The cloning and overexpression of E. coli acyl carrier protein (ACP)

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Acyl carrier protein (ACP) is a small (9kDa) protein which is essential for fatty acid synthesis. In bacteria [1] and plants [2] which use type I1 dissociable fatty acid synthetases, acyl chains are esterified to ACP through the 4'-phosphopantetheine prosthetic group, and ACP acts as a carrier during fatty acid synthesis. ACPs from bacteria and plants are very similar. All are small acidic proteins with conserved regions particularly around the serine residue through which the prosthetic group is attached.

The similarity of E. coli ACP to many plant ACPs is such that E. coli ACP can be used as a substitute for plant ACP in many assays for plant fatty acid synthetase activity; indeed it sometimes proves more active in vitro than the native plant ACP [3]. This is useful because such large quantities of plant material are required to isolate relatively small amounts of protein. It is therefore easier to isolate E. coli ACP. E. coli contains only one ACP isoform and also has the advantage of being active in many plant systems, whereas plant ACPs often do not work in other plant systems, and different and specific isoforms occur in different tissues [4].

It is possible to isolate 20-30mg of ACP from 200g of E. coli wet cell paste in 3 days. However, assaying plant fatty acid synthesis enzymes requires large quantities of ACP and, therefore,

![Sequence and translation of a DNA fragment coding for E. coli ACP](image)

![Figure 1](image)

The primer regions are heavily underlined and the restriction enzyme sites built onto the end of the primers are single underlined.

Rawlings and Cronan [7] confirmed our original assumption that the initial Met residue would be lost after translation but that no other amino acid residues would be processed from the N-terminus. The Ser residue through which the 4' phosphopantetheine group is attached is underlined.

The cloning of the DNA sequence of E. coli ACP was published earlier as a template for a PCR using degenerate oligonucleotide primers which were designed on the basis of available protein sequence data [5,6] encoding the N- and C-terminal regions of the protein with additional 5' extensions containing appropriate cloning sites (Fig. 1). When genomic E. coli DNA was used as a template for a PCR containing these primers a product whose size corresponded to that predicted for the ACP gene was generated. Cloning and sequencing of the PCR product showed it to encode an ACP type protein. However in all clones sequenced a frame shift was identified due to the incorporation of an additional base in the N-terminal region of the gene. The latter addition was removed by PCR overlap extension mutagenesis. The product of this reaction was cloned into the expression vector pKK 223-3 but overexpression of the ACP could not be detected. Furthermore, a protein product of the appropriate size was not detected when in vitro translation of the plasmid was performed. Sequencing of the PCR mutagenesis product in M13 revealed two families of clones, the full length sequence (Fig. 1) and a deletion mutant in which nucleotide 41 was absent. It is possible that the failure to overexpress the gene was caused by the cloning of the latter mutant into pKK 223-3.

The DNA sequence of E. coli ACP was published earlier this year [7] and agrees exactly with the sequence we have reported apart from the areas predetermined by our PCR primers. Furthermore, translation of both open reading frames produces identical protein products which disagree with the two previously reported protein sequences at residues 24 and 43. Vanaman et al. [5] reported Asp (24) and Val (43) and Jackowski and Rock [6] Asn (24) and Ile (43). We found position 24 to be Asn and position 43 to be Val (Fig. 1) as did Rawlings and Cronan [7].

One other very interesting observation of Rawlings and Cronan [7] is that the natural gene when cloned into M13 had a high frequency of spontaneous deletions. This is consistent with the high frequency of mutations seen in the fragment we isolated containing the ACP gene. They also suggest that DNA segments encoding ACP are toxic to E. coli and their synthetic construct with high levels of ACP expression did indeed prove lethal to the organism [8].

Other expression systems to overexpress E. coli ACP are currently being evaluated.

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