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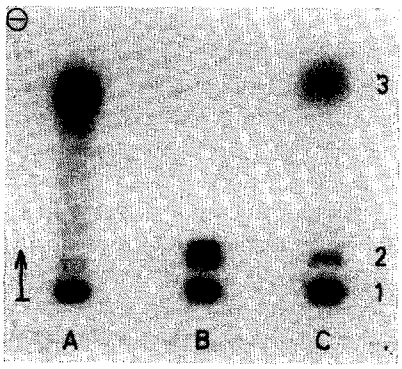
# The conversion of lysophosphoglycerides by homogenates of spinach leaves

In connection with differences in the positional distribution of various fatty acids between several types of phosphoglycerides from photosynthetic tissues[1–3], a study was made on the uptake of labeled fatty acids into the lipids of green leaves *in vitro*. In the course of this work it was observed that the addition of glycero-3-phosphate stimulated predominantly the incorporation into di- and triglycerides, while the presence of monoacyl phosphoglycerides enhanced the esterification of fatty acids into phospholipids. The latter phenomenon may be due to an acylation of monoacyl phosphoglycerides, a reaction first reported by LANDS[4] to occur in liver tissue. Acylation of lyso compounds is now known to be catalyzed by many animal tissues and it was thought to be of interest to verify whether this reaction occurs in the plant domain as well.

To this end [$^{32}$P]lysolecithin and [$^{32}$P]lysophosphatidyl ethanolamine were incubated with homogenates of spinach leaves. These substrates were obtained by enzymatic hydrolysis of lecithin and phosphatidyl ethanolamine from rat liver as described before[5], and both preparations furnished one spot on paper chromatograms and thin-layer chromatograms developed in various systems. An amount of 100 g fresh spinach leaves was homogenized in a Waring blendor with 60 ml of ice-cold 0.02 M Tris buffer (pH 7.4), containing 0.002 M $MgSO_4$. To 1 ml of the filtered homogenate were added: 10 $\mu$moles ATP, 0.2 $\mu$mole CoA and 1 ml of 0.1 M Tris buffer (pH 8) containing a trace amount of [$^{32}$P]lysolecithin or [$^{32}$P]lysophosphatidyl ethanolamine and 0.1 $\mu$mole of oleic acid. The incubation was carried out under a $N_2$ atmosphere at room temperature. The reaction was stopped by pouring the incubation mixture into methanol so as to give a final methanol concentration of 80%. The lipids were extracted according to the method of BLIGH AND DYER[6]. The water layer was found to contain less than 4% of the total radioactivity and was discarded. The lipids were separated by thin-layer chromatography on silica G with chloroform–methanol–25% ammonia (70:20:2, v/v/v) as developer. The radioactive spots were counted in a liquid-scintillation counter. The homogenate of spinach leaves was found to convert lysolecithin into lecithin (Fig. 1B). The same proved to be true for the ethanolamine analogue. The formation of lecithin from lysolecithin reached a maximum after 20–30 min and about 90% of the substrate was converted (Fig. 2). The conversion of lysophosphatidyl ethanolamine proceeded more slowly and the extent of acylation was more limited. When ATP and CoA were not added to the system the conversion of the lyso compounds into the diacyl analogues was significantly impaired. The present results support the conclusion that spinach leaves are capable of forming diacyl phosphoglycerides by an acylation of monoacyl analogues.

In the experiments dealt with above, [$^{32}$P]lecithin or [$^{32}$P]phosphatidyl ethanolamine were the only newly formed radioactive compounds detectable in the lipid fraction after incubation with the corresponding lyso compounds. However, in earlier experiments, in which the acylation reaction was stopped by the addition of only one equal volume of methanol to the incubation mixture, another radioactive spot

was observed which even dominated over that of the lecithin or phosphatidyl ethanolamine (Fig. 1C, Spot 3). This product was not formed when the incubation mixture was heated at 100° for 5 min before the addition of methanol. The spot did not stain with ninhydrin or the Dragendorff reagent and exhibited in several chromatographic



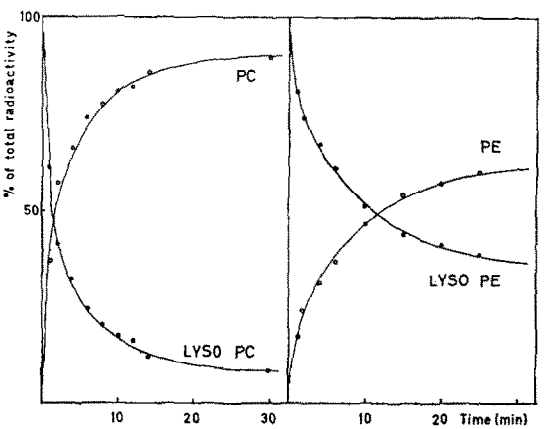




Fig. 1. Autoradiograph of the products of conversion of phosphoglycerides by homogenates of cabbage and spinach leaves. (A) Conversion of lecithin by a homogenate of cabbage leaves in the presence of ether and methanol (for conditions, see text). (B) Acylation of lysolecithin to lecithin by a homogenate of spinach leaves. The incubation was stopped by the addition of methanol to a final concentration of 80%. (C) As for B, but in this case the incubation was stopped by the addition of methanol to a final concentration of 50%. 1A, phosphatidic acid, 1B and 1C lysolecithin; 2, lecithin; 3, phosphatidyl methanol.

Fig. 2. Time-course curve of the conversion of lysolecithin (lyso PC) to lecithin (PC) and of lysophosphatidyl ethanolamine (lyso PE) to phosphatidyl ethanolamine (PE) by a homogenate of spinach leaves.

systems a $R_F$ value which differed from that of phosphatidic acid or its salts, as well as from that of any other known phospholipid. No significant quantities of this compound were detectable when the acylation of lysolecithin or lysophosphatidyl ethanolamine was impaired by the omission of ATP and CoA. These facts suggested that during the lipid extraction under given conditions, lecithin or phosphatidyl ethanolamine can be enzymatically converted into an artificial phospholipid. The elucidation of the nature of this compound was greatly facilitated by the recent work of BENSON,

FREER AND YANG[7] on the phosphatidyl transferase activity of phospholipase D (EC 3.1.4.4). These investigators characterized phosphatidyl ethanol as a major lipid in ethanol extracts of vascular tissues of lettuce and cabbage and also demonstrated the transferase activity in plant tissue by the formation of phosphatidyl isopropanol and phosphatidyl methanol. The alcohol concentrations required for optimal conversion of lecithin to these esters were less than 3%; good yields were, however, obtained with 50% ethanol. That a transphosphatidylation is catalyzed by plant extracts was confirmed in this laboratory by P. P. M. BONSEN and this reaction was found to be very suitable for preparative purposes.

Since in our earlier experiments with homogenates of spinach leaves we tried to stop all enzymic reactions by the addition of methanol, to a final concentration of only 50%, the unknown radioactive compound was suspected to be identical with phosphatidyl methanol. In order to make a comparison with the experiments of BENSON, FREER AND YANG[7], we incubated [$^{32}$P]lecithin from rat liver with 1 ml of an extract of cabbage leaves in 0.1 M sodium acetate buffer (pH 5.8) containing $5 \times 10^{-3}$ M $CaCl_2$ in the presence of 0.05 ml of methanol and 1 ml of ether for 2 h at room temperature. A compound was formed (Fig. 1A, Spot 3) having the same $R_F$ value as the unknown compound detected during the earlier experiments on the formation of lecithin from lysolecithin (Fig. 1C, Spot 3). Chromatographic comparisons with synthetic preparations of phosphatidyl methanol, phosphatidic acid and phosphatidyl glycerol, kindly donated by Dr. BONSEN, confirmed the view that phosphatidyl methanol was formed in the experiments with homogenates of both cabbage and spinach leaves. A considerable conversion of lecithin and phosphatidyl ethanolamine by homogenates of spinach leaves was demonstrated 15 min after the addition of methanol to a final concentration of 50% by volume. The formation of the artifact was not observed at 80% methanol. In this respect it is of interest to note that the hydrolytic action of phospholipase D from spinach chloroplasts was found by KATES[8] to be only partly inactivated under given conditions at 45% methanol, whereas 60% methanol caused a complete inactivation.

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