Re-evaluation of plant sulpholipid labelling from UDP-[^{14}C]glucose in pea chloroplasts

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The sulphoquinovosyldiacylglycerol (sulpholipid) zone obtained by TLC of pea chloroplast lipids yields, on hydrolysis, not only sulphoquinovose but also galactose and glucose. Following incorporation from UDP-[^{14}C]glucose, the percentages of the total radioactivity in these three sugars were typically 1, 5 and 85%, respectively. The occurrence of the glucolipid causes difficulties in elucidating further the biosynthetic pathway for sulphoquinovose.

Key words: biosynthesis, galactolipid, glucolipid, sulphoquinovose, sulphoquinovosyldiacylglycerol.

INTRODUCTION

Pugh et al. [1] showed that chloroplasts isolated from seedlings of pea, *Pisum sativum*, incorporated radioactivity from UDP-[^{14}C]glucose into the plant sulpholipid, sulphoquinovosyldiacylglycerol (SQDG). A continuation of this work has shown an unexpected complication: namely, that in the 'SQDG' separated by TLC from chloroplast lipids [1,2], most of the radioactivity is associated with glucose and only relatively small amounts with sulphoquinovose. This occurrence of a glucolipid was unexpected and appears not to have been noted previously. Its presence compromises experiments aimed at elucidating further the details of sulphoquinovose biosynthesis.

EXPERIMENTAL

Radioactivities were measured by liquid scintillation counting in Optifluor (Canberra Packard, Pangborne, Berks., U.K.) with quench-correction by the external-standard ratios method. Chloroplasts were prepared from 12-day-old pea seedlings [2] exactly as described by Mills and Joy [3] and suspended in incubation buffer (see below) to give a chlorophyll concentration of about 800 μg/ml [4]. When required, chloroplast membranes were damaged by two cycles of freezing and thawing of this suspension.

Incubation buffer contained 0.33 M sorbitol, 0.033 M tricine, 2 or 20 mM $MgCl_2$ (as detailed below), 2 mM NaH_2PO_4 and 0.1 mM K_2SO_4, adjusted to pH 7.9 with KOH. This buffer was used to suspend the isolated chloroplasts and to dissolve any further reagents.

Intact or damaged chloroplasts (about 200 μg of chlorophyll) were kept for 1 h at 25 °C in 0.5 ml of incubation buffer containing 0.5 mM dithiothreitol, 2 mM ATP and 9.25 kBq of UDP-[^{14}C]glucose (carrier free, 11 GBq/mmol; Nycomed Amersham, Little Chalfont, Bucks., U.K.). The reaction was stopped and the lipids were extracted using the Garbus system [5] as previously described [1].

For preparative work, the lipids from eight such reaction mixtures were combined for TLC [2] on silica gel 60 plates (Merck, Darmstadt, Germany), activated for 1 h at 110 °C immediately before use, using the solvent system chloroform/methanol/acetic acid/water (85:15:10:3.5, by vol.) [6]. Radioactive zones were located by autoradiography.

The SQDG zone was removed, transferred to a small column and the lipid eluted by washing successively with small volumes of chloroform/methanol (2:1, 1:1 then 1:2, by vol.). The combined extracts were taken to dryness by rotary evaporation at 25 °C to give a small residue (about 1 mg), some of which was obviously silica.

When required (see below), the lipid was purified by TLC, as described above, but in the solvent system chloroform/methanol/water (70:30:4, by vol.) [7]. The major zone was located, eluted and taken to dryness as before.

The lipid so obtained was then hydrolysed in 2 ml of 2.5 M HCl under reflux for 3 h on a boiling-water bath. After taking to dryness on a rotatory evaporator at 25 °C, the residue was dissolved in 1 ml of water, two drops of 18 M ammonium hydroxide were added to neutralize any sulphosugar and the solution was again taken to dryness.

The small residue was dissolved in a few microlitres of water and samples (containing about 1500 d.p.m. of ^{14}C) were taken for TLC on silica gel 60 plates, impregnated with phosphate and activated as described by Hansen [8], but using the solvent system propan-2-ol/acetone/0.1 M formic acid (2:2:1, by vol.) [9]. The latter solvent, with formic acid replacing the lactic acid used by Hansen [8], allows alkaline $KMnO_4$ to be used to detect reducing compounds. Radioactive compounds were located by autoradiography and reducing sugars by reaction with aniline diphenylamine in phosphoric acid. Authentic sulphoquinovose [9] was used as a standard.

RESULTS AND DISCUSSION

The rate of incorporation of ^{14}C from UDP-[^{14}C]glucose into the SGDQ zone (R_F 0.12; compare with digalactosyldiacylglycerol, R_F 0.21), measured exactly as described previously [1], by nine preparations of chloroplasts from separate batches of seedlings, was 940 ± 290 d.p.m./h per mg of chlorophyll (mean ± S.D.). In experiments using $^{35}SO_4^{2-}$ [2], the sole radioactive band coincided with the putative SQDG zone labelled from UDP-[^{14}C]glucose, confirming that this contained sulpholipid.

In an attempt to increase this rate of incorporation, the use of carrier-free UDP-[^{14}C]glucose was investigated, as described

Abbreviation used: SQDG, sulphoquinovosyldiacylglycerol.

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Table 1 The distribution of radioactivity in hydrolysates of the SQDG zone from chloroplast lipids labelled with UDP-[^{14}C]glucose and separated by TLC

Values are given for sulphoquinovose, galactose, glucose, residual lipid and material remaining at the origin of the TLC. Each section, located by autoradiography, was counted in quadruplicate and the values are means ± S.D., corrected for background (18.9 ± 4.5 d.p.m.; $n = 8$). The amount of hydrolysate corresponded to 60 and 140 μg of chlorophyll in experiments 1 and 2, respectively, each using a separate preparation of chloroplasts.

	Radioactivity (d.p.m.)	
	Experiment 1	Experiment 2
Origin	5.7 ± 5.2	3.7 ± 9.5
Sulphoquinovose	10.6 ± 6.1	14.2 ± 9.4
Galactose	51.1 ± 6.0	42.0 ± 6.8
Glucose	840.6 ± 28.0	1006.4 ± 18.1
Lipid	85.3 ± 9.7	98.8 ± 10.6
Total	993	1165

Table 2 The effect of incubation conditions on the distribution of radioactivity in hydrolysates of the SQDG zone from chloroplast lipids labelled with UDP-[^{14}C]glucose and separated by TLC

Values are given as in Table 1. Each section was counted in triplicate and the values are means ± S.D., corrected for background (16.8 ± 2.5 d.p.m.; $n = 6$). The amounts of hydrolysate corresponded to 60, 50 and 40 μg of chlorophyll, respectively, in columns 1–3. A single preparation of chloroplasts was used.

	Radioactivity (d.p.m.)		
	Intact chloroplasts	Damaged chloroplasts	
Concentration of Mg^{2+} ...	2 mM	2 mM	20 mM
Origin	13.6 ± 6.2	11.4 ± 3.6	12.5 ± 4.9
Sulphoquinovose	11.0 ± 4.5	64.5 ± 6.3	193.3 ± 7.6
Galactose	154.1 ± 9.1	303.4 ± 4.0	246.2 ± 12.1
Glucose	914.9 ± 23.4	836.2 ± 16.8	874.6 ± 43.1
Lipid	94.6 ± 5.0	116.3 ± 5.9	149.6 ± 4.2
Total	1188	1332	1476

above. Intact chloroplasts in incubation buffer containing 20 mM Mg^{2+} gave rates of incorporation of ^{14}C into the SQDG zone of 40000 and 23000 d.p.m./h per mg of chlorophyll in two separate experiments. Rechromatography of these lipids gave two or three very minor components and one major band, which was eluted and hydrolysed.

The sugars in the hydrolysate were identified by TLC on silica gel 60 plates impregnated with phosphate. Comparison with standards run on the same plates showed that almost all the radioactivity had a mobility identical with that of glucose (R_F 0.25): only small amounts were present in galactose (R_F 0.17) and even less in sulphoquinovose (R_F 0.10). Some unhydrolysed lipid (R_F 0.73) remained and small amounts of radioactivity did not move from the origin. Reaction with aniline diphenylamine showed traces of glucose together with smaller amounts of galactose and sulphoquinovose. The identification of glucose was confirmed by treatment with glucose oxidase, which gave a product with the same mobility (R_F 0.08) as that, detected by alkaline $KMnO_4$, produced from glucose.

Quantitative data, obtained by scraping the silica gel from the appropriate areas of the TLC plates and measuring the amount of associated radioactivity, are given in Table 1.

These results confirm that ^{14}C from UDP-[^{14}C]glucose is incorporated into sulphoquinovose by isolated chloroplasts from the pea. They also show that quantitative interpretation of such data is difficult because the SQDG zone, separated by TLC, contains not only this lipid but also gluco- and galacto-lipids. Glucolipids have been reported from several plant sources [7], but apparently not from the chloroplasts of higher plants. The present compound (or compounds) has not been characterized and it is not known whether glucose and galactose occur in stoichiometric proportions because the specific radioactivities in the reaction mixture of UDP-[^{14}C]glucose and of the UDP-[^{14}C]galactose (presumably derived from it) are unknown. The mobility of the lipid is such that it cannot be monoglucosyldiacylglycerol, reported as a minor lipid in cyanobacteria [10], or diglucosyldiacylglycerol, both of which have rather higher mobilities than the corresponding galactolipids, at least in the solvent system chloroform/methanol/water (65:25:4, by vol.) [11], but it could be a more highly glycosylated lipid. Another possibility is an *O*-sulpho glycolipid. These seem not to have been found in plants but 3′-*O*-sulphogalactosyldiacylglycerol occurs in rat brain and it has a mobility, in the above solvent system, similar to digalactosyldiacylglycerol [11,12]. Such sulphate esters, unlike sulphoquinovose which contains a *C*-sulpho group, are labile [13] under the conditions of lipid hydrolysis and would not routinely be detected.

When the lipid in the SQDG zone was not purified by a second TLC, the radioactivity associated with sulphoquinovose was rather greater: 7 and 8 % of the total in duplicate determinations compared with about 1 % in Table 1. This may reflect only the considerable variation in the activities of individual chloroplast preparations.

Table 2 shows the effect of changing the Mg^{2+} concentration in the incubation buffer and of disrupting the chloroplast membranes on the utilization of UDP-[^{14}C]glucose. The incorporation into sulphoquinovose by chloroplasts was considerably increased when their membranes were damaged by freezing and thawing. Lowering the Mg^{2+} concentration in the incubation buffer to 2 mM, as used in previous work [1], decreased the incorporation. The effect of the concentration of Mg^{2+} on the incorporation into the gluco- and galacto-lipids was less obvious but the effects on the sulpholipid labelling were consistent with the observation [14] that the UDP-sulphoquinovose:diacylglycerol sulphoquinovosyltransferase (which catalyses the final step of the synthesis of SQDG in chloroplasts) is envelope-bound and highly sensitive to the concentration of Mg^{2+}.

The incorporation of the available ^{14}C into the isolated sulphoquinovose is small: with 20 mM Mg^{2+} and broken chloroplasts it amounts only to about 0.15 %. The incorporation into glucose is some two orders of magnitude greater. In terms of their mass, the glucolipid(s) must be quantitatively insignificant. However, the above work shows that they may cause unsuspected difficulties for investigations of the metabolism of UDP-[^{14}C]glucose by pea chloroplasts and, especially, in further elucidation of the sulphoquinovose biosynthetic pathway.

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