LIPID BIOSYNTHESIS IN ISOLATED BARLEY PROTOPLASTS

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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 $H^{14}CO_3^-$ and (1-^{14}C) acetate into lipids of barley protoplasts was examined. CO_2-dependent O_2 evolution rates of the protoplasts were around 180 µmol O_2/h/mg Chl and intactness was also ascertained by phase contrast microscopy. Incubating protoplasts with 1mM $H^{14}CO_3^-$ or 50 µM (1-^{14}C) acetate resulted in 146.2 and 17 nmol/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 nmol/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 nmol acetate/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 2½ 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.

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TABLE OF CONTENTS

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 sulphonic acid
MES	2[N-morpholino] ethane sulphonic 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	phoshatidic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PEP	phosphoenolpyruvate
PG	phosphatidyglycerol
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

LIST OF FIGURES

Figure | Page

Figure | Page

LIST OF TABLES