

Effects of Phospholipids in the Action of Acetyl-CoA Carboxylase from Rat Liver

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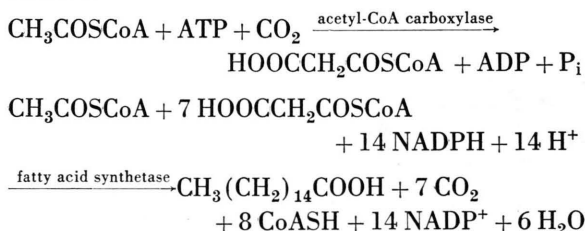
Acetyl-CoA carboxylase (E.C. 6.4.1.2) was isolated from rat liver. The purified enzyme contains phospholipids with a rather large amount of phosphatidylinositol (26%). Incubation of the purified acetyl-CoA carboxylase with phospholipase A₂ (E.C. 3.1.1.4) or with phospholipase D (E.C. 3.1.1.4) diminishes the phospholipid content by 70%, this treatment leading to a complete inactivation of the enzyme. After removal of the phospholipases, the lipid-depleted enzyme can be reactivated to a certain degree by incubation with a phospholipid extract from rat liver, with phosphatidylinositol alone, or with serum albumin.

Introduction

There have been many reports of enzymes which require phospholipids for their activity (reviewed by Coleman¹, Hallinan²). Removal of phospholipids from these enzymes, usually by detergents or phospholipases, led to a loss in activity. In many cases, it has been shown that enzyme activity could be restored by adding back dispersions of phospholipids, e.g. for the Na,K-ATPase from different sources^{3–8}, the Ca-ATPase from *E. coli*⁹, the acetylcholinesterase from human erythrocytes¹⁰, or the aminopyrine demethylase from liver endoplasmic reticulum². It has often been reported that only a special phospholipid or a special group of phospholipids was able to restore the activity of the lipid-depleted enzyme. For example, phosphatidylserine was found to be essential for the Na,K-ATPase^{3–6}, phosphatidylethanolamine for the Ca-ATPase from *E. coli*⁹, and phosphatidylcholine for the aminopyrine demethylase from liver endoplasmic reticulum². The molecular mechanism of phospholipid action with the enzymes is not yet completely understood, although it seems to be well established that phospholipids not only function as effectors which can be reversibly bound to the enzyme, but also as integral part of the enzyme itself, by forming a lipoprotein-complex (e.g.^{9,10}).

We investigated the role of phospholipids in acetyl-CoA carboxylase, which catalyzes the first step of fatty acid synthesis according to the following

scheme:



Materials and Methods

Purification of the enzyme

The acetyl-CoA carboxylase from rat liver (strain DA) was purified according to the method of Inoue and Lowenstein¹¹ with modifications. The specific activity of our preparation was 3.2 units/mg protein, expressed in μmol malonyl-CoA formed at 25 °C per min. The purified enzyme preparation exhibited only one peak on a column of Sepharose 4B or Sephadex A 25.

Measurement of activity

The activity of the acetyl-CoA carboxylase was determined in an Eppendorf photometer at 334 nm by measuring the decrease in NADPH₂ in the presence of fatty acid synthetase from yeast. The reaction mixture contained 16.5 mM NADPH₂, 13 mM MgCl₂, 16.5 mM imidazole pH 7.1, 0.1 mM EDTA, 33 mM KHCO₃, 33 mM sodium citrate, 60 mU fatty acid synthetase, 0.33 mM acetyl-CoA and 2 mM ATP. The total volume was 310 μl .

Treatment with phospholipase A₂ and phospholipase D

The enzyme preparation was incubated with phospholipase A₂ or phospholipase D in a medium con-

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taining 0.1 mM EDTA, 10 mM $MgCl_2$, 50 mM $CaCl_2$ in a total volume of 1.07 ml. After incubation for the indicated times, the phospholipases had been removed by ion-exchange chromatography with Sephadex A 25.

Protein assay

The protein content was determined according to the method of Schacterle and Pollack¹². Bovine serum albumin served as standard.

Phospholipid extraction and analysis

All samples were lyophilized. The phospholipids were extracted with chloroform/methanol (2:1, v/v) as described by Folch *et al.*¹³. The determination of the individual phospholipids was performed by quantitative thin-layer chromatography according to the method of Peter and Wolf¹⁴.

Reagents

Acetyl-CoA was synthesized in our laboratory according to the method of Simon and Shemin¹⁵. Fatty acid synthetase was purified from baker's yeast according to the method of Lynen¹⁶. Phospholipids were purchased from Serva, Heidelberg, Germany. Bovine serum albumin was obtained from Behringwerke, Marburg, Germany. The phospholipases A_2 and D and coenzyme A were from Boehringer, Mannheim, Germany as well as NADPH₂ and ATP. DEAE-Sephadex and Sepharose 4B were from Pharmacia, Uppsala, Sweden. Silica gel plates and all other reagents were purchased from Merck, Darmstadt, Germany.

Results

Although the purification of the acetyl-CoA carboxylase from rat liver involved 26 steps, including ammonium sulfate fractionations and column chromatography with DEAE-Sephadex and with Sepharose 2B, the isolated enzyme contained phospholipids. The total amount was 796 $\mu g/mg$ protein, and the relative distribution of the individual phospholipids was 26% phosphatidylinositol, 42% phosphatidylethanolamine, 25% phosphatidylcholine, 4.6% sphingomyelin and 2.4% phosphatidylserine. In comparison with the phospholipid composition of the rat liver, with 3.8% phosphatidylinositol, 22.9% phosphatidylethanolamine, 63% phosphatidylcholine, 5.2% sphingomyelin, 3.2% phosphatidylserine and 1.9% phosphatidic acid, a striking enrichment of phos-

phatidylinositol took place, which is obviously bound more tightly to the acetyl-CoA carboxylase than the other phospholipids. In order to check whether or not the phospholipids bound to the acetyl-CoA carboxylase preparation are essential for the enzyme activity, we destroyed them with phospholipase A_2 or phospholipase D. As shown in Figs 1 and 2, this treatment causes a loss in the activity.

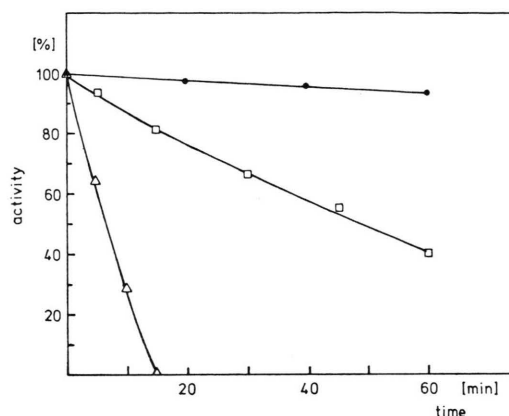


Fig. 1. Influence of phospholipase D on the acetyl-CoA carboxylase preparation. The conditions for incubation are described in 'materials and methods'. □ 400 mU phospholipase D; △ 600 mU phospholipase D; ● control without phospholipase.

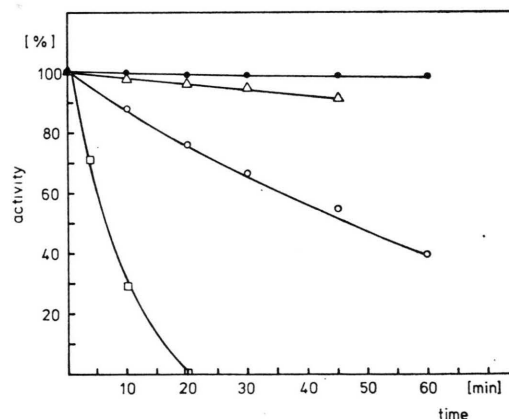


Fig. 2. Influence of phospholipase A_2 on the acetyl-CoA carboxylase preparation. The conditions for incubation are described in 'materials and methods'. △ 100 mU phospholipase A_2 ; ○ 200 mU phospholipase A_2 ; □ 500 mU phospholipase A_2 ; ● control without phospholipase.

We also analyzed the phospholipid composition after the phospholipase treatment. From these results, which are presented in Fig. 3, one can see that the two phospholipases act in nearly the same manner.

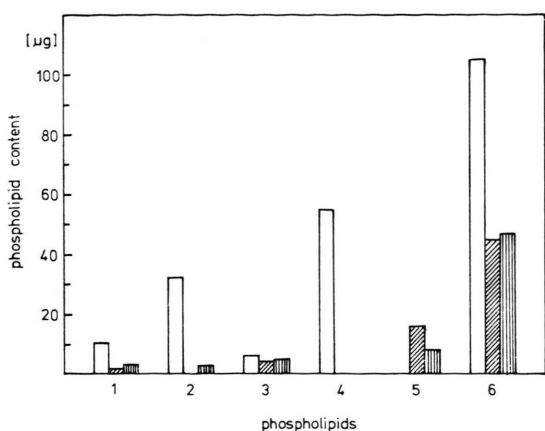


Fig. 3. Phospholipid composition of the acetyl-CoA carboxylase preparation before □ and after incubation with phospholipase D ▨ and phospholipase A₂ ▩. 1 Sphingomyelin, 2 phosphatidylcholine, 3 phosphatidylserine, 4 phosphatidylinositol, 5 phosphatidic acid, 6 phosphatidylethanolamine.

In both cases, phosphatidylinositol had been destroyed completely. The phospholipases did not contain any proteases, so that the fall in acetyl-CoA carboxylase activity could only be due to the reduced phospholipid content.

After the removal of the phospholipases (see 'Materials and Methods') we tried to reactivate the

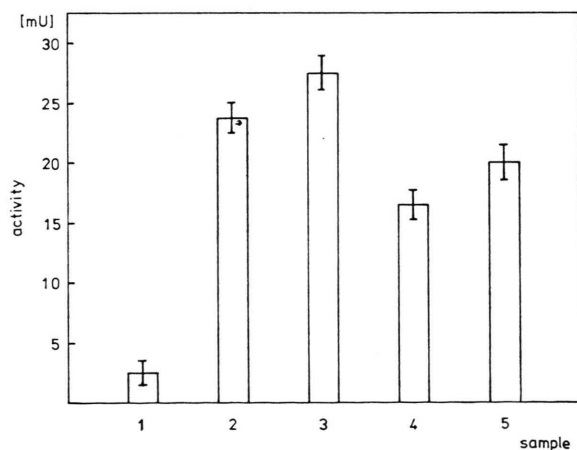


Fig. 4. Reactivation of the acetyl-CoA carboxylase which had been phospholipid-depleted with a mixture of phospholipase A₂ and phospholipase D. The data are averages of 4 experiments. 1 Activity of the lipid-depleted enzyme. The activity before lipase-treatment was 49 mU. 2 Incubation for 5 hours with a phospholipid extract from rat liver (225 µg phospholipids/ml). 3 Incubation for 3 hours with 138 µg phosphatidylinositol/ml. 4 Incubation for 3 hours with 0.5 mg bovine serum albumin/ml. 5 Incubation for 3 hours with 1 mg bovine serum albumin/ml.

lipid-depleted enzyme with a phospholipid extract from rat liver, with individual commercial phospholipids and with bovine serum albumin. It can be seen from Fig. 4 that the phospholipid extract is able to reactivate the enzyme. Of all the individual phospholipids, only phosphatidylinositol applied in concentrations of 200 nmol/mg protein could reactivate the enzyme. All other phospholipids, namely sphingomyelin, phosphatidylcholine, phosphatidic acid, were ineffective or, in the cases of phosphatidylethanolamine and phosphatidylserine, were slightly inhibitory (Fig. 5). Interesting-

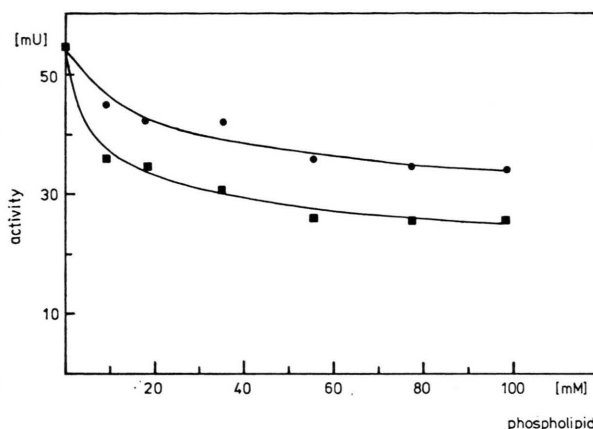


Fig. 5. Influence of phosphatidylethanolamine ● and phosphatidylserine ■ on the acetyl-CoA carboxylase, which had been partially phospholipid-depleted with phospholipase A₂ and D.

ly, serum albumin, which is usually added to the test medium for measuring acetyl-CoA carboxylase activity, was also able to reactivate the phospholipid-depleted enzyme to a certain degree (Fig. 4). In this connection it seems worth mentioning that we also found phospholipids in the serum albumin, with a rather high percentage of phosphatidylinositol: 20.9% phosphatidylinositol, 21.3% phosphatidylcholine, 11.9% phosphatidylethanolamine, 47% phosphatidylserine, 4.4% sphingomyelin, 37.2% phosphatidic acid. The total amount of phospholipids was 4 µg/mg protein.

Discussion

Our results show that the acetyl-CoA carboxylase from rat liver is a lipoprotein with phosphatidylinositol as the phospholipid component. There is

one indication in a previous work of a relationship between acetyl-CoA carboxylase and phospholipids: Forster and McWhorter¹⁷ found that the fatty acid synthesis can be stimulated by a phospholipid extract from microsomes, this stimulation being due to an activation of the acetyl-CoA carboxylase.

It seems to be reasonable to assume that the acetyl-CoA carboxylase 'in vivo' is membrane-bound, the phosphatidylinositol component of the enzyme being also a part of the membrane itself. This theory is supported by our finding that the purified acetyl-CoA carboxylase contained all the phospholipids which occur in the membrane. How-

ever, we cannot exclude the possibility that these phospholipids other than phosphatidylinositol may be impure elements.

At present, we are not able to interpretate the molecular function of phosphatidylinositol in acetyl-CoA carboxylase. Possibly it favours the polymeric structure of the acetyl-CoA carboxylase, which is known to be the active form of the enzyme, or it may function as an allosteric effector.

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