conformation promoted by its acylation with palmitic acid could be attributable to a disruption of normal intramolecular hydrophobic interactions in the protein in favor of protein–lipid interactions with the saturated acyl group. Presumably, *cis*-vaccenic acid is unable to mediate a comparable level of disruption of these intramolecular interactions. In any event, the existence of different acyl-ACP conformers could be of extreme physiological significance in determining the types and amounts of fatty acids utilized for cellular phospholipid synthesis. In this regard, it is noteworthy that, in *R. sphaeroides*, *cis*-vaccenic acid comprises greater than 90% of the total cellular fatty acids.

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