Detection of Glucose-Phosphate Isomerase Deficiency by a Screening Procedure

By Karl-Georg Blume and Ernest Beutler

A method for rapid detection of red cell glucose-phosphate isomerase deficiency is described. The procedure is based on the appearance of fluorescence, caused by TPNH, that is generated in the linked glucose-phosphate isomerase/glucose-6-phosphate dehydrogenase reaction.

GLUCOSE-PHOSPHATE ISOMERASE (GPI) controls the equilibrium between glucose-6-phosphate (G-6-P) and fructose-6-phosphate (F-6-P). Though GPI does not seem to have a rate-limiting function in red cell glycolysis, deficiencies of this enzyme cause hereditary nonspherocytic hemolytic anemia. Twelve cases from nine different families have been reported suffering from hemolytic anemia caused by GPI-deficiency. All cases were detected during the last 4 yr.

By the method reported herein, large populations can be screened for this particular enzyme deficiency. The procedure is based on an ultraviolet spot test principle that was reported for the rapid detection of the following red cell enzyme deficiencies: galactose-1-phosphate uridyltransferase, glucose-6-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione reductase, pyruvate kinase, triose phosphate isomerase, glutathione peroxidase, and NADH diaphorase.

The method that has been developed depends on the following reaction:

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\begin{align*}
\text{GPI} & \quad \text{G-6-PD} \\
F-6-P & \quad \text{G-6-P} \\
\text{TPN} & \quad 6-	ext{PG} \\
& \quad \text{TPNH}
\end{align*}
\]

When GPI is present in the hemolyzate F-6-P is converted to G-6-P, which is subsequently metabolized to 6-phosphogluconate by glucose-6-phosphate dehydrogenase (G-6-PD) with concomitant reduction of triphosphopyridine nucleotide (TPN) to reduced triphosphopyridine nucleotide (TPNH). The TPNH formed fluoresces when activated with ultraviolet light.

MATERIALS AND METHODS

All reagents were obtained from the Sigma Chemical Co., St. Louis, Mo. The reaction mixture consisted of the following: 1.0 ml Tris-HCl pH 8.0, 1.0 M; 1.0 ml MgCl₂, 0.1 M; 2.0 ml TPN, 2.0 mM; 1.0 ml G-6-PD from Torula yeast, 10 EU/ml; 4.0 ml H₂O; and 1.0 ml F-6-P (20 mM) in 10 mM KH₂PO₄ is added prior to use.

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The hemolyzate was prepared with 5 μl of blood, collected in EDTA, heparin, or ACD, and mixed with 100 μl of water. In the test procedure, 5 μl of hemolyzate were mixed with 100 μl of reaction mixture and incubated at room temperature. A drop of this incubation mixture was spotted on Whatman No. 1 chromatography paper or washed filter paper at intervals. Dry spots were examined under long-wave ultraviolet light.

RESULTS

If normal GPI activity is present in the hemolytes, bright fluorescence of TPNH can be observed after 15 min. This was found in 91 samples from normal subjects and from hospital patients with varying degrees of anemia. As described for the screening test for G-6-PD deficiency,12 the test reported herein is self compensating for anemia; in samples with low hematocrit the decreased enzyme activity is counterbalanced by the decreased quenching effect of hemoglobin. Patients with clinically significant GPI deficiency do not show complete fluorescence for 60 min or more. Fluorescence occurred at an intermediate rate when two heterozygotes were studied. If the residual activity in enzyme-deficient samples is relatively high, preincubation of the hemolyzates from normals and heat-labile variants8,10 at 48°C for 1 hr gives better discrimination. A typical result with two controls, two not yet published cases,1 a G-6-PD-deficient sample, and blanks without substrate and without hemolyzate is shown in Fig. 1.

The diagnosis of GPI deficiency can be made by the use of the spot test reported here. Since ample G-6-PD is present in the reaction mixture, G-6-PD deficiency does not lead to false positive results. G-6-PD from Torula yeast, which is used as an auxiliary enzyme in this assay, is stable even in the frozen

Fig. 1. Ultraviolet spot test for GPI deficiency with two normal samples (27.9 and 29.1 EU GPI/g Hb), two GPI-deficient samples (8.1 and 3.9 EU GPI/g Hb), and a G-6-PD-deficient sample (35.9 EU GPI/g Hb). Controls were performed without F-6-P and without hemolyzates.
state. All commercial F-6-P preparations contain small amounts of G-6-P; therefore, fluorescence can be noticed to a minor extent even if no hemolyzate is added to the assay. As F-6-P is spontaneously converted to G-6-P in alkaline media, separate storage of the substrate in slightly acid solution is necessary. The reaction mixture without F-6-P is stable for at least 17 wk when stored at —20°C. The relatively large number of cases of GPI deficiency that have been described recently suggests that after G-6-PD deficiency and pyruvate kinase deficiency it may be the third most common cause of enzyme-deficient nonspherocytic hemolytic disease. For this reason, it may be useful to screen all patients with unexplained nonspherocytic hemolytic anemias for GPI activity. Because the screening reagent described is stable for a long period and the procedure is very simple, this screening test should be a useful tool in the differential diagnosis of hemolytic diseases.

REFERENCES

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