Modification of Glyceraldehyde 3-Phosphate Dehydrogenase Activity by Adsorption on Phospholipid Vesicles

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(Received 24 June 1976)

1. The adsorption of [^{14}C]carboxymethylated glyceraldehyde 3-phosphate dehydrogenase to negatively charged liposomes of phosphatidic acid/phosphatidylcholine (3:7, w/w) was investigated. The apparent association constant at $I/2 = 60$, pH 7.6, was $0.4 \times 10^6 \text{ M}^{-1}$. Adsorption decreased as ionic strength and pH were increased. 2. In the presence of negatively charged liposomes, the K_m value for glyceraldehyde 3-phosphate of glyceraldehyde 3-phosphate dehydrogenase was increased and V_{max} decreased. In the presence of positively charged liposomes, the K_m value for glyceraldehyde 3-phosphate decreased and there was no significant change in V_{max} . Addition of Triton X-100 abolished the effect of both positively and negatively charged liposomes on the kinetic properties of the enzyme.

Glyceraldehyde 3-phosphate dehydrogenase is one of several glycolytic enzymes that are found associated with isolated cell components (De Duve *et al.*, 1962; Green *et al.*, 1965). A considerable fraction of the total cell content of glyceraldehyde 3-phosphate dehydrogenase is retained in the isolated membrane 'ghosts' of mammalian erythrocytes (Kant & Steck, 1973; MacDaniel *et al.*, 1974) and is also found adsorbed to actin in rat and rabbit muscle homogenates (Arnold & Pette, 1968). Association of the enzyme with human erythrocyte plasma membranes appears to be specific and reversible, and depends on the pH and ionic strength of the medium used for isolation (Kant & Steck, 1973). Kant & Steck (1973) report that binding of the enzyme is also influenced by micromolar amounts of NAD^+ , NADH and glyceraldehyde 3-phosphate. In a previous paper (Wooster & Wrigglesworth, 1976), we have shown that [^{14}C]carboxymethylated glyceraldehyde 3-phosphate dehydrogenase from rabbit muscle binds to condensed charged monolayers by multiple electrostatic interactions and that binding is strongly influenced by the concentration of surface charge and the presence of Ca^{2+} .

The significance of enzyme binding studies to the situation *in vivo* is as yet a matter for conjecture. It has been suggested that association of glyceraldehyde 3-phosphate dehydrogenase with cellular membranes may be an important factor in various transport processes, either directly in the transport of phosphate or indirectly (coupled with phosphoglycerate kinase) in generating localized concentrations of ATP for active transport (Schrier, 1963; Kant & Steck, 1973;

MacDaniel *et al.*, 1974). Alternatively, modification of catalytic properties has been shown to occur in several water-soluble enzymes when they are immobilized on artificial membranes (Katchalski *et al.*, 1971) and the catalytic properties of glyceraldehyde 3-phosphate dehydrogenase could be affected by adsorption to cell membranes. Alteration in the surface micro-environment of the membrane by ions or charged molecules could then regulate enzyme activity. Concentration changes in specific metabolites could also regulate activity by altering the degree of association of the enzyme with the membrane. In the present paper we present evidence that the kinetic properties of rabbit muscle glyceraldehyde 3-phosphate dehydrogenase is modified by adsorption to a charged surface. We have used phospholipid vesicles as adsorbing surfaces, since these provide a useful model membrane system of defined composition and charge.

Materials and Methods

Glyceraldehyde 3-phosphate [Sigma (London) Chemical Co., London S.W.6, U.K.] was prepared from the diethylacetyl form by hydrolysis. Phospholipids from egg yolk (Lipid Products, Nutfield Nurseries, Nutfield, Surrey, U.K.) were tested for purity by t.l.c. Stearylamine (Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.) was purified by recrystallization from ethanol.

Rabbit skeletal muscle glyceraldehyde 3-phosphate dehydrogenase [Boehringer Corp. (London) Ltd., Ealing, London W.5, U.K.] was labelled with iodo-[^{14}C]acetate (The Radiochemical Centre, Amer-

sham, Bucks., U.K.) of specific radioactivity 51 mCi/mmol, as described by Wooster & Wrigglesworth (1976). Hand-shaken liposomes were prepared by the method of Redwood & Patel (1974) and isolated by centrifugation at 100000g for 1 h. Liposomes of greater surface area to volume ratio were prepared by sonication under N₂ in an ice-cooled vial by using an MSE 100W sonicator. For binding studies, enzyme and liposomes were incubated in 3 ml polycarbonate centrifuge tubes for 1 h at 4°C. After centrifugation the supernatants were carefully removed and portions of both the resuspended pellets and supernatant were counted for radioactivity by using liquid-scintillation methods.

The reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase was followed by monitoring the reduction of NAD⁺ at 340 nm by using a Perkin-Elmer SP.356 dual-wavelength spectrophotometer in its dual-wavelength mode with a reference wavelength of 370 nm at 20°C.

Results

Adsorption of enzyme to liposomes

Fig. 1 shows the extent of adsorption of various concentrations of [¹⁴C]carboxymethylated glyceraldehyde 3-phosphate dehydrogenase to unsonicated phospholipid liposomes (phosphatidic acid/phosphatidylcholine, 3:7, w/w) after incubation for 1 h at 4°C. Enzyme adsorption increases with increasing concentration of free enzyme but decreases as the ionic strength of the incubation medium is raised by the addition of KCl. No changes in the amount of enzyme bound were observed after longer incubation times at any of the protein concentrations used. Included in Fig. 1 are adsorption data on resuspended liposome-enzyme complexes. For these studies, the liposomes were incubated with the labelled protein for 1 h and then centrifuged at 100000g for 1 h to separate the liposome-enzyme complex from unbound protein. The supernatant was removed and the pellet resuspended in an equal amount of buffer. After further incubation for 1 h, the mixture was re-centrifuged and the pellet and supernatant were counted for labelled protein radioactivity. The similarity in binding data between the two types of experiment indicates that the formation of the liposome-enzyme complex is reversible and that equilibrium occurs in less than 1 h.

An apparent association constant (K_{app}) of $0.4 \times 10^6 \text{ M}^{-1}$ at $I/2 = 0.06$ is obtained when the results are subjected to a Scatchard (1949) plot (Fig. 1b). The linearity of the Scatchard plot over the range of protein concentrations used indicates that there is little effect on binding by any electrostatic interaction between binding sites (Scatchard, 1949).

The pH-dependence of adsorption of enzyme to liposomes (phosphatidic acid/phosphatidylcholine,

3:7) was essentially the same as for the adsorption of enzyme to mixed monolayers of phosphatidic acid and phosphatidylcholine (Wooster & Wrigglesworth, 1976). Adsorption increased between pH 6 and pH 7.5, but above pH 7.5 the amount adsorbed decreased with increasing pH up to pH 10, where no adsorption could be detected.

Fig. 1. Characteristics of [¹⁴C]carboxymethylated glyceraldehyde 3-phosphate dehydrogenase adsorption to negatively charged liposomes of phospholipid

(a) Binding of enzyme to liposomes (phosphatidic acid/phosphatidylcholine 3:7, w/w) at 4°C as a function of free enzyme concentration in 10 mM-Tris/HCl buffer, pH 7.6, plus 1 mM-EDTA, 1 mM-cysteine and (○) 50 mM-KCl or (■) 250 mM-KCl. ●, Binding data for resuspended enzyme-liposome complexes (see the text) with 50 mM-KCl in the buffer mixture, (b) Scatchard (1949) plot of data with 50 mM-KCl in the mixture.

Fig. 2. Effects on the kinetic properties of glyceraldehyde 3-phosphate dehydrogenase by adsorption to negatively charged liposomes

Plots of $[S]/v$ against $[S]$ using glyceraldehyde 3-phosphate as variable substrate in the absence of liposomes (○) and in the presence of liposomes (phosphatidic acid/phosphatidylcholine, 3:7) at final phosphatidic acid concentrations of 2.6 mM (●) and 2.6 mM with 2 min sonication of liposomes before addition (■). The reaction medium contained 10 mM-potassium phosphate buffer, pH 7.6, 1 mM-EDTA, 1 mM-cysteine, 50 mM-KCl, 0.3 mM-NAD⁺ and 0.7 mM-glyceraldehyde 3-phosphate dehydrogenase.

Effect of adsorption on enzyme activity

Fig. 2 shows a plot of $[S]/v$ against $[S]$, where $[S]$ is the concentration of glyceraldehyde 3-phosphate and v the initial velocity of the enzyme reaction, for enzyme in free solution and in the presence of various preparations of negatively charged liposomes. Adsorption of enzyme to the liposomes inhibits enzyme activity, $V_{\max}$ is decreased and the K_m for glyceraldehyde 3-phosphate is increased. The effects of adsorption on the kinetic activity of the enzyme depend on the area of charged surface available for adsorption. Addition of unsonicated liposomes increases the K_m from 20 to 33 μM and decreases $V_{\max}$ from 48 to 26 $\mu\text{mol/min per mg}$. Addition of sonicated liposomes of the same phosphatidic acid concentration increases the K_m for glyceraldehyde 3-phosphate to 39 μM and decreases $V_{\max}$ to 16 $\mu\text{mol/min per mg}$. Interaction of glyceraldehyde 3-phosphate dehydrogenase with the charged liposome surface did not appear to cause any irreversible change in catalytic activity. Full activity was regained after desorption of the adsorbed enzyme by dilution. Addition of 0.5% Triton X-100 to enzyme adsorbed to liposomes decreased the K_m to 22 μM , close to the value found for enzyme in free solution.

Addition of enzyme to a suspension of positively charged liposomes (stearylamine/phosphatidic acid, 3:17, w/w) was found to activate enzyme activity

(Fig. 3). The K_m for glyceraldehyde 3-phosphate decreases from 123 to 62 μM on the addition of unsonicated positively-charged liposomes and to 45 μM on the addition of sonicated liposomes to the same stearylamine concentration. In these experiments the concentration of phosphate had to be decreased to 1 mM for a significant effect of adsorption on enzyme activity to occur. K_m for glyceraldehyde 3-phosphate of the enzyme in the presence of sonicated positively-charged liposomes plus 0.5% Triton X-100 was 120 μM . As noted previously (Shin & Carraway, 1973) Triton X-100 decreased the $V_{\max}$ of glyceraldehyde 3-phosphate dehydrogenase both in free solution and in the presence of either positively charged or negatively charged liposomes.

Discussion

Previous studies on the adsorption characteristics of the enzyme to condensed monolayers of phospholipid (Wooster & Wrigglesworth, 1976) have shown that glyceraldehyde 3-phosphate dehydrogenase will bind to charged surfaces by multiple electrostatic interactions. In the present experiments the effects of ionic strength and pH on adsorption of the enzyme to charged liposomes indicate that a similar mechanism of binding is likely. The results also indicate that P_i can compete with the enzyme for binding to positively charged liposomes. Significant effects of these

Fig. 3. Effects on the kinetic properties of glyceraldehyde 3-phosphate dehydrogenase by adsorption to positively charged liposomes

Plots of $[S]/v$ against $[S]$ with glyceraldehyde 3-phosphate as variable substrate in the absence of liposomes (○) and in the presence of unsonicated liposomes (●) and liposomes after 2 min sonication (■). Liposome composition was stearylamine/phosphatidylcholine (3:17, w/w) at a final concentration of stearylamine of 2.6 mM. Conditions were as given in Fig. 2, except that 1 mM-potassium phosphate and 9 mM Tris/HCl buffer, pH 7.6, were used in place of 10 mM-potassium phosphate.

liposomes on enzyme activity were only seen when the P_i concentration in the medium was decreased to 1 mM. The apparent association constant of $0.4 \times 10^6 \text{ M}^{-1}$ for negatively charged liposomes is comparable with that found for binding of the enzyme to monolayers (Wooster & Wrigglesworth, 1976) and close to the range of 10^6 – 10^7 M^{-1} reported for binding to biological membranes (Kant & Steck, 1973; MacDaniel *et al.*, 1974).

The changes in K_m for glyceraldehyde 3-phosphate produced by adsorption of glyceraldehyde 3-phosphate dehydrogenase to both negatively and positively charged liposomes were greater when sonicated liposomes were used. Sonication increases the available surface area for adsorption and will shift the equilibrium in favour of the adsorbed form of the enzyme. It was also found that addition of Triton X-100 to the liposome–enzyme complex abolishes the effects of adsorption on K_m for glyceraldehyde 3-phosphate. This non-ionic detergent does not directly influence ionic interactions between lipids and proteins (Mazia & Ruby, 1968; Maretzki *et al.*, 1974; Hallam & Wrigglesworth, 1976) but would disrupt the low surface curvature of the liposome bilayer to form mixed micelles of surfactant and phospholipid of smaller radius and higher surface curvature (Yedgar *et al.*, 1974). The results would therefore suggest that modification of enzyme activity by adsorption to a charged surface is a function of the surface area and curvature of the adsorbing surface.

The effects of adsorption to charged liposomes on the kinetic behaviour of glyceraldehyde 3-phosphate dehydrogenase can be explained in terms of an unequal distribution of glyceraldehyde 3-phosphate between the surface phase and the bulk phase. For negatively charged surfaces, the electrostatic potential of the surface will decrease the local concentration of negatively charged substrates in the micro-environment of the bound enzyme molecules. This would have the effect of shifting the apparent K_m for the substrate to higher values (Goldstein *et al.*, 1964). An opposite effect will prevail for positively charged surfaces. In the present experiments, adsorption of glyceraldehyde 3-phosphate dehydrogenase to negatively charged liposomes increases the apparent K_m for the negatively charged glyceraldehyde 3-phosphate whereas adsorption of the enzyme to positively charged liposomes decreases the apparent K_m compared with the K_m values for glyceraldehyde 3-phosphate in bulk solution.

The electrostatic field at the surface of a liposome will also affect the local concentration of H^+ , depending on the size and magnitude of the surface charge. Keleti (1970) has suggested that phosphorylation of glyceraldehyde 3-phosphate constitutes the rate-limiting step of the reaction and that the ionization of phosphate in glyceraldehyde 3-phosphate and in the P_i will influence the pH-dependence of the reaction.

Keleti & Batke (1967) have shown that below approx. pH 8, V_{max} for glyceraldehyde 3-phosphate dehydrogenase in free solution is decreased as the pH is decreased. The decreased V_{max} observed in the present experiments for enzyme adsorbed to negatively charged liposomes is unlikely to result from simple inactivation of a fraction of the enzyme molecules on binding since full activity is regained on desorption and no significant effect on V_{max} was found on adsorption of the enzyme to positively charged liposomes, but could be a consequence of the more acid pH in the micro-environment of the enzyme at the negatively charged surface compared with the 'bulk' pH of 7.6. Above pH 7.6, the pH-dependence of V_{max} for glyceraldehyde 3-phosphate dehydrogenase is complex, and depends on the relative concentrations of the three substrates (Keleti & Batke, 1967). These cannot yet be measured at the surface of the liposomes and an analysis of the effect of adsorption on V_{max} at alkaline pH values is not possible.

The present results show that the activity of glyceraldehyde 3-phosphate dehydrogenase can be modified by adsorption to a charged surface. The inner surface of the erythrocyte membrane, to which the enzyme binds *in vitro* with a K_{ass} value of 10^6 – 10^7 M^{-1} , carries many groups of both positive and negative charge (Hallam & Wrigglesworth, 1976). The adsorption of the enzyme to the inner surface of the erythrocyte membrane is sensitive to micromolar concentrations of its substrates as well as to the ionic strength of the suspending medium (Kant & Steck, 1973). Preliminary results reported by Wrigglesworth *et al.* (1976) suggest that when glyceraldehyde 3-phosphate dehydrogenase is adsorbed to the inner surface of the erythrocyte membrane, the kinetic properties of the enzyme are modified. The possibility arises that attachment of the enzyme to the membrane *in vivo* and the subsequent modification of the activity of the enzyme are regulated by changes in substrate concentrations.

M. S. W. was supported by a grant from the Wellcome Trust. J. M. W. acknowledges a grant from the Central Research Fund, University of London.

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