ACETYL-CoA CARBOXYLASE IN PHOTOSYNTHETIC TISSUE

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at MASSEY UNIVERSITY

BASIL JOHN NIKOLAU

1981

ABSTRACT

Acetyl-CoA carboxylase catalyses the rate-limiting reaction in de novo fatty acid biosynthesis in a wide variety of organisms. In plants however, the significance of this enzyme in regulating de novo fatty acid biosynthesis is unknown.

In this investigation acetyl-CoA carboxylase of maize, barley and spinach leaves has been studied in order to compare some features of this enzyme in the three plants. In maize, acetyl-CoA carboxylase is located in chloroplasts, where it occurs as a soluble enzyme in the stromal fraction. Both mesophyll and bundle sheath cells also exhibit acetyl-CoA carboxylase activity. Activities of acetyl-CoA carboxylase in isolated chloroplasts have been compared to rates of lipid synthesis from acetate in order to investigate the role of acetyl-CoA carboxylase in regulating de novo fatty acid biosynthesis from acetate. Although acetyl-CoA carboxylase activity was higher than that expected from the rate of acetate incorporation into lipids of isolated chloroplasts of maize and barley, the opposite was found for chloroplasts isolated from spinach. In chloroplasts from the emerging leaves of maize seedlings, lipid synthesis was maximal with chloroplasts isolated from the leaf segment corresponding to leaf greening. However, a relationship between leaf development and acetyl-CoA carboxylase activity was less apparent. Consequently a regulatory role for acetyl-CoA carboxylase in de novo fatty acid biosynthesis could not be directly established from a comparison of the rates of lipid synthesis and acetyl-CoA carboxylase activities.

An alternative approach to the establishment of a regulatory role for acetyl-CoA carboxylase was to purify the enzyme from maize and barley leaves, and study its response to variable concentrations of substrates, products and certain cellular metabolites. Purification of both enzymes was per-

formed by identical procedures, including polyethylene glycol fractionation, hydrophobic chromatography and gel filtration. Acetyl-CoA carboxylase from both maize and barley leaves appeared to be an integral enzyme, as no evidence for its dissociation was found, contrary to the findings of the bacterial enzyme. The kinetic properties of the partially purified enzyme from maize and barley were very similar. The apparent Michaelis constants for the substrates, acetyl-CoA and HCO_3^-, were about 0.1mM and 2mM, respectively for both enzymes. The enzymically active form of the substrate, ATP, was found to be Mg.ATP. Furthermore, free ATP inhibited enzymic activity, while free Mg^{2+} activated the enzyme from both plant sources. Monovalent cations, particularly K^+, were positive effectors of acetyl-CoA carboxylase, on the other hand, the products of the acetyl-CoA carboxylase reaction, malonyl-CoA and ADP were inhibitors. ADP inhibition was competitive with respect to ATP, but uncompetitive with respect to acetyl-CoA. Inhibition of acetyl-CoA carboxylase activity by CoA was noncompetitive with respect to acetyl-CoA, while palmitoyl-CoA inhibition was uncompetitive with respect to acetyl-CoA.

From the view point of regulation of acetyl-CoA carboxylase activity, the observed response of activity to changes in pH, and the concentrations of Mg^{2+}, K^+, ATP and ADP *in vitro*, may be of significance. Changes in the chloroplastic levels of these effectors of acetyl-CoA carboxylase activity have been reported to occur during light-dark transition of chloroplasts, and would be consistent in regulating acetyl-CoA carboxylase activity in the light and dark.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr J C Hawke, for his invaluable advice, encouragement and assistance throughout the course of this study. I am also indebted to Dr C R Slack of the Plant Physiology Division, DSIR, for his advice and assistance, particularly during the absence of Dr Hawke.

I would like to thank Mr I J Warrington of the Plant Physiology Division, DSIR, for helpful advice in the use of the climate control rooms for the growing of maize and barley plants.

In addition, particular thanks is extended to Ms Joy Watson for typing this manuscript.

LIST OF ABBREVIATIONS

ACP	acyl-carrier protein
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
BCCP	biotin carboxyl-carrier protein
BSA	bovine serum albumin
chl	chlorophyll
ρ-CMB	ρ-chloromercuribenzoate
CoA	coenzyme A
DTT	dithiothreitol
EDTA	ethylenediamine tetraacetic acid
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
Mes	2[N-Morpholino] ethane sulphonic acid
NADH	β-nicotinamide adenine dinucleotide, reduced form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
NEM	N-ethylmaleimide
PEG	polyethylene glycol
PEP	phosphoenolpyruvate
2-PGA	2-phosphoglyceric acid
3-PGA	3-phosphoglyceric acid

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
RuDP	ribulose 1,5-diphosphate
TEMED	N,N,N',N'-Tetramethylethylenediamine
TLC	thin-layer chromatography
Tricine	N-tris[Hydroxymethyl]-methyl glycine
Tris	tris (hydroxymethyl) aminomethane

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES