ISOLATION AND CHARACTERIZATION OF A PROTEIN CONTAINING 4'-PHOSPHOPANTETHEINE FROM E. COLI K-12[1]

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Recent studies have demonstrated that acyl carrier protein plays an important role in a number of biosynthetic pathways (Goldman et al., 1961; Lennarz et al., 1962; Goldman et al., 1962; Alberts et al., 1963; Goldman et al., 1963a; Goldman et al., 1963b; Majerus et al., 1964; Wakil et al., 1964; Sauer et al., 1964; Overath and Stumpf, 1964; Majerus et al., 1965; Brooks and Stumpf, 1966; Nagai and Bloch, 1966; Brock et al., 1966; Goldfine, 1966; Ailhaud and Vagelos, 1966; Simoni et al., 1967). Acyl carrier protein is a conjugated protein having 4'-phosphopantetheine as the prosthetic group (Majerus et al., 1965; Pugh and Wakil, 1965). Very recently, we suggested that probably two species of protein containing 4'-phosphopantetheine may occur in spinach leaf tissue (Matsumura and Stumpf, 1967). This observation raises several important problems such as a possible pro-acyl carrier protein or another 4'-phosphopantetheine binding protein which has a different physiological role from acyl carrier protein. This communication deals with the isolation and properties of a protein different from E. coli ACP[2] but having 4'-phosphopantetheine.

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[2]The abbreviation used is: ACP, acyl carrier protein.

EXPERIMENTAL PROCEDURE. The heat stable protein fraction from 1 kg of E. coli K-12, Hfr, Thia$^-$ cells was prepared according to the procedure of Lennarz et al. (1962). Protein concentration was determined spectrophotometrically by measuring the difference in optical densities at 215 mμ and 225 mμ according to the procedure of Murphy and Kies (1960). ACP activity was determined by enzymatic assay in the malonyl-CoA CO_2 exchange reaction as described by Alberts et al. (1963). The heat stable protein was fractionated essentially according to the procedure of Simoni et al. (1967). A flow rate of approximately 30 ml per hour was achieved by gravity. Fractions of 15 ml were collected.

Amino Acid Analyses. Approximately 8 mg of the protein was oxidized with performic acid according to the method of Hirs (1956). The oxidized protein was hydrolyzed anaerobically in 6 N HCl for 24 hours, and the hydrolysate was analyzed with the Phoenix Amino Acid Analyzer Model-K 8000 VG.

Phosphate Determination. Analysis for organic phosphate in protein and peptide was carried out according to Ames and Dubin (1960).

Determination of Sulfhydryl Group. The sulfhydryl content of the protein was assayed by the procedure of Ellman (1959) and ^{14}C-iodoacetamide derivatives of the purified proteins were made as described previously (Simoni et al., 1967).

Pantetheine Determination. Pantetheine was determined by gas chromatographic analysis of the pantolactone which is liberated from protein c by digestion with Pronase according to Majerus et al. (1965).

Peptic Hydrolysis of S-^{14}C-Carbamoylmethyl-Protein. S-^{14}C-Carbamoylmethyl-protein was digested with pepsin as described previously (Matsumura and Stumpf, 1967). The enzymatic hydrolysates were fractionated by high-voltage electrophoresis at pH 6.5 in pyridine-acetate. Electrophoresis was carried out at 3000 volts for 1 hour. Radioactive peptides were eluted with 5% acetic acid from paper.

RESULTS. When the heat stable protein fraction was subjected to DEAE cellulose column, a pattern of protein peaks and acyl carrier protein activity appeared as shown in Fig. 1.

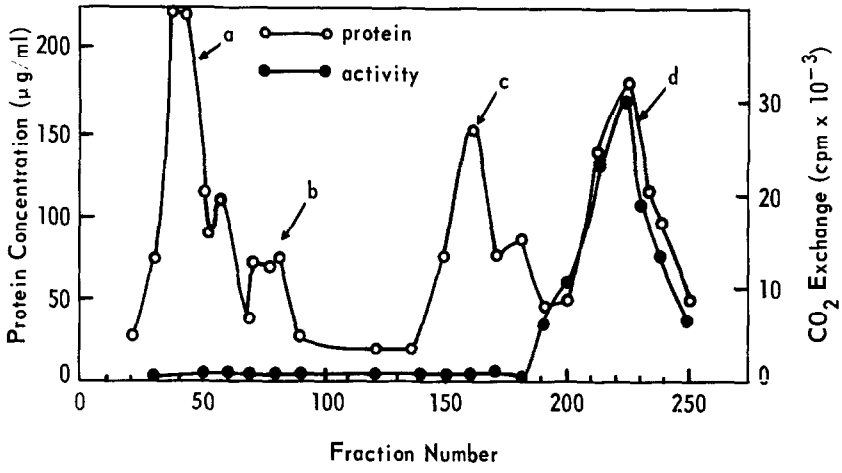

Fig. 1. Chromatography of E. coli heat-stable protein on DEAE cellulose column (4x50 cm).

The four peaks of protein were arbitrarily designed a, b, c and d. Peak d showed ACP activity in the malonyl-CoA CO_2 exchange reaction, and its elution position corresponds to that of ACP as described previously by Vagelos et al. (1964) and Wakil et al. (1964). However, protein in peaks a, b and c showed no malonyl-CoA CO_2 exchange reaction. This procedure yielded approximately 20 mg of protein c and 70 mg of 51-fold purified ACP. The elution pattern of protein c from the second DEAE cellulose column gave a single symmetrical protein peak. The protein c was homogeneous in the analytical ultracentrifuge and on disc gel electrophoresis.

Amino Acid Composition. As shown in Table 1, the amino acid compositions of ACP and protein c are quite similar. They differ slightly in that protein c has one more lysine, one more proline and one more aspartic acid but two less glutamic acid than ACP. Furthermore, there is little difference in neutral amino acids between ACP and protein c. It is interesting to note that protein c has one cysteamine and one β-alanine

TABLE I

Amino acid composition of protein c and ACP

Amino acid analyses were performed as described under "EXPERIMENTAL PROCEDURE". The values given above represent the estimated number of residues per molecule of protein. No corrections were made for hydrolytic losses of serine and threonine.

Amino Acid	ACP[a]	Protein c[b] residue/mole	Nearest Integer
Lysine	4	5.2	5
Histidine	1	1.4	1
Arginine	1	0.9	1
Aspartic acid	10	11.0	11
Threonine	6	5.9	6
Serine	3	2.8	3
Glutamic acid	22	20.3	20
Proline	1	1.6	2
Glycine	4	5.2	5
Alanine	8	9.1	9
Valine	8	7.0	7
Methionine	1	0.8	1
Isoleucine	8	6.6	7
Leucine	6	7.0	7
Tyrosine	1	0.7	1
Phenylalanine	2	2.6	3
β-Alanine	1	1.0	1
Taurine	1	0.9	1
Cysteic acid	0	0.2	0
Sulfhydryl group	1	0.8	1
Organic phosphate	1	1.3	1

[a] The amino acid values given for ACP were obtained from data of Vagelos et al. (1966).

[b] The number of residues of each amino acid in protein c was calculated on the assumption that one β-alanine residue is present per molecule of protein.

residue per mole of protein. The determination of sulfhydryl groups indicates that it contains one SH group per mole of protein. Protein c contains one mole of organic phosphate.

Peptic Digestion of ACP and Protein c. As indicated in Fig. 2, radioactive peaks were obtained by paper electrophoresis of the pepsin hydrolysates of S-^{14}C-Carbamoylmethyl-ACP and protein c at pH 6.5. These radioactive peptides were eluted and further purified by paper chromatography. The chromatographic behavior of radioactive peptic peptides from protein c was quite similar to that of the peptic peptides from ACP. In addition,

the amino acid composition of peptides containing the prosthetic group, appears to be identical in ACP and protein c (Matsumura and Stumpf, 1967).

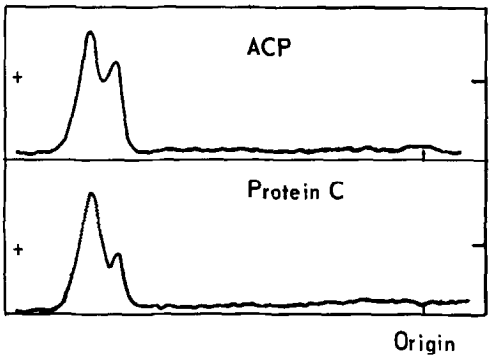

Fig. 2. Radioactivity tracings of paper electrophoresis of peptic peptides formed from ^{14}C-iodoacetamide treated ACP and protein c at pH 6.5.

DISCUSSION. The function of ACP as the carrier of the growing fatty acid chain during fatty acid synthesis and of long chain fatty acids for transfer to complex phospholipids is well established in various bacteria and plant systems. Despite the similarity of amino acid compositions of both ACP and protein c, and despite the presence in both ACP and protein c of 4'-phosphopantetheine, and the occurrence in both proteins of a very similar amino acid core around the prosthetic group, nevertheless, protein c is unable to replace ACP in the malonyl-CoA CO_2 exchange reaction. In fact, as shown in Fig. 3, a higher concentration of protein c inhibited the malonyl-CoA CO_2 exchange reaction.

The implication of these observations are of considerable interest. Recent work of Majerus (1967) indicated that the enzymes involved in fatty acid biosynthesis must interact with the ACP molecule over a large portion of the protein rather than only with the prosthetic group. Very recently, Goldfine (1967) has observed that ACP regenerated from palmityl-ACP inhibits the malonyl-CoA CO_2 exchange reaction of ACP and the transfer of acyl group from acyl-ACP to phosphoglyceride. It is quite conceivable that protein c may function as an acyl carrier for a series of reaction other than that involved in fatty acid synthesis.

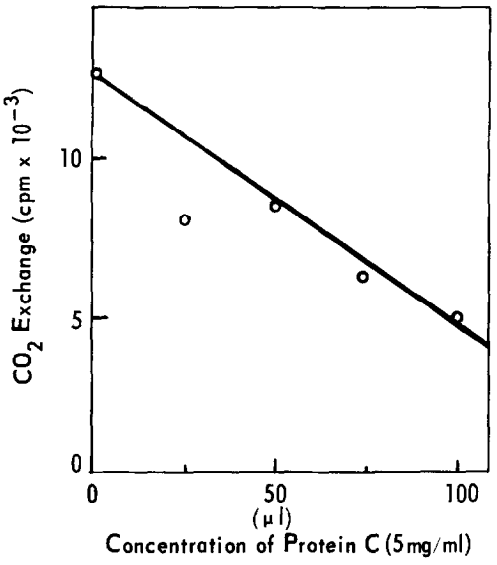

Fig. 3. The inhibition of malonyl-CoA CO_2 exchange reaction of ACP by protein c. The reaction mixtures contained potassium phosphate buffer, pH 6.0, 50 µmoles; dithiothreitol, 20 µmoles; malonyl-CoA, 0.70 µmoles; caproyl-CoA, 0.15 µmoles; $KH^{14}CO_3$, 10 µmoles ($3x10^6$cpm); E. coli synthetase, 2 mg and E. coli ACP 10 µg.

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