Hereditary Nonspherocytic Hemolytic Anemia and Hexokinase Deficiency

By E. Beutler, P. G. Dyment, and F. Matsumoto

An 11-yr-old child with mild chronic hemolytic anemia was found to have decreased red cell hexokinase activity in spite of the reduced mean age of her red cell population. Similar decreases in red cell hexokinase activity were documented in the patient's parents and in one sib. The red cells were morphologically normal. Red cell 2,3-DPG levels were normal and ATP and glucose-6-phosphate levels were diminished. The kinetic properties, electrophoretic mobility, and thermal stability of the residual red cell hexokinase were normal or nearly so. Glucose consumption of the hexokinase-deficient cells was not appreciably decreased, probably because less of the potent inhibitor glucose-6-phosphate was present in the erythrocytes. It is likely, although not certain, that in this patient nonspherocytic hemolytic anemia resulted from hexokinase deficiency.

HEXOKINASE is the first enzyme in the glycolytic pathway. It catalyzes the phosphorylation of glucose by ATP:

\[
\text{Glucose} + \text{ATP} \rightarrow \text{glucose-6-phosphate} + \text{ADP}
\]

Hereditary hexokinase deficiency appears to be among the least common of the hereditary defects of glycolysis associated with nonspherocytic hemolytic anemia.

CASE REPORT

L.F. was first noted to be anemic at age 11 yr. When first examined at the Cleveland Clinic at age 12 yr, her only symptom was that of mild fatigue of 1 yr duration. There was no history of neonatal jaundice or of anemia during early childhood. Physical examination was normal; no hepatomegaly or splenomegaly was present. The hemoglobin concentration was 11.6 g/dl, hematocrit 34", and red cell count $3.6 \times 10^6$/cu mm. Red cell morphology appeared normal on the blood film. Reticulocyte count was 5.1", total bilirubin 1.0 mg/dl with direct reacting bilirubin 0.5 mg/dl, hemoglobin electrophoresis normal, haptoglobin less than 10 mg/dl, direct and indirect Coombs' tests negative, and heat stability test for unstable hemoglobin and autohemolysis test normal. Eight months later the hemoglobin concentration was 12.2 g/dl with a reticulocyte count of 7.4". The whole blood $P_{50}$ was normal at 25.5 mm Hg. Examination of the blood of family members gave the following results: mother, hemoglobin 12.5 g/dl, reticulocytes 0.7"; father, hemoglobin 17.1 g/dl; brother, hemoglobin 13.9 g/dl, reticulocytes 1.4"; sister, hemoglobin 12.3 g/dl, reticulocytes 0.2".

MATERIALS AND METHODS

Refrigerated blood samples and frozen neutralized perchloric acid extracts prepared from whole blood were shipped from Cleveland to Duarte. A few drops of blood were added to 1% buffered glutaraldehyde and mixed for 18 hr, washed, and examined by scanning electron microscopy. Red
cell enzyme assays and measurements of metabolic intermediates were carried out using standard methodology. Kinetic measurements of the enzyme were carried out using a freshly prepared hemolysate stabilized by the addition of glucose in a final concentration of 0.5 mM. The $K_m$ for ATP was determined at a glucose concentration of 10 mM and the $K_m$ for glucose at an ATP concentration of 10 mM. Thermal stability of the enzyme was investigated at 42° and 45°C using hemolysate stabilized by the addition of 0.5 mM glucose or at 42°C without added glucose. Hexokinase activity was determined at different pH levels using the Tris glycine phosphate system described by Kirkman et al. Starch gel electrophoresis of red cell hexokinase was carried out as previously described.

Glucose consumption and lactate production were measured on the red cells from the patient's, family's, and control blood samples refrigerated in acid citrate dextrose (ACD) solution for 72 hr. The blood was filtered through an α-cellulose microcrystalline cellulose mixture as previously described, and the cells were washed in saline and suspended in 4 volumes of type-AB plasma. Incubation was carried out under an air-CO$_2$ mixture, varying the carbon dioxide continuously so as to maintain the pH at 7.4. Glucose consumption and lactate production were linear for 4–6 hr, and computation of the hourly rate was based on the change after 4 hr incubation.

**RESULTS**

The patient's blood film and the appearance of her red cells on scanning electron microscopy were normal.

The results of glycolytic enzyme assays on the patient and family members are shown in Table 1. In spite of a reduced mean age of the patient's red cell population, as indicated by the elevated reticulocyte count and the increase in activity of age-dependent enzymes such as glucose-6-phosphate dehydrogenase (G-6-PD) and glutamate oxaloacetate transaminase, hexokinase activity was below or at the lower limit of normal. The slightly lower than normal activity of phosphofructokinase was due to the delay in carrying out assays on shipped blood samples; it is the least stable of the red cell glycolytic enzymes. Figure 1 compares the hexokinase activity of the red cells of the propositus with those of 12 consecutive patients with hemolytic anemia studied in this laboratory who had reticulocyte counts ranging to 25%. The patient's parents and one of her sibs also manifested distinctly reduced activity of hexokinase—well below the lower limit of normal.

The levels of red cell metabolic intermediates of the patient and of family members are shown in Table 2. The patient's red cell ATP level appeared to be somewhat lower than normal, but the 2,3-diphosphoglycerate (2,3-DPG) level was normal. The rate of red cell glucose utilization was 1.73 μmoles/hr/ml of cells, compared with a concurrent normal control rate of 1.46 μmoles/hr/ml, and values of 1.56 and 1.41 μmoles/hr/ml for her brothers and sister, respectively. The rates of lactate production in these studies were 3.28, 2.68, 2.36, and 2.91 μmoles/hr/ml of red cells, respectively.

On starch gel electrophoresis both bands I and III of hexokinase activity were visualized in their normal positions. The $K_m$ for ATP was 1.8 mM, compared with a normal control of 2.4 mM, and the average of three determinations of the $K_m$ for glucose was 0.096 mM, compared with control values of 0.074 mM.

The thermal stability of the patient's hexokinase measured in three separate samples was either slightly diminished or normal. The pH optimum of the enzyme was normal at pH 7.9. The capacity of the mutant enzyme to utilize nucleotides other than ATP was difficult to determine because of the low hexokinase activity observed with these analogues, but the rate observed with 1
HEXOKINASE DEFICIENCY

Table 1. Glycolytic Enzyme Assays

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mother</th>
<th>Father</th>
<th>Sister</th>
<th>Brother</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>high S</td>
<td>0.83, 1.05</td>
<td>0.81</td>
<td>0.72</td>
<td>0.88, 0.76</td>
</tr>
<tr>
<td></td>
<td>low S</td>
<td>49.3%, 57%</td>
<td>42%</td>
<td>47%</td>
<td>58.5%, 47%</td>
</tr>
<tr>
<td>GPI</td>
<td>high S</td>
<td>60.7, 68.9</td>
<td>58.67</td>
<td></td>
<td>60.8 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>low S</td>
<td>53%, 56%</td>
<td>50%</td>
<td></td>
<td>62.4 ± 2.4%</td>
</tr>
<tr>
<td>PFK</td>
<td>high S</td>
<td>6.64</td>
<td>9.76</td>
<td></td>
<td>9.05 ± 1.89</td>
</tr>
<tr>
<td></td>
<td>low S</td>
<td>38.2%</td>
<td>33%</td>
<td></td>
<td>16.5% ± 2.01%</td>
</tr>
<tr>
<td>low S+</td>
<td></td>
<td>53.2%</td>
<td>41.4%</td>
<td></td>
<td>29.9% ± 4.15%</td>
</tr>
</tbody>
</table>

"Low-S" values represent enzyme activities measured with less-than-saturating substrate concentration. These values are presented as the percentage of the "high-S" value, which is the activity of the substrate-saturated enzyme, presented as IU/g Hb.

DISCUSSION

As first reported by Valentine et al.5 in 1967, hexokinase deficiency still appears to be a very rare cause of hemolytic anemia. This apparent rarity may be due in part to the difficulty of establishing the diagnosis. Since hexokinase is among the most age-dependent of red cell enzymes, the residual activity present in the red cells of deficient individuals may be as high as the activity in normal red cells. It is usually only when hexokinase activity is considered in relation to the reticulocyte count or the activity of other age-dependent red cell enzymes that it becomes apparent that hexokinase deficiency may be present. Family studies may be particularly helpful in confirmation of the diagnosis. The red...
cells of heterozygotes for the deficiency who do not have hemolytic disease generally have hexokinase activity as low or lower than that of affected individuals. When qualitative abnormalities of hexokinase can be demonstrated, the diagnosis is simplified;\(^6\) however, in some cases, such as in the one we report, the residual enzyme appears to be kinetically and electrophoretically normal.\(^5\) The diagnosis of hexokinase deficiency in our patient was strongly suggested by the finding that the average red cell hexokinase activity was only 74% of mean normal, in spite of a reticulocyte count of 7\(\%\) and elevated activities of age-dependent enzymes such as G-6-PD and glutamate oxaloacetate transaminase. The hereditary nature of the defect was established by family studies, and its functional significance is implied by the lowered levels of glucose-6- and fructose-6-phosphate in the red cells.

### Table 2. Metabolic Intermediates in Patient's and Family Members' Red Blood Cells (nmoles/ml RBC)

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Sister</th>
<th>Brother</th>
<th>High Reticulocyte Controls</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>1112</td>
<td>1245</td>
<td>1405</td>
<td>1281</td>
<td>1508</td>
</tr>
<tr>
<td></td>
<td>1508</td>
<td>1438±99</td>
<td>1438±99</td>
<td>1438±99</td>
<td>1438±99</td>
</tr>
<tr>
<td>2,3-DPG</td>
<td>4521</td>
<td>4068</td>
<td>4157</td>
<td>3657</td>
<td>3181</td>
</tr>
<tr>
<td></td>
<td>3181</td>
<td>4171±63</td>
<td>4171±63</td>
<td>4171±63</td>
<td>4171±63</td>
</tr>
<tr>
<td>G-6-P</td>
<td>17.7</td>
<td>24.4</td>
<td>61±16</td>
<td>61±16</td>
<td>30.5±5.3</td>
</tr>
<tr>
<td>F-6-P</td>
<td>3.7</td>
<td>9.6</td>
<td>22±6.7</td>
<td>22±6.7</td>
<td>9.6±1.5</td>
</tr>
<tr>
<td>DHAP</td>
<td>13.4</td>
<td>18.2±4.6</td>
<td>9.6±1.5</td>
<td>9.6±1.5</td>
<td>9.6±1.5</td>
</tr>
<tr>
<td>FDP</td>
<td>4.0</td>
<td>3±0.9</td>
<td>2.1±0.3</td>
<td>2.1±0.3</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>3-PGA</td>
<td>42.5</td>
<td>60.7±11.9</td>
<td>56.7±5.5</td>
<td>56.7±5.5</td>
<td>56.7±5.5</td>
</tr>
<tr>
<td>2-PGA</td>
<td>6.5</td>
<td>10.7±3.6</td>
<td>5.5±14</td>
<td>5.5±14</td>
<td>5.5±14</td>
</tr>
<tr>
<td>PEP</td>
<td>13.0</td>
<td>20±5.9</td>
<td>11.6±1.8</td>
<td>11.6±1.8</td>
<td>11.6±1.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>1580</td>
<td>1103±260</td>
<td>964.7±132</td>
<td>964.7±132</td>
<td>964.7±132</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>99.6</td>
<td>68.6±28.2</td>
<td>56.3±8.8</td>
<td>56.3±8.8</td>
<td>56.3±8.8</td>
</tr>
</tbody>
</table>
In some cases of hexokinase, deficiency diminished 2,3-DPG levels were reported. In our patient, however, 2,3-DPG levels were normal. This finding was somewhat unexpected in view of the brisk reticulocyte response in the face of a hemoglobin concentration in the lower range of normal. The reason for this apparent discrepancy is not clear. Unexplained lack of correspondence between hematologic response and 2,3-DPG levels was also observed in patients with hereditary spherocytosis.

The normal intracellular concentrations of most red cell metabolic intermediates are sufficiently low so that the enzymes that metabolize them operate well under their maximum velocities. A deficiency in most glycolytic enzymes causes an increase in the concentration of their substrate. This increases the effective rate of the reaction and results in a normal or near-normal metabolic flow of glucose through the erythrocyte. Hexokinase, in contrast to the other glycolytic enzymes, is normally saturated with its substrates, glucose and ATP; increasing the concentration of glucose does not appreciably increase its activity. Moreover, since red cell hexokinase accounts for only a trivial proportion of blood glucose consumption, a decrease in the activity of this red cell enzyme would hardly be expected to influence blood sugar levels. One might therefore anticipate that the consumption of glucose by hexokinase deficient cells would be compromised, and this has indeed been the case in previously studied patients. In our patient, however, the rate of glucose consumption was slightly greater than normal, although perhaps somewhat lower than is usually found in red cells obtained from blood samples with an equivalent reticulocyte count.

In order to understand how this apparent anomaly might come about, it is important to realize that although hexokinase is normally saturated with substrate, it is not functioning at its maximum velocity. The hexokinase reaction at maximal velocity would be sufficient to consume approximately 25 μmoles glucose/ml RBC/hr, approximately 15 times the rate of normal glucose consumption by red cells. Even the reduced hexokinase activity present in our patient's red cells would be capable of phosphorylating approximately 18 μmoles glucose/ml RBC/hr, or approximately 10 times the rate observed. Hexokinase does not operate at its maximal velocity intracellularly because it is strongly inhibited by 2,3-DPG and by glucose-6-phosphate. Since the red cells of the patient contained only about 58% of normal glucose-6-phosphate levels, they reflected a greater proportion of the enzyme's capacity to phosphorylate glucose than do normal erythrocytes. That glucose consumption in the red cells of this patient was normal provides another example of the flexibility of red cell glucose metabolism and the capacity of metabolic pathways to adjust to changes in the activity of even the first enzyme in a chain to maintain normal glucose flow.

Although it seems clearly established that our patient had hereditary erythrocyte hexokinase deficiency, it is prudent to be cautious in assuming a cause-and-effect relationship between the enzyme and the hemolytic anemia. In the case of certain other red cell enzyme defects, notably glutathione reductase deficiency, glutathione peroxidase deficiency, and glyceraldehyde-3-phosphate dehydrogenase deficiency, such a relationship was assumed on the basis of individual
cases. However, in these instances it subsequently became clear that some individuals with a similar degree of enzyme deficiency had no shortening of red cell lifespan. Only when several family members share the defect and all manifest hemolysis, while those whose red cells do not have the defect are hematologically normal, can one be reasonably certain that the enzyme defect does produce the hemolytic anemia. In view of the similarity of this patient’s findings to those reported originally by Valentine et al. and subsequently by others, it seems likely that our patient’s hemolysis was due to hexokinase deficiency. Nonetheless, as in any individual case, a cause-and-effect relationship is not firmly established.

REFERENCES

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