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EFFECT OF METHANOL ON ENZYMATIC SYNTHESIS OF PHOSPHATIDYLINOSITOL

M. M. Abdullaeva

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The effect of methanol on enzymatic synthesis of phosphatidylinositol catalyzed by phospholipase D was studied. It was found that methanol enhances the transferase reaction under in vitro conditions to form the unnatural phospholipid phosphatidylmethanol.

Key words: enzymatic synthesis, phospholipids, inositol, phosphatidylinositol.

Inositol is produced via hydrolytic cleavage of phytin by an activated form of phytase [1]. More than 20% of metabolized inositol is found in phospholipids as phosphoinositides and polyphosphoinositides [2]. It is thought that the transformation of inositol into phosphatidylinositol (PI) occurs via its reaction with cytidinediphosphoacylglycerol [3]. However, based on the fact that phospholipase D (PL-D) can catalyze transalkylation of phospholipids in the presence of alcohols [4, 5], it was proposed that this enzyme may be involved in the biosynthesis of PI [1]. According to this hypothesis, inositol formed from phytin should enter into exchange reactions with cotton phospholipids and replace alcohols in the polar part of the phospholipids to form PI and the corresponding alcohols. Direct proof of this hypothesis has not been published.

Compounds that can compete with inositol in transalkylation reactions catalyzed by PL-D must be selected in order to accomplish this.

Therefore, we studied the role of methanol, which can compete with inositol in transalkylation reactions.

The investigations showed that methanol was the best alcohol substrate in model experiments with PL-D. It can compete with inositol in transalkylation reactions to form phosphatidylmethanol (PM), a phospholipid that does not occur naturally. This hypothesis can be proved if methanol does actually affect enzymatic synthesis of PI, which is formed from phospholipid and inositol, in model experiments.

Incubation of phosphatidylcholine (PC), inositol, and PL-D enzymatic preparation formed in the reaction mixture the new phospholipid PI. Next we studied the effect of methanol, which can effectively react by enzymatic transalkylation of phospholipids to synthesize PI. Fig. 1a shows that the amount of PI formed without methanol is less than 12% of its starting amount if the enzymatic preparation is incubated with PC and inositol.

Adding methanol to the reaction mixture led to the formation of the new phospholipid PM. Methanol at concentrations of 0.6-0.8% increased the amount of synthesized PI to 45-50%. PM was formed in the reaction mixture in addition to PI (Fig. 1a, 2). The synthesis of PI was suppressed with predominant formation of PM if the methanol concentration was increased above 2%.

Unidentified P-containing compounds were also formed in the reaction mixture at higher methanol concentration (Fig. 1a, 5). It is possible that these are inositoldi- and inositolpolyphosphatides. It is interesting that the content of phosphatidic acid (PA) under these conditions decreased to 5-7% of the initial value (Fig. 1a, 4), i.e., practically only transferase reactions occurred under the selected experimental conditions.

If inositol is added to the reaction mixture with methanol, PI does not form (Fig. 1b, 1). Furthermore, inositol in small quantities stimulated the formation of PM (2). The PC content decreased practically to zero (3). High inositol concentrations slightly inhibited the formation of PM. However, this is due to a hydrolytic reaction (Fig. 1b, 4).

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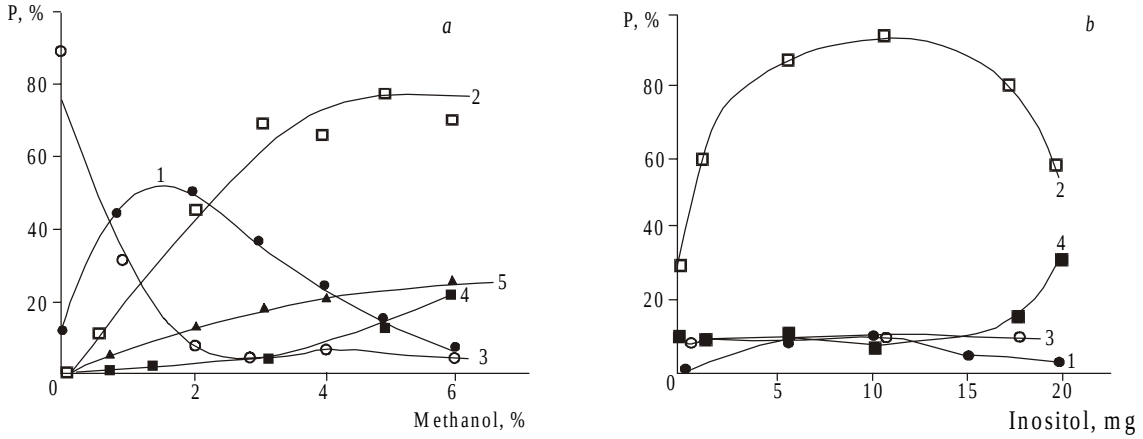

Fig. 1 Effect of methanol (a) and inositol (b) on phospholipid content during transferase reaction. PI (1), PM (2), PC (3), PA (4), unidentified P-containing products (5).

The results lead to the conclusion that the transalkylation occurs under various conditions. PI can be effectively synthesized from inositol and other phospholipids. Methanol can compete with inositol in transferase reactions in various model systems. Methanol itself is the most effective compound that can be recommended as a specific substrate that can affect the transferase reaction under in vivo conditions to form the unnatural phospholipid PM.

EXPERIMENTAL

We used PL-D isolated from cotton seeds of variety 108-F [1]. Partially purified PL-D preparation was prepared by treating dormant seeds with liquid nitrogen, removing the husk, grinding, and defatting with anhydrous acetone and diethylether. The resulting powder was suspended in a 10-fold excess of Tris-HCl buffer (0.01 M) at pH 9.2 and incubated for 4 h at 0-4°C. The extract was separated by centrifugation (6000 g, 15 min) and treated with twice the volume of acetone cooled to -10°C. The solid was separated by centrifugation (2000 g, 15 min), washed with anhydrous acetone, and suspended in doubly distilled water. The extract was separated (18000 g, 15 min) and lyophilized.

Transferase activity of PL-D was determined from the rate of PC transalkylation in the presence of methanol, inositol, or other alcohol substrates. The standard reaction mixture (total volume 2.5 mL) contained inositol (5 mM), PC, absolute methanol, $CaCl_2$ (30 mM), silica gel (40 mg), sodium-acetate buffer (200 μM, pH 5.6), and enzyme preparation (50 mg) and was incubated at 30°C for 20 min. The course of the reaction was monitored by two-dimensional TLC [1] using first the solvent system $CHCl_3$:CH_3OH:NH_4OH (25%) (13:5:1) and then $CHCl_3$:$(CH_3)_2CO$:CH_3OH:CH_3CO_2H:H_2O (10:4:2:2:1). We used pure PC, PI, PM, and PA synthesized using PL-D from radish [6, 7] as chromatographic standards. Phospholipid compounds on chromatograms were developed using color reactions [8]. Quantitative analysis of the isolated phospholipids was based on P [9].

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