Glycerol-3-Phosphate Dehydrogenase Activity in the Red Cells of Patients With Thalassemia

By Phaedon Fessas, Nick P. Anagnou, and Dimitris Loukopoulos

L-α-Glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) has been reported to be absent in the erythrocytes of normal adults, but can be found in those of cord blood and of thalassemia major. The aim of this study was to investigate whether there is any relation between GDH and γ-chain synthesis. Erythrocyte GDH activity was determined on 118 different blood samples. It was undetectable in normal adult erythrocytes and definitely high in cord blood cells (23.6 IU/10^11 RBC). Considerable GDH activity was also noted in patients with thalassemia major (11.0 IU/10^11 RBC) as well as in cases with pronounced reticulocytosis (11.4 IU/10^11 RBC). Red cells from β-thalassemia heterozygotes exhibited moderate but distinct GDH activity (5.2 IU/10^11 RBC). After fractionation into young and old erythrocyte populations, clearly higher GDH activity was found in the younger cells; however, there was no significant correlation with the reticulocyte count. Presence of reticulocytes alone appears insufficient to explain the values obtained in cord blood and the thalassemias, especially heterozygous. Furthermore, no direct correlation between GDH and fetal hemoglobin (HbF) was obtained in cord blood.

In view of the rather fragmentary and, in part, conflicting data of the literature on GDH of human erythrocytes, we were interested in obtaining more information concerning the behavior of the cytoplasmic form of this enzyme in various types of red cells; in particular, because the reported presence of GDH in erythrocytes with high levels of HbF, such as occur in cord blood and in thalassemia, and its absence in normal adult erythrocytes, could imply a possible association between the two phenomena.

MATERIALS AND METHODS

Blood samples from 118 subjects, belonging to the following six groups, were examined.

**Group A.** Adult controls fulfilling the following criteria: normal hematologic parameters, reticulocytes less than 2%, normal hemoglobin pattern.

**Group B.** Patients with pronounced reticulocytosis of various etiology; mean reticulocyte count was 15% (387.7 × 10^9/liter), ranging from 4.2% (169.0 × 10^9/liter) to 44% (981.2 × 10^9/liter).

**Group C.** Fifteen cases of healthy carriers of β-thalassemia. Thirteen had classical high A2 β-thalassemia (Hb A2 >3.4%); the other two had αβ-thalassemia with increased Hb F (>2.5%).

**Group D.** This group was comprised of 23 cases of clinically severe or intermediate β-thalassemia; of these, 7 had been splenectomized. Eleven patients had never been transfused, while the rest were transfused rather irregularly; on the latter, the blood samples were drawn at least 60 days after their last transfusion.

**Group E.** Cord blood samples obtained from 32 normal, full-term neonates at delivery.

**Group F.** A small number of samples from various other conditions was also studied.

Blood samples (15 ml approximately) were drawn into heparinized tubes. Red cell parameters and reticulocyte counts were determined by standard methods. A 3-ml aliquot of blood was processed...
Red cell GDH and thalassemia

Fig. 1. The 1-α-glycerol-3-phosphate cycle. Cytoplasmic GDH (cGDH) links glycolysis and phospholipid metabolism by converting dihydroxyacetone phosphate to 1-α-glycerol-3-phosphate. Simultaneously, (cGDH) links glycolysis and phospholipid metabolism by converting dihydroxyacetone phosphate to 3-α-glycerol-3-phosphate. Simultaneously, (mGDH) for the transfer of reducing equivalents from the cytoplasmic NADH to the respiratory chain.

RESULTS

GDH Activity of Unfractionated Blood

The activity of GDH of the unfractionated blood of the five groups, expressed both as IU/g Hb and as IU/10^11 RBC, is summarized in Table 1. No activity was found in the unfractionated blood of normal adults. The highest activity was found in the cord blood erythrocytes and to a lesser degree in the cells of thalassemia major and in cases with pronounced reticulocytosis. Moderate but distinct activity was detected constantly in β-thalassemia heterozygotes. Comparison of the mean values of unfractionated blood of the five groups by pairs, showed statistically significant differences in all cases when the activity was expressed as IU/g Hb. When expressed in IU/10^11 RBC, all p values were significant except between groups B and D (p < 0.45).

Enzymatic activity in the unfractionated blood of the thalassemia major group did not seem to be influenced by the presence or absence of the spleen or by the transfusional history.

The GDH activity in the heterogroupic group F is shown in Table 2. In the three cases of sickle cell-thalassemia, a measurable activity was detected. No activity was found in one case of β-thalassemia silent gene. Interestingly enough, very high activities were found in two cases of heterozygous β-thalassemia with a concomitant deficiency of G6PD but no evidence of increased hemolysis. Some activity was also detected in patients with α-thalassemia syndromes, in particular one case with α-thalassemia/Hb Athinae syndrome (7.1 IU/g Hb).

GDH Activity After Fractionation of Erythrocytes

The aliquots of 118 samples were separated into young and old erythrocytes as outlined in Materials and Methods. The findings are presented in Fig. 2 and Table 1. Highly significant differences between the MCHC (mean corpuscular hemoglobin concentration) and reticulocyte percentage in all groups (p < 0.0005) between the two extreme layers testify to the efficiency of the separation; very significant differences in the percentage of HbF were also obtained in...
expressed in lU/g RBC. all were significant except between groups B and D (p < 0.05).

The reticulocyte-rich layer of normal controls.

By this procedure it was possible to detect a slight activity in two extreme layers were highly significant. By this the unfractionated sample; the differences between the two fractions of the three groups presenting this

Although it is clear that higher GDH activity is a constant finding in the younger erythrocyte populations, when reticulocyte percentages of the unfractionated samples or of the top layers were plotted against the respective enzyme activity, no correlation was found in any of the groups. Within the high reticulocytosis group B, five cases presented a stable-state retic-

<table>
<thead>
<tr>
<th>Group</th>
<th>Disease</th>
<th>GDH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF</td>
<td>1010^11 RBC</td>
<td>IU/g Hb</td>
</tr>
<tr>
<td>Top</td>
<td>30.2 ± 2.8</td>
<td>14.9 ± 12.2</td>
</tr>
<tr>
<td>Bottom</td>
<td>34.0 ± 3.9</td>
<td>3.2 ± 7.0</td>
</tr>
<tr>
<td>Top</td>
<td>26.6 ± 4.6</td>
<td>31.2 ± 23.7</td>
</tr>
<tr>
<td>Bottom</td>
<td>34.0 ± 3.9</td>
<td>3.2 ± 7.0</td>
</tr>
<tr>
<td>Top</td>
<td>27.9 ± 2.6</td>
<td>6.6 ± 6.2</td>
</tr>
<tr>
<td>Bottom</td>
<td>32.8 ± 2.8</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Top</td>
<td>26.9 ± 4.8</td>
<td>20.0 ± 13.8</td>
</tr>
<tr>
<td>Bottom</td>
<td>33.2 ± 4.5</td>
<td>1.7 ± 3.6</td>
</tr>
<tr>
<td>Top</td>
<td>27.4 ± 2.4</td>
<td>13.2 ± 7.0</td>
</tr>
<tr>
<td>Bottom</td>
<td>34.3 ± 3.0</td>
<td>0.2 ± 0.2</td>
</tr>
</tbody>
</table>

UF, unfraccionated blood.

*Comparison (p) between GDH activity of top and bottom layer.

† Values obtained from the comparison of the mean values of UF of each group and the rest were all significant when expressed in IU/g Hb. When expressed in IU/10^11 RBC, all were significant except between groups B and D (p < 0.45).

the two fractions of the three groups presenting this hemoglobin. GDH activity was always higher in the top fraction compared to the bottom fraction and to the unfractionated sample; the differences between the two extreme layers were highly significant. By this procedure it was possible to detect a slight activity in the reticulocyte-rich layer of normal controls.

Table 2. Characteristics of Fractionated Erythrocytes and GDH Activity of the Three Layers of Erythrocytes From the Five Groups (Mean ± SD)

<table>
<thead>
<tr>
<th>No.</th>
<th>Disease</th>
<th>Fraction</th>
<th>MCHC (g/dL)</th>
<th>Reticulocytes (%)</th>
<th>HbF (%)</th>
<th>GDH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IU/g Hb UF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IU/10^11 RBC</td>
</tr>
<tr>
<td>1</td>
<td>M/β-thal/HbS</td>
<td>Top</td>
<td>28.8 ± 2.5</td>
<td>3.0 ± 1.2</td>
<td>—</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>34.3 ± 2.1</td>
<td>0.0 ± 0.0</td>
<td>—</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>M/β-thal/HbS</td>
<td>Top</td>
<td>30.7 ± 0.8</td>
<td>1.0 ± 0.4</td>
<td>—</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>34.3 ± 2.1</td>
<td>0.0 ± 0.0</td>
<td>—</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>F/β-thal/HbS</td>
<td>Top</td>
<td>30.2 ± 2.8</td>
<td>14.9 ± 12.2</td>
<td>—</td>
<td>3.7 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>34.0 ± 3.9</td>
<td>3.2 ± 7.0</td>
<td>—</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>F/HbH/Hb Athinae</td>
<td>Top</td>
<td>26.6 ± 4.6</td>
<td>31.2 ± 23.7</td>
<td>—</td>
<td>7.9 ± 2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>34.0 ± 3.9</td>
<td>3.2 ± 7.0</td>
<td>—</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>5</td>
<td>M/HbH</td>
<td>Top</td>
<td>27.9 ± 2.6</td>
<td>6.6 ± 6.2</td>
<td>0.7 ± 0.6</td>
<td>5.8 ± 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>32.8 ± 2.8</td>
<td>0.1 ± 0.3</td>
<td>3.7 ± 3.2</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>6</td>
<td>M/HbH</td>
<td>Top</td>
<td>26.9 ± 4.8</td>
<td>20.0 ± 13.8</td>
<td>39.8 ± 29.4</td>
<td>10.3 ± 3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>33.2 ± 4.5</td>
<td>1.7 ± 3.6</td>
<td>65.9 ± 29.0</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>7</td>
<td>F/HbH</td>
<td>Top</td>
<td>27.4 ± 2.4</td>
<td>13.2 ± 7.0</td>
<td>60.3 ± 8.5</td>
<td>10.0 ± 2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>34.3 ± 3.0</td>
<td>0.2 ± 0.2</td>
<td>89.5 ± 6.2</td>
<td>4.6 ± 1.9</td>
</tr>
</tbody>
</table>

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ulocytosis but here again, no good correlation between percentage of reticulocytes and enzyme activity was found.

**GDH and Fetal Hemoglobin**

The possible relation between HbF percentage and GDH activity was investigated. No significant correlation was found between these two parameters on both unfractionated thalassemia major \(r = 0.08, p < 0.40\) and cord erythrocytes \(r = -0.27, p < 0.15\). To obviate the influence of the presence of young erythrocytes, the same correlation was tested on the bottom layer of thalassemia major and cord erythrocytes and was found lacking. In cord blood, the top layer, which represents the younger but also the more HbA-containing erythrocytes, displayed double enzyme activity than the layer of aged erythrocytes, which contains predominantly fetal hemoglobin. This is illustrated in Fig. 3.

**DISCUSSION**

Our study confirms the absence of detectable GDH activity in the normal adult erythrocytes, as stated in the literature; on the contrary, some activity was constantly detected in the reticulocyte-rich layer of normal controls and was clearly present in the cases with pronounced reticulocytosis of various origin. Dependence of GDH activity on cell age is further supported by all cell separation studies, GDH activity being 4–6 times higher in the lighter compared to the denser fraction of red cells; in cord blood, the difference is less pronounced, possibly because of the younger mean cell age of the cord erythrocyte population. In spite of the obvious connection of GDH activity to cell age in all groups, no good correlation with reticulocyte percentage could be obtained; apparently, cell age cannot be the sole factor determining enzyme activity. This is best illustrated by the data obtained on thalassemia heterozygotes, where reticulocyte percentages are not far from normal and erythrocyte lifespan is only minimally reduced, if at all: they present GDH activity comparable to those of the high reticulocytosis group B.

High GDH activities were observed in the cells of cord blood and of thalassemia major, which is in agreement with the initial studies of Löhr and Waller and their coworkers. In thalassemia major or intermedia, the activity is generally lower than in cord blood, particularly when expressed as enzyme activity per erythrocyte. Assuming a homogenous initial enzyme distribution over the red cell population, which is not necessarily correct, a thalassemic cell has a lower GDH complement than a cord erythrocyte; whether the much smaller size of the thalassemic cell (mean cell volume 72.8 ± 13.4 fl compared to 102.5 ± 7.5 fl) has a role in this difference cannot be deduced.
from the data. Statistical exploration showed no good correlation between GDH and fetal hemoglobin level in both groups; however, the rapid decay of GDH activity with progressing maturation and the presence of a higher quota of HbF in the denser fraction, because of preferential survival of the HbF-containing cells or because of the ongoing switch of γ- to β-chain synthesis,23,24 may cause difficulties in obtaining a quantitative correlation (if such exists) between the two parameters. Good evidence that GDH activity is not connected to HbF synthesis is provided by the β-thalassemia heterozygotes who, while having practically no HbF, present on the average half the enzyme activity of thalassemia major cells. It also appears unlikely that GDH is connected to one particular type of thalassemia, because enzyme activity could be detected in some α-thalassemias without a very pronounced reticulocytosis; furthermore, the group of β-thalassemias studied is not genetically homogeneous. That the phenomenon may not be specific for thalassemia is suggested from the positive findings on other erythrocytic abnormalities, congenital or acquired; the numbers tested, however, are too limited to permit conclusions on the full spectrum of the GDH alterations, physiologic or pathologic.

Increases of the activities of a large number of enzymes in thalassemia have been reported in the literature;8,11,25 27 these increases occur in both homozygotes and heterozygotes, but are somewhat higher in the former. Only few enzymes are found within normal limits. The increases reported for 10 enzymes by Vovan et al.27 range from 1.2 to 2.9 times the normal value for homozygotes and 1.1 to 1.6 for heterozygotes when activities are expressed per number of erythrocytes. The figures given by Grignani et al.14 and by Belfiore et al.11 are also within this range; in the latter study, the values are probably lower if referred to red cell number rather than volume. An exception may be erythrocyte arginase; it has been reported as showing a 2–6-fold increase of the normal mean in thalassemia homozygotes and a modest increase above the upper normal in 3 of 12 cases of thalassemia minor, presumably healthy heterozygotes;28 however, a mean increase of only 30% has been reported by others11 for this enzyme. Thus, apart from some inconsistencies of the literature, the general pattern of enzymatic activities in the thalassemic cell, especially of the heterozygote, appears to be one of a rather modest increase, which may be spuriously high if not referred to number of erythrocytes. In contrast, GDH activity, normally undetectable in the adult red cell, virtually reappears in thalassemia.

At present, we conclude that cytoplasmic GDH activity is a characteristic of human fetal erythrocytes; it is also detectable in adult reticulocytes and persists in the thalassemic erythrocyte to a degree disproportionate to the maturity of the red cell and independent of the hemoglobin F level. The mechanism, as well as the physiologic significance, if any, of this persistence of GDH remains unknown; some aspects of these problems are under current study.

REFERENCES


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