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ACETYL-CoA CARBOXYLASE IN THE PHOTOSYNTHETIC

TISSUE OF MAIZE

A thesis presented in partial fulfilment of the
requirement for the degree of Master of Science
in Biochemistry at
MASSEY UNIVERSITY

SHANE McARTNEY RUTHERFURD

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ABSTRACT

The aim of this study was, a). to examine further, aspects of the role of acetyl-CoA carboxylase in the regulation of fatty acid synthesis in the provision of acyl lipid for plastid development, and b). to purify acetyl-CoA carboxylase from maize leaves using the affinity methods which have been used successfully to purify the enzyme from animal tissues.

In a constant weight of tissue, carboxylase activity decreased 7.6-fold over the period of 4 to 12 days after sowing, while total acetyl-CoA carboxylase activity increased 9-fold in maize seedlings over the period of 4 to 8 days with no further increase up to day 12. Protein levels decreased 3-fold over the growth period examined, while specific activity was constant at 27.2 to 28.3nmol/min/mg of protein between 4 and 6 days, before increasing to a maximum of 33.2nmol/min/mg of protein at day 7, then decreasing to one third of the maximum value on day 12. Chlorophyll levels in a constant weight of tissue increased 260-fold over the period of 4 to 11 days.

The changes in the level of acetyl-CoA carboxylase activity paralleled changes in fatty acid levels in tissue along the length of the 9-day-old maize leaf. The levels of both biochemical parameters increased in the region from the leaf

base to 15mm along the leaf. After which they both decreased to a minimum at 25-30mm along the leaf before increasing to a maximum at 60mm along the leaf, and finally decreasing towards the leaf tip.

A 5-fold increase in acetyl-CoA carboxylase activity was observed from the least favourable chloroplast stromal concentrations of ATP, ADP, Mg^{2+} and H^+ in the dark, to the most favourable concentrations of these metabolites present in the chloroplast stroma during light periods.

These findings are consistent with, 1). a role for acetyl-CoA carboxylase in the regulation of fatty acid synthesis in maize photosynthetic tissue and, 2). control of acetyl-CoA carboxylase activity via light-dependent changes in the pH and concentrations of ATP, ADP and Mg^{2+} found in the stroma of chloroplasts.

Several attempts were made to purify acetyl-CoA carboxylase using avidin-affinity chromatography. However, after the initial, apparently successful attempt, active enzyme could not be recovered from the avidin-affinity column upon elution with biotin. Changes were made to several chromatographic conditions, and although ionic strength in the range of 0.1 to 1.0M KCl, did not affect the elution of active acetyl-CoA carboxylase from the column; lowering the column flow rates from 1.5ml/hr/ml of gel to 0.15-0.3ml/hr/ml of gel did appear to enhance the binding of the enzyme to the column. Using this flow rate, a 62 000 dalton protein and a 54 500 dalton protein were eluted in a fraction found to contain biotin-containing

proteins. Since it is feasible that the 62 000 dalton is biotin-containing and since this protein has a similar molecular weight to 60 000-62 000 dalton biotin-containing subunit of maize leaf acetyl-CoA carboxylase, the potential for purifying acetyl-CoA carboxylase from maize leaves using avidin-affinity chromatography seems to exist. However, further investigation is necessary in order to facilitate the recovery of active carboxylase from the avidin-affinity column.

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LIST OF ABBREVIATIONS

| | |
|----------|--|
| ACC | acetyl-CoA carboxylase |
| ADP | adenosine 5'-diphosphate |
| AMP | adenosine 5'-monophosphate |
| ATP | adenosine 5'-triphosphate |
| BCCP | biotin carboxyl-carrier protein |
| BSA | bovine serum albumin |
| CoA | coenzyme A |
| DGDG | digalactosyl diglyceride |
| DMCS | dimethyl dichlorosilane |
| DTT | dithiothreitol |
| EDTA | ethylenediamine tetraacetic acid |
| Hepes | N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid |
| Mes | 2[N-morpholino] ethane sulphonic acid |
| MGDG | monogalactosyl diglyceride |
| PBS | phosphate buffer-saline |
| PEG | polyethylene glycol |
| POPOP | 1,4-bis[2(5-phenyloxazolyl)]benzene |
| ppGpp | guanosine 5'-diphosphate-3'-diphosphate |
| PPO | 2,5-diphenyloxazole |
| pppGpp | guanosine 5'-triphosphate-3'-diphosphate |
| RNA | ribonucleic acid |
| Rubisco | ribulose 1,5-bisphosphate carboxylase oxygenase |
| SDS | sodium dodecyl sulphate |
| TEMED | N,N,N',N'-tetramethylethylenediamine |
| Tricine | N-tris[hydroxymethyl]-methyl glycine |
| Tris | tris (hydroxymethyl) aminomethane |
| Tween 20 | polyoxyethylene sorbitan monolaurate |

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