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Erythrocyte glucose-6-phosphate dehydrogenase activity assay and affinity for its substrate under "physiological" conditions

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) activity and the affinity for its substrate glucose-6-phosphate were investigated under conditions similar to the physiological environment in terms of ionic strength (I: 0.188), cation concentration, pH 7.34, and temperature (37°C). A 12.4, 10.4 and 21.4% decrease was observed in G6PD B, G6PD A^+ and G6PD A^- activities, respectively. A Km increase of 95.1, 94.4 and 95.4% was observed in G6PD B, G6PD A^+ and G6PD A^-, respectively, leading to a marked decrease in affinity. In conclusion, the observation of the reduced activity and affinity for its natural substrate reflects the actual pentose pathway rate. It also suggests a much lower NADPH generation, which is crucial mostly in G6PD-deficient individuals, whose NADPH availability is poor.

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Key words
- Glucose-6-phosphate dehydrogenase
- Red blood cells
- G6PD activity and affinity

Erythrocyte glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) is a pentose shunt enzyme involved in the maintenance of adequate concentrations of reduced nicotinamide adenine dinucleotide phosphate (NADPH). This nucleotide, together with glutathione reductase, keeps glutathione in its reduced form (GSH), protecting the red cell against oxidative stress. There are two normal G6PD variants, G6PD B and G6PD A^+,and other deficient polymorphic mutants such as G6PD A^-, besides dozens of rare ones (1), some of which have been described by our group (2,3).

The standard methods used to assay G6PD activity and affinity (Michaelis-Menten constant - Km) for its substrate are currently performed using reaction conditions of 145 mOs and ionic strength I: 0.06, pH 8.0, at 37°C for activity assay and 25°C for Km determination. In the present study the enzyme activity as well as its affinity were determined under nearly physiological conditions regarding osmolarity (290 mOs) and ionic strength (I: 0:188), 3 mM $MgCl_2$, pH 7.34, at 37°C. The activity assay was performed with NADP and glucose-6-phosphate (G6P) in excess. Under physiological conditions (37°C and 290 mOs) there was a 10-12% decrease in the activity assay, as well as an affinity decrease of 50% when compared to the standard methods (25°C and 145 mOs).

Blood was collected in the preservative

solution ACD (citric acid, citrate and dextrose), and filtered through 1:1 microgranular cellulose:alpha-cellulose. The red cells were washed in buffered saline at 4°C and lysed and the enzymatic assays were carried out according to standard methods (4,5). The "physiological" reagent system was: 250 mM Tris-HCl, pH 7.34, 3 mM $MgCl_2$, 0.2 mM NADP, which, together with the hemolysate and the substrate in excess (glucose-6-phosphate - 0.6 mM G6P) gave a final osmolarity of 290 mOs with I: 0.188 ionic strength at 37°C. G6PD purification and variant characterization were performed at 25°C according to standard methods (4,5) (activity against substrates, electrophoresis, Km for G6P and NADP, Ki for NADPH, pH curve, and thermal stability) and the Km was subsequently determined again under "physiological" conditions.

Other authors have focused on the effect of isolated factors on G6PD activity and affinity (6-9). In a different approach, the present study was planned to investigate how the activity and affinity would work in a nearly "physiological" environment involving several factors, a fact that impairs comparison of our data with those reported by others.

According to the results shown in Table 1, the studied variants showed different values in enzyme activity under "physiological" conditions (<10.4% in G6PD B, <12.4% in G6PD A^+ and <21.5% in G6PD A^-). The deficient variant G6PD A^- appeared to be more sensitive to "physiological" conditions than the normal variants, a fact that, allied to its decreased activity, may enhance its difficulty in carrying out its metabolic functions, with impaired red cell performance.

The enzyme affinity for its physiological substrate glucose-6-phosphate was decreased when the "physiological" reagent system was employed, although the higher temperature used should theoretically facilitate the affinity. The ionic strength seems to play an important role in determining the affinity, impairing the access of the substrate to the active site of the enzyme.

In conclusion, the reduced activity of G6PD A^-, taken together with its diminished affinity for its substrate, obtained under "physiological" conditions, in fact reflects the actual pentose shunt rate. This suggests a lower actual NADPH generation as well, which is crucial mostly in G6PD-deficient individuals, whose NADPH availability is poor.

Table 1 - Glucose-6-phosphate dehydrogenase (G6PD) activity and affinity for its substrate under standard (WHO) and "physiological" conditions.
G6P, glucose-6-phosphate. *IU/g Hb, **µM/l.

	Osmolarity mOs	Ionic strength (I)	G6PD activity (37°C)*	G6PD Km for G6P**	
				25°C	37°C
G6PD type B					
WHO	145	0.086	12.6 ± 1.3 (100%)	47.6 ± 2.6 (100%)	62.3 ± 6.3 (131%)
"Physiological"	290	0.188	11.3 ± 1.2 (89.6%)		95.1 ± 10.3 (200%)
G6PD type A^+					
WHO	145	0.086	11.7 ± 1.6 (100%)	49.1 ± 1.2 (100%)	62.9 ± 4.4 (128%)
"Physiological"	290	0.188	10.2 ± 1.3 (87.2%)		94.4 ± 4.4 (192%)
G6PD type A^-					
WHO	145	0.086	1.4 ± 0.4 (100%)	50.0 ± 1.3 (100%)	63.5 ± 4.8 (127%)
"Physiological"	290	0.188	1.1 ± 0.1 (78.5%)		95.4 ± 10.5 (191%)

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