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THE INTERACTION OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE WITH BOVINE ERYTHROCYTE MEMBRANES

An analysis of the binding of glyceraldehyde 3-phosphate dehydrogenase to bovine erythrocyte membranes by studying the purified components association was undertaken. The effect of this binding on the enzyme catalytic activity and the influence of some chemical factors on the release of the membrane bound enzyme were also studied.

Though glycolytic enzymes are considered to be soluble cytoplasmic constituents, several glycolytic enzymes have been found to bind in vitro and in vivo to membranes and other subcellular structures. But the physiological significance of these interactions remains obscure.

Materials and methods

Enzyme activity was measured by the method of C o r i et al. [1]. Bovine erythrocyte ghosts were prepared by hypotonic haemolysis according to the method of D o d g e et al. [2] with slight modifications.

Ghosts had about 1.1 activity units of glyceraldehyde 3-phosphate dehydrogenase (G3PD) per mg of membrane protein, it means about 85 per cent of the whole cell enzyme activity.

G3PD was isolated from the bovine erythrocyte membranes and purified on (G-100) Sephadex column. The purified bovine enzyme showed about 35 activity units/mg of protein.

Binding assay was performed in low ionic strength medium, pH

7.4, with depleted ghosts (it means that ghosts devoid of G3PD by washing with phosphate buffered saline). Following an one--hour incubation on ice, the membranes were pelleted and the supernatant G3PD assayed. Membrane-bound G3PD was estimated by subtracting the supernatant activity from the total activity in the suspension before centrifugation.

Results

The constant amount of membranes (60 μg/ml) was incubated with an increasing amount of G3PD. For the higher concentrations of G3PD the saturation of binding sites of the ghosts by the enzyme was observed.

The capacity and affinity for the ghosts - G3PD interaction were estimated by plotting of equilibrium binding data according to E a d i e and H o f s t e e [3, 4] as $B = B_{max} - k_d(B/F)$, where B is G3PD bound per g of membrane protein, F is the concentration of free G3PD and k_d is the dissociation constant.

A curve of concave shape was obtained indicating that there are two classes of binding sites: $(0.30 \pm 0.13) \times 10^{18}$ G3PD sites per g of membrane protein were of high affinity with the dissociation constant $k_d = (6.3 \pm 3.9) \times 10^{-9}$M and $(7.21 \pm 1.18) \times 10^{18}$ sites per g were of low affinity, the dissociation constant for these sites was $k_d = (5.65 \pm 2.16) \times 10^{-7}$M.

It was also found that the binding of G3PD to the membrane caused the reversible loss of enzyme activity.

Activity of the enzyme was measured using ionic strength, pH and substrate levels which did not cause the elution of membrane-bound enzyme because the binding of G3PD is very sensitive to these factors.

Constant amount of G3PD (10 μg/ml) was incubated with increasing amounts of membrane protein. There was a good correlation between enzyme inactivation and enzyme binding at the same incubation conditions. The bound enzyme did not show any activity.

As it was told the interaction of G3PD with membrane ghosts is very sensitive to medium ionic strength and pH. The release of bound G3PD increased sigmoidally with ionic strength, with a

50% elution at about 70 μM ionic strength. Similar effect was observed with the increasing pH. The increase of the ionic strength of the medium shifted the pH elution curve to the left, potentiating the dissociation of the enzyme. These data suggested an electrostatic component in the binding reaction.

The metabolite specifity for the desorption of G3PD from the membranes was also studied. Strong effect on the enzyme release from the membrane showed such metabolites as: NADH, NADPH, ATP and ADP; the strongest enzyme release showed NAD with 0,1 mM 3-phosphate glyceraldehyde. The metabolite concentration was 2 mM. It is of interest that while the reduced forms of NAD and NADP cause the specific enzyme release, their oxidated forms do not cause this.

Discussion

It was found that there are two classes of binding sites for G3PD in bovine erythrocyte membranes. Binding of the enzyme to the membrane inactivates the enzyme reversibly and though probably the electrostatic component in the binding reaction was found, it seems very likely that the interaction is highly specific. The data obtained support the hypothesis that the interaction of glycolytic enzymes with membranes plays a role in the regulation of the enzyme activity and that the G3PD of bovine erythrocyte may partition between the cytoplasm and specific membrane sites in manner which is responsive to local variation in pH, ionic strength and metabolite concentrations.

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ODDZIAŁYWANIE DEHYDROGENAZY ALDEHYDU 3-FOSFO-D-GLICERYNOWEGO Z BŁONAMI ERYTROCYTÓW KROWY

Dokonano analizy wiązania dehydrogenazy aldehydu 3-fosfo-D--glicerynowego do błon erytrocytów krowy, badając asocjację izolowanego czystego enzymu. Zbadano także wpływ tego wiązania na aktywność katalityczną enzymu i wpływ niektórych czynników chemicznych na uwalnianie związanego z błoną enzymu.