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LIPID BIOSYNTHESIS IN ISOLATED CHLOROPLASTS

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University

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1979

ABSTRACT

Two notable features of previous work on lipid biosynthesis by isolated chloroplasts have been:- (a) The inability of chloroplasts to incorporate more than small amounts of acetate into the main constituent fatty acids of the chloroplast lipids, namely linoleic (18:2) and linolenic (18:3) acids. (b) The poor incorporation of fatty acids synthesized into galactolipids, which are the main chloroplast lipids. Both of these aspects of lipid biosynthesis were investigated using chloroplasts isolated from spinach, maize and sweetcorn. Initial attempts to improve the synthesis of polyunsaturated fatty acids from [1-^{14}C]acetate were not successful. Consequently the main object of the investigation was directed towards increasing the incorporation of long chain fatty acids into galactolipids in the hope that increased galactolipid synthesis might also lead to increased desaturation of oleate to linoleate and linolenate.

Factors affecting the rates of acetate incorporation into lipids by spinach, maize and sweetcorn chloroplasts were investigated. Optimum concentrations of acetate, ATP and CoA were found to be about 0.5mM-acetate (spinach somewhat higher at 0.75mM-acetate), 0.5mM-ATP and 0.25mM-CoA under the incubation conditions used in the present study. Acetate concentration had a major effect on the rate of incorporation; optimisation of ATP and CoA concentrations gave only small enhancements of acetate incorporation. The effect of divalent cations was also investigated for spinach chloroplasts. Optimum Mg^{++} was 3.0mM; addition of 1mM-Mn^{++} in the presence of 1mM-Mg^{++} gave a comparable stimulation of acetate incorporation. Acetate incorporation by spinach chloroplasts was also enhanced by the addition of Triton X-100, sn-glycerol-3-phosphate and UDP-galactose.

Maximum incorporation rates obtained for maize and sweetcorn chloroplasts were 20-30nmol of acetate/mg chlorophyll/h which are up to 10-fold higher than previously reported rates for maize. Rates of up to 500nmol of acetate/mg chlorophyll/h were obtained for spinach chloroplasts which compare favourably with the rates obtained by other workers using chloroplasts isolated from younger leaf tissue.

Oleic and palmitic acids with small amounts of stearic

acid were the main fatty acids synthesized from acetate by isolated chloroplasts from all three sources. Little synthesis of linoleic and linolenic acids was achieved and changes in acetate, ATP and CoA concentrations had no significant effect on the synthesis of polyunsaturated fatty acids from acetate. Triton X-100 and divalent metal ion concentrations also had little effect on the synthesis of polyunsaturated fatty acids by spinach chloroplasts.

The synthesis of diglycerides (DG) by isolated chloroplasts from spinach, maize and sweetcorn was enhanced by the addition of sn-glycerol-3-phosphate (G-3-P). Synthesis of monogalactosyldiglyceride (MGDG) was enhanced by the addition of UDP-galactose particularly if G-3-P was also present. Triton X-100 greatly enhanced the synthesis of DG and also (in the presence of UDP-galactose) MGDG by spinach chloroplasts. Spinach chloroplasts gave higher rates of DG and MGDG synthesis than either maize or sweetcorn chloroplasts.

The synthesis of MGDG from DG by spinach chloroplasts was investigated by double-labelling experiments, using [1(3)-^{3}H]sn-glycerol-3-phosphate and [1-^{14}C]acetate, fatty acid analysis and positional distribution of the incorporated fatty acids. The synthesis of MGDG was shown to occur without prior modification of the fatty acid composition of the DG.

It was evident from the incorporation of oleate and palmitate into DG (and subsequently into MGDG) and from the positional distribution of these two fatty acids that a specific acylation of G-3-P occurred synthesizing mainly 1-oleoyl, 2-palmitoyl-sn-glycerol. The effects of altering the proportions of oleate and palmitate synthesized on the relative amounts of these fatty acids incorporated into DG (and MGDG) were investigated. The results suggested that palmitate was incorporated into position 2 first followed by oleate into position 1. If there was more palmitate than oleate synthesized some palmitate could be also incorporated into position 1.

The rates of DG synthesis calculated from [1(3)-^{3}H]-sn-glycerol-3-phosphate incorporation were considerably greater than those calculated from [1-^{14}C]acetate incorporation indicating that a considerable dilution of the label from [1-^{14}C]acetate had occurred and that a major proportion of the fatty acid carbon had come from an alternative source.

Bicarbonate, present in the reaction medium, was found to be utilized by spinach chloroplasts for the synthesis of fatty acids and lipids. Thus bicarbonate was probably the alternative source of fatty acid carbon. The fatty acids and lipids synthesized by spinach chloroplasts from exogenous acetate and bicarbonate were very similar.

Although high rates of DG and MGDG synthesis have been achieved in the course of the present study by the addition of appropriate metabolites, stimulation of synthesis of these lipids did not alter the rates of synthesis of linoleic and linolenic acids from acetate. Other attempts to increase polyunsaturated fatty acid synthesis from acetate by isolated chloroplasts were also unsuccessful. The use of chloroplasts isolated from developing maize leaf sections had little effect on the rates of linoleic and linolenic acids synthesized from acetate. The addition of a 100,000 X g particulate preparation from leaf homogenate to isolated maize and spinach chloroplasts though stimulating overall incorporation of acetate, gave only minor increases in the proportion of linoleic and linolenic acids synthesized. The stimulation of phosphatidylcholine synthesis by the particulate fraction, in the presence of isolated chloroplasts, failed to result in any dramatic increases in the proportions of polyunsaturated fatty acids synthesized.

These findings are discussed in relation to the current understanding of fatty acid and lipid synthesis and recent *in vivo* and *in vitro* studies of plant lipid synthesis.

ACKNOWLEDGEMENTS

I wish to express my appreciation to my supervisor, Dr. J. C. Hawke, for his advice and encouragement during this study. I am also indebted to Dr. G. G. Pritchard for his supervision and encouragement during the absence of Dr. Hawke.

I would like to thank Drs. I. Warington and A. Hardacre, of the Plant Physiology Department of the DSIR, for the use of the climate control rooms for the growing of the maize and sweetcorn plants used in this study; Mr. D. Hopcroft, of the DSIR, for the electron microscopy work and Dr. R. L. Prestidge for the help in the preparation of *E. coli* acyl carrier protein.

In preparation of the thesis, thanks are due to Mrs. J. Trow for the preparation of the figures.

Finally, I am grateful to my parents for their support and encouragement.

To my Parents

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

ACP	acyl carrier protein
ATP	adenosine 5'-triphosphate
A_{645}	absorbance at 645nm
BCCP	biotin carboxyl carrier protein
BSA	bovine serum albumin
^{o}C	degrees Celsius
CDP-choline	cytidine 5'-diphosphate choline
Ci	Curie
cm	centimetre
cm^3	cubic centimetre
CoA	coenzyme A
DCCD	N,N'-dicyclohexylcarbodiimide
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DEGS	diethylene glycol succinate
DG	diglyceride (or diacylglycerol)
DGDG	digalactosyldiglyceride (or digalactosyl-glycerol)
d.p.m.	disintegrations per minute
DTT	dithiothreitol
E. coli	*Escherichia coli*
FCCP	4-trifluoromethoxyphenylhydrazone
FFA	free fatty acids
ft-candle	foot-candle (1 ft-candle = 10.7639 lx)
g	gram
g	force of gravity
G-3-P	*sn*-glycerol-3-phosphate
g.l.c.	gas-liquid chromatography
h	hour
i.d.	internal diameter
kv	kilovolt
l	litre
lyso-PA	lyso-phosphatidic acid (or monoacyl-*sn*-glycerol-3-phosphate)
lx	lux
M	molar
mg	milligram
MG	monoglyceride (or monoacylglycerol)
MGDG	monogalactosyldiglyceride (or monogalactosyl-diacylglycerol)

MGMG	monogalactosylmonoglyceride (or monogalactosyl-monoacylglycerol)
min	minute
mM	millimolar
mmol	millimole
mol	mole
NADH	nicotinamide adenine dinucleotide, reduced
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
nm	nanometre
nmol	nanomole
p., pp.	page, pages
PA	phosphatidic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PEP	phosphoenolpyruvate
PG	phosphatidylglycerol
2-PGA	2-phosphoglyceric acid
3-PGA	3-phosphoglyceric acid
POPOP	1,4 bis[2-(5-phenyloxazolyl)]-benzene
PPO	2,5-diphenyloxazole
s	second
sn	stereospecific numbering
SQDG	sulphoquinovosyldiglyceride (or sulphoquinovosyl-diacylglycerol)
TG	triglyceride (or triacylglycerol)
t.l.c.	thin-layer chromatography
Tricine	N-Tris(hydroxymethyl) methylglycine
Tris	Tris(hydroxymethyl) aminomethane
UDP-galactose (UDP-gal)	uridine 5'-diphosphate D-galactose
UK	unknown compound (see Methods, p. 36)
v	volume
wt	weight
µ	micro

NOMENCLATURE

For the specific structural designation of complex lipids containing a glycerol moiety, the nomenclature suggested by the IUPAC-IUB Commission on Biochemical Nomenclature (Eur. J. Biochem. (1967) 2, 127-131) has been followed. However, the trivial names of complex lipids are used when it is more appropriate. Widely used abbreviations, e.g. MGDG for monogalactosyldiglyceride, have also been used for the sake of brevity. These are defined on pp. xxi-xxii.

Fatty acids are designated by the shorthand notation of number of carbon atoms:number of double bonds, e.g. 18:3 refers to linolenic acid.

Other abbreviations and the format for the figures and tables in this thesis followed the guide lines set down by the Biochemical Journal (Biochem. J. (1975) 145, 1-20).