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Radioactively labeled ether lipids by biotransformation of symmetrical alkylglycerols in cell suspension cultures of rape

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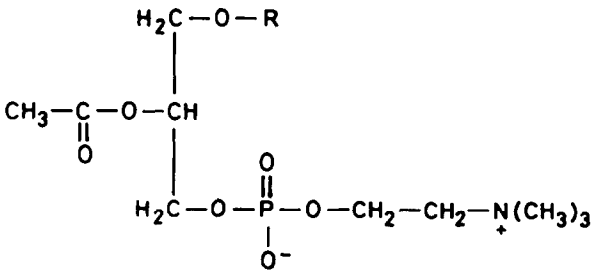
Biotransformation of 2-*O*-[1′-^{14}C]hexadecylglycerol by photomixotrophic rape *(Brassica napus)* cells in suspension culture leads in appreciable yield (>60%) to 1-acyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholines. Alkaline hydrolysis of the acyl moiety in these phospholipids yields 2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholine which is acetylated to 1-acetyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholine, i.e. a positional isomer of platelet-activating factor.

Ether lipid; Platelet-activating factor; Lyso-PAF; Cell culture; PAF analog; Lyso-PAF analog

1. INTRODUCTION

Some physiologically active ether lipids are of great current interest in biomedical and clinical research [1,2]. These substances include platelet-activating factor (PAF), i.e. 2-acetyl-1-*O*-alkyl-*sn*-glycero-3-phosphocholines (I), a potent mediator of allergic reactions such as inflammation, asthma and anaphylaxis [1,3]. Numerous studies aimed at elucidating the role of PAF in the cell and at developing antagonists of this deleterious ether lipid are in progress [3,4]. The corresponding lyso compounds, i.e. 1-*O*-alkyl-*sn*-glycero-3-phosphocholines (lyso-PAF), are used as antineoplastic agents [2].

Plant cells in culture are eminently useful for the semi-synthetic preparation of I as we reported recently [5–7]. Here, we describe a procedure for the semi-synthesis of 1-acetyl-2-*O*-[1′-^{14}C]alkyl-*sn*-glycero-3-phosphocholines (II) via the lyso compound, two radiolabeled ether lipids, that may be helpful in a variety of biomedical investigations [8].



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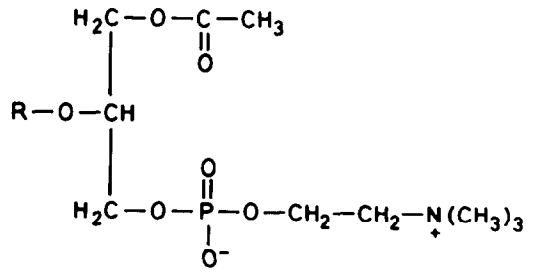

II

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2. MATERIALS AND METHODS

2-*O*-[1′-^{14}C]Hexadecylglycerol (0.98 GBq/mmol) was prepared from [1-^{14}C]palmitic acid (Amersham Buchler, Braunschweig) by the reaction of the corresponding methanesulfonate with 1,3-benzylideneglycerol followed by acid-catalyzed hydrolysis of the protecting group. The radiochemical purity of the labeled 2-*O*-hexadecylglycerol was better than 98%.

Photomixotrophic cell suspension cultures of rape were propagated in modified MS medium [9]. The cultures were shaken under continuous illumination at 25°C and subcultured every 10–14 days. The dry weight of the cells (900 mg dry material/10 g cells fresh wt) was determined as in [5].

Photomixotrophic rape cells, 2 g cells/4 ml preconditioned medium, were first incubated for 1 h under anaerobic conditions (argon atmosphere) and then added to 37 kBq (38 nmol) 2-*O*-[1′-^{14}C]hexadecylglycerol in 0.02 ml of 80% aqueous ethanol/diethyl ether (1:2, v/v). The cells were incubated at 25°C for 4–48 h, under nearly anaerobic conditions. Aliquots of about 0.4 g rape cells each were withdrawn after 4, 8, 24, 36 and 48 h of incubation. The cells were homogenized and the lipids extracted according to [10]. The radioactivity of the extracts was determined by liquid scintillation counting of aliquots.

The total lipids extracted from the incubation mixtures were fractionated by TLC on silica gel with chloroform/methanol/water (65:25:4, by vol.), and the distribution of radioactivity in the various fractions was determined by scanning. The fractions of diradylglycerophosphocholines (R_f 0.25) and neutral lipids (R_f 0.8–1.0) were each isolated. The neutral lipids were further resolved by TLC on silica gel with hexane/diethyl ether (7:3, v/v), twice, and the radioactivity in the fractions of diradylglycerols (R_f 0.20–0.30) and triradylglycerols (R_f 0.80) was determined by scanning. The radioactive lipid fractions were purified and characterized as described [6].

Aliquots of the fractions of diradylglycerophosphocholines, diradylglycerols and triradylglycerols were reduced with lithium aluminium hydride. The resulting radioactive long-chain alcohols and hexadecylglycerol were analyzed and characterized [6]. An aliquot of labeled diradylglycerophosphocholines isolated from rape cells was hydrolyzed with phospholipase C. The radioactivity of the resulting products, diradylglycerols and phosphocholine, was determined after TLC [6].

For preparative purposes, 20 g rape cells were incubated with 370 kBq (380 nmol) 2-*O*-[1′-^{14}C]hexadecylglycerol for 48 h, and the total lipids were isolated and fractionated by TLC as described above.

1-Acyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholines (100 kBq) isolated from rape cells were subjected to alkaline hydrolysis. The resulting 2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3- phosphocholine (analogue of lyso-PAF; 95% yield based on labeled 1-acyl-2-*O*-hexadecyl-*sn*-glycero- 3-phosphocholines) (R_f 0.23) was purified by TLC on silica gel with chloroform/methanol/water (70:35:7, by vol.), isolated, and acetylated [5]. The final product, 1-acetyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholine (analogue of PAF; 95% yield based on the lyso compound) (R_f 0.22) was purified by TLC on silica gel using chloroform/methanol/water/acetic acid (70:35:5:5, by vol.).

The purity of II was checked by cochromatography with a standard on silica gel layers with chloroform/methanol/ammonia (70:35:7, by vol.) as well as by two-dimensional TLC on silica gel using chloroform/methanol/water (65:35:7, by vol.) in the first dimension and chloroform/methanol/ammonia (65:35:7, by vol.) in the second. Radioactivity was detected by scanning and evaluated using a perspective TLC program (BF-Vertriebsgesellschaft, Wildbad, FRG). Aliquots of the labeled product were reduced with lithium aluminium hydride, subjected to alkaline hydrolysis or hydrolyzed with phospholipase C, and the reaction products analyzed and characterized as described above.

3. RESULTS AND DISCUSSION

Higher plants do not contain appreciable amounts of ether lipids, if any [11]. Yet, photomixotrophic rape cells in suspension culture transform exogenous 2-*O*-[1′-^{14}C]hexadecylglycerol to 1-acyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholines, 1(3)-acyl-2-*O*-[1′-^{14}C]hexade-

cylglycerols and 1,3-diacyl-2-*O*-[1′-^{14}C]hexadecylglycerols.

The cells readily take up the radioactive precursor from the incubation medium (>97% within 4 h). The time course of biotransformation of 2-*O*-[1′-^{14}C]hexadecylglycerol over a period of 48 h is shown in fig.1. The radioactive substrate is incorporated predominantly into diradylglycerophosphocholines. After 48 h, more than 50% of the radioactivity is found in these ionic ether lipids, whereas other radiolabeled polar lipids amount to around 3% only. Neutral ether glycerolipids, 1(3)-acyl-2-*O*-hexadecylglycerols and 1,3-diacyl-2-*O*-hexadecylglycerols contain about 20 and 10%, respectively, of the total radioactivity.

The radioactive acyl moieties of lipids that had been isolated from rape cells were analyzed after reduction with lithium aluminium hydride. It was found that at maximum 3% of the radioactivity was located in long-chain alcohols derived from acyl moieties of diradylglycerophosphocholines, diradylglycerols and triradylglycerols. Obviously, under the conditions described only small proportions of the radioactive substrate were degraded by oxidative cleavage, even after 48 h of incubation. This finding was confirmed by the results of an analysis of the products obtained by alkaline hydrolysis of radioactive diradylglycerophosphocholines. Radioactive compounds formed by oxidation of the substrate were not detected in the phosphocholine moiety of diradylglycerophosphocholines liberated by enzymatic cleavage at the phosphodiester linkage with phospholipase C.

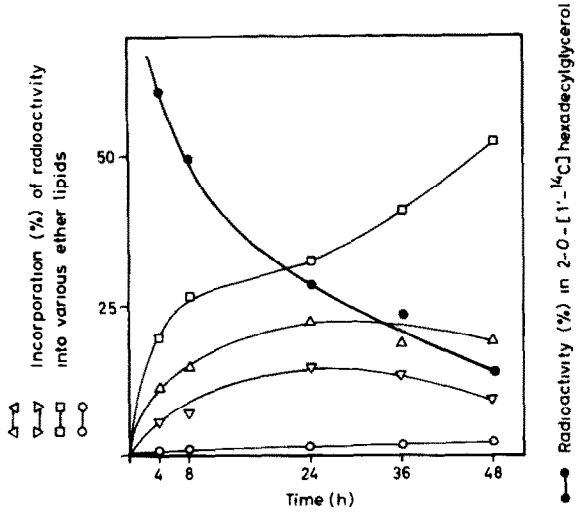

Fig.1. Incorporation of radioactivity from 2-*O*-[1′-^{14}C]hexadecylglycerol into ether lipids of rape cells in suspension culture as well as the decrease of radioactive substrate during incubation (●—●, 2-*O*-[1′-^{14}C]hexadecylglycerol, substrate; △—△, diradylglycerols; ▽—▽, triradylglycerols; □—□, diradylglycerophosphocholines; ○—○, other polar lipids).

Recently, we observed that cholinephosphotransferase of cultured plant cells esterifies phosphocholine moieties to 1,2-diradyl-*sn*-glycerols with high stereospecificity, whereas the corresponding enantiomers, i.e. 2,3-diradyl-*sn*-glycerols, are not utilized as substrates at all [7]. The chiral purity of 1-acyl-2-*O*-hexadecyl-*sn*-glycero-3-phosphocholines, prepared by biotransformation from 2-*O*-hexadecylglycerol using rape cell cultures, was checked by determination of the optical rotation of derivatives, as described in [5].

The chemical synthesis of 2-O-alkyl-1-radyl-*sn*-glycero-3-phosphocholines starts from optically active glycerol derivatives [12]. Syntheses using 2-*O*-alkylglycerols as starting material lead to racemic mixtures. Separation of the two enantiomers is difficult and lowers the yield of the desired stereoisomer having the 'natural' configuration. We have prepared such an analogue of PAF containing a 2-*O*-[1′-^{14}C]hexadecyl moiety by incubation of rape cells with radioactive 2-*O*-hexadecylglycerol. This semi-synthesis leads in remarkably high yield (overall yield 42%) to 1-acyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholines; the method does not require the separation of enantiomers. The radioactive substrate incorporated into alkylacylglycerols and alkyldiacylglycerols can be easily recovered after alkaline hydrolysis and recycled together with the unmetabolized substrate in further incubations to increase the overall yield; an additional 10–15% of 1-acyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholines are thus obtained.

Alkaline hydrolysis of radioactive diradylglycerophosphocholines followed by acetylation of the resulting lyso ether lipid leads to 1-acetyl-2-*O*-[1′-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholine in an overall yield of about 50% (with respect to [1-^{14}C]palmitic acid used). The specific activity (0.98 GBq/mmol) of the added precursor and the resulting metabolites is the same because these

plant cells do not contain ether lipids. The radiochemical purity of the isolated PAF analogue II was better than 98%.

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