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Metabolism of lysolecithin and lecithin in a yeast supernatant

Lysolecithin was found to cause a very limited ionic leakage from yeast cells, this in contrast to the strong action of synthetic detergents of the quaternary amine type. With the aid of chromatography on silica-impregnated paper it was demonstrated that after a short incubation time the amount of egg lysolecithin added was greatly diminished, while lecithin was found to be present in the supernatant fluid or adsorbed at the cell surface. This conversion of lysolecithin into lecithin was also effected by a supernatant freed from yeast cells by centrifugation, and this ability appeared to be lost after heating the supernatant for 10 min at 100°. Theoretically the formation of lecithin might proceed by an acylation of lysolecithin as observed by LANDS[1] and WEBSTER[2] to occur in the microsomal and mitochondrial fractions of animal tissues. Since phospholipase C (EC 3.1.4.3.)[3] and choline phosphate cytidyl transferase (EC 2.7.7.15.)[4] have been reported to be present in yeast another pathway may play a part as well. Presuming that phospholipase C-formed monoglyceride is converted into diglyceride, lecithin might be formed according to a synthesis *de novo* as established by KENNEDY[5]. However, our experiments showed that the noticed formation of lecithin from lysolecithin in the yeast supernatant involves a quite different mechanism.

An amount of 100 g of bakers yeast (Koningsgist, Delft) was suspended in 300 ml of water and stored at 0° for 48 h. A tracer amount of [^{32}P]lysolecithin was emulsified with 0.5 ml of the clear yeast supernatant fluid together with 0.2 ml of a 0.1 M phosphate buffer (pH 7.4) and the mixture was incubated under shaking at 37°. At various time intervals adequate samples were subjected to chromatography on silica-impregnated paper[6]. Lysolecithin was found to give rise to the formation of labeled lecithin and a radioactive water-soluble compound, which was demonstrated to be identical to glycerylphosphorylcholine (R_F 0.44, propanol - ammonia - water;

6:3:1, v/v). The latter compound and the fatty acids liberated dominated the amount of the produced lecithin and that of the lysolecithin remaining in the incubation mixture (Fig. 1A). Incubation of the yeast supernatant with glyceryl [^{32}P]phosphorylcholine or [^{32}P]phosphorylcholine together with inactive lysolecithin resulted into the formation of non-radioactive lecithin only. This finding indicates that both water-soluble compounds do not act as intermediates in the conversion of lysolecithin to lecithin. Apparently glycerylphosphorylcholine is formed by an enzyme resembling the action of a lysolecithin acyl-hydrolase (EC 3.1.1.5.). The formation of lecithin, however, appeared not to be brought about by an acylation of lysolecithin with fatty acids liberated during its enzymic breakdown. After incubation of lysolecithin together with 1-^{14}C-labeled fatty acids (palmitate, oleate and linoleate) the formed lecithin did not exhibit any detectable radioactivity. This was also true when the labeled fatty acids were pre-incubated with the yeast supernatant and ATP and CoA were added. The lecithin produced was shown to contain fatty acids identical to those of the lysolecithin used, *viz.* mainly palmitic acid and stearic acid, also when myristic acid was added to the medium. Hence, the conversion of lysolecithin into lecithin does not appear to proceed via an acylation with exogenous fatty acids. This points to an enzymic transacylation involving the transfer of a fatty acid constituent from one lysolecithin molecule to another with the simultaneous production of one molecule of lecithin and glycerylphosphorylcholine. However, the production of glycerylphosphorylcholine turned out to exceed the formation of lecithin (Fig. 1A). On the other hand, if a degradation of newly formed lecithin *e.g.* by the action of phospholipase A (EC 3.1.1.4.) occurred, the proposed mechanism would still agree with the experimental facts.

Indeed, incubation of [^{32}P]lecithin with the yeast supernatant produced both radioactive lysolecithin and glycerylphosphorylcholine. The conversion–time curves strongly suggest the enzymically formed lysolecithin to act as a precursor of glycerylphosphorylcholine (Fig. 1B), although the additional action of a diacyl hydrolase cannot be completely precluded.

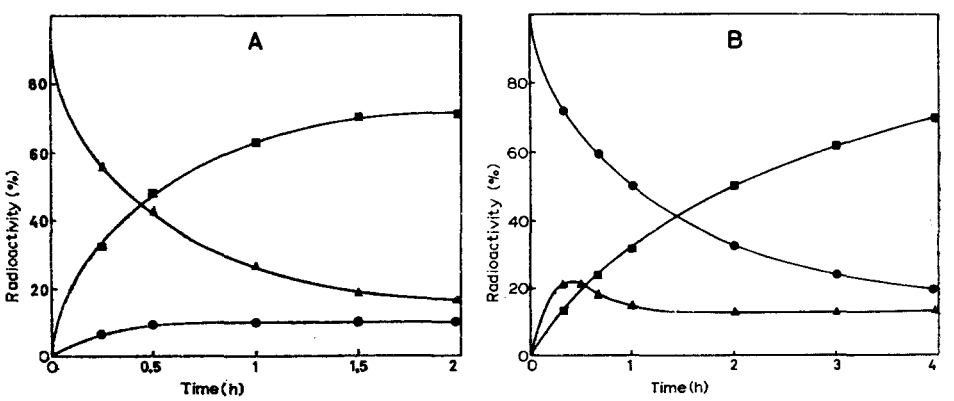

Fig. 1. Demonstration of the metabolism of lysolecithin and lecithin by a yeast supernatant A, time course of the conversion of [^{32}P]lysolecithin (lysophosphatidylcholine; L-PC) (▲—▲) into glycerylphosphorylcholine (GPC) (■--■) and lecithin (phosphatidylcholine, PC) (●—●). B. Time course of the conversion of [^{32}P]lecithin (PC) (●—●) into lysolecithin (L-PC) (▲—▲) and glycerylphosphorylcholine (GPC) (■--■).

The following reactions may account for the observed conversions:

$$\text{Lecithin} \xrightarrow{\text{phospholipase A}} \text{lysolecithin} + \text{fatty acid}$$

$$\text{Lysolecithin} \xrightarrow{\text{acyltransferase}} \text{lecithin} + \text{glycerylphosphorylcholine}$$

In summary, it appears that in the studied yeast supernatant a lysolecithin–lecithin cycle functions, which involves phospholipase A activity and the action of a lysolecithin-acyl transferase. Comparable results were obtained with kephalins as substrates, whereas sphingomyelin was not converted at all. It is worth noting that the conversion of lysolecithin into lecithin was inhibited by the addition of free fatty acids to the yeast supernatant.

A conversion of lysolecithin into lecithin catalyzed by a transacylase was recently supposed by ERBLAND AND MARINETTI[7] to happen in certain cell fractions of rat liver. Furthermore, GLOMSET[8] recently demonstrated the presence of an acyl transferase in plasma, catalyzing the transfer of fatty acids from lecithin to cholesterol. A renewal of fatty acids of phospholipids from red cells was suggested to involve a transerterification of diacyl phosphoglycerides[9].

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R. KOKKE
G. J. M. HOOGHWINKEL
H. L. BOOIJ

Laboratory for Medical Chemistry, State University, Leyden (The Netherlands)

H. VAN DEN BOSCH
L. ZELLES
E. MULDER
L. L. M. VAN DEENEN

Department of Biochemistry, Laboratory of Organic Chemistry, State University, Utrecht (The Netherlands)

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