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BINDING OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE TO PHOSPHOLIPID LIPOSOMES

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Summary

The binding of glyceraldehyde-3-phosphate dehydrogenase prepared from rabbit muscle to phospholipid model membranes (liposomes) as a function of pH, ionic strength, and the influence of the binding on specific activity of the enzyme was studied. The binding decreases the specific activity of the enzyme. The binding was studied by the method of association of the enzyme with liposomes during centrifugation. The existence of a dominant interaction of electrostatic character was found.

Introduction

The interaction between lipids and proteins as structural components of biological membranes is an important and still unsolved problem in studies of the structure and function of the membranes. Not only structural unsolvable proteins are important for membrane function but also water-soluble proteins, which can bind to membranes. During the last few years some glycolytic enzymes regarded as typically cytoplasmic proteins have been found associated with cellular membranes [1,2]. Glyceraldehyde-3-phosphate dehydrogenase is an example of erythrocyte membrane associated protein [3]. Its association with the membranes depends on the ionic strength and pH [4]. Moreover, these enzymes have been found to change their activity on liberation from membrane [4,5].

It is also of interest to investigate the interaction of the enzymes with artificial membranes. As model membranes phospholipid liposomes were used because many of their properties imitate the properties of biological membranes [6,7]. In this investigation we studied the binding of glyceraldehyde-3-phosphate dehydrogenase to liposomes made of various pure phospholipids as a function of various conditions: pH, salt concentration, protein concentra-

tion and changes of specific activity of the enzyme when being adsorbed to liposomes.

Materials and Methods

Chemicals. The glyceraldehyde-3-phosphate dehydrogenase was prepared from rabbit muscle according to Kochman and Rutter [8]. It was three times recrystallized from ammonium sulphate solutions. Phosphatidylcholine was extracted from egg yolks and purified on aluminium oxide and silicic acid columns according to Dawson [9]. The commercial preparations of phosphatidylinositol and phosphatidylserine extracted from bovine brain by Koch-Light Laboratories Ltd. were used without further purification. Purity of all phospholipid preparations was tested by thin layer chromatography. Small amounts of contaminants were detected in commercial preparations.

Preparation of phospholipid suspensions. To prepare the phospholipid suspensions, dry phospholipids were suspended in buffered medium with KCl of appropriate concentration by mechanically shaking with glass beads for 45 min at room temperature. The procedure produces multilamellar liposomes of varying sizes, mostly within the range of 500 Å to a few micrometers [10]. The solution of 10 mM Tris/Cl 1 mM β-mercaptoethanol was used as buffer. To ensure stabilization the suspensions were prepared 24 h before using. Lipid suspensions obtained by sonication for 20 min (MSE ultrasonic disintegrator, 20 kHz) were used for activity measurements.

Determination of lipid concentration. In all our experiments the lipid concentration was calculated from phosphorus determination according to Bartlett [11] assuming 4% as average phosphorus content in the weight of phospholipids.

Activity measurements. Specific activity of the enzyme was determined spectrophotometrically by measuring the increase of the NADH extinction at 340 nm as a function of time [12]. The measurements were made on the SP-800 Unicam spectrophotometer. The liposomes were incubated with the enzyme for 30 min at 20° C in pH 7.5 Tris/Cl buffer. The incubated mixtures contained liposomes of 1 mg/ml concentration in 0.08 M KCl and appropriate amounts of protein. For control, the activity was measured for each protein concentration value.

Determination of protein concentration. In centrifugation studies the concentration of the protein was determined by the Lowry's method [13] in supernatant. Glyceraldehyde-3-phosphate dehydrogenase of the known concentration (determined by extinction measurement at 280 nm) was used as the standard.

Adsorption of the enzyme to liposomes. The enzyme and the liposomes were incubated at room temperature for 30 min, then centrifuged for 60 min at 100 000 $\times g$, the MSE-50 centrifuge being used. After centrifugation lipid and protein concentration were determined in supernatant. Lipid and protein contents of the pellets were calculated from the difference between the initial concentration and that in the supernatant. For control, centrifugation of enzyme alone was made and no amounts of the enzyme were found in the pellet. The dependence of the adsorption on protein concentration, pH and

KCl concentration was studied. To obtain proper pH values, 10 mM Tris/Cl, 1 mM EDTA, 1 mM β-mercaptoethanol buffers adjusted with 1 M HCl were used in all experiments.

Results and Discussion

Recently, Wooster and Wrigglesworth [14] have published their studies on the influence on specific activity of glyceraldehyde-3-phosphate dehydrogenase adsorption to liposomes. They studied liposomes made of a mixture of phosphatidic acid and phosphatidylcholine. The authors have suggested the occurrence of only multiple electrostatic interactions. Effect of adsorption on enzyme activity was explained as being the result of local changes in the microenvironment of adsorbed enzyme molecules. On the basis of reversibility of interaction the authors excluded conformational changes of the enzyme. In our work we have studied the adsorption of the rabbit muscle glyceraldehyde-3-phosphate dehydrogenase to liposomes made of pure phospholipids negatively charged and zwitterionic in water systems. The amount of the enzyme in the pellet after centrifugation with liposomes as a function of the initial amounts of protein reveals the difference between the effects of negative and zwitterionic liposomes (Fig. 1). Phosphatidylserine and phosphatidylinositol carry the same net negative charge per molecule in this pH range, but the binding ability of the enzyme to phosphatidylserine is rather similar to that of phosphatidylcholine. Almost all of the added amount of the enzyme goes to the pellet during centrifugation together with phosphatidylinositol, whereas in the case of phosphatidylserine the amount of the precipitating protein is about 67% for concentrations below saturation of the liposomes. The amount of protein saturating the liposomes are quite different for phosphatidylinositol and phosphatidylserine. It may not be explained on the basis of existence only multielectrostatic interactions. Increasing pH in the range 6—9 results in the

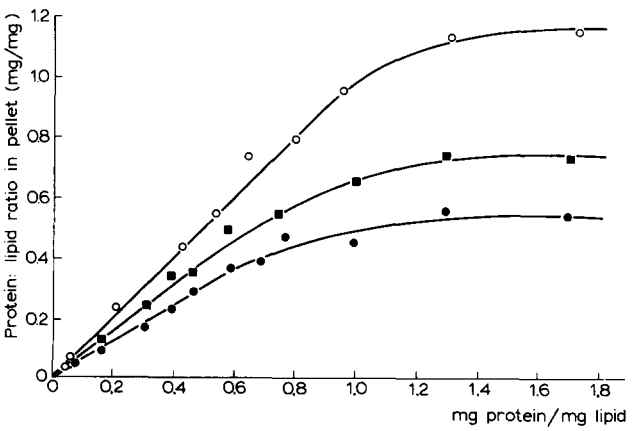

Fig. 1. Binding of glyceraldehyde-3-phosphate dehydrogenase to liposomes as a function of the protein amount. Phospholipid concentration was 1 mg/ml, pH 7.5, KCl concentration 0.08 M, volume 3.5 ml. ○———○, phosphatidylinositol; ■———■, phosphatidylserine; ●———●, phosphatidylcholine, unsonicated liposomes.

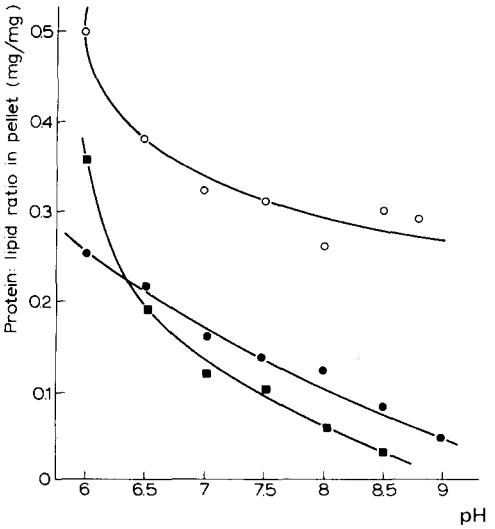

Fig. 2. Influence of pH on the binding of glyceraldehyde-3-phosphate dehydrogenase to liposomes. Phospholipid concentration was 1 mg/ml, protein concentration 0.85 mg/ml, KCl concentration 0.08 M. ○———○, phosphatidylinositol; ■———■, phosphatidylserine; ●———●, phosphatidylcholine, unsonicated liposomes.

decrease of the amount of the precipitating protein together with phospholipids (Fig. 2). The properties of the enzyme are stable in that pH range [15]. No influence of the enzyme on the quantity of sedimenting liposomes was found at pH values in that region. Here again unexpected behaviour of phosphatidylserine can be observed. Its binding ability decreased quickly with

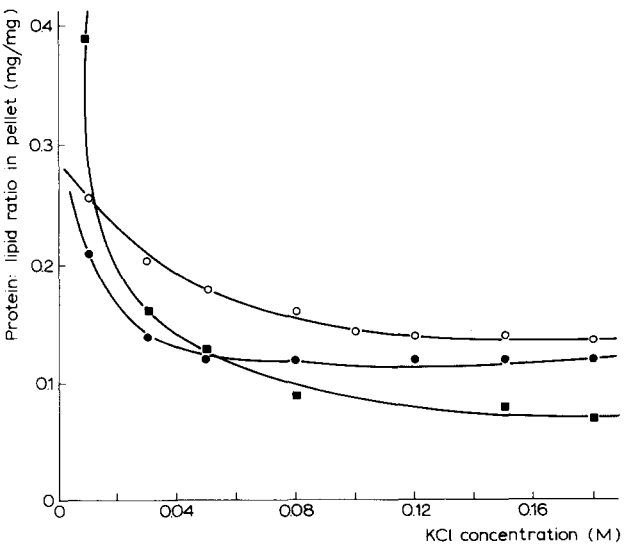

Fig. 3. Influence of KCl concentration on the binding of glyceraldehyde-3-phosphate dehydrogenase to liposomes. Phospholipid concentration 0.85 mg/ml, protein concentration 0.7 mg/ml, pH 7.5. ○———○, phosphatidylinositol; ■———■, phosphatidylserine; ●———●, phosphatidylcholine, unsonicated liposomes.

increasing pH and above pH 6.5 decreased below the values for phosphatidylcholine, although its negative net charge did not disappear. The dependence on salt concentration of enzyme binding to liposomes is shown in Fig. 3.

Adsorption of the protein to liposomes decreases with increasing the KCl concentration up to 0.04 M and 0.1 M for phosphatidylcholine and phosphatidylinositol or phosphatidylserine, respectively, and is constant for higher KCl concentrations. The data proved the existence of binding of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase to liposomes, both neutral and electrically charged. Dependence of the association on pH, salt concentration and surface charge of liposomes indicates the existence of coulombic type of interaction as dominant. Increasing the salt concentration results in the decrease of the electric surface potential of liposomes, causing decrease in association of the protein with liposomes (Fig. 3). The dependence curves of association of the enzyme with liposomes on salt concentration is typical for coulombic interactions, but not in the whole range of concentrations. Over 0.08 M KCl concentration increase does not influence the binding of the enzyme to liposomes. The specific activity decreases hyperbolically with the increasing amount of lipid per mg of the enzyme (Fig. 4). Activity of free enzyme in studied concentration was assumed as 100%. Acidic phospholipids, phosphatidylinositol and phosphatidylserine, diminished the activity almost identically, whereas the effect of neutral phosphatidylcholine was considerably smaller.

The inactivation of glyceraldehyde-3-phosphate dehydrogenase by its association on liposomes may result from several effects: (1) the change in conformation of the enzyme adsorbed to liposomes, (2) less accessibility of active sites of the enzyme for substrates, (3) local changes in microenvironment of the enzyme due to electrostatic interactions, e.g. changes of local concentrations of substrate, pH etc. A combination of these effects may take place too.

In the light of these experiments it is evident that net charge of the liposomes plays the dominant role in the interactions. However, it seems that occurrence of the other interactions (e.g., hydrogen bonds, charge transfer) should be taken into consideration. Specificity of chemical groups in the polar

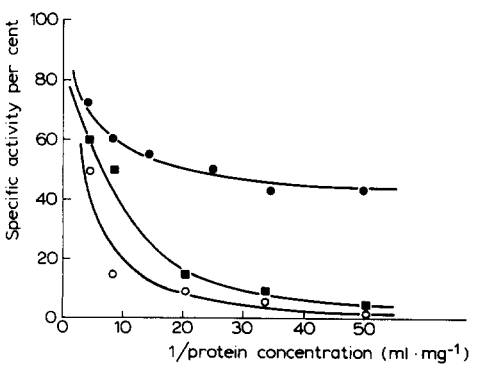

Fig. 4. Inactivation of glyceraldehyde-3-phosphate dehydrogenase in the presence of phospholipid as a function of protein concentration. Phospholipid concentration was 1 mg/ml, KCl concentration 0.08 M, pH 7.5. ○———○, phosphatidylinositol; ■———■, phosphatidylserine; ●———●, phosphatidylcholine, liposomes were sonicated for 20 min.

part of the lipids is responsible for occurrence of the interactions. It may explain different binding abilities of charged lipids. Further studies involving spectroscopic techniques are necessary to resolve this problem in detail.

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References

1 Green, D.I., Murer, E., Hulton, H.O. and Baum. H. (1965) Arch. Biochem. Biophys. 112, 635–647
2 Mitchell, C.D., Mitchell, W.B. and Hanahan, D.J. (1965) Biochim. Biophys. Acta 104, 348–358
3 Nilson, O. and Ronquist, G. (1969) Biochim. Biophys. Acta 183, 1–9
4 Duchon, G. and Colier, H.B. (1971) J. Membrane Biol. 6, 138–157
5 Yu, J. and Steck, T.L. (1975) J. Biol. Chem. 250, 9176
6 Bangham, A.D. and Horne, R.W. (1964) J. Mol. Biol. 8, 660–668
7 Bangham, A.D., Standish, M.M. and Watkins, J.C. (1965) J. Mol. Biol. 13, 238–252
8 Kochman, M. and Rutter, W.J. (1968) Biochemistry 7, 1671–1677
9 Dawson, R.M.C. (1963) Biochem. J. 88, 414–423
10 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–638
11 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
12 Velick, S.F. (1955) Methods Enzymol. 1, 401
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
14 Wooster, N. and Wrigglesworth, J.M. (1976) Biochem. J. 159, 627–631
15 Elödi, P., Jécsai, Gy. and Mozolowsky, A. (1960) Acta Phys. Acad. Sci. Hung. XVII, 165–173