G-6-PD ÇORUM: A NEW VARIANT OF GLUCOSE -6- PHOSPHATE DEHYDROGENASE

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SUMMARY: A new variant of glucose-6-phosphate dehydrogenase (G-6-PD) is reported. Severe enzyme deficiency (1% of normal) was found in a 16-year-old Turkish school boy. This enzyme deficiency was associated with chronic haemolytic anaemia. The partially purified enzyme had a slow electrophoretic mobility in both polyacrylamide slab gel and cellulose acetate strip system, normal Km for G-6-P, decreased Km for $NADP^+$, normal heat stability, biphasic pH curve and slightly increased utilization of 2-deoxy glucose-6-P, galactose-6-p, and deamino-$NADP^+$. The utilization of NAD^+ by the variant enzyme was increased sixfold. The variant is named G-6-PD Çorum, after the place of origin.

Key Words: Glucose-6-phosphate dehydrogenase, Glucose-6-phosphate dehydrogenase deficiency, Glucose-6-phosphate dehydrogenase variant, Haemolytic anaemia.

INTRODUCTION

As the catalyst for the initial step in the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G-6-PD) has a key position in red blood cell metabolism. G-6-PD deficiency is the most common enzyme abnormality associated with haemolytic anaemia. More than 300 G-6-PD variants had been recognized up to 1983 (1). Most of these variants do not cause clinical problems. The variability of the clinical expression of G-6-PD deficiency is paralleled by the differing biochemical characteristics of the variant enzymes. The vast majority of individuals deficient in G-6-PD activity are haematologically normal and only exhibit haemolysis when exposed to exogenous agents such as drugs, infections fava beans (2). These variant types are very common in Mediterranean and Negro populations.

In this report, results of the studies of a G-6-PD variant from Turkey are described. The variant enzyme was purified and characterized in accordance with WHO recommendations (2). It was designated G-6-PD Çorum in compliance with the WHO recommendations on nomenclature (2). The characteristics of this G-6-PD variant are described.

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MATERIALS AND METHODS

Freshly drawn heparinized venous blood was used for this biochemical study. The blood samples of the Turskish patients were maintained at near 4°C and transported to Finland by air. Red cell G-6-PD activity was determined by a method recommended by the WHO scientific group (2). The samples were stored at 4°C and determinations carried out within 4-5 hours. The G-6-PD enzyme was partially purified and characterized by the method recommended in the WHO scientific report (2). Dialysis was performed within 8-9 hours in a cold room with two changes of dialysing solution (for its composition see WHO scientific report) (2). Michaelis constants, utilization of various substrate analogues, thermal stability and the effect of pH on enzyme activity were determined fluorometrically (a Farrand fluorometer A-3 and a Transcon 102 FN nephelofluorometer) at 25°C in an assay medium consisting of 0.1 mol/l Tris-HCI buffer pH 8.0,5 mmol/l $MgCl_2$, 0.01 % bovine serum albumin, 0.2 mmol/l $NADP^+$ and 1 mmol/l glucose-6-phosphate (G-6-P). In Km measurements, the G-6-P concentration was varied between 0.002 and 1 mmol/l and the $NADP^+$ concentration between 0.001 and 0,2 mmol/l. The thermal stability test was performed at 45°C and the incubation medium contained 10 μmol/l $NADP^+$. The effect of pH on G-6-PD activity rates was measured in 0.1 mol/l potassium phosphate (pH 6.0-7.2), 0.1 mol/l Tris-HCI (pH 7.1-9.0), and 0.1 mol/l AMP-HCI (pH 9.0-10.0) buffers.

The electrophoretic characterization was carried out in two different systems, polyacrylamide gel and cellulose acetate. The activity of the partially purified deficient enzyme was too low to see an electrophoretic band. Hence, an Amicon 4104 Centrifree Micropartition System was used (1000 xg, 30 min) in order to concentrate the deficient enzyme (about 10 times) before electrophoresis. The normal enzyme was also concentrated with the Amicon Centrifree device after dilution (1:10) with the dialysing solution prior to electrophoresis. In vertical polyacrylamide slab gel electrophoresis (LKB 2001 Vertical Electrophoresis System) the gel buffer was Tris-citrate (pH 8.8, 76 mmol/l Tris, 12.5 mmol/l citric acid). The concentration of acrylamide was 4% in the upper gel and 7% in the separation gel. Both the upper and the lower tank were filled with borate buffer (pH 8.8, 30 mmol/l borate, 0.011 mmol/l NaOH), and contained 10 mmol/$NADP^+$. A constant current of 15 mA was passed until the samples had just migrated into the gel. Then electrophoresis was continued at a constant current of 30mA. The total time taken for a run was 120 min (3). For staining, the polyacrylamide gels were incubated for 45 min at 37°C in a medium consisting of 0.1 mol/l Tris-HCl buffer pH 8.0, 0.4 mg/ml nitroblue tetrazolium (NBT), 1 mg/ml phenazine methosulphate (PMS), 0.5 mmol/l $NADP^+$ and 6 mmol/l G-6-P (4,5).

In the second system for the electrophoretic separation on cellulose acetate strips, Tris-barbital buffer (pH 8.6, 0.05 mol/l) was used. Each of the outer compartments of the electrophoretic chamber contained 10 μmol/l $NADP^+$. The system was run for 30 min at 200 volts. After electrophoresis, the strips were stained for G-6-PD activity by incubating in 2 ml of incubation solution for 20 min at 37°C (The incubation solution was the same as above).

Protein was determined according to the method of Lowry (6).

RESULTS AND DISCUSSION

The Michaelis constants and substrate analogue utilization data and the results of heat stability, electrophoretic mobility, and pH optima for the partially purified enzyme are presented in Table 1. Also, comparison of the characteristics of the new variant with G-6-PD Mediterranean (7,8) and normal G-6-PDs is shown in Table 1. The

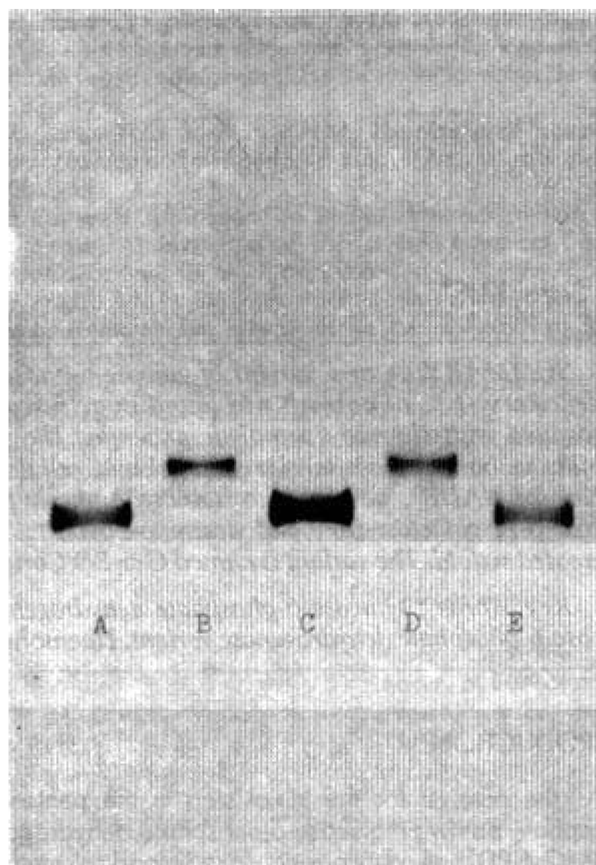

Figure 1: Electrophoretic mobility of partially purified G-6-PDs. Separation was carried out in vertical polyacrylamide slab gel electrophoresis (see Materials and Methods). A: normal G-6-PD (after dialysis the enzyme was diluted) 1:10 -the activity applied on gel was 2 nmol/mil), B and D: deficient G-6-PD (new variant) (after dialysis the enzyme was concentrated in an Amicon Centrifree device -the activity applied 2 nmol/min), C: normal G-6-PD (after dialysis the enzyme was diluted 1:10 and then concentrated-the activity applied 4 nmol/min), E: normal G-6-PD (after dialysis the enzyme was diluted 1:10 and then concentrated - the activity applied 2 nmol/min).

Table 1: Comparison of the characteristics of the new variant G-6-PD Çorum with G-6-PD Mediterranean and normal G-6-PD B.

Variant	Enzyme Activity (%)	Km (μmol/l)		Substrate Utilization (%)				Heat Stability	Electro phoretic Mobility	pH optima
		G-6-P	$NADP^+$	2d G-6-P	Gal-6-p	Deamino $NADP^+$	NAD^+			
Normal B (Turkish)	100	33.7	5.1	9.7	5.5	32.8	4.4	Normal	100	Normal
Normal B (WHO)	100	50-70	2.9-4.4	4	5	55-60	-	Normal	100	Normal
Mediter-anean	0-7	19-26	1.2-1.6	23-37	-	350	-	Low	100	Biphasic
Çorum (New Variant)	1.0	34.7	1.3	14.8	7.5	45.3	25.2	Normal	85	Biphasic

Km of the variant enzyme for G-6-P was normal, but reduced for $NADP^+$. The utilization of 2-deoxy glucose-6-phosphate (2 d G-6-P), galactose-6-phosphate (Gal-6-P), and deamino-$NADP^+$ by the variant enzyme was studied, and slightly increased activity towards all substrate analogues was observed. The utilization of NAD^+ by the variant enzyme was increased six times when compared with the normal enzyme. Thermal stability of the variant enzyme was studied and shown to be normal. The variant enzyme showed a biphasic pH-activity curve. The electrophoretic mobility of the variant enzyme on polycrylamide gel and cellulose acetate was reduced to 85% of normal (Figure 1).

The Km values for $NADP^+$ have often been criticized as inaccurate, and are not in themselves sufficient as criteria for distinguishing G-6-PD variants (9). However, when I used partially purified enzyme and fluorometric measurements, Lineweaver-Burk plots always formed a straight line. In fluorometric measurements the sensitivity is about 100 times higher than in the spectrophotometric methods, the amount of enzyme needed is much smaller and the impurities consequently interfere with the reaction less than in the spectrophotometric technique (10). Therefore, I believe that the observed decrease in Km for $NADP^+$ in G-6-PD Çorum is real.

When I compared the new variant with G-6-PD Mediterranean (7,8), there was no similarity regarding heat stability and electrophoretic mobility. Also, when the new variant and previously known Turkish variants (11, 12, 13) are compared, most of the characteristics of the new variant were different from previously known variants in Turkey. These findings suggest a new G-6-PD variant. In conclusion, the characteristics of G-6-PD Çorum are uniquely different from the other known variants (1,11,12,13). I wish to name this variant G-6-PD Çorum, in accordance with the WHO recommendations on G-6-PD variant nomenclature (2).

The prevalence of G-6-PD deficiency has been calculated to be between 1 and 11% in Turkey (14). The highest frequency (10.4%) of G-6-Pd deficient parents occurs among the Eti-Turks of the Adana region (15). There may be other variants not yet discovered in Turkey. Therefore all cases, of chronic haemolytic anaemia without apparent cause in Turksih patients, should be evaluated with regard to possible G-6-PD variants of red cells.

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