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PROGRESS REPORT

Grant No. DE-FG02-87ER13729

ROLE OF ACYL CARRIER PROTEIN ISOFORMS IN PLANT LIPID METABOLISM

Examination of acyl carrier protein (ACP) isoforms in evolutionarily diverse species, in different tissues and under various environmental conditions.

Previous research from my lab has revealed that several higher plant species have multiple isoforms of acyl carrier protein (ACP) and therefore this trait appears highly conserved among higher plants. This level of conservation suggests that the existence of ACP isoforms is not merely the result of neutral gene duplications. To more carefully examine the occurrence and evolutionary development of ACP isoforms, we have developed techniques to examine a wider range of species. Acyl carrier proteins can be labelled very specifically and to high specific activity using H^3-palmitate and the E. coli enzyme acyl-ACP synthetase. Isoforms were then resolved by Western blotting and native PAGE of H^3-palmitate labelled ACP's. Multiple isoforms of ACP were observed the leaf tissue of the monocots Avena sativa and Hordeum vulgare and dicots including Arabidopsis thalliana, Cuphea wrightii, and Brassica napus . Lower vascular plants including the cycad, Dioon edule, Ginkgo biloba, the gymnosperm Pinus, the fern Anernia phyllitidis and Psilotum nudum, the most primitive known extant vascular plant, were also found to have multiple ACP isoforms (Fig. 1 and Fig 2) as were the nonvascular liverwort, Marchantia and moss, Polytrichum (Fig. 2). Therefore, the development of ACP isoforms occurred early in evolution. However, the unicellular algae Chlamydomonas and Dunaliella and the photosynthetic cyanobacteria Synechocystis and Agmnellum have only a single electrophoretic form of ACP (Fig. 3). Thus, multiple forms of ACP do not occur in all photosynthetic organisms but may be associated with multicellular plants.

We have also examined light and tissue control over the expression of ACP isoforms. The expression of multiple forms of ACP in leaf of Spinacia and Avena is altered very little by light. Rather, the different patterns of ACP isoforms are primarily dependant on tissue source.

A manuscript describing the above work is in preparation.

Determination of three dimensional structure of plant ACP.

By scaling up our E. coli cultures which express spinach ACP-I to 100 liter fermentations, we have now purified over 100 mg of this protein. Most of this protein has been sent to Dr. James Prestegard of the Chemistry Department at Yale. Dr. Prestegard has determined a 3-D model for E. coli ACP using 2-dimensional NMR and he is now extending these studies to spinach ACP-I. Comparison of the 3-D structures for bacterial and plant ACP will provide a powerful method to discover the essential structural features of ACP which have been conserved through evolution.

FIGURE 1

MULTIPLE ISOFORMS OF ACYL-CARRIER PROTEIN HAVE BEEN FOUND IN ALL HIGHER PLANTS EXAMINED

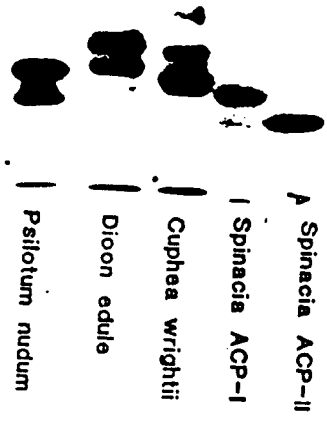


FIGURE 2

MULTIPLE ISOFORMS OF ACYL-CARRIER PROTEINS ARE FOUND IN VASCULAR AND NON-VASCULAR PLANTS

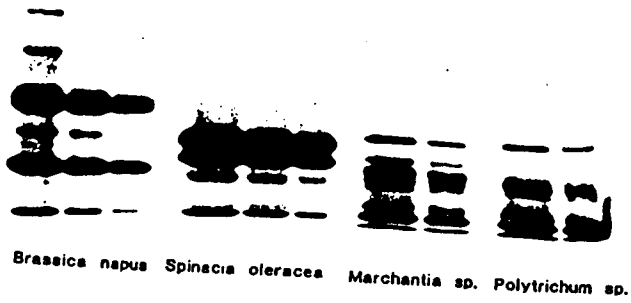
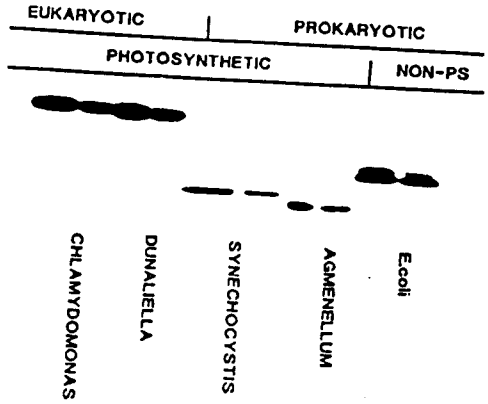


FIGURE 3

THE APPEARANCE OF MULTIPLE ISOFORMS OF ACYL-CARRIER PROTEIN DOES NOT OCCUR AT THE PROKARYOTIC/EUKARYOTIC OR PHOTOSYNTHETIC/NON-PHOTOSYNTHETIC BOUNDARY



Preparation of antibodies specific for ACP-I and ACP-II.

In collaboration with Calgene of Davis, California we have contracted with the firm, Immusine, to prepare monoclonal antibodies to ACP-I. Monoclonals reactive with ACP-I have now been obtained and we are in the process of screening these to determine which (if any) of the clones react specifically with ACP-I. This screening is initially taking place with western blots of ACP-I and ACP-II resolved on native polyacrylamide gel electrophoresis. The availability of antibodies specific to ACP-I will be of great value for a number of applications. In particular, we will use them initially to evaluate the forms of acyl ACP-I which exist in vivo. This has not been possible using our polyclonal antibodies because of complex patterns on gel electrophoresis resulting from both ACP-I and ACP-II.

In addition to the above progress with ACP-I, we have also recently succeeded in obtaining a cDNA clone for ACP-II from spinach. Therefore, we have now for the first time in any plant species, clones for the two major isoforms of ACP. We are in the process of subcloning the ACP-II cDNA into an E. coli expression vector without its transit peptide. This will allow us to prepare large (mg) quantities of this rare protein (as we have already done with ACP-I) and thereby to raise antibodies to it.

Site directed mutagenesis of the spinach ACP-I prosthetic group attachment site.

Site-directed mutagenesis was used to change the phosphopantetheine attachment site of spinach acyl carrier protein-I (ACP-I) from a serine to a threonine or cysteine residue. When expressed in Escherichia coli, the resulting ACP-I mutants were found to be completely devoid of the phosphopantetheine group. In addition, expression of the apoACP-I analogs did not alter the growth rate of the bacterial cells. Compared to holoACP-I, the mutant apoACP-I analogs had: a) altered mobility in SDS and native gel electrophoresis, b) altered binding to anti-spinach ACP-I antibodies, and c) altered isoelectric points. The mutant ACPs were inactive as substrates for and were strong inhibitors of spinach holoACP synthase. In contrast, the mutant ACPs were weak or ineffective as inhibitors of spinach fatty acid synthesis and spinach oleoyl-ACP hydrolase. The combined physical, immunological, and enzyme inhibition data support the concept that apoACP-I has a different conformation than holoACP-I.