EFFECT OF DIVALENT CATIONS ON THE STRUCTURE OF HUMAN ERYTHROCYTE GLUCOSE 6-P DEHYDROGENAS

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SUMMARY

The balance between the two active oligomeric forms of human G6PD, namely dimers and tetramers, is regulated by a number of environmental conditions: the dissociating effect displayed by high ionic strength and alkaline pH values is counteracted by several divalent cations resulting in formation of tetramers. Binding of Mg^{2+} ions to the protein determines association of two dimers through an allosteric transition.

Glucose 6-phosphate dehydrogenase from human erythrocytes exists in two catalytically active states, namely tetramers and dimers (1-5). The two forms are interconvertible according to environmental conditions largely accounting for previous discrepancies on the molecular weight of active G6PD (see ref. 2). The present paper is concerned with an investigation on factors affecting the equilibrium between tetramers and dimers: at alkaline pH and (or) high ionic strength dissociation prevailed, in agreement with results by Cohen and Rosemeyer (1, 2). Conversely, Mg^{2+} ions and other divalent cations were found to displace the balance toward tetramers through a concentration-dependent effect. Moreover, data are reported suggesting that the association process involves an allosteric transition induced by Mg^{2+} ions.

MATERIALS AND METHODS

G6PD was purified by the procedure of Bonsignore et al. (5), yielding homogeneous enzyme protein. Before use G6PD was extensively dialyzed against the same buffer employed in the experiment. The standard enzyme assay was in 1.0 ml of 0.05 M Tris-HCl, pH 9.0, containing 0.05 M NaCl, 2 mM G-6-P and 0.5 mM NADP at 25° C in an Optica CF 4 Spectrophotometer. One unit of G6PD activity was defined

as the amount catalyzing reduction of 1 μmole of NADP at 340 mμ under the standard assay conditions. Protein was evaluated by the method of Lowry et al. (6).

Sucrose gradient centrifugation was performed according to Martin and Ames (7), analytical ultracentrifugation and electrophoresis on polyacrylamide gel were as previously reported (5).

Table I

Effect of pH and ionic strength on the dimer-tetramer equilibrium of erythrocyte G6PD

Buffer	pH	I	$s_{20°,w}$	ϕ
Phosphate	6.5	0.08	8.46	0.21
Phosphate	6.5	0.12	8.15	0.30
Phosphate	7.5	0.08	7.0	0.63
Phosphate	7.5	0.23	6.42	0.80
Tris-HCl	8.0	0.03	6.65	0.74
Tris-HCl	8.0	0.15	6.57	0.76
Tris-HCl	7.5	0.23	6.51	0.78
Tris-HCl	8.6	0.15	6.40	0.81
Glycine-NaOH	7.8	0.18	7.1	0.61
Glycylglycine-NaOH	7.5	0.08	6.62	0.75

Sedimentation coefficients are average values of several experiments performed with 1.2 I.U. of dialyzed enzyme at different stages of purification. Centrifugation was at 49,000 rpm for 12 hours at 5° C in 5%-20% sucrose gradients containing the actual buffer of each experiment, 0.1 mM EDTA and 5 μM NADP (SW 50 rotor). COHb was used as marker protein. Values of ϕ calculated on the basis of $s_{20°,w}$ values, as follows (2,5):

$$\phi = \frac{s_t - \bar{s}}{s_t - s_d}$$

where s_t (9.18 S) and s_d (5.75 S) are the sedimentation coefficients of the tetramers and of the dimers, respectively (5), and $\bar{s}$ is the actual $s_{20°,w}$. ϕ defines the extent of dissociation of the dimer-tetramer system: at values of ϕ approaching 1.0 the active enzyme would be 100% dimers, whereas $\phi \rightarrow 0$ would indicate homogeneous tetramers.

RESULTS

Effect of buffers, pH and ionic strength on G6PD structure - Several environmental parameters, including the buffer, the pH and the salt concentration, affect markedly the dimer/tetramer ratio, as evaluated by actual sedimentation coefficients of G6PD. Dissociation is favoured at alkaline pH and high ionic strength (Table I). These variations support earlier evidences provided by Cohen and Rosemeyer (1, 2) concerning dependence of the quaternary structure of the erythrocyte enzyme on solvent conditions.

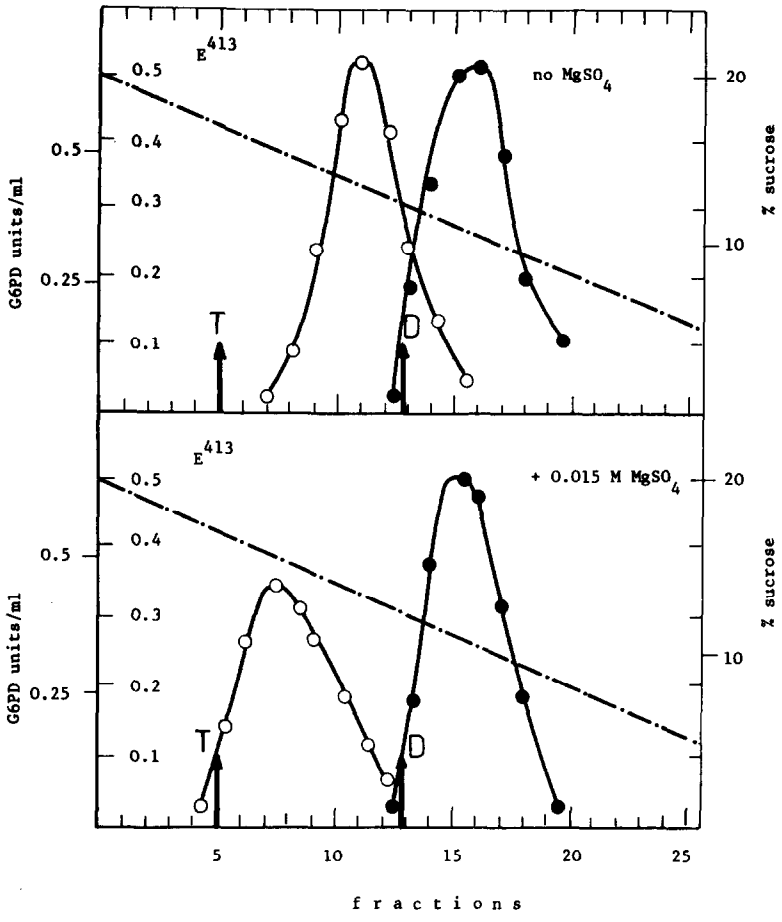

Figure 1: Effect of $MgSO_4$ on sedimentation of active G6PD -

Linear 5%-20% sucrose gradients contained 0.02 M phosphate buffer, pH 7.8, 0.1 mM EDTA, 5 µM NADP, 0.1% β-mercaptoethanol (vol/vol) and $MgSO_4$ where indicated. Centrifugation at 49,000 rpm for 12 hours at 5° C in SW 50 rotor. Arrows indicate theoretical migrations of homogeneous tetramers and dimers along the tube, as related to actual migration of marker COHb (filled circles). The open circles refer to G6PD activity and the dotted line to the sucrose gradient.

Effect of Mg^{2+} ions - Replacement of KCl by $MgSO_4$ to increase the ionic strength resulted unexpectedly in an active G6PD species sedimenting more rapidly than the control enzyme (Fig. 1). Although this behaviour could be accounted for by any conformational change leading to an increased density of the protein, a shift of the dimer-tetramer system toward the heavier species was more likely due to the marked extent of the effect. Moreover $MgSO_4$ produced an increase of the tetramer peak occurring at the expense of the dimers, as shown with the schlieren optics system at the Spinco model E ultracentrifuge(5).Finally, tetramerization was evident under these conditions at the electron microscope (Wrigley, Bonsignore and De Flora, manuscript in preparation).

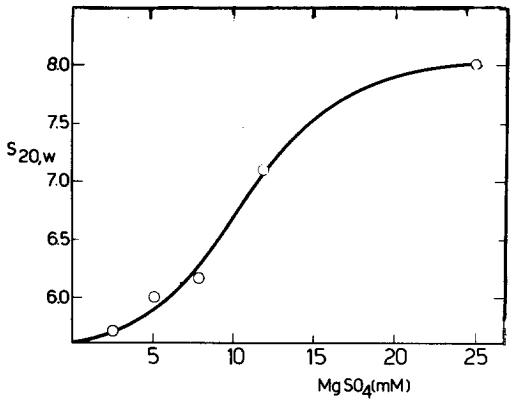

Figure 2: Formation of tetramers as a function of Mg^{2+} concentration - 5%-20% sucrose gradients contained 0.04 M phosphate buffer, pH 7.8, 0.1 mM EDTA, 5 μM NADP, 0.2% β-mercaptoethanol (vol/vol) and $MgSO_4$ at the concentration shown in abscissa. Centrifugation at 26,500 rpm for 46 hours at 6° C in a SW 27 rotor (long tubes).

The effect displayed by $MgSO_4$ was observed with other Mg^{2+} salts; on the contrary K_2SO_4, Na_2SO_4 and $(NH_4)_2SO_4$ slightly increased dissociation of the active enzyme as compared with the controls, because of the higher ionic strength.

The extent of association of the dimers, as expressed by the increase of actual sedimentation coefficients, depends on the concentration of Mg^{2+} according to a sigmoid-shaped relationship (Fig. 2). Higher $MgSO_4$ concentrations (above 0.05 M) result in an opposite effect, as indicated by a decrease of the tetramer/dimer ratio: this may be accounted for by a more rapid inactivation of the tetramers than of the dimers (see below) and (or) by the prevailing effect

of the high ionic strength leading to dissociation. Plotting the centrifuge data according to the Hill equation (Fig. 3) yielded interaction coefficients significantly above 1.0: this is consistent with the view that binding of the cation to at least two positively interacting sites promotes a face to face aggregation of dimers across the same plane of symmetry which is inversely affected by high ionic strength and alkaline pH.

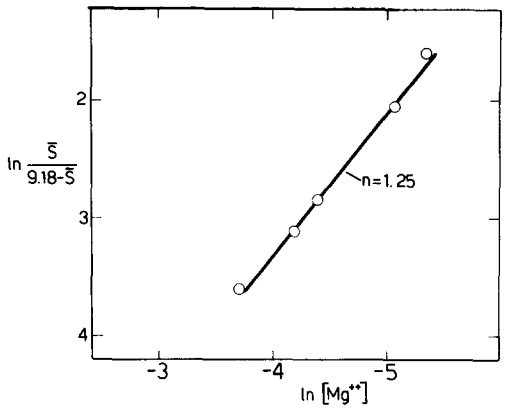

Figure 3: Hill plot of the Mg^{2+} effect on the dimer-tetramer equilibrium - 5%-20% sucrose gradients contained 0.02 M phosphate buffer, pH 7.5, 0.1 mM EDTA, 5 μM NADP, 0.2% β-mercaptoethanol (vol/vol) and different concentrations of $MgSO_4$. Centrifugation at 26,500 rpm for 41 hours at 6° C in a SW 27 rotor (long tubes).

Effect of other divalent cations - A number of divalent cations produce a shift of the dimers toward tetramers; these are listed in Table II, showing that their effectiveness decreases in the following order: $Co^{2+} > Ca^{2+} > Ba^{2+} > Mn^{2+} > Mg^{2+}$. Moreover, the divalent cations show additive effects, a mixture of Ca^{2+}, Ba^{2+} and Mg^{2+} leading to an intermediate extent of tetramerization with respect to those determined by the individual metals. Among the cations tested, Zn^{2+} and Cu^{2+} produced an immediate inactivation, making it impossible to evaluate the dimer/tetramer ratio.

DISCUSSION

The divalent cations producing tetramerization of G6PD do not reach in the erythrocyte the concentrations that appear to be effective in our experiments. The only exception is provided by Mg^{2+} which at the physiological concentrations of 2-4 mM (8) might shift to some extent the dimer-tetramer equilibrium toward

Table II

Effect of divalent cations on the dimer-tetramer equilibrium

Salt	M	$s_{20°,w}$	ϕ
$MgSO_4$	0.05	8.31 (6.34)	0.25
$MnSO_4$	0.05	8.37 (6.31)	0.21
$MgCl_2$	0.025	7.93 (6.52)	0.36
$CoCl_2$	0.025	9.03 (6.53)	0.04
$BaCl_2$	0.025	8.19 (6.48)	0.29
$CaCl_2$	0.025	8.9 (6.52)	0.08
$CaCl_2$ + $BaCl_2$ + $MgCl_2$	0.025	8.30 (6.52)	0.25

Centrifugations were in 5%-20% sucrose gradients containing 0.04 M glycylglycine-NaOH, pH 7.5, 0.1 mM EDTA and 10 μM NADP (SW 50 rotor).
In parentheses $s_{20°,w}$ values of controls are reported.

the latter form. In this connection, it must be emphasized that the physiological values of pH and ionic strength are such as to favour slightly dissociation to dimers (2); therefore Mg^{2+} ions could re-equilibrate in vivo the balance between the two active species, out of which the dimer only undergoes the dissociation to inactive monomers following catalytic operation of the enzyme (3).

Although the detailed mechanism of the effect of divalent cations still escapes recognition, it is noteworthy that the dimer-dimer association involves cooperative interactions between distinct Mg^{2+}-binding sites. This represents a further evidence for the allosteric nature of erythrocyte G6PD which had already emerged as regards binding of NADP (4, 9).

The face to face association of G6PD dimers is highly reminiscent of the aggregation of double hexagonal units of "tightened" E.coli glutamine synthetase

occurring upon addition of divalent cations (10). The similarity is even more striking since the tendency of individual molecules of glutamine synthetase to aggregate is obscured by high ionic strength (11).

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