# CONFORMATION OF ACYL CARRIER PROTEIN FROM MYCOBACTERIUM PHLEI[1]

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## SUMMARY

The molecular weight of $ACP_{M.\ phlei}$[2] as determined by physical measurements is 10,450. Optical rotatory dispersion measurements indicate that this protein has a high degree of α-helical structure. The function of $ACP_{M.\ phlei}$ was examined in the E. coli fatty acid synthetase system.

We have reported the isolation and some properties of an acyl carrier protein from M. phlei which is a required component for one of the two fatty acid synthetases in this organism (1, 2). In the present paper, some physical properties and catalytic activities of $ACP_{M.\ phlei}$ are described and compared with those of $ACP_{E.\ coli}$.

## METHODS

$ACP_{M.\ phlei}$ was isolated from extracts of Mycobacterium phlei ATCC-356 as described (2). Guanidine HCl was prepared from guanidine carbonate (Eastman Kodak Co.) and was recrystallized from ethanol.

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[2] The abbreviations used are: ACP, acyl carrier protein; $ACP_{E.\ coli}$ and $ACP_{M.\ phlei}$ denote their origin from Escherichia coli and Mycobacterium phlei, respectively.

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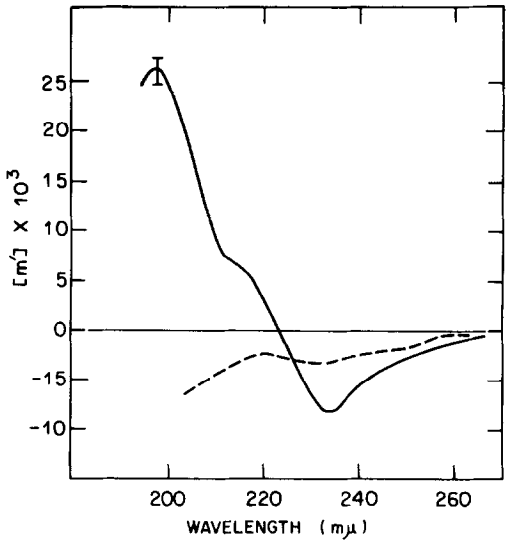


Figure 1. Optical rotatory dispersion pattern of $ACP_{\underline{M.\ phlei}}$ in 0.04 M NaCl (pH 5.8) (——) and in 4.2 M guanidine HCl (pH 5.6) (----) at 27° and at a protein concentration of 0.94 mg/ml; 1.00 mm cell.

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Optical rotatory dispersion was measured with a Cary Model 60 spectropolarimeter. Sedimentation velocity measurements were carried out in a Spinco Model E analytical ultracentrifuge after reduction of the protein with dithiothreitol (Calbiochem). A double sector synthetic boundary cell of the capillary type was used. ACP activity was assayed enzymatically both in the malonyl-CoA $CO_2$ exchange reaction (3) and in total fatty acid synthesis (4).

## RESULTS AND DISCUSSION

The partial specific volume of $ACP_{\underline{M.\ phlei}}$ was calculated from the amino acid composition (2) to be 0.731 ml per gram. The sedimentation coefficient of $ACP_{\underline{M.\ phlei}}$ as determined by extrapolation to zero protein concentration was $S^{o}_{20,W} = 1.49$; this value and the diffusion constant ($13.10 \times 10^{-7}$ $cm^2$/sec) yielded a calculated molecular weight of 10,450

in good agreement with the value of 10,600 obtained from the amino acid composition (2). The values for the frictional coefficient and the minimum frictional coefficient were $3.11 \times 10^{-8}$ and $2.78 \times 10^{-8}$, respectively. The value of $f/f_{min} = 1.12$ indicates that $ACP_{\underline{M. phlei}}$ in solution is present as a typical globular protein (5). As shown in Fig. 1, the optical rotatory dispersion spectrum of $ACP_{\underline{M. phlei}}$ displays typical α-helical features; namely, a negative trough with a minimum at 233 mμ, a cross-over point at 224 mμ, a shoulder between 210 and 215 mμ, and a positive Cotton effect with a maximum at 198 mμ. These features disappear in 4.2 M guanidine HCl. The mean residue rotation [m'] at 233 mμ was found to be -7800. A large, negative Cotton effect with a trough at 233 mμ has been shown to be characteristic of α-helical conformation in polypeptides.

The $b_o$ value (- 289°) calculated from the Moffitt equation (6) corresponds to a helix content of 46%.

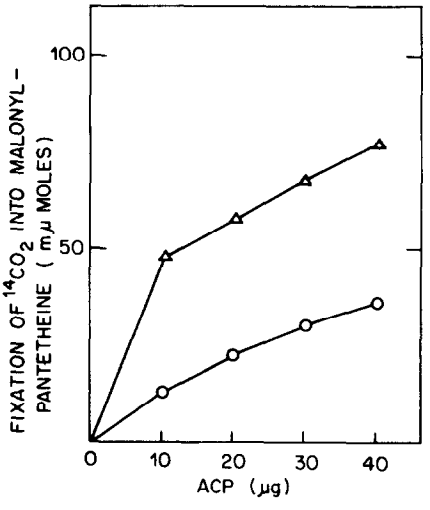


Figure 2. Effect of ACP on the $CO_2$-malonyl-CoA exchange reaction in E. coli extracts. (-o-o-) $ACP_{\underline{M. phlei}}$ and (-Δ-Δ-) $ACP_{\underline{E. coli}}$. Assays were performed according to Alberts et al. (3).

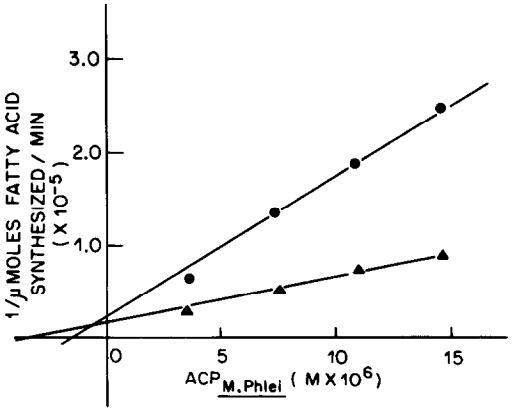


Figure 3. Dixon plot of $ACP_{M.\ phlei}$ inhibition of total fatty acid synthesis by E. coli enzymes; (-●-●-) 1.7 x $10^{-6}$ M $ACP_{E.\ coli}$ and (-▲-▲-) 3.4 x $10^{-6}$ M $ACP_{E.\ coli}$. The reaction mixture contained potassium phosphate, pH 7.0, 50 μmoles; acetyl-CoA, 20 mμmoles; TPNH, 0.07 μmoles; DTT, 10 μmoles; glucose-6-phosphate, 0.3 μmoles; glucose-6-phosphate dehydrogenase, 0.1 unit; malonyl-2-$C^{14}$-CoA, 40 mμmoles (SA=2.5); and enzyme (1 mg) in a total volume of 0.5 ml. Incubations were carried out at 37° for 30 min

Biological Activity

$ACP_{M.\ phlei}$ supports the malonyl-CoA - $CO_2$ exchange reaction in E. coli extracts with an activity about half that of $ACP_{E.\ coli}$ (Fig. 2). It is surprising, on the other hand, that $ACP_{M.\ phlei}$ is altogether unable to replace $ACP_{E.\ coli}$ in the fatty acid synthetase system of E. coli and, in fact, inhibits this process.

This inhibition was examined by varying the $ACP_{M.\ phlei}$ concentration at a fixed $ACP_{E.\ coli}$ concentration. The data plotted according to the method of Dixon (7) (Fig. 3) suggest that $ACP_{M.\ phlei}$ or possibly acyl $ACP_{M.\ phlei}$ inhibits ($K_I$ = 0.5 x $10^{-6}$ M) the utilization of $ACP_{E.\ coli}$ in E. coli fatty acid synthesis, presumably in a step or steps following transacylation and condensation. It is noteworthy, on the other hand, that in the transformation of stearoyl-CoA to longer-chain acids by M. phlei extracts both $ACP_{E.\ coli}$ and $ACP_{M.\ phlei}$ are active as acyl carriers (1). Thus,

the species specificity of $ACP_{\underline{M.\ phlei}}$ is much less broad than that of $ACP_{\underline{E.\ coli}}$. The explanation may well be the presence of four proline residues (2) – compared to one in $ACP_{\underline{E.\ coli}}$ – and the large content of α-helix in $ACP_{\underline{M.\ phlei}}$. Recent studies of Majerus suggest that two regions in $ACP_{\underline{E.\ coli}}$ determine its activity, one that carries the acyl moiety and the other located in some portion of the polypeptide chain which interacts with the fatty acid synthetase enzymes of E. coli (8). According to this view, the differences in biological activity between bacterial and plant ACP's are likely to be related to differences in the amino acid sequences at some distance from the prosthetic group (9-12). For these reasons, the elucidation of the primary structure of $ACP_{\underline{M.\ phlei}}$ may provide insight into the structure-function relationships of ACP molecules.

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## REFERENCES

1. Brindley, D. N., Matsumura, S., and Bloch, K., Nature (in press).

2. Matsumura, S., Brindley, D. N., and Bloch, K., Biochem. Biophys. Res. Commun. (in press).

3. Alberts, A. W., Goldman, R., and Vagelos, P. R., J. Biol. Chem., 238, 557 (1963).

4. Kass, L., and Bloch, K., Proc. Nat. Acad. Sci., U. S., 58, 1168 (1967).

5. Takagi, T., and Tanford, C., J. Biol. Chem., 243, 6432 (1968).

6. Moffitt, W., and Yang, J. T., Proc. Nat. Acad. Sci., U. S., 42, 596 (1956).

7. Dixon, M., Biochem. J., 55, 170 (1953).

8. Majerus, P. W., J. Biol. Chem., 242, 2325 (1967).

9. Simoni, R. D., Criddle, R. S., and Stumpf, P. K., J. Biol. Chem., 242, 573 (1967).

10. Vanaman, T. C., Wakil, S. J., and Hill, R. L., J. Biol. Chem., 243, 6420 (1968).

11. Matsumura, S., and Stumpf, P. K., Arch. Biochem. Biophys., 125, 932 (1968).

12. Matsumura, S., Biochem. Biophys. Res. Commun., 29, 437 (1967).