Inhibition by ATP of Erythrocyte Glucose-6-Phosphate Dehydrogenase Variants[1] (37034)

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It is a general experience that different types of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency of the erythrocytes result in variable clinical expression, ranging from a silent carrier to the extreme of chronic hemolysis in patients with congenital nonspherocytic hemolytic anemia (CNSHA) (1). The severity of the deficiency as measured in the ordinary assay does not always correlate with the clinical syndrome. This is most evident in the variants associated with CNSHA, where some have higher activities than some of the clinically silent variants (2–4). Several biochemical characteristics of the variants such as *in vivo* lability, low affinity for the substrate glucose-6-phosphate (G-6-P) (5), abnormal affinity for NADP perturbing the monomer–dimer equilibrium (4) and differing sensitivity to the inhibitory effect of NADPH (6, 7), have all been suggested as explanations for these discrepancies.

Avigad (8) and others (9) have shown that ATP and certain other nucleoside triphosphates, inhibit G-6-PD competitively with the substrate, G-6-P. Smith and Anwer (10) have found an uncharacterized variant with a low inhibition constant and suggested that this could explain some of the observed discrepancies between G-6-PD activity and clinical manifestations. To test the possibility that this might be the explanation for the occurrence of CNSHA we undertook a study of the inhibition by ATP of several G-6-PD variants, some of which are associated with CNSHA.

Materials and Methods. Red cell enzymes were partially purified using methods recommended by the WHO and previously described in detail (2). All partially purified enzymes were dialyzed for 3 hr. For the determinations of the inhibition constants (K_i) for ATP the following modifications of the standard assay (2) were used: $MgCl_2$ was omitted and the pH of the Tris–HCl buffer was lowered to 7.3 instead of 8.0. Neutralized 20 m*M* ATP solution was added to a final concentration of 2 and 4 m*M*. All assays were carried out with the Gilford model 2400 spectrophotometer at 37°. The Michaelis constants (K_m) for G-6-P with and without ATP were calculated using the least square fit equation. K_i-ATP was calculated from equations given by Dixon and Webb (10) for competitive inhibition.

Results and Discussion. In preliminary experiments we confirmed that ATP inhibits erythrocyte G-6-PD by competition with G-6-P. This is partially relieved by magnesium ions and is very sensitive to the pH of the reaction mixture: it is almost inapparent at the WHO standard pH of 8.0 and increases as the pH falls. At the adopted pH of 7.3, a concentration of 0.2 m*M* NADP and in the absence of magnesium, G-6-PD B has an apparent K_i-ATP of 2.11 ± 0.6 m*M*. The K_m-G-6-P and K_i-ATP of the variants studied are given in Table I.

In some of the variants the observed K_m for the G-6-P differ from the reported values (12–15), especially for G-6-PD Alhambra, because of the different conditions of the assay (pH 7.3, no Mg^{2+}). As shown, none of the studied variants had K_i-ATP value below normal, while G-6-PD Alhambra, G-6-PD Canton and to a lesser degree G-6-PD

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TABLE I. K_m-G-6P and K_i-ATP of Erythrocyte G-6PD Variants.[a]

	K_m-G-6P μM	K_i-ATP mM
Gd B (6)	32	2.1 ± 0.6
Gd A (2)	34	2.5
Gd Mediterranean (2)	14	2.6
Gd Chicago	37	2.1
Gd Canton	39	4.7
Gd Alhambra	28	4.0
Gd Charleston	25	1.9

[a] The number of samples is given in parentheses.

Mediterranean, were found to have higher K_i values. As none of the variants manifested undue sensitivity to ATP, inhibition by the physiologic levels of intracellular ATP is probably not a major factor in the pathogenesis of hemolysis in patients with those variants. These determinations did not distinguish variants associated with CNSHA like G-6-PD Alhambra, G-6-PD Chicago and G-6-PD Charleston from those unassociated with chronic hemolysis like G-6-PD A-, G-6-PD Mediterranean and G-6-PD Canton.

Recently Yoshida and Lin (7) studied several variant enzymes at physiologic conditions of pH, substrate and inhibitor concentrations and found that enzymes from subjects with hemolysis are strongly inhibited by physiologic concentrations of NADPH, while variants not associated with CNSHA, are far less sensitive to inhibition by NADPH. The latter are also more resistant to the inhibition of ATP. In an assay system which contains physiologic concentrations of NADP and NADPH, the variants G-6-PD Union, G-6-PD Markam and G-6-PD Mediterranean, not associated with CNSHA were less inhibited by ATP compared to the normal enzyme (7). We, too, found a slightly higher K_i for ATP in G-6-PD Mediterranean.

It will be useful to study more variants by these methods in order to establish the role of this biochemical characteristic in G-6-PD deficient erythrocytes.

Summary. ATP is a competitive inhibitor of erythrocyte glucose-6-phosphate dehydrogenase. Inhibition of the normal enzyme and the enzyme of six variants, four of them associated with nonspherocytic congenital hemolytic anemia was investigated. None of the variants manifested undue sensitivity to ATP inhibition. It is concluded that inhibition by physiologic levels of ATP is probably not a major factor in the pathogenesis of hemolysis in these variants.

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