STUDY OF METABOLITE COMPARTMENTATION IN ERYTHROCYTE GLYCOLYSIS

Peter FRIEDRICH, Veronika A. APRÓ-KOVÁCS and Magda SOLTI
Enzymology Department, Institute of Biochemistry, Hungarian Academy of Sciences, Karolina u. 29, 1113 Budapest, Hungary

Received 29 August 1977
Revised version received 3 October 1977

1. Introduction

The glycolytic enzymes of the erythrocyte seem to be supramolecularly organized. Several enzymes, above all glyceraldehyde 3-phosphate dehydrogenase [1–4], aldolase [4,5] and phosphoglycerate kinase [6,7], bind fairly strongly to the inner side of the membrane under hypotonic conditions. The kinetics of incorporation of extracellular inorganic phosphate into ATP also suggest that certain enzymes are located close to each other at the erythrocyte membrane [8–10]. Indeed, elaborate schemes for the putative enzyme–enzyme interactions in the red cell have been presented [7,11].

One of the possible consequences, and at the same time physiological significance, of multienzyme organization is the direct transfer of metabolite(s) from one enzyme to the other, i.e., the so-called channelling effect observed with rigid enzyme aggregates [12]. It has been proposed that such phenomena might also occur in a system of enzymes that are usually isolated as separate entities [13]. Recent kinetic data obtained with crystalline muscle enzymes suggest that GAP is directly transferred from aldolase to GAPD [14].

In the present work an approach has been devised to tackle the question of metabolite compartmentation (channelling) in human erythrocyte glycolysis at the GAPD reaction. To this end α-glycerophosphate dehydrogenase, an enzyme absent from mature human erythrocytes, was added to red cell sonicates and its interference with the production of lactate from glucose was measured.

Abbreviations: GAPD, glyceraldehyde 3-phosphate dehydrogenase; GDH, α-glycerophosphate dehydrogenase; LDH, lactate dehydrogenase; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; α-GP, α-glycerophosphate

2. Materials and methods

Freshly drawn human blood was obtained from the National Institute of Haematology and Blood Transfusion, Budapest. Red cells were washed three times in 0.9% NaCl–1 mM sodium phosphate, pH 7.4, and once in Krebs-Ringer solution (121 mM NaCl, 4.8 mM KCl, 1.21 mM KH_2PO_4, 16.5 mM Na_2HPO_4, 1.21 mM $MgSO_4$, pH 7.0) at 4°C.

GDH (EC 1.1.1.8) was prepared from rabbit muscle and recrystallized four times [15]. The enzyme crystals were dissolved and dialyzed against Krebs-Ringer to remove ammonium sulfate. LDH–M_4 (EC 1.1.1.27) for the lactate assay was prepared from pig muscle and recrystallized four times [16]. Enzyme concentrations were determined spectrophotometrically by using the coefficients, $E_{280}^{1\,mg/ml}$, 1.0 and 1.29 for GDH and LDH, respectively. The molar activity of GDH was 310 kat/mol enzyme in 0.1 M glycine–NaOH–1.3 mM EDTA buffer, pH 10, and 80 kat/mol enzyme in 5 mM sodium phosphate buffer, pH 7.5, containing 110 mM KCl, 10 mM NaCl and 2.5 mM $MgCl_2$, at 37°C with 47 mM α-GP and 2.5 mM NAD as substrates.

Packed red cells were sonicated in an MSE 150 W ultrasonic disintegrator at high power for 30 s at 0°C. Haemolysis was >98%. Lactate production from 5 mM glucose was measured in sonicates of 90% and 30% nominal haematocrit values at 37°C. All additions to the packed red cell sonicate were introduced with vigorous mixing on a Vortex-Genie. It did not affect

the results if GDH was mixed in by sonication. The pH of sonicates was 7.3 to 7.5 and did not change detectably during the incubation. Lactate was determined enzymatically from the trichloroacetic acid-supernatant of aliquots, essentially as described [17].

3. Results

The time courses of lactate production in erythrocyte sonicates in the presence and absence of GDH are shown in fig.1. The progress curves were linear within the period examined. It is seen that in the concentrated sonicate (90% haematocrit) GDH did not influence lactate formation, whereas it reduced the rate to about one-half after three-fold dilution of the system. It should be noted that lactate production in sonicates varied from 2.9–4.2 μmol lactate/ml red cell × h with different blood specimens, but the relative differences with and without GDH were similar to those in fig.1 in all experiments (altogether 5). Furthermore, the same result was obtained if, instead of Krebs-Ringer, the three-fold dilution was made with 1 mM or 5 mM sodium phosphate buffer, pH 7.5, containing 110 mM KCl, 10 mM NaCl and 2.5 mM $MgCl_2$ (4 experiments).

A possible reason of the ineffectiveness of GDH at 90% haematocrit might be that the enzyme is inhibited by the concentrated sonicate. However, this seems not to be the case, as indicated by the following comparison (fig.2): the amount of lactate formed from α-GP in the presence of GDH in the sonicate fairly agreed with the amount of α-Gp oxidized by GDH under comparable conditions in a buffer medium, up to the point (about 1 μg/ml GDH) where the glycolysing ability of the sonicate became rate-limiting (fig.2, inset).

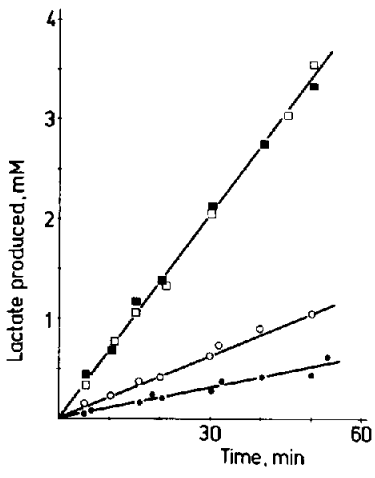

Fig.1. Time course of lactate production in erythrocyte sonicates in the presence and absence of GDH. (A) To 2.7 ml packed red cell sonicate glucose and NAD were added in 0.3 ml Krebs-Ringer to give final concentrations 5 mM and 0.5 mM, respectively (90% haematocrit). An 0.7 ml aliquot of this mixture was then diluted with 1.4 ml Krebs-Ringer (30% haematocrit). Both 90% (□) and 30% (○) haematocrit samples were incubated at 37°C and at the times indicated 0.2 ml aliquots were taken for lactate determination. (B) The same as A, except that the 0.3 ml addition also contained rabbit muscle GDH to give a final concentration of 0.5 mg/ml. 90% (■) and 30% (●) haematocrit, respectively. (The data of two parallel runs made from the same blood specimen are shown.)

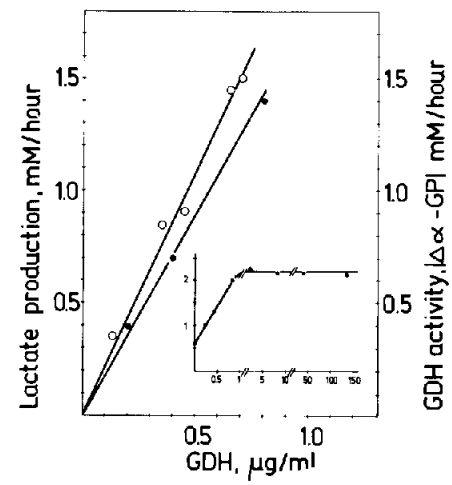

Fig.2. Comparison of GDH activity and lactate production from α-GP in sonicate. Lactate (●) was produced in concentrated sonicate (90% haematocrit) with 47 mM α-GP, 0.5 mM NAD and GDH as indicated, at 37°C. The progress curves of lactate production were linear up to at least 40 min. From the actually measured rates the 'blank' rate, i.e., lactate formed without any GDH added (0.6 mM/h), was subtracted. GDH activity (○) was determined in initial velocity measurements in 5 mM sodium phosphate buffer, pH 7.5, containing 110 mM KCl, 10 mM NaCl and 2.5 mM $MgCl_2$, with 47 mM α-GP and 0.5 mM NAD as substrates at 37°C.
Inset: Total lactate production in the sonicate as a function of GDH concentration. Conditions: cf. above. Coordinates are the same as the corresponding ones in the main figure.

4. Discussion

GDH catalyzes the following reaction:

$$DHAP + NADH + H^+ \rightleftharpoons \alpha\text{-GP} + NAD^+$$

The other triose phosphate, GAP, may also serve as substrate instead of DHAP [18]. Near neutral pH the equilibrium is far to the right. Accordingly, this enzyme can divert the metabolite flow in glycolysis by converting triose phosphates and NADH to α-GP and NAD. Rabbit muscle GDH when added to human erythrocyte sonicate acts as an 'enzyme-probe', testing whether the relevant metabolites are available in the bulk medium: if they are, lactate production will be diminished; if they are not, lactate production will remain unaffected. A crucial point in assessing the response of such an 'enzyme-probe' is the effect of diluting the system. Conceivably, any kind of metabolite compartmentation (channelling) created by weakly interacting enzymes is bound to be concentration-dependent, inasmuch as it should disappear on dilution.

In the 90% haematocrit sonicate, in which concentrations are close to those in the intact cell, GDH had no effect on the rate of lactate formation, whereas in the 30% haematocrit sonicate it markedly reduced lactate production. This experimental finding is consistent with expectations if triose phosphates and/or NADH are compartmented within erythrocyte glycolysis as a result of loose macromolecular associations.

Specific differences between the substrate saturation curves of the enzymes involved would also explain the above phenomenon by simple enzyme competition, without any resort to compartmentation. Namely, if the K_m of GAP were lower for GDH than for GAPD, and/or the K_m of NADH were lower for GDH than for LDH, then the dilution of metabolites would promote the formation of α-GP. The same effect would ensue, if the K_m of GAP and DHAP for GDH were lower than the K_m of pyruvate for LDH. However, these relations apparently do not hold. In the physiological pH-range K_m^{GAP} is 0.16 mM for GDH [19] and 0.13 mM for human erythrocyte GAPD [20], i.e., they are practically identical; K_m^{NADH} is 30 μM for GDH (with GAP substrate) [19] and about 3 μM for LDH-H_4 [21], i.e., dilution should favour lactate production; K_m^{Pyr} is 0.04 mM for LDH-H_4 [21], whereas K_m^{GAP} is 0.16 mM [19] and K_m^{DHAP} is 0.35 mM for GDH [22,23], which again would rather promote lactate production on dilution. All substrate saturation curves are hyperbolic, which is a prerequisite of comparison.

Figure 2 demonstrates that the GDH 'enzyme-probe' is not inhibited in the concentrated sonicate. In addition, the independence of lactate production from GDH concentration above 1 μg/ml, and the fact that this rate is close to the glycolytic rate of intact cells (about 2 μmol lactate/ml red cells × h [24]) point to the importance of the lower part of glycolysis in flux control, in contrast with current views [25].

Work is in progress to describe the response of the 'enzyme-probe' more rigorously and to extend this approach to the study of muscle glycolysis. At present we suggest that there is metabolite compartmentation at the GAPD reaction in the glycolytic pathway of the human erythrocyte.

References

[1] Tanner, M. J. A. and Gray, S. R. (1971) Biochem. J. 125, 1109–1117.
[2] Carraway, K. L. and Shin, B. C. (1972) J. Biol. Chem. 247, 2102–2108.
[3] Kant, J. A. and Steck, T. L. (1973) J. Biol. Chem. 248, 8457–8464.
[4] Solti, M. and Friedrich, P. (1976) Mol. Cell. Biochem. 10, 145–152.
[5] Strapazon, E. and Steck, T. L. (1976) Biochemistry 15, 1421–1424.
[6] Schrier, S. L., Ben-Bassat, I., Junga, I., Seeger, M. and Grumet, F. C. (1975) J. Lab. Clin. Med. 85, 797–810.
[7] Tillmann, W., Cordua, A. and Schröter, W. (1975) Biochim. Biophys. Acta 382, 157–171.
[8] Gourley, D. R. H. (1952) Arch. Biochem. Biophys. 40, 1–12.
[9] Latzkovits, L., Szentistványi, I. and Fajszi, Cs. (1972) Acta Biochim. Biophys. Acad. Sci. Hung. 7, 55–66.
[10] Niehaus, W. G. and Hammerstedt, R. H. (1976) Biochim. Biophys. Acta 443, 515–524.
[11] Green, D. E., Murer, E., Hultin, H. O., Richardson, S. H., Salmon, B., Brierley, G. P. and Baum, H. (1965) Arch. Biochem. Biophys. 112, 635–647.
[12] Ginsburg, A. and Stadtman, E. R. (1970) Ann. Rev. Biochem. 39, 429–472.
[13] Friedrich, P. (1974) Acta Biochim. Biophys. Acad. Sci. Hung. 9, 159–173.
[14] Keleti, T., Batke, J., Ovádi, J., Jancsik, V. and Bartha, F. (1977) in: Adv. Enzyme Regul. (Weber, G. ed) Vol. 15, pp. 233–265, Pergamon Press, Oxford, New York.

[15] Telegdi, M. (1964) Acta Physiol. Hung. 25, 177–180.
[16] Jécsai, G. (1961) Acta Physiol. Hung. 20, 339–346.
[17] Beutler, E. (1971) in: Red Cell Metabolism, pp. 109–111, Grune and Stratton, New York, London.
[18] Telegdi, M. (1968) Biochim. Biophys. Acta 159, 227–235.
[19] Telegdi, M. and Keleti, T. (1968) Acta Biochim. Biophys. Acad. Sci. Hung. 3, 131–140.
[20] Maretzki, D. and Rapoport, S. (1972) Acta Biol. Med. Germ. 29, 207–221.
[21] Schwert, G. W. and Hakala, M. T. (1952) Arch. Biochem. Biophys. 38, 55–65.
[22] Young, H. L. and Pace, N. (1958) Arch. Biochem. Biophys. 75, 125–141.
[23] Blanchaer, M. C. (1965) Can. J. Biochem. 43, 17–24.
[24] Reimann, B., Küttner, G., Maretzki, D. and Rapoport, S. (1975) Acta Biol. Med. Germ. 34, 1777–1785.
[25] Heinrich, R., Rapoport, S. M. and Rapoport, T. A. (1977) Progr. Biophys. Molec. Biol. 32, 1–82.