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Original Investigations

G6PD-Puerto Limón: A New Deficient Variant of Glucose-6-Phosphate Dehydrogenase Associated with Congenital Nonspherocytic Hemolytic Anemia

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Summary. A new glucose-6-phosphate dehydrogenase (G6PD) variant with total deficiency associated with congenital nonspherocytic hemolytic anemia was found in a Costa Rican family. The study of the partially purified enzyme revealed thermal instability, increased G6P affinity, abnormal pH optimum, increased utilization of analogues, and a chromatographic behavior that differs from all the variants previously described. Thus, this new variant was designated G6PD Puerto Limón.

Introduction

Deficiency of glucose-6-phosphate dehydrogenase (G6PD) is the most common genetically determined enzyme abnormality in humans. Until now, over one hundred variants of human G6PD have been described with very variable clinical expression including episodic hemolysis, favism, neonatal hyperbilirubinemia, and congenital nonspherocytic hemolytic anemia (CNSHA) (World Health Organization 1972; Beutler and Yoshida 1973; Yoshida and Beutler 1978). These variants differ from normal in activity, electrophoretic mobility, kinetic characteristics, or chromatographic behavior (Beutler 1978; Luzzatto and Testa 1978). This paper describes a new deficient variant with fast electrophoretic mobility and CNSHA found in a Costa Rican family.

Materials and Methods

Blood from the patients and from control subjects was collected in alsever solution and transported and stored at 4° C. The diagnosis of G6PD deficiency was made by screening procedures (Brewer et al. 1962; Sass and Caruso 1970). Red cell G6PD and 6PGD activity was measured according to the method described by Beutler (1975). G6PD was partially purified by absorption on DEAE-Cellulose (Yoshida 1966) and DEAE Sephadex A-50 (G. Battistuzzi, personal communication). Starch gel electrophoresis was carried out using a phosphate buffer system pH 7.0 (Yunis 1969). Michaelis constant (Km), substrate analogues (2dG6P, deamino NADP), thermal stability, and pH optimum were carried out as described by the World Health Organization (1967).

These studies were performed on semipurified samples, after dialysis, with a solution consisting of 2.7 m*M* EDTA and 7 m*M* β-mercaptoethanol for three hours at 4° C as described by Yunis (1969). The thermostability test was performed by incubation of partially purified enzyme either for 20 min at different temperatures or at 42° C for different times. The residual enzyme activity was measured.

The resolution of G6PD variant by chromatography on DEAE-Sephadex columns was made according to the methods of Usanga et al. (1975) and Luzzatto and Testa (1978). The samples were previously dialysed against a 5 m*M* phosphate buffer containing 1 m*M* EDTA, 1 m*M*-aminocaproic acid, 50 m*M* KCl, and 20 m*M* NADP, and the activity was adjusted to 0.5 Luzzatto units.

Case Report

The propositus J.M. is a 21-year-old male born in Costa Rica of Spanish ancestry. The diagnosis of G6PD deficiency was made at the age of 4 years. He had chronic persistent hemolytic anemia with acute crises during infectious diseases accompanied by jaundice and pallor. He also had biliary lithiasis and no splenomegaly. Recent laboratory data were: hemoglobin 13.2 g/dl, reticulocyte count 15%, packed cell volume 40%, total bilirubin 3.4 mg%, slightly increased autohemolysis, normal osmotic fragility, and negative Coomb's test. During the last eight years the hemoglobin level has remained between 10.3–15.2 g/dl with the reticulocyte count between 8.8–24%. His growth and development are normal.

The family study showed that the mother was a heterozygote with hemoglobin 13.4 g/dl, reticulocyte count 2.5%, packed cell volume 42%, total bilirubin 0.5 mg%, normal osmotic fragility, and normal autohemolysis. Sass and Caruso's test, Brewer's test, and the Ascorbate-cyanide test were positive. One sibling (female) died during the neonatal period and another one also has the same variant (R.M.). He has a history of CNSHA, jaundice, and pallor but no splenomegaly. His hemoglobin concentration has remained between 7.2–14.6 g/dl with a reticulocyte count of 12.2–22%. Blood count values at the time of the study were: hemoglobin 12.9 g/dl, reticulocyte count 14%, and packed cell volume 39%.

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Table 1

	Red cell enzyme activity (% of normal)	Electrophoretic mobility (% of normal)	Km G6P (μM)	2-d-G6P utilization (% of G6P)	Deamino-NADP utilization (% of NADP)	Heat stability	pH Optima
G6PD B	100	100 (ph)	48 -62.0	0 - 4	55 -60	Normal	Normal
G6PD Puerto Limón	0	150 (ph)	32.1–33.3	10.5–12.3	84.8–87.7	Labile	Biphasic 7.6, 8.5

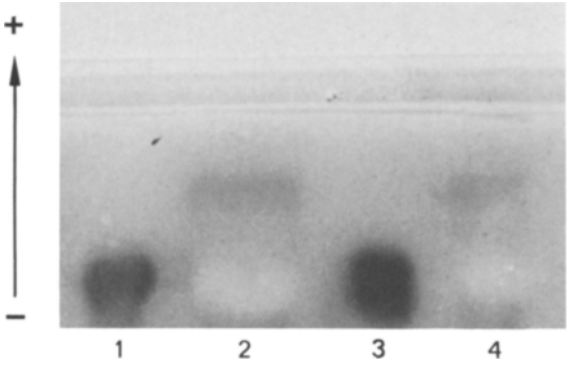

Fig. 1. Starch gel electrophoresis in phosphate buffer: 1,3 G6PD-B and 2,4 G6PD-Puerto Limón

Results and Discussion

The electrophoretic and kinetic properties of the G6PD variant are shown in Table 1. G6PD activity was zero in the hemolysate and the electrophoretic mobility was 150% in semipurified samples (Fig. 1). The thermal stability of this variant was substantially decreased and its pH activity curve was biphasic with a peak at 7.6 and 8.5. The Km for G6P was lower than that of the normal enzyme, and the utilization of substrate analogues was greater than G6PD B. The peak of the elution profile of G6PD Puerto Limón in DEAE-Sephadex columns was observed 5.2 ml after the peak of G6PD B (Fig. 2).

Red cell G6PD Puerto Limón shows deficiency, thermal instability, abnormal pH optimum, low Km for G6P, increased utilization of analogues, and electrophoretic mobility faster than

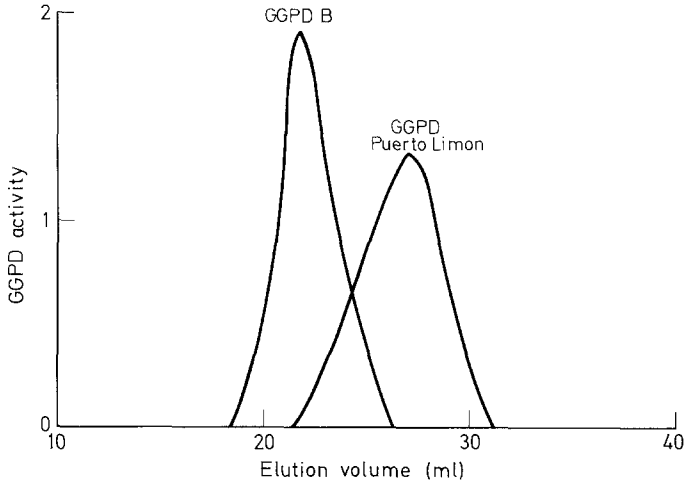

Fig. 2. Elution profile obtained with G6PD Puerto Limón and G6PD B in DEAE Sephadex columns

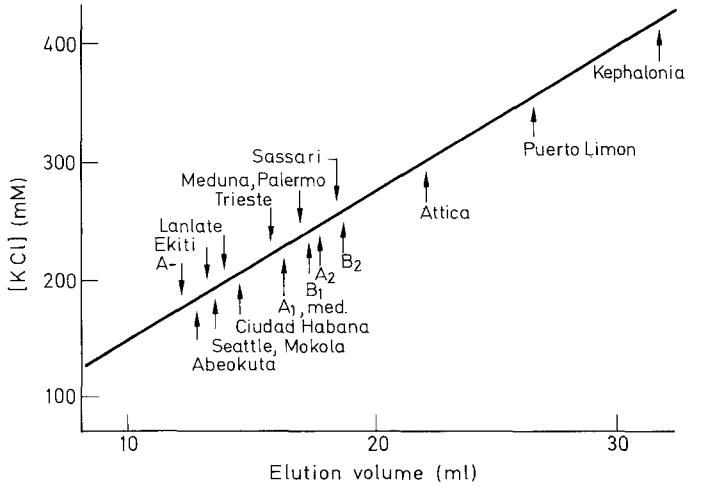

Fig. 3. Resolution of G6PD variants including G6PD Ciudad de La Habana by chromtography on DEAE Sephadex

G6PD B. Among the known G6PD variants (class 1) with fast electrophoretic mobilities, the G6PD Puerto Limón variant is unique. Also, the elution profile obtained for G6PD Puerto Limón (Fig. 3) indicates that it is probably a new variant.

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