ELSEVIER

Biochimica et Biophysica Acta 1236 (1995) 299–305

BBA Biochimica et Biophysica Acta

Interaction of bovine heart pyruvate kinase with phospholipids

Anna Dąbrowska [a,*], Grzegorz Terlecki [a], Elżbieta Czapińska [a], Jan Gutowicz [b]

[a] Department of Biochemistry, Wrocław University of Medicine, Chałubińskiego 10, 50-368 Wrocław, Poland
[b] Department of Biophysics, Wrocław University of Medicine, Chałubińskiego 10, 50-368 Wrocław, Poland

Received 7 February 1995; accepted 22 February 1995

Abstract

The interaction between bovine heart pyruvate kinase and liposomes was investigated for various phospholipids as function of pH, and salt concentration using steady-state kinetics and ultracentrifugation. Liposomes made from erythrocyte total lipid fraction and individual phospholipids were used. Pyruvate kinase specific activity increases upon the interaction with the phospholipids. The activation is specifically sensitive to presence of phosphatidylserine in liposomes. L-serine, and phospho-L-serine which are main components of phosphatidylserine head group show also some activation effect. Efficient adsorption of pyruvate kinase to phosphatidylserine liposomes occurs in the pH range 6.0–8.0 and at low ionic strength. Interaction with phosphatidylserine liposomes results in the change of V_{max} and K_m values for phospho*enol*pyruvate without marked effect on K_m value for ADP, and Hill coefficients for both substrates. The interaction does not seem to influence the cooperativity between binding sites.

Keywords: Pyruvate kinase; Phosphatidylserine; Liposome; (Bovine heart)

1. Introduction

Pyruvate kinase (ATP:phosphotransferase, EC 2.7.1.40) is one of the key regulatory enzymes of the glycolytic pathway directly involved in ATP formation in cytosolic and nuclear compartments [1–8].

A number of reports have proved that pyruvate kinase should be considered as one of the glycolytic enzymes having the ability of reversible association with subcellular membrane structures (see reviews [9–11]). The association is probably very important for the regulation of glycolysis, as the enzymes adsorbed on a membrane reveal modified kinetic properties. Since the complex composition and heterogeneity of membrane preparations hinder the elucidation of mechanisms of the regulation the study of lipid–protein interactions in model membrane systems is an alternative approach. Monolayers, bilayers, and liposomes are commonly used in this type of investigation [12]. Phospholipid lamellar systems reveal many physical properties in common with biological membranes. These membrane models are pretty well defined systems what allows for controlling the operational parameters and for more detailed analysis compared to biological membranes [13]. Even if we assume that there are specific protein binding sites on membranes for the glycolytic enzymes, as shown, e.g., for glyceraldehyde-3-phosphate dehydrogenase in erythrocytes [14,15], the mosaic structure of cellular membranes provides adsorptive domains due to multielectrostatic and other non-specific interactions. Surface of phospholipid liposomes is an adequate model of such domains. In several recent studies some glycolytic enzymes like aldolase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase and phosphoglycerate kinase have been found to be able to associate in vitro with phospholipid bilayers [16–21]. The enzymes have been shown to change some of their molecular properties upon the association like activity, conformation, thermostability, accessibility for proteinase attack etc. [22–24].

In this work we found out that the liposomes made of lipid fraction of erythrocyte membranes activated the bovine heart pyruvate kinase. In the light of the above-cited literature data, dealing with the interaction of other glycolytic enzymes with cell membranes and phospholipid model membranes this finding was unexpected. Most of the previously studied glycolytic enzymes have been shown to reveal an inactivation upon the interaction with membranes or phospholipids. Results of our investigations of the interaction between pyruvate kinase and lipid components of membranes and attempts to find out which com-

* Corresponding author. Fax: +48 71 225415.

SSDI 0005-2736(95)00061-5

ponent of the lipid fraction is responsible for the activation are presented in this paper.

2. Materials and methods

2.1. Chemicals

Phospho*enol*pyruvate, ADP (potassium salts), and NADH (inhibitor free) were purchased from Boehringer, Tris and all used natural and synthetic phospholipids, from Sigma; L-serine, from Calbiochem. All other chemicals were obtained from Polskie Odczynniki Chemiczne (Poland) and were of analytical grade. For all solutions and preparations deionized water was used routinely.

2.2. Bovine heart pyruvate kinase

The pure enzyme in ammonium sulfate solution was purchased from Polskie Odczynniki Chemiczne (Poland). These preparations have a specific activity of more than 200 U/mg in 50 mM Tris-HCl buffer, pH 7.5 and gave one band upon polyacrylamide gel electrophoresis.

Before use, appropriate amount of the pyruvate kinase suspension in ammonium sulfate solution was centrifuged down and the pellet was dissolved in 20 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5 or just in 50 mM Tris-HCl, pH 7.5 in the binding experiments. Then the solutions were dialyzed for 20 h against the same buffer at temperature of 4° C and centrifuged again to remove denatured protein.

2.3. Enzyme assay

The amount of pyruvate kinase was determined according to the method of Bücher and Pfleiderer [25]. For the measurements of the activity and kinetic parameters the assay mixture contained 50 mM Tris-HCl buffer, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.15 mM NADH, 0.03 U of pyruvate kinase and 5 U of lactate dehydrogenase. Value of pH in each sample was checked before and after the assay. It was preincubated for 20 min at 25° C in a thermostated bath before starting the reaction by addition of either PEP or ADP to final concentration at 0.8 mM PEP or 1.5 mM ADP. All values in the Tables 1 and 2 as well as the points in Figs. 1 to 3 represent mean values from the determination in at least three separate samples. The determinations with variations less than 15% were taken into consideration.

2.4. Determination of protein concentration

Protein concentration was measured by the method of Lowry et al. [26] or alternatively by biuret method [27]. The biuret method gave better reproducibility for the samples in the presence of phospholipids. For estimation of the enzyme concentration in samples without modifiers a value of $E^{0.1\%}_{280nm} = 0.55$ was used as has been established for pyruvate kinases from various sources [28,29].

2.5. Preparation of erythrocyte lipids

Lipids were extracted from freshly harvested bovine erythrocyte with n-butanol according to the method described by Zahler et al. [30]. The extract of the lipids was evaporated to dryness under a nitrogen stream and thin-layer chromatography (TLC) of the lipids was performed on the plates coated with Silica Gel H. Chromatograms were developed with chloroform-methanol-water (65:25:4, v/v). Since the separation of PI from PS in this solvent system was not good enough two-dimensional TLC was used. Chromatograms were developed on Silica Gel H plates with two systems of solvents: (1) chloroform-methanol-ammonium hydroxide (70:35:8, v/v) and then (2) chloroform-methanol-formic acid (53:10:6, v/v). Positions of phospholipid spots were visualised under UV light by spraying the plates with dichlorofluoresceine. Standards were used for the identification of phospholipids on the plates. Each phospholipid spots was scraped into a vial and its extraction from silica gel was performed with Blight and Dyer's extraction solvent [31] after addition of 2–3 drops of NH_4OH for better separation of dichlorofluorescein. Vigorous vortexing was necessary for good extraction. Homogeneity of each separated fraction was checked by analytical TLC with the same solvent systems as described above and additionally with the system of hexane-diethyl ether-glacial acetic acid (85:15:1, v/v). Routinely, appropriate phospholipid standards were parallel run on the same plates. Spraying the plates with sulfuric acid and heating were applied for staining of the chromatograms. The obtained preparations of lipids were not contaminated by proteins as determined by the biuret method.

2.6. Determination of lipid concentration

The concentration of lipids was calculated from the weight of dried matter or indirectly by phosphorus determination according to Bartlett [32].

2.7. Formation of liposomes

Erythrocyte lipid fraction or phospholipids were suspended in cold 50 mM Tris-HCl buffer, pH 7.5. The mixture was flushed with nitrogen, then shaken mechanically for 10 min to generate liposomes. Liposomes obtained by this method were completely separated from the solution by centrifugation at $100\,000 \times g$ for 30 min as checked by phosphorus determination in the supernatants. Liposomes used in the activation experiments were subjected to ultrasonic irradiation for 10 min to reduce liposome size heterogeneity. State of aggregation and size of liposomes strongly depend on conditions of their formation. In order to get similar populations of liposomes in

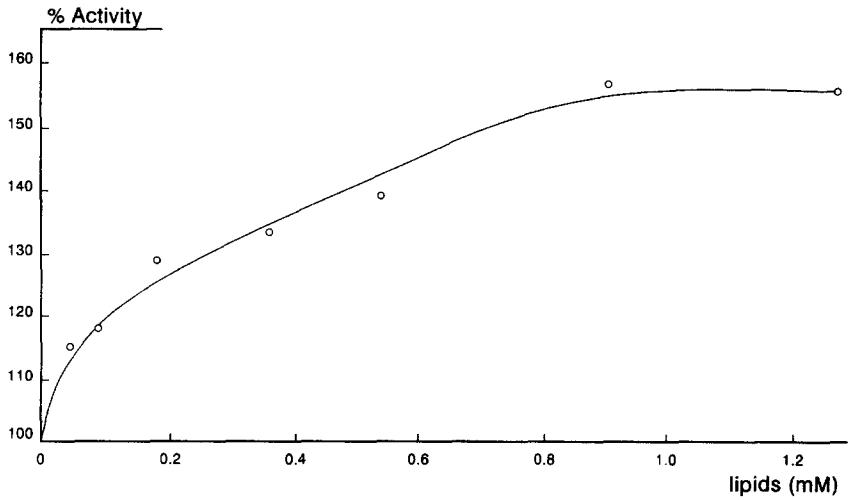

Fig. 1. Effect of liposomes prepared from bovine erythrocyte membrane lipids on the activity of bovine heart pyruvate kinase. Activity is expressed as percent of that in a control sample without liposomes. Experimental conditions are described in Materials and methods.

each experiment the same experimental conditions of the formation were kept. It was important to us that liposomes provided well-defined surface of phospholipid bilayer for interaction with the enzyme. Hence, the liposome preparations were not characterised in terms of their size or aggregation state. Such properties are believed not to be effective qualitatively in this type of studies.

2.8. Binding experiments

Ultracentrifugation of preincubated mixtures of liposomes and pyruvate kinase were used for the binding studies. Phospholipid dispersion and the enzyme solution were mixed in 10 ml polycarbonate centrifuge tubes and total volume of samples was adjusted to the same value by 50 mM Tris-HCl buffer, pH 7.5. The mixtures were gently swirled for 30 s, incubated at 4° C for 30 min, then centrifuged at $100\,000 \times g$ for 30 min. Decreasing the temperature to 4° C markedly improved the reproducibility of the data. After centrifugation, the supernatants were carefully removed without disrupting the tightly packed pellets, and transferred to separate tubes. In the control samples, with the enzyme alone, no decrease in protein concentration was observed after centrifugation. All liposome solutions were freshly prepared for each binding experiment. Protein contents in the pellets were calculated from the difference between the initial concentration (total protein concentration before centrifugation) and that in the supernatant (after centrifugation). In the plots (Figs. 4 and 5) each point represents the mean value of at least three independent determinations.

3. Results

3.1. Effect of phospholipids on pyruvate kinase activity and kinetics

Addition of the liposomes made of erythrocyte lipid fraction to the solution of pyruvate kinase results in increasing of the enzyme specific activity (Fig. 1). The activity increased with increase of the concentration of liposomes reaching the saturation around 1 mM of lipid. The saturating lipid/protein ratio appears to be relatively high but its exact value has rather no quantitative meaning since only limited number of lipid molecules in liposomes are accessible for the interaction and formation of a complex. From the above-cited previous studies on the interac-

Table 1
Kinetic parameters of pyruvate kinase from bovine heart in the absence and in the presence of activators

		Enzyme alone	Enzyme + activators		
			phosphatidylserine (0.007 mM)	phospho-L-serine (10 mM)	L-serine (3 mM)
PEP	K_m (mM)	0.045	0.08	0.07	0.07
	n_H	1.00	1.06	0.97	0.99
	V_{max} (relative)	1.00	1.62	1.20	1.37
ADP	K_m (mM)	0.30	0.30	0.30	0.30
	n_H	0.95	0.97	0.99	0.93
	V_{max} (relative)	1.00	1.60	1.38	1.30

tion between some glycolytic enzymes and liposomes it was reasonable to suspect that phospholipids were those effective components of the erythrocyte lipid extract. Thus, the erythrocyte lipid extracts used in the above experiments were analysed and fractions were separated by thin-layer chromatography. The main phospholipid components of the erythrocyte lipid extract were found to be: phosphatidylethanolamines, phosphatidylcholines, phosphatidylinositols and phosphatidylserines. Effect of each of these fractions at the concentrations up to 0.5 mM on the heart pyruvate kinase activity was investigated. No influence was detected for phosphatidylcholines, phosphatidylethanolamines and phosphatidylinositols. No changes in pyruvate kinase activity were also observed in the presence of phosphatidic acid and phosphatidylinositol/phosphatidic acid mixture (molar ratio of 5:2) while phosphatidylserines showed an activating effect. Similar effect was observed when we used commercial preparations of purified bovine brain phosphatidylserine and synthetic dipalmitoyl-DL-phosphatidylserine. Liposomes made of other type of phospholipids: phosphatidylcholines, phosphatidylcholine + phosphatidylinositol mixture (9:1, w/w) or phosphatidylcholine + phosphatidylethanolamine mixture (9:1, w/w) did not affect the activity in the pH range of 6–9 (not shown). Effect of the commercial preparation of bovine brain phosphatidylserines on bovine heart pyruvate kinase activity compared with that of L-serine and of phosphoserine was shown in Fig. 2. The concentrations of phosphatidylserines, serine and phosphoserine corresponding to half of the maximal activity ($K_{1/2}$) were 0.4 μM, 0.5 mM and 2.2 mM, respectively, in this experiment, however, the value for the phosphatidylserine liposomes can be only qualitatively compared with the values for L-serine because of supramolecular structure of liposomes as discussed above. Nevertheless, it is clear that the interaction with phosphatidylserines is much more effective than with L-serine. For phosphoserine we could see similar effect which is saturated in an order higher concentration than that for

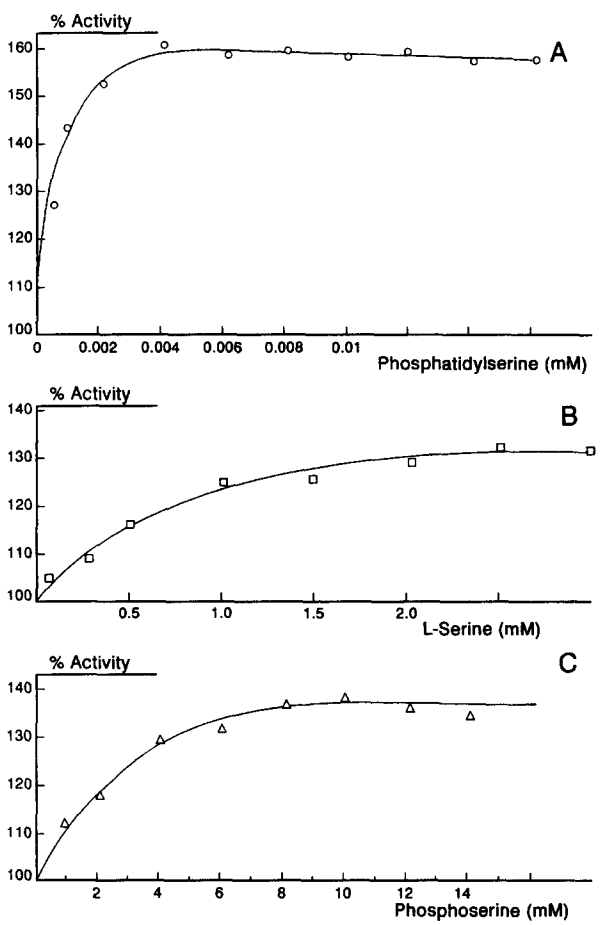

Fig. 2. Effect of phosphatidylserines (A), serine (B) and phosphoserine (C) on the activity of pyruvate kinase. Activity is expressed as percent of that in a control sample without a modifier. Experimental conditions are described in Materials and methods. Sample volume was 1.5 ml. Protein concentration was 1 μg/ml.

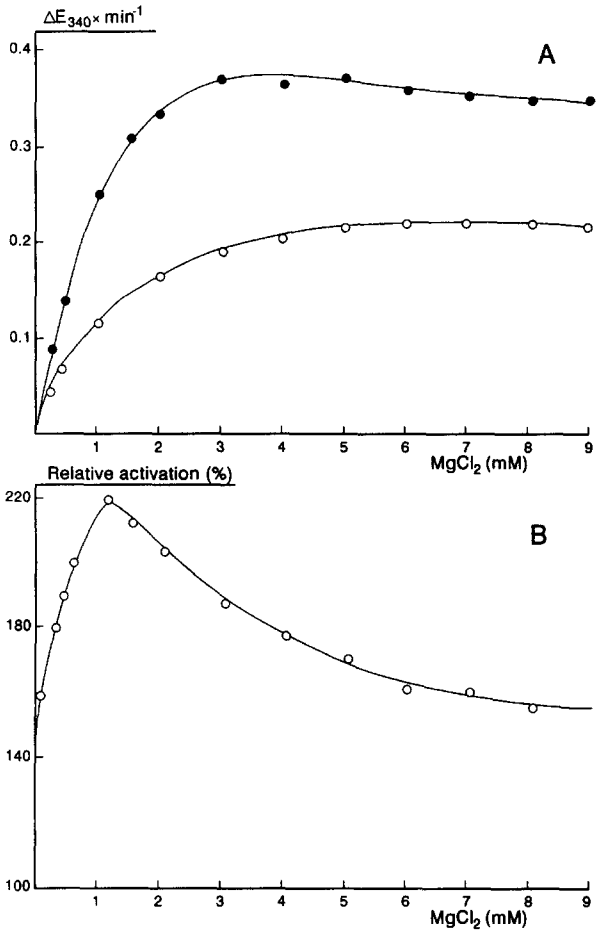

Fig. 3. (A) Activation of pyruvate kinase by $MgCl_2$ without any modifier (open circles) and in the presence of phosphatidylserine liposomes (dark circles). The concentration of phospholipid was 0.004 mM. Assay conditions were described in Materials and methods. (B) Relative activation of pyruvate kinase by $MgCl_2$ in the presence phosphatidylserine liposomes. The curve was calculated using the data from Fig. 3A. Each point represents the activity of the enzyme in the presence of liposomes as percent of that in sample of the enzyme alone.

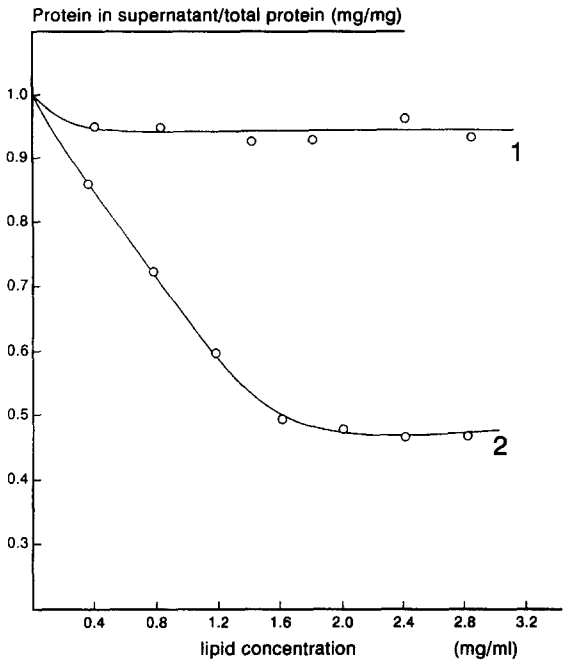

Fig. 4. Centrifugation of the bovine heart pyruvate kinase with liposomes made of phosphatidylcholine (1) and phosphatidylcholine + phosphatidylserine mixture (weight ratio 9:1) (2). Each 3.2 ml sample contained 0.35 mg/ml of pyruvate kinase and indicated concentration of phospholipid in 50 mM Tris-HCl buffer, pH 7.5.

L-serine. Addition of phosphatidylcholine or phosphatidylethanolamine liposomes into the solution of serine or phosphoserine did not change the effect of L-serine or phosphoserine (not shown). It is clear from these results that the charge of the effector is not a crucial factor for the interaction. Some kinetic parameters for pyruvate kinase

Table 2
Influence of magnesium ions on K_m value of the enzyme for PEP in the presence and in the absence of phosphatidylserine liposomes at various pH

pH	$MgCl_2$ (mM)	K_m for PEP (mM)	
		enzyme alone	enzyme + 0.007 mM PS
6.0	1.0	0.192	0.055
	10.0	0.159	0.122
7.0	1.0	0.051	0.051
	10.0	0.052	0.055
8.0	1.0	0.058	0.083
	10.0	0.058	0.077

reaction measured in the presence and in the absence of the studied modifiers are given in Table 1. Without a modifier, K_m values for PEP and ADP were 0.045 mM and 0.3 mM respectively and Hill's coefficient (n_H) for the both substrates was close to 1.0. Hyperbolic saturation curves for the both substrates suggest a simple Michaelis-Menten kinetics in the used experimental conditions (Tris-HCl buffer, pH 7.5). In the presence of the modifiers the maximal velocity of the enzyme at saturating concentration of PEP or ADP and K_m for PEP are increased. Pyruvate kinase shows no cooperativity of binding sites for PEP or ADP and this is not changed by the interaction. Since pyruvate kinases are known to require monovalent and divalent ions for their catalytic activity [5] an investigation of the effect of the liposomes on the activation of the enzyme by potassium and magnesium ions seemed to be of interest. Effect of the increasing concentration of $MgCl_2$ on the enzyme activity in the presence of bovine brain

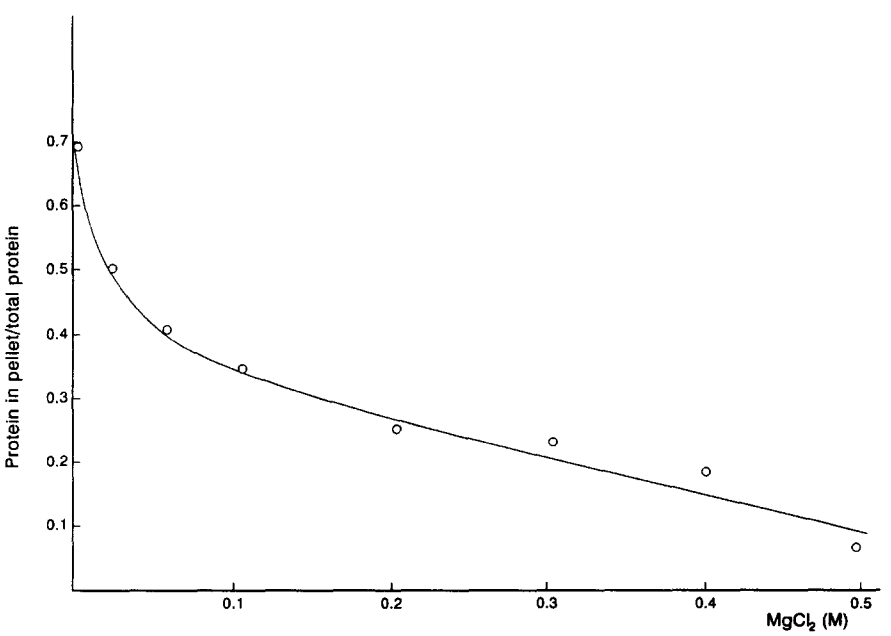

Fig. 5. Effect of $MgCl_2$ on the binding of bovine heart pyruvate kinase to phosphatidylcholine/phosphatidylserine (weight ratio 9:1) liposomes in 50 mM Tris-HCl buffer, pH 7.5. Protein and lipid concentration were 0.35 mg/ml and 2.0 mg/ml, respectively. Sample volume was 3.2 ml.

phosphatidylserine liposomes compared to the enzyme alone as a control can be seen in Fig. 3A. The relative activation curve Fig. 3B) is complex likely due to the interaction of magnesium ions with both the enzyme and phospholipids. Increasing concentration of KCl results in increase of the enzyme activity in the presence of phosphatidylserine liposomes monotonically up to the value of 0.2 M; at higher concentrations of the salt the activity started to decrease (not shown). Michaelis constant (K_m) for PEP was determined at $MgCl_2$ concentrations of 1 mM and 10 mM at pH 6, 7, and 8 in the presence and in the absence of 7 μM of phosphatidylserine (PS). In the absence of PS liposomes no marked changes of K_m (PEP) were found at pH 7, and 8, but at pH 6 K_m value increased 3–4-fold to that in standard conditions (Table 2). In the presence of phosphatidylserine at pH 7 and 8 the results were quite similar. Whilst at pH 6 K_m (PEP) increased 2.5-fold at $MgCl_2$ concentration of 10 mM but no change in K_m (PEP) was found in 1 mM $MgCl_2$ (Table 2). It suggests a strong influence of the interaction with the phospholipid on the conformation of PEP binding site. Phosphatidylserine is probably able to protect the site from the effect of lower pH when magnesium concentration is relatively low. We did not observe such an effect at higher pH.

3.2. Binding studies

Results of our binding studies are shown in Fig. 4. Centrifugation of the phospholipid liposomes in the presence of soluble pyruvate kinase resulted in lowering of the concentration of the pyruvate kinase in the supernatant. For liposomes made of phosphatidylcholine alone the decrease was less 7%, whilst incorporation of 10% phosphatidylserine into the phosphatidylcholine liposomes resulted in pelleting up 52% of protein upon the centrifugation. There was only slight effect for liposomes made of phosphatidylcholine-phosphatidylinositol mixture (9:1, w/w) and phosphatidylcholine-phosphatidylethanolamine mixture (9:1, w/w) – not shown. In control samples, without liposomes no detectable precipitation of the enzyme was observed in the used conditions.

Increasing concentration of $MgCl_2$ decreased the proportion of the enzyme adsorbed to liposomes (Fig. 5).

Table 3
Dependence of bovine heart pyruvate kinase adsorption to phosphatidylcholine/phosphatidylserine (9:1, w/w) liposomes on pH values

pH	% protein in supernatant
6.0	85
6.5	76
7.0	63
7.5	49
8.0	40

Protein and lipid concentration were 0.35 mg/ml and 2.0 mg/ml, respectively, sample volume 3.2 ml.

Similarly, increasing concentration of KCl or NaCl dissociated the binding (not shown). At the concentrations of the salts higher than 0.5 M no binding was detected. The binding appeared to be also sensitive for change of charge on the phospholipid and the enzyme molecules upon changing pH. Effect of pH on the binding was shown in Table 3. The binding increases monotonically with increasing pH value between 6 and 8 though the expected changes in net electric charge upon the raising of pH are not supposed to promote of this increase since positive charge is titrated on both phospholipid and enzyme molecules. It can only be explain if one assume a conformational adaptation of binding site(s) upon the increase of pH.

4. Discussion

First conclusion arising from the results is that bovine heart pyruvate kinase can be bound to and activated by liposomes made of overall membrane lipid fraction and thus also in membranes in vivo by lipid domains. However, the binding and the activation show some specificity and occur only when phosphatidylserines are present in a bilayer. There are two possible types of the interaction: (1) adsorption of the enzyme molecules on surface of liposome bilayers and/or (2) formation of molecular lipid-enzyme complex. Bovine heart preparation of pyruvate kinase contains only M_1 type isozyme with pI value being 8.9 [33]. Hence, at least initiation role of the electrostatic interaction between negative charge of phosphatidylserines and positive net charge of the enzyme at the studied pH range should be expected. It is clear from the binding studies that the first type of the interaction occurs here. Nevertheless, a contribution of the latter type of the interaction can not be excluded. Components of phosphatidylserine head polar group: L-serine and phospho-L-serine used separately also activate the enzyme, however, the activation is much smaller (Fig. 2, Table 1). This indicates that there is a highly specific phosphatidylserine binding site(s) on the enzyme molecule. It seems, however, that the adsorption of the enzyme on liposome surface and the binding of phosphatidylserine molecules to binding site(s) promote each other. Since addition of L-serine, phosphoserine or phosphatidylserines results in similar modification of the affinity of PEP for the enzyme they probably involve the same binding site(s). Besides that the phosphoserine moiety of phosphatidylserine molecule likely takes part in the occupation of the binding site on the protein there must be other factors, provided by entire phospholipid molecule, that increase the strength of the binding and simultaneously causing the activation. Simple model of the enzyme molecule adsorbed on liposome surface by non-specific multielectrostatic attractions is not valid here since liposomes made of other acidic phospholipids (phosphatidic acid, phosphatidylinositol) neither bind the enzyme nor alter its activity. State of ionization and/or

hydration of the phospholipid must play a role in the interaction as well since high salt concentration diminishes the interaction but it does not seem to be the only necessary factor. Effect of magnesium ions at lower concentrations on the activation is complex likely because of superposition of their interaction with phospholipid and with the enzyme (Fig. 3B). Increase of the concentration of divalent ions diminishes electric negative charge of the phospholipid ionic groups very effectively and, on the other hand, the divalent ions bound to the enzyme are strictly necessary for its activity. Likely, the binding of the ions to the enzyme molecules promotes the phospholipid binding site since the effect of phospholipid on the activation increases in the presence of magnesium but at higher concentrations of magnesium ions the effect on the phospholipid charge (and on binding at the same time) dominate.

Considering both kinetic and binding studies it can be postulated that the phosphatidylserine binds to specific binding site(s) on the enzyme molecule and conformationally modifies the catalytic site. Effect of pH on the binding and kinetic parameters is more unstable if a conformational change extending on catalytic region is assumed upon this interaction. The postulate of conformational changes upon the interaction is supported by the 'strange' effect of magnesium ions on the K_m at pH 6 (Table 2) if we assume that the effect can be a result of complex superposition of conformational changes due to change in pH, divalent ion concentration and binding of phospholipid.

Acknowledgements

We are grateful to Prof. Janina Kwiatkowska-Korczak for helpful discussions and criticism and for her help in the English version of the manuscript. This work was supported by of Polish Committee of Science Research (Grant No. 0.07).

References

[1] Susor, W.A. and Rutter, W.J. (1968) Biochem. Biophys. Res. Commun. 30, 14–20.
[2] Imamura, K. and Tanaka, T. (1972) J. Biochem. 71, 1043–1051.
[3] Carbonell, J., Feliu, J.E., Marco, R. and Sols, A. (1973) Eur. J. Biochem. 37, 148–156.
[4] Strandholm, J.J., Dyson, R.D. and Cardenas, J.M. (1976) Arch. Biochem. Biophys. 173, 125–131.
[5] Nowak, T. and Suelter, C. (1981) Mol. Cell. Biochem. 35, 65–75.
[6] Noguchi, T., Yamada, K., Inoue, H., Matsuda, T. and Tanaka, T. (1987) J. Biol. Chem. 29, 14366–14371.
[7] Wong, S.C., Wu, S.W.N. and Yeung, D.C.Y. (1988) Int. J. Biochem. 2, 167–174.
[8] Guminska, M., Stachurska, M.B. and Ignacak, I. (1988) Biochim. Biophys. Acta 966, 207–213.
[9] Duchon, G. and Collier, H.B. (1971) J. Membr. Biol. 6, 138–157.
[10] Masters, C.J. (1978) Trends Biochem. Sci. 3, 206–209.
[11] Masters, C.J. (1981) CRC Crit. Rev. Biochem. 2, 105–137.
[12] Ehrenstein, G., Lecar, H. and Nossal, R. (1970) J. Gen. Physiol. 55, 119–133.
[13] Thompson, T.E. and Henn, F.A. (1970) in Membranes of Mitochondria and Chloroplasts (Racker, E., ed.), pp. 1–52, Van Nostrand Reinhold, New York.
[14] Yu, J. and Steck, T.L. (1975) J. Biol. Chem. 250, 9176–9184.
[15] Kliman, K.J. and Steck, T.L. (1980) J. Biol. Chem. 255, 6314–6321.
[16] Wooster, M.S. and Wrigglesworth, J.M. (1976) J. Biochem. 159, 627–731.
[17] Gutowicz, J. and Modrzycka, T. (1978) Biochim. Biophys. Acta 512, 105–110.
[18] Gutowicz, J. and Modrzycka, T. (1979) Biochim. Biophys. Acta 552, 358–363.
[19] Sidorowicz, A., Golebiowska, J. and Siemieniewski, H. (1986) Gen. Physiol. Biophys. 5, 307–313.
[20] Dabrowska, A. and Gutowicz, J. (1986) Biochim. Biophys. Acta 855, 99–104.
[21] Dabrowska, A., Terlecki, G. and Gutowicz, J. (1989) Biochim. Biophys. Acta 980, 357–366.
[22] Gutowicz, J. and Kosmider-Schmidt, A. (1987) Biophys. Chem. 27, 97–102.
[23] Dabrowska, A. and Czapinska, E. (1990) Biochim. Biophys. Acta 1027, 301–303.
[24] Michalak, K., Gutowicz, J. and Modrzycka, T. (1992) Gen. Physiol. Biophys. 11, 545–554.
[25] Bcher, T. and Pfleiderer, G. (1955) Methods Enzymol. 1, 1–50.
[26] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[27] Layne, E. (1957) Methods Enzymol. 3, 447–457.
[28] Cardenas, J.M. and Dyson, R.D. (1973) J. Biol. Chem. 248, 6938–6944.
[29] Parkinson, J. and Easterby, J. (1977) Biochim. Biophys. Acta 481, 471–480.
[30] Zahler, P. and Niggli, V. (1977) Membr. Biol. 1, 1–50.
[31] Blight, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[32] Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
[33] Cardenas, J.M., Dyson, R.D. and Strandholm, J.J. (1973) J. Biol. Chem. 248, 6931–6937.