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LIPID BIOSYNTHESIS IN ISOLATED
BARLEY PROTOPLASTS

A THESIS PRESENTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN BIOCHEMISTRY AT
MASSEY UNIVERSITY

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1983

ABSTRACT

In most studies of fatty acid and lipid synthesis in plants there has been poor incorporation of radioactive label from acetate into linoleic (18:2) and linolenic (18:3) acids. Consequently the amounts of these fatty acids found in the galactolipids in such studies are much less than their observed endogenous levels.

In the present study incorporation of $\text{H}^{14}\text{CO}_3^-$ and $(1\text{-}^{14}\text{C})$ acetate into lipids of barley protoplasts was examined. CO_2 -dependent O_2 evolution rates of the protoplasts were around $180 \mu\text{mol O}_2/\text{h}/\text{mg Chl}$ and intactness was also ascertained by phase contrast microscopy. Incubating protoplasts with $1\text{mM H}^{14}\text{CO}_3^-$ or $50 \mu\text{M } (1\text{-}^{14}\text{C})$ acetate resulted in 146.2 and $17 \text{ nmol}/\text{mg Chl}$ being incorporated into lipids respectively after 1 hour. A concentration of 10 mM was optimal for HCO_3^- incorporation and up to $580 \text{ nmol}/\text{mg Chl}$ was incorporated into lipids at the end of 1 hour. Mg^{++} and P_i ions used at 2 mM had little effect on HCO_3^- incorporation while PP_i appeared to be slightly inhibitory. Acetate assimilation and its incorporation into lipids was markedly affected by pH and pH 5.8 was chosen for the assay medium. In 20 hour incubations $162 \text{ nmol acetate}/\text{mg Chl}$ was incorporated. About 33% of label from acetate was found in each of palmitic (16:0) and oleic (18:1) acids with less than 9% in each of stearic (18:0), linoleic and linolenic acids. There was little or no incorporation of acetate into DGDG and less than 10% into each of PG, MGDG, PE and U (unknown lipid). Incorporation into PC after $2\frac{1}{2}$ hours was 36.8% then decreased to 8.9%. Acetate incorporation was most significant into U_{SF} (another unknown

lipid), being 73.4%. Although acetate was incorporated into a range of glycerolipids, incorporation into constituent 18:2 and 18:3 of these lipids was not significant.

ACKNOWLEDGEMENTS

I am especially grateful to my supervisor Dr J.C. Hawke for his guidance and encouragement during this study.

Thanks are due to Dr I. Warrington of the Plant Physiology Dept of the DSIR for the use of the climate control rooms for the growing of the maize and barley plants.

I wish to thank Mr J. Reid very much for the preparation of the figures in this thesis.

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LIST OF ABBREVIATIONS

ACP	acyl-carrier protein
ATP	adenosine 5'-triphosphate
BSA	bovine serum albumin
Chl	chlorophyll
CoA	coenzyme A
DAG (or DG)	diacylglycerol (or diglyceride)
DGDG (or DDG)	digalactosyldiacylglycerol (or digalactosyldiglyceride)
EDTA	ethylenediamine tetraacetic acid
FA	fatty acid
FFA	free fatty acid
fr. wt	fresh weight
g.l.c.	gas-liquid chromatography
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethane sulphonie acid
MES	2[N-morpholino] ethane sulphonie acid
MG	monoacylglycerol (or monoglyceride)
MGDG (or DGD)	monogalactosyldiacylglycerol (or monogalactosyldiglyceride)
NADH	nicotinamide adenine dinucleotide, reduced form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
PA	phosphatidic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PEP	phosphoenolpyruvate
PG	phosphatidylglycerol
PGA	phosphoglycerate
PI	phosphatidylinositol
POPOP	1,4-bis[2(5-phenyloxazolyl)] benzene
PP _i	pyrophosphate
PPO	2,5-diphenyloxazole
TG	triacylglycerol (or triglyceride)
TLC	thin-layer chromatography
U, U _{SF}	unknown compounds (see Section 3.6)
UDP -gal	uridine 5'-diphosphate D-galactose

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