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INTERACTION OF RABBIT MUSCLE ALDOLASE WITH PHOSPHOLIPID LIPOSOMES

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Summary

The interaction between rabbit muscle fructose diphosphate aldolase and phospholipid model membranes (liposomes) was studied by measurement of the tryptophan fluorescence of the enzyme. Interaction with liposomes decreases intrinsic fluorescence intensity of the enzyme and shifts the emission wavelength maximum to higher values. The effects appear to be strongly dependent on the nature of the phospholipid polar group and on ionic strength. Also, a reversible modification of specific activity of aldolase upon interaction with liposomes was found. It is postulated that aldolase binds to liposomes mainly by electrostatic interactions and that the binding causes a change in the conformation of the enzyme.

Fructose diphosphate aldolase is one of several glycolytic enzymes that are known to bind to cellular membranes [1,2]. For example, aldolase has been found to be associated with human erythrocyte membranes [3] and microsomal membrane fraction from rat brain [4]. Association of the enzyme with membranes appears to be reversible and dependent on pH, ionic strength of medium and concentration of substrate. The enzyme increases its activity on liberation from membranes [3,5]. The phenomenon may be important in the regulatory processes of cells. Some recent studies have suggested that the protein fraction of membranes is the binding site for the enzyme [5,6]. However, it seems that the significance of the interaction with phospholipids for binding of the enzyme to membranes is not yet sufficiently explained.

A number of investigators have recently shown that various water-soluble proteins can also bind to phospholipid model membranes [7—11].

In this paper we report results from measurements of the changes in the enzymic activity and tryptophan fluorescence of aldolase adsorbed on phosphatidylcholine and phosphatidylinositol liposomes.

The phospholipid liposomes were prepared according to Brunner et al. [12]. Dry phospholipids were suspended in 10 mM Tris-HCl/1 mM EDTA buffer (pH 7.5) by mechanically shaking with glass beads for 30 min. To the milky phospholipid dispersions potasium cholate was added to final concentration of 2%.

After overnight incubation the mixtures were passed through Sephadex G-50 column to remove cholate. This procedure produces single-shelled liposomes to uniform size (diameter approx. 300 Å) [12].

The commercial preparations of egg yolk phosphatidylcholines and of bovine brain phosphatidylinositol (Koch-Light Laboratories Ltd.) were used. The phosphatidylcholines were chromatographically pure and neglible amounts of contamitants were detected by thin-layer chromatography in phosphatidylinositol preparation. Concentration of the phospholipids was calculated from phosphorus determination by the method of Bartlett [13].

Fructose-1,6-bisphosphate aldolase was prepared from rabbit muscle according to Penhoet et al. [14]. In all experiments, the concentration of aldolase was determined spectrophotometrically using $E_{280}^{1\%} = 0.91$, an extinction coefficient according to Baranowski et al. [15].

The specific activity of aldolase was determined spectrophotometrically by measuring the increasing absorbance at 240 nm according to the hydrazine sulphate test of Jaganathan et al. [16]. Control samples of the mixture of the protein and lipid did not change their absorbance at 240 nm after incubation time.

Fluorescence measurements were performed on Perkin-Elmer MPF-3L spectrofluorimeter. All spectra were automatically corrected and performed at 20° C with an excitation wavelength of 290 nm.

Fig. 1 shows the changes in specific activity of aldolase with increasing

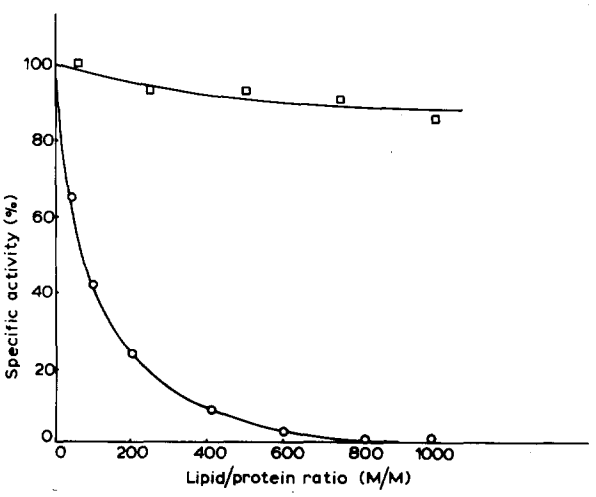

Fig. 1. Inactivation of aldolase in the presence of phospholipids as a function of molar lipid/protein initial ration. The enzyme in concentration 0.16 mg/ml was incubated with the appropriate amounts of liposomes for 30 min in 10 mM Tris-HCl/1 mM EDTA buffer (pH 7.5) at room temperature. ○———○, phosphatidylinositol; □———□, phosphatidylcholine.

amounts of the phospholipids. The activity of free enzyme at the studied concentration was assumed as 100%.

Negatively charged phosphatidylinositol liposomes strongly diminished the activity of aldolase with an increasing initial lipid/protein ratio, whereas in the case of zwitterionic phosphatidylcholines the effect was considerable smaller. Addition of 0.5% Triton X-100, nonionic detergent which disrupts the liposome structure [17] caused full restoration of the enzyme activity. Control addition of Triton X-100 to the solution of free aldolase (without phospholipids) caused only slight increase of the activity.

Next, we studied the effect of the phospholipids on tryptophan fluorescence of the enzyme (Fig. 2). The intensity of the light scattered by control samples of free liposomes was subtracted from fluorescence intensities in order to avoid the errors due to some turbidity of the samples.

As can be seen in Fig. 2, the interaction of aldolase with the two kinds of liposome results in different quenching of tryptophan fluorescence of the enzyme. The wavelength of the emission maximum shifts to higher values as the lipid to protein initial ratio increases (Fig. 3) still revealing the large differences between the effects of phosphatidylcholine and phosphatidylinositol. Addition of KCl decreased the quenching and the 'red' shifts (Figs. 2, 3). A higher concentrations of KCl (above 0.5 and 0.1 M for phosphatidylinositol and phosphatidylcholines respectively) fully abolished the effect of the phospholipids on the fluorescence parameters.

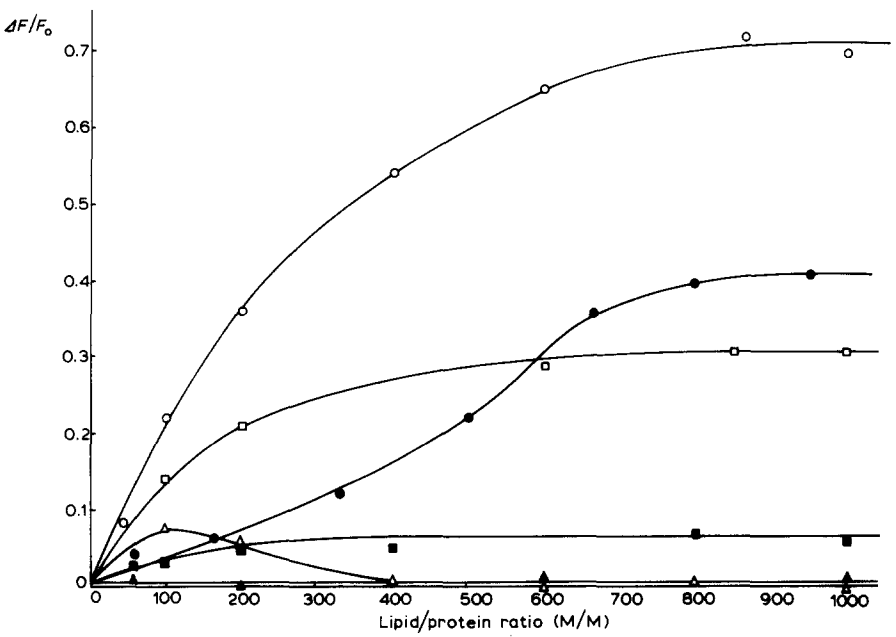

Fig. 2. Quenching of the tryptophan fluorescence of aldolase by phospholipid liposomes as a function of molar lipid/protein initial ratio in various KCl concentrations. The samples contain 0.07 mg/ml of phospholipids. F_0, fluorescence intensity of the enzyme alone; F, fluorescence intensity of the enzyme in the presence of liposomes; $\Delta F = F - F_0$. ○——○, □——□, △——△, phosphatidylinositol with 0, 0.05 and 0.5 M KCl, respectively; ●——●, ■——■, ▲——▲, phosphatidylcholine with 0, 0.05 and 0.5 M KCl, respectively.

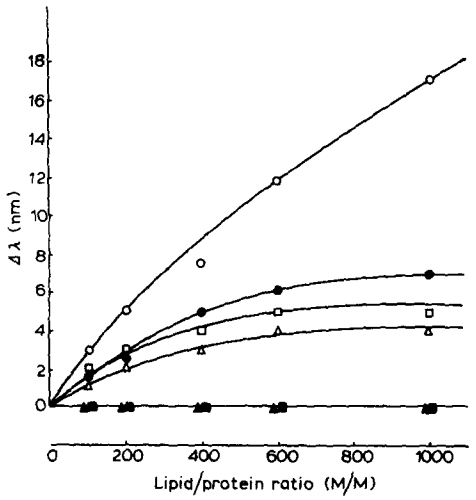

Fig. 3. Influence of lipid/protein molar ratio and KCl concentration on red shift of emission λ_{max}. Experimental conditions as in legend of Fig. 2. ○———○, □———□, △———△, phosphatidylinositol with 0, 0.05, 0.5 M KCl, respectively; ●———●, ■———■, ▲———▲, phosphatidylcholine with 0, 0.05 and 0.5 M KCl, respectively.

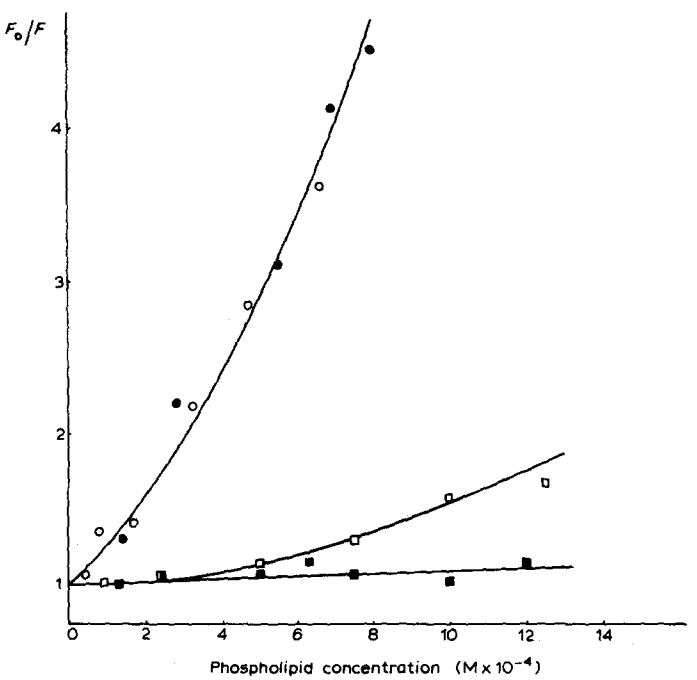

Fig. 4. Stern-Volmer plots of the fluorescence quenching with phospholipids of the tryptophan and aldolase. Tryptophan and aldolase concentrations were $1.2 \cdot 10^{-5}$ and $0.1 \cdot 10^{-5}$ M, respectively. Fluorescence was measured at emission maximum. F_0, fluorescence intensity of the enzyme alone; F, fluorescence intensity of the enzyme in the presence of liposomes. ○———○, aldolase + phosphatidylinositol; □———□, aldolase + phosphatidylcholine; ●———●, tryptophan + phosphatidylinositol; ■———■, tryptophan + phosphatidylcholine.

The dependence of the fluorescence quenching of aldolase and tryptophan on phospholipid concentration is shown as Stern-Volmer plots in Fig. 4. Aldolase contains 12 tryptophanyl residues per molecule [18,19], hence the 12-fold higher tryptophan concentration than that of the enzyme was used in the experiment. The degree of the quenching of tryptophan fluorescence by phospholipids was quite similar to that of the enzyme but no wavelength shifts was observed.

The obtained data proved the existence of binding of rabbit muscle aldolase to phosphatidylinositol and, to a considerably smaller extent, to phosphatidylcholine liposomes.

Quenching processes fall into general types: (a) collisional quenching and (b) static quenching, i.e. the quenching by formation of nonfluorescent complex. Various intermediate states between a and b are possible. It seems that the static mechanism is dominant in the quenching of the aldolase fluorescence by phospholipids. The conclusion arises from the fact that Stern-Volmer plots (Fig. 4) appear to be upward curved ones.

According to studies of Eftink and Ghiron [20] such positive deviation of Stern-Volmer plot indicates that a static mechanism is involved in the quenching. Only binding of the enzyme to liposomes may involve such a mechanism of the quenching. This conclusion is confirmed by the dependence of the quenching on KCl concentration (Fig. 2) and occurrence of 'red' shifts in emission spectra (Fig. 3). The large difference between effects of zwitterionic and negatively charged liposomes and diminishing of the quenching with increasing KCl concentration indicate that the binding of aldolase to liposomes is mainly dependent on electrostatic forces. It seems that the hydrophobic interactions between apolar groups of the protein and hydrocarbon core of liposomes are not involved in that binding. These interactions would cause increase of fluorescence and 'blue' shift of λ_{max}, which is not observed in our investigations.

Interaction of aldolase with phospholipids causes reversible inactivation of the enzyme (Fig. 1). The inactivation of the enzyme by liposomes may result from several effects but our fluorescence data strongly suggest that there are some changes in conformation of the enzyme upon the association.

Rabbit muscle aldolase is a multitryptophan protein [19]. The wavelength of fluorescence maximum (about 320 nm) indicates a nonpolar environment of tryptophanyl residues in aldolase molecule. It has been shown by Eftink and Ghiron [21], that all its tryptophanyl residues are nonexposed to quenching and such a typical quencher as acrylamide minimally quenches their fluorescence. Furthermore, simple quenching of multitryptophan protein fluorescence usually results in 'blue' shifts of λ_{max} since the short-wavelength fluorescence of nonexposed, non-quenched tryptophanyl residues becomes dominant [21]. In our experiments, phosphatidylinositol strongly quenched the aldolase fluorescence with a marked 'red' shift of wavelength maximum. We obtained similar data for quenching of glyceraldehyde-3-phosphate dehydrogenase fluorescence by the phospholipids. These results can be explained if it is assumed that the enzyme modifies its conformation by associating on liposomes. With the modification, tryptophanyl residues become more exposed to polar environment.

Since the phosphatidylinositol liposomes effectively quench the fluorescence of free tryptophan in aqueous solution, it may be concluded that the quenching observed for the enzyme results not only from increasing accessibility of fluorogenic residues to water molecules but also from indirect interactions with phospholipid molecules. Specific interactions of individual residues, including such forces as hydrogen bonding, may be important. The reversibility of inactivation upon addition of Triton X-100 indicates that the presence of phospholipids is not sufficient for conformation and activity modification of the enzyme; the characteristic structure of liposomes and their adsorptional properties are probably essential for the phenomena.

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