G-6-PD Bangkok: A New Variant Found in Congenital Nonspherocytic Hemolytic Disease (CNHD)

By Phumara Talalak and Ernest Beutler

RED CELL glucose-6-phosphate dehydrogenase deficiency consists of a heterogeneous group of disorders in which mutations affecting the structure of the enzyme give rise to various clinical manifestations. These range from the totally asymptomatic state to drug-induced hemolytic anemia, neonatal jaundice and congenital nonspherocytic hemolytic disease (CNHD). In cases of CNHD eleven distinct variants of this enzyme have already been characterized and differentiated, based on kinetic properties, such as affinity for its substrate, glucose-6-phosphate, and its analogue 2-deoxyglucose-6-phosphate, its coenzyme, NADP, pH optimum, thermal stability and electrophoretic mobility. These variants include Oklahoma, Chicago, Ohio, Albuquerque, Duarte, Mediterranean, Torrance, Hong Kong, Paris, Beaujon and Milwaukee. Other, possibly distinct, variants include "Eyssen," "Tübingen," and "Berlin."

In Thailand, glucose-6-phosphate dehydrogenase deficiency occurs quite commonly. The distribution is, however, uneven with an incidence of 6.5 per cent—7.4 per cent in central Thailand and one as high as 12.6 per cent—15.7 per cent in the northeastern parts. The electrophoretic mobility of the enzyme of deficient Thai subjects has been almost exclusively the normal type "B." Only a few subjects had an enzyme electrophoretic mobility faster than type "A." The manifestations encountered clinically were hemolytic anemia with hemoglobinuria induced by drugs, infections, and occasional neonatal jaundice. Congenital nonspherocytic hemolytic anemia due to this enzyme deficiency has never previously been described in Thailand. We now report biochemical studies of the enzyme from such a patient. These demonstrate existence of a new variant, G-6-PD Bangkok. Clinical and hematological findings are presented in greater detail elsewhere.

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G-6-PD BANGKOK

Table 1.

<table>
<thead>
<tr>
<th>Enzyme Activity (% of normal)</th>
<th>Electro-</th>
<th>Thermal Lability 20° at 46°C</th>
<th>$K_m$ (μM)</th>
<th>$K_m$ (μM)</th>
<th>2dG6P Utilization (% of G6P)</th>
<th>Deamino NADP Utilization (% of NADP)</th>
<th>NAD Utilization (% of NADP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>75-125</td>
<td>10%</td>
<td>40-60</td>
<td>&lt;4</td>
<td>57-63</td>
<td>&lt;3</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>5</td>
<td>90%</td>
<td>60</td>
<td>5.3</td>
<td>8.4</td>
<td>40</td>
<td>14</td>
</tr>
</tbody>
</table>

* Kindly reported to us by Dr. A. Yoshida.

MATERIAL AND METHODS

About 200 ml. of the patient's blood collected in ACD formula A was shipped by air in a plastic bag in ice from Bangkok to Duarte for biochemical studies. G-6-PD was partially purified and the enzyme was characterized using standard methods. A sample of blood was also sent to Dr. A. Yoshida at the International Reference Laboratory for G-6-PD, Seattle, Washington.

CLINICAL FINDINGS

K.T. was an 11 year old boy who was found to be markedly anemic and required blood transfusion at the age of 5 months. Although not noticed during neonatal period, jaundice became apparent at the age of 4 years. He had two episodes of acute hemolysis associated with high fever at the age of 5 and 10. On the latter occasion the hemolysis was more severe, methemoglobinemia and methemoglobinuria were present. The hemoglobin level during the attack was 5.6 Gm. per cent, the red count was 1.85 million/mm.\(^3\), the direct reacting bilirubin was 0.8 mg. per cent, total bilirubin was 2.6 mg. per cent. Two units of packed red blood cells restored his hemoglobin to 10.5 Gm. per cent. Fever and methemoglobinuria subsided after active treatment for five days, and recovery was then uneventful. During his steady state he had a hemoglobin of 10.3 Gm. per cent, a red cell count of 3.4 million/mm.\(^3\); a reticulocyte count of 25.2 per cent, and an increased nonconjugated bilirubin of 1.5 mg. per cent. The red cell osmotic fragility was normal. Screening for G-6-PD\(^20\) immediately after acute hemolysis and blood transfusion was normal; this, however, revealed enzyme deficiency several months later.

The patient's father was Chinese, born in Thailand; the mother was of Thai ancestry. The parents and other siblings had no history of anemia, jaundice or gallstone.

BIOCHEMICAL FINDINGS

The results of biochemical characterization of G-6-PD Bangkok are compared with the normal in Table 1 and in Figure 1. It is apparent that the enzyme was markedly unstable when heated to 40 degrees for twenty minutes. Most of the activity was lost within twenty minutes, while normal enzyme under identical conditions lost only 10 per cent of its activity. There was also a modest increase in the utilization of 2-deoxyglucose-6-phosphate and of the coenzyme analogue deamino-NADP and NAD. The pH optimum curve was slightly displaced toward the more acid side.

DISCUSSION

From the biochemical data it is evident that G-6-PD Bangkok is different
from other previously described mutant enzymes in several respects. The pH optimum of the enzyme is 8-8.5, which is lower than normal, and higher than Duarte, resembling Oklahoma, Albuquerque, and Milwaukee. It is not bimodal as is the pH activity curve of the Mediterranean variant. The electrophoretic mobility of Bangkok is normal type “B,” clearly differentiating it from the Ohio, Eyssen, Milwaukee, and Beaujon variants. The affinity for substrate and coenzyme of G-6-PD Bangkok are normal, while Oklahoma, Albuquerque, Ohio, Milwaukee, Beaujon, and Paris have a high $K_m$ for G-6-P and/or NADP. Tübingen, Mediterranean, and Hong Kong have a low $K_m$. Utilization of 2-deoxyglucose-6-phosphate is higher than normal but lower than that of the Mediterranean variant. Oklahoma, Chicago, Ohio have a normal rate of utilization of 2-deoxyglucose-6-phosphate. G-6-PD Bangkok is very labile to heat. Ninety per cent of the enzyme activity is lost after 20 minutes at 46° C., whereas only 10 per cent of the normal enzyme is lost under the same conditions. This is a common property found in almost every variant giving rise to CNHD. Although some of the individual differences between G-6-PD Bangkok and other variants are too small to be clearly significant, there appears to be no reasonable doubt that this enzyme is unique on the basis of all of the findings collectively.

It is becoming increasingly apparent that among subjects with CNHD tremendous diversity exists in G-6-PD types. This is probably the case because severe selection occurs against this serious disorder. As a consequence, existing mutations are not widely propagated in the population and most of the cases that are detected represent relatively new mutations which have arisen independently of previously described patients.
SUMMARY

A new variant of G-6-PD was found in a Thai boy who had typical clinical and hematologic findings of congenital nonspherocytic hemolytic anemia. This mutant has normal electrophoretic mobility, normal affinity for G-6P and NADP, and is very labile to heat. It has an activity of about 5 per cent of normal and shows increased utilization of 2-deoxyglucose-6-phosphate and NAD and decreased utilization of deamino NADP. It is different from previously described mutants, and is named G-6-PD Bangkok.

SUMMARIO IN INTERLINGUA

Un nove variante de G-6-PD esseva trovate in un puero thai qui habeva typic manifesta- tiones clinic e hematologic de congenite nonsperocytic anemia hemolytic. Le mutante in question ha un normal mobilitate electrophoretic e un normal affinitate pro G-6-P e NADP e es multo thermolabile. Illo ha un activitate de approximativemente 5 pro cento del norma e monstra un augmentate utilisation de 2-deoxyglucosa-fi-phosphato e NAD e un reducite utilisation de deamino-NADP. Illo differe ab previemente de describite mutantes e es designate como G-6-PD Bangkok.

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REFERENCES


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