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Studies on the lecithinase from *Penicillium notatum*

FAIRBAIRN[1] reported that *Penicillium notatum* was an excellent source of a lysophospholipase but it showed no activity towards lecithins and phosphatidylethanolamines. However, according to the studies of DAWSON and his colleagues[2-5] the enzyme preparation from *P. notatum* attacked not only lysolecithin but also 'lecithin' and produced 1 mole of glycerophosphorylcholine, and 1 or 2 mole(s) of fatty acids, respectively. In the latter case, however, 'lecithin' particles must be charged negatively by addition of some anionic amphipathic substances, for example, monophosphoinositide or dicetylphosphoric acid.

Recently, KATES and coworkers[6,7] demonstrated that phospholipase B from *P. notatum* hydrolyzed purified lecithin in the absence of any activators when the substrate was well dipersed, but did not show the effect of such amphipathic activators as dicetylphosphoric acid on the enzyme activity.

The present study was intended to ascertain whether these anionic amphipathic substances are really essential to the enzymatic deacylation of purified lecithin by the crude and partially purified enzyme preparations from *P. notatum*. It was found that both enzyme preparations originally possessed the enzyme activity which catalyzed the deacylation of lecithin and lysolecithin in the absence of these anionic amphipathic activators and without ultrasonically dispersing the substrates. The lecithinase activity, however, was much less than that of lysolecithinase.

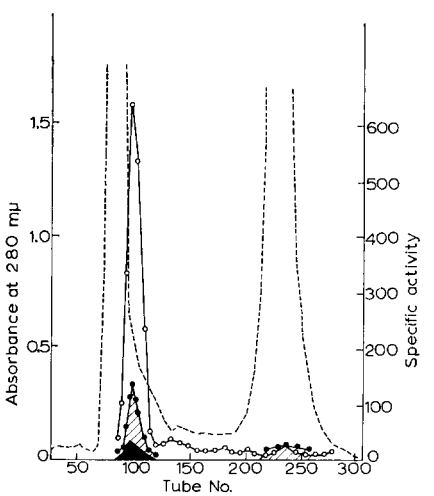
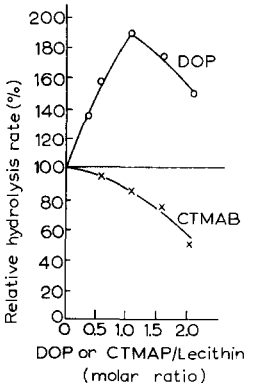


Fig. 1. Purification of lecithinase and lysolecithinase from *P. notatum* on Sephadex G-100. The frozen cells of *P. notatum* (50 g) were homogenized and the supernatant (2.1 g) was applied to the column. Column size was 5.5 cm × 85 cm. Rate of flow was 1.4 ml/min and volume of eluate in each tube was 10 ml. The water used contained EDTA in a final concentration of 10^{-3} M and all procedures were carried out at 4°. Enzyme protein was measured by optical absorption at 280 mμ. Lecithinase and lysolecithinase activities were assayed principally by the method of DAWSON[3], and a unit of these enzymes was expressed as μg phosphorus liberated per mg protein per min and h, respectively. — — —, protein; ———, lysolecithinase; hatched area, lecithinase (without dioleoyl phosphate); black area, lecithinase (with dioleoyl phosphate, in a molar ratio of 1:1).

The partial purification of lecithinase and lysolecithinase from *P. notatum* was undertaken by gel filtration on a Sephadex G-100 column and it was observed that both enzymes were eluted simultaneously from the column and could not be separated from each other (Fig. 1).

The enzyme reaction between ovolecithin and crude enzyme was really activated by dioleoyl phosphate or sodium lauroyl sulfate and inhibited by cetyltrimethylammonium bromide (Fig. 2). On the other hand, in the case of partially purified enzyme



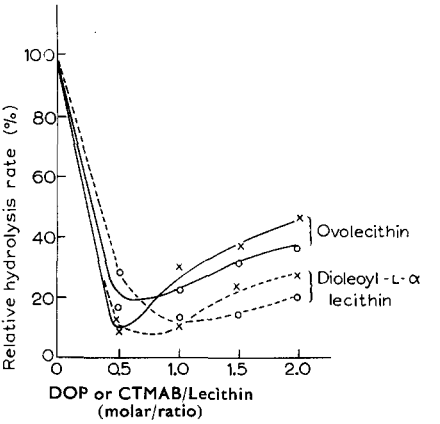


Fig. 2. Effect of dioleoyl phosphate (DOP) and cetyltrimethylammonium bromide (CTMAB) on ovolecithin hydrolysis by crude enzyme preparation from *P. notatum*.

Fig. 3. Effect of dioleoyl phosphate (DOP, ○—○) and cetyltrimethylammonium bromide (CTMAB, ×—×) on the enzymatic hydrolysis of synthetic dioleoyl-L-α-lecithin (- - - -) and ovolecithin (———) by partially purified enzyme preparation from *P. notatum*.

preparation, dioleoyl phosphate no longer activated the enzyme activity towards natural and synthetic lecithins but actually inhibited it as did cetyltrimethylammonium bromide (Fig. 3).

The substrates used here were synthetic dioleoyl-L-α-lecithin, prepared by Drs. F. C. REMAN and B. WITTELS, and ovolecithin, which was prepared using aluminum oxide[8] and silicic acid[9]. Lysolecithin was prepared enzymatically by the technique reported before[10].

As to the mode of action of the enzyme preparation from *P. notatum*, which catalyzed the deacylation of lecithin, it is not yet completely resolved, but the enzymatically liberated water-soluble phosphate compound was identified as glycerophosphorylcholine by paper chromatography, *i.e.*, the incubation mixture, which consisted of lecithin, partially purified enzyme and acetate buffer (pH 4.0) was treated with cationic ion exchange and the filtrate was concentrated and applied to the paper. A single spot was detected and identified as glycerophosphorylcholine by the method of HANES AND ISHERWOOD[11] and the procedures of CHARGAFF, LEVENE AND GREEN[12].

With regard to the differences in these results, particularly on the anionic amphipathic substances, as compared with those of the previous workers, it would be possible to infer that some inhibitory factor(s) to the initiation of the enzyme reaction which was present in the crude preparation would be displaced electro-

statically by these anionic amphipathic activators. Such a factor was removed by partial purification on Sephadex column.

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Fluoroacetyl-CoA as a substrate for malate synthase

The toxicity of fluoroacetate to warm-blooded animals has been traced to a lethal synthesis of fluorocitrate, a potent inhibitor of aconitase[1]. The K_m for fluoroacetyl-CoA in the reaction with oxaloacetate catalyzed by citrate synthase was found[2] to be $2.5 \cdot 10^{-5}$ M, the same as that for acetyl-CoA in this reaction.

Acetyl-CoA is a substrate for an analogous reaction catalyzed by malate synthase (L-malate glyoxylate-lyase (CoA-acetylating), EC 4.1.3.2), in which glyoxylate is the acetyl acceptor. DIXON, KORNBERG AND LUND[3] in examining the substrate specificity of this enzyme, observed that fluoroacetyl-CoA was cleaved at a rate "approximately one-quarter of the rate observed with similar concentrations of acetyl-CoA".

We report here the results of experiments in which the kinetics of the reaction with fluoroacetyl-CoA are compared to those with acetyl-CoA as substrate. The malate synthase employed was that present in glyoxysomes prepared from the endosperm tissue of germinating castor beans[4], with a specific activity of 0.6–1.4 μmoles/min per mg protein.

Enzyme activity was measured by following the rate of appearance of SH resulting from the cleavage of acetyl-CoA in the presence of glyoxylate[5], with correction, as necessary, for the rate observed before addition of glyoxylate. In the ab-