THE BIOSYNTHESIS OF GALACTOLIPIDS IN CHLOROPLAST ENVELOPES

A Thesis Presented as Partial Fulfillment for the Degree of Master of Science in Biochemistry

by

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1979

ACKNOWLEDGEMENTS

I would like to thank my Supervisor Dr. J.C. Hawke for his invaluable guidance and advice during the practical work and the writing of this thesis.

Thanks are also extended to Mr D.H. Hopcroft for the electronmicrographs, and my mother, Mrs A.F. Benny, for typing this thesis.

ABSTRACT

The procedure of Douce et al (1973) was employed for the isolation of envelopes from purified chloroplasts of spinach (Spinacia oleracea) and maize (Zea maize var. Wis. 235). Maize chloroplasts gave very low yields of envelope protein and low incorporation of radioactivity from UDP-^{14}C-Galactose into galactolipids. However the use of spinach chloroplasts resulted in higher yields of envelope protein and high levels of a galactosyltransferase that synthesised galactolipids from endogenous lipid substrates and added UDP-^{14}C-Galactose. The products of galactosyltransferase were identified as MGDG and DGDG by comparison with standard lipids on thin layer chromatography. The procedure for the isolation of chloroplast envelopes reported by Poincelot and Day (1973) gave a higher yield of less contaminated envelope membranes and an increased specific activity of galactosyltransferase compared to the results obtained using envelopes isolated by the method of Douce et al (1973)

Total incorporation of radioactivity from 0.3 μM UDP-^{14}C-Galactose by galactosyltransferase was dependent on the time and temperature of incubation and the nature of the incubation buffer. Maximum incorporation (about 72% of the added radioactivity) was obtained upon incubation at 30°C for 30 min, in 50 mM HEPES-NaOH at pH 8.0. MGDG was identified as the major labelled lipid (MGDG:DGDG ratio 1.7:1). Lower pH values gave higher incorporation into DGDG.

A cation dependence of galactosyltransferase was observed and incorporation was stimulated by addition of Ca^{2+}, Mg^{2+} or Ba^{2+}. Maximum incorporation was obtained with 5 mM Ba^{2+}. In contrast 5 mM Cu^{2+} completely inhibited incorporation.

The sulphydryl nature of the chloroplast galactosyltransferase (Chang, 1970; Mudd et al 1971) was confirmed with galactosyltransferase of the chloroplast envelope.

Linoleic acid at 0.72 μM completely inhibited transferase activity. The inhibition by linoleate could be partially removed by addition of about 10 mM Ca^{2+} or Ba^{2+} but 10 mM Mg^{2+} and BSA (30 μg per ml) were without effect.

UMP, UDP and UTP at 1 mM inhibited incorporation by transferase. UDP was the most effective inhibitor and gave 50% inhibition of incorporation at about 5 μM. NADH and PP_i did not significantly affect incorporation.

The addition of exogenous diacylglycerol (1-palmitoyl, 2-oleoyl glycerol or 1, 2-di-linoleoyl glyerol) did not increase the incorporation of radioactive galactose into galactolipids. Incorporation was inhibited by 0.3% Triton X-100 and 6 mM sodium cholate. No radioactivity from added ^{14}C-diacylglycerol was incorporated into MGDG by chloroplast envelopes.

Preincubation of the chloroplast envelopes with phospolipase C or D reduced the total amount of radioactivity incorporated by galactosyltransferase. Transferase activity was detectable after preincubation of the envelopes with trypsin and protease.

The fatty acid composition of MGDG, DGDG and DG from whole tissue, chloroplasts and chloroplast envelopes of spinach is presented. The characteristic highly unsaturated nature of the fatty acids of MGDG and DGDG is in contrast to the relatively saturated fatty acid content of DG isolated from whole tissue and chloroplasts. However, DG isolated from chloroplast envelopes contained predominantly 16:0, 18:1 and 18:3.

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ATP	adenosine-5'-triphosphate
Tris	tris(hydroxymethyl)aminomethane
BSA	bovine serum albumin
cm	centimetre
CoA and acylCoA	coenzyme A and its acyl derivative
cpm	counts per minute
C_i	curie (3.7×10^{10} s^{-1})
^oC	degrees celsius
DG	1,2-diacylglycerol (diglyceride)
DEGS	di-ethylene glycol succinate
DGDG	digalactosyldiacylglycerol
EDTA	ethylenediaminetetraacetic acid
E	extinction ($\log \frac{I_o}{I}$)
GLC	gas-liquid chromatography
G-3-P	sn-glycerol-3-phosphate
g	gram
x g	x gravitational force
h	hour
l	litre
K_m	Michaelis constant
µg	microgram (10^{-6} g)
p (prefix)	micromicro (10^{-12} x)
µmole	micromole (10^{-6} M)
n (prefix)	millimicro (10^{-9} x)
M	molar (moles per litre)
mM	millimolar (millimoles per litre)
min	minute
MGDG	monogalactosyldiacylglycerol
NADH	nicotinamide-adenine dinucleotide, reduced
P_i	orthophosphate (inorganic)
PP_i	pyrophosphate (inorganic)
%	per cent
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PI	phosphatidylinositol
rpm	revolutions per minute
s	second
sn	stereospecific numbering

TLC	thin layer chromatography
PA	phosphatidic acid
lyso-PA	lyso-phosphatidic acid
TTGDG	tetragalactosyldiacylglycerol
TGDG	trigalactosyldiacylglycerol
UMP	uridine-5'-phosphate
UDP	uridine-5'-pyrophosphate
UTP	uridine-5'-triphosphate
UDP-^{14}C-Galactose	uridine-5'-diphosphate-D-U-^{14}C-Galactose
UDP-Galactose	uridine-5'-diphosphate-D-galactose
vol.	volume
wt.	weight
dpm	disintegrations per minute