Interaction of Phosphoglycerate Kinase with Phosphatidylserine Liposomes

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Abstract. The interaction of 3-phosphoglycerate kinase from bovine heart with natural phosphatidylserine (I) and synthetic dipalmitoyl phosphatidylserine (II) in form of liposomes was ivestigated by measuring fluorescence and activity of the enzyme. The addition of increasing amounts of I resulted in progressive quenching of protein fluorescence with no shift in the emission maximum. In contrast, II did not cause any change in the fluorescence. In the presence of low amounts of I and II (lipid/protein molar ratio 10—40) full enzymatic activity of 3-phosphoglycerate kinase was observed even after 80 min of incubation, whereas without phospholipids the activity considerably decreased. At higher lipid concentrations I strongly inactivated the enzyme and the inactivation by II was only insignificant. It was concluded that the phospholipid membrane protects the enzyme against thermal denaturation, whereas the inactivation is mainly due to phospholipid impurities.

Key words: Phosphoglycerate kinase — Phosphatidylserine — Enzyme-lipid interaction — Fluorescence quenching — Liposomes

Introduction

The interaction of glycolytic enzymes aldolase and glyceraldehyde-3-phosphate dehydrogenase with model phospholipid membranes decreases their enzymatic activities, which is associated with a red shift and quenching of intrinsic protein fluorescence. Changes in the fluorescence parameters and the inactivation have been intepreted as resulting from conformational changes of the proteins after binding to the phospholipid membrane. Inactivation seems to be reversible, suggesting a participation of phospholipid components of the natural membranes in the regulatory processes of cells (Gutowicz and Modrzycka 1978, 1979).

However, at least two problems concerning the mechanism of the incativation of glycolytic enzymes by phospholipids still remain unsolved. First, aldolase and glyceraldehyde-3-phosphate dehydrogenase both are tetrameric enzymes having

inactive subunits (Gerschitz et al. 1977; Jaenicke 1982) and it is unknown whether the phospholipid membrane causes dissociation of the tetramers. Second, nothing is known about a possible participation of single molecules of the phospholipids and their impurities in the inactivation process, as well as in the fluorescence quenching. For these reasons it was of interest to investigate the effect of phospholipids on the activity and fluorescence properties of monomeric enzyme.

3-phosphoglycerate kinase (E.C.2.7.2.3) seems to be the only glycolytic enzyme, which is not composed of subunits (Kulbe et al. 1975). It catalyses the reversible transfer of a phosphoryl group from $MgATP^{2-}$ to 3-phosphoglycerate and is a key enyzme for ATP generation in muscles. Similarly like glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase interacts with erythrocyte ghost membranes with a dominant participation of electrostatic forces, as follows from the effect of increasing ionic strength on the binding of the two enzymes (De and Kirtley 1977). It is unknown whether the enzyme binds to the phospholipid or to a protein component of the membrane. Phosphatidylserine (PS) was chosen as a model anionic phospholipid due to its strong inhibitory effect on tetrameric enzymes (Gutowicz and Modrzycka 1978).

Materials and Methods

Crystalline 3-phosphoglycerate kinase from bovine heart (specific activity 480 U/mg at 25°C) and ATP (disodium salt) were purchased from Polish Chemical Reagents. NADH and 3-phosphoglycerate (trisodium salt) were purchased from Boehringer (FRG). Glyceraldehyde-3-phosphate dehydrogenase from bovine heart (specific activity 80 U/mg at 25°C) was obtained in our laboratory according to Kochman et al. (1975) Three preparations of phosphatidylserine (PS) of different purity were used. Synthetic, dipalmitoyl-DL-α-PS and pure bovine brain PS were from Serva (FRG). The Folch fraction III PS from bovine brain was a product of Koch-Light Laboratories Ltd. (England). The purity of the phospholipids was tested by thin layer chromatography. Single spots were obtained for synthetic PS, whereas Folch fraction III contained small amounts of phosphatidylinositol and lysophosphatidylethanolamine. Traces of these phospholipids were also detected in natural PS from Serva (solvent: $CHCl_3/MeOH/H_2O = 65:25:4$, R_f 12 and 20, respectively).

Commercial phospholipid solutions were evaporated under reduced pressure of nitrogen until constant weight. Dry phospholipids were suspended in 10 mmol/l Tris-HCl buffer pH 7.6 (unless specified otherwise) and sonicated over 20—40 min in an ice bath using a MSE 150W sonifier. According to literary data, suspensions of PS prepared as described above contain mainly unilamellar vesicles of the average diameter about 16 nm (Hauser and Phillips 1973). Phospholipid concentration in the suspensions, calculated from phosphorus determination (Bartlett 1959), was about 2.5 mmol/l. Fluorescence labeling of the liposomes by the reaction with o-phthaldialdehyde was performed in borate buffer pH 8, as described elsewhere (Sidorowicz and Michalak 1984). The lipid suspensions were mixed with a solution of 3-phosphoglycerate kinase in Tris-HCl buffer pH 7.6, and incubated for 30 min before fluorescence was measured. Final concentration of the enzyme in the mixtures was about 5 μmol/l and PS concentration ranged within 0.05 and 1 mmol/l.

In order to measure the enzymatic activity of 3-phosphoglycerate kinase, the lipid-protein mixtures were diluted 20-fold with 10 mmol/l Tris-HCl buffer. The activity measurements were performed immediately after the diluttion and after subsequent incubation of the diluter samples at

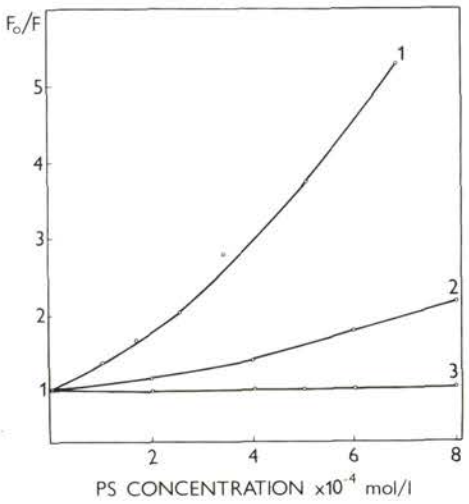

Fig. 1. Fluorescence quenching of 3-phosphoglycerate kinase by phosphatidylserines. 1 — Folch fraction III, 2 — purve bovine brain PS, 3 — synthetic PS.

room temperature over 30 and 80 min. The enzymatic activity was determined by the method of Bücher (1955), using 3-phosphoglycerate as a substrate and glyceraldehyde-3-phosphate dehydrogenase as a coupling enzyme. In a volume of 3 ml of 10 mmol/l Tris-HCl buffer, the substrate concentrations were (in mmol/l): 3-phosphoglycerate 6.2: NADH 0.2: ATP 1.1: $MgCl_2$ 2, and 8 units of the coupling enzyme. The reaction was started by the addition of 0.2 μg 3-phosphoglycerate kinase with or without the phospholipid, and followed by the measurement of absorption decrease at 340 nm. The protein concentration was determined by measuring absorbance at 280 nm, using $A_{280}^{0.1\%} = 0.67$, and the molar concentration of the enzyme was calculated using the relative molecular mass of 47.000 (Kulbe et al. 1975). The spectrophotometric measurements were done using a SPECORD-UV-VIS spectrophotometer. Fluorescence emission spectra were recorded on a Perkin-Elmer MPD-3 spectrofluorimeter, with an excitation wavelength of 290 nm for the protein and 340 nm for the isoindole probe. Fluorescence polarization of the probe was measured at 25°C with a precision of ±0.01.

Results

Fluorescence measurements

The tryptophanyl fluorescence of 3-phosphoglycerate kinase displayed an emission maximum at 339 nm when excited at 290 nm. The addition of increasing amounts of natural phospholipids was followed by progressive quenching of the fluorescence with no shift of the emission maximum. On the other hand, neither fluorescence quenching nor a shift was observed in the presence of synthetic PS. The relationship between fluorescence quenching and phospholipid concentration is shown in Fig. 1 in terms of Stern-Volmer plots. The curves 1, 2, and 3 represent the

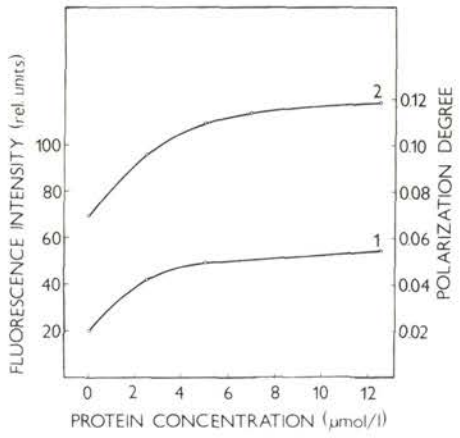

Fig. 2. Dependence of the fluorescence intensity (curve 1) and the polarization degree (curve 2) of the isoindole probe bound to the liposomes on the concentration of 3-phosphoglycerate kinase.

quenching effect of the Folch fraction III, pure bovine brain PS and synthetic PS, respectively. As can be seen, there is a large difference in quenching following natural phosphatidylserines, which strongly suggests that the effect is due to impurities in the phospholipid. The lack of fluorescence changes in the presence of synthetic PS means that, in this case, there is no interaction of the protein with the liposomes, or that the interaction is not followed by changes in the environment of the protein emitters. The latter case seems possible however an evidence is required that binding indeed occurs. To solve this problem, a fluorescence isoindole probe was introduced on the liposome surface by the reaction of a part of the lipid amino groups with *o*-phthaldialdehyde reagent (Sidorowicz and Michalak 1984). The probe exhibits maximum fluorescence at 450 nm when excited at 340 nm. The addition of increasing amounts of the enzyme to the labeled liposomes resulted in a significant increase in the probe fluorescence (Fig. 2, curve 1) and a continuous increase in its polarization degree from a value of 0.07 without the protein to 0.12 in the presence of 12.5 μmol/l of the enzyme (Fig. 2, curve 2). No shift in the emission maximum was observed. Since the fluorescence parameters exhibit a saturation effect at a lipid/protein molar ratio of about 20, this may be interpreted as resulting from an increase in microviscosity of the probe environment due to protein binding. In addition to this effect, the lipid-protein interaction resulted in sensitized fluorescence of the probe when protein tryptophans were excited.

Table 1. Effects of phosphatidylserine liposomes on the activity of 3-phosphoglycerate kinase

	Lipid/protein molar ratio	% of enzymatic activity after different times of incubation		
		0 min	30 min	80 min
No addition	—	100	69	57
Synthetic PS added	10	100	100	112
	20	100	100	100
	40	100	100	—
	60	75	75	—
	80	75	75	—
	100	68	68	—
Bovine brain PS added	13	115	93	—
	26	107	95	—
	50	55	—	—
	78	54	—	—
	115	26	—	—

Activity measurements

Table 1 shows the effect of bovine brain PS (Folch fraction III) and synthetic PS on the activity of 3-phosphoglycerate kinase, measured after different times of incubation of the diluted lipid-enzyme mixtures. The initial activity of the enzyme (480 U/mg) was taken for 100% and the other results were related to this value. As can be seen, at low lipid/protein molar ratios (10—40) full enzymatic activity of 3-phosphoglycerate kinase was observed in the presence of both phospholipids. With natural PS the activities were even somewhat higher as compared with the control. At higher concentrations of the natural PS (lipid/protein molar ratios 50—115) the activity strongly decreased whereas similar concentrations of synthetic PS induced considerably lower inactivation. The higher purity of the latter phospholipid suggests an important role of impurities in the inactivation process.

Another effect observed at low lipid concentrations was stabilisation of the enzymatic activity of 3-phosphoglycerate kinase in the diluted protein solution (0.25 μmol/l). In the presence of synthetic PS full enzymatic activity was observed even after 80 min of incubation of the diluted enzyme at room temperature, whereas it markedly decreased in the absence of PS. It seems that the phospholipid membrane protects the enzyme against thermal denaturation, which occurs at experimental conditions used.

Discussion

The results presented above suggest that the interaction of 3-phosphoglycerate kinase with PS liposomes is not associated with significant conformation changes of the protein. As follows from experiments with synthetic PS, adsorption of the enzyme on the lipid membrane may occur without any changes in the protein fluorescence or enzymatic activity. Hence, it seems obvious that the protein fluorescence quenching and the inactivation both result from an interference of impurities in the phospholipid. This conclusion is strengthened by different quenching effects obtained for three PS preparations. The quenching may be due to some oxidation products of the phospholipid; similar products have been shown to be efficient quenchers of melittin fluorescence (Duforcq and Faucon 1977). Also the inactivation of the enzyme seems to be, at least partially, due to impurities present in the phospholipid, although impurities of a different type. It has been shown previously that the enzyme from yeasts can bind a variety of organic anions, in particular the highly charged ones (Wrobel and Stinson 1978). The yeast enzyme was also shown to be inhibited by ATP^{4-} (Larsson-Raźnikiewicz and Schierbeck 1977) and by salicylates (Larsson-Raźnikiewicz and Wiksell 1978). Hence, it seems possible that water soluble products of phospholipid degradation carrying negative charge may act as potent inhibitors of the enzyme.

High activities of 3-phosphoglycerate kinase observed at low lipid concentrations and the stabilization of the activity by the liposomes also suggest a mechanism of the inactivation other than protein-membrane interaction. The latter effect allows to suppose a possible practical application of PS liposomes as a protective agent for 3-phosphoglycerate kinase.

Obviously, it remains unknown whether the mechanism of incativation of tetrameric glycolytic enzymes is similar to that of 3-phosphoglycerate kinase. The effects of phospholipids and their impurities are probably highly specific and depend on the protein structure. It seems however that the problem of impurities should be considered in any case of lipid-protein interaction.

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