PARTIAL REVERSIBLE INACTIVATION OF ENZYMES DUE TO BINDING TO THE HUMAN ERYTHROCYTE MEMBRANE

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Summary

Hypotonic human erythrocyte ghosts, devoid of the original glyceraldehyde-3-phosphate dehydrogenase content of the red cell, bind added glyceraldehyde-3-phosphate dehydrogenases, isolated from human erythrocytes, rabbit and pig muscle, as well as rabbit muscle aldolase. There are only slight differences in the affinities towards the various glyceraldehyde-3-phosphate dehydrogenases. On the other hand, glyceraldehyde-3-phosphate dehydrogenases are bound much stronger than aldolase; in an equimolar mixture the former can prevent the binding of the latter, or replace previously bound aldolase at the membrane surface. Binding is always accompanied by the partial reversible decrease of enzyme activity encountered at membrane surfaces in vivo. An outstanding example is hexokinase, which has been claimed to be associated with the mitochondrial membrane and this results in its preferential utilization of intra-mitochondrial ATP.

The relation of glycolytic enzymes to the erythrocyte membrane might also be of physiological interest. It has been shown that glyceraldehyde-3-phosphate dehydrogenase is strongly associated to hypotonic ghosts so that it may be regarded as a peripheral membrane protein. Moreover, tracer kinetic data suggested that GAPD was located in or at the membrane, possibly together with other enzymes for the generation of ATP and transport. Recently Kant and Steck put forward evidence that various metabolites seem to influence the binding of GAPD specifically, there are a limited number of equivalent binding sites for the enzyme, and these are located on the inner side of the membrane.

In the present work we studied the interaction of purified human erythrocyte, rabbit and pig muscle GAPD's, as well as rabbit muscle aldolase, with the red cell membrane. We have found that hypotonic ghosts bind large amounts of these enzymes, GAPD's are bound much stronger than aldolase, and whenever binding occurs there is a partial reversible decrease of enzyme activity.

* Abbreviations: GAPD = glyceraldehyde-3-phosphate dehydrogenase; HE = human erythrocyte; RM = rabbit muscle.
Materials and Methods

*Human blood* was obtained from the National Institute of Haematology and Blood Transfusion, Budapest. To all experiments freshly drawn blood was used. Results were not affected by the type of blood.  

*Human erythrocyte GAPD* was prepared according to Maretki and Rapoport\(^1\),\(^2\), but the crystallization step was omitted and the eluate from CM-Sepharose was concentrated on an Amicon Model 202 ultrafiltration apparatus with an XM50 membrane.

In this solution, about 1 mg/ml, the enzyme could be stored in the presence of 1 mM dithiothreitol for several weeks without activity loss. The specific enzyme activity was:

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0.58 \text{ kat} \times \text{kg}^{-1}.
\]

(The crystalline enzyme was reported to have a specific activity of 0.83 kat \times kg\(^{-1}\).) The preparation contained some contaminants; the major one was hemoglobin (cf. Fig. 1).

*Rabbit and pig muscle GAPD* [D-glyceraldehyde-3-phosphate: NAD oxidoreductase (phosphorylating) \(\text{EC 1.2.1.12}\)] were prepared and three times recrystallized according to Eldén and Szőrényi\(^1\),\(^3\),\(^4\). The molar activity of GAPD was 250 to 330 kat \times mole\(^{-1}\).

GAPD activity was assayed by Warburg’s optical test at 25 °C in the following reaction mixture: 2 mM NAD, 1.9 mM D-glyceraldehyde-3-phosphate, 5 mM NaHASO\(_4\), 50 mM Tris. HCl-0.3 mM EDTA buffer, pH 7.5, with about 1.5 \(\mu\)g/ml (10 \(\text{S} \times 10^{-5}\) mm) GAPD.

*Rabbit muscle aldolase* (Fructose-1,6-bisphosphate: D-glyceraldehyde-3-phosphate lyase, \(\text{EC 4.1.2.13}\)) was prepared and three times recrystallized by the method of Taylor et al.\(^1\),\(^5\),\(^6\),\(^7\). Aldolase activity was assayed according to Jagannathan et al.\(^8\), Aldolase activity was assayed according to Jagannathan et al.\(^8\),\(^9\) in the following reaction mixture: 1.7 mM fructose-1,6-bisphosphate, 10 mM hydrazine sulphate, 50 mM Tris. HCl-0.3 mM EDTA buffer, pH 7.5, with about 4 \(\mu\)g/ml (2.5 \(\times 10^{-5}\) mm) aldolase. The specific activity of aldolase, as defined by Friedrich et al.\(^10\), was 45, which corresponds to a molar activity of 50 kat \times mole\(^{-1}\) in the dehydrogenase-coupled assay system.

Fructose-1,6-bisphosphate and NAD were REANAL (Hungary) products. Glyceraldehyde-3-phosphate was prepared from fructose-1,6-bisphosphate according to Szewczuk et al.\(^11\).

\(^{(14C)}\text{Iodoacetate acid* was purchased from Radiochemical Center, Amersham; specific radioactivity was 33 mCi/m mole. Radioactivity was measured in a Packard TriCarb Model 2420 liquid scintillation spectrometer, by using a toluene-based scintillation cocktail. Radioactive material was recovered from polyacrylamide gels by cutting the gel rod (5 mm \(\phi\)) into 2 mm-slices, followed by incubation of each gel slice in 0.7 ml of Soluene 100 in the counting vial at 60 °C for 2 hours. After cooling, the scintillation cocktail was added to the vials.  

Carboxymethylation of GAPD’s was performed at 1 mg/ml protein and 0.2 mM iodoacetate (unlabelled or \(^{14C}\)-labelled) concentration in standard hypotonic buffer at 20 °C. The reaction was monitored by following the decrease of enzyme activity. When residual activity reached 5%, cysteine was added to 2 mm final concentration. The mixture was dialyzed against several changes of standard hypotonic buffer. In the case of HE GAPD, which was not a homogeneous preparation, we ascertained that under the conditions applied only the enzyme became radioactively labelled (Fig. 1).

Protein concentration of HE GAPD preparations was determined according to Lowry et al.\(^12\), whereas that of RM aldolase and RM and pig muscle GAPD solutions was measured spectrophotometrically by using the extinction coefficients \(A_{280} = 0.74\) and \(A_{280} = 1.0\), respectively. Hemoglobin was assayed by the cyanmethemoglobin method\(^21\).

Preparation and properties of standard ghosts. Human blood was washed three times in 0.9% NaCl-20 mM sodium phosphate buffer, pH 7.2. Erythrocyte ghosts were prepared by hypotonic hemolysis according to the principles described by Dodge et al.\(^22\). One volume of packed cells was added to 14 volumes of 10 mM sodium phosphate buffer, pH 7.4, at 0 °C. Ghosts were collected by centrifuging at 20,000 \(\times g\) in a Janetzki K-24 centrifuge for 40 minutes at 0 °C. Ghosts were washed three times in 7 mM sodium phosphate buffer, pH 7.4. (In the following this buffer will be referred to as “standard hypotonic buffer”.)

The final ghost suspension was slightly pink and contained 8 to 10 mg dry weight per ml (4 to 5 \(\times 10^7\) ghosts per ml). Of the total amount of GAPD activity present in the hemolysate 8 to