IDENTITY OF SULFATE AND PHOSPHATE ACTIVATION OF THE PHOSPHOFRUCTOKINASE FROM ERYTHROCYTES

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1. Introduction

It has been repeatedly observed that the phosphofructokinase (EC 2.7.1.11) from various sources is more active in the presence of ammonium sulfate as compared with ammonium chloride [1–3]. It occurred to us that this difference might be due to an activating effect of the sulfate ions similar to that of phosphate ions. A stimulating effect of sulfate ions on the phosphofructokinase had been suggested earlier on the basis of an increase of glycolysis of erythrocytes after addition of sodium sulfate [4, 5]. Some measurements of the phosphofructokinase activity in hemolysates support the assumption of a sulfate activation of the enzyme.

The purpose of the present study was an investigation of the effect of sulfate in comparison with phosphate ions on the properties of a purified phosphofructokinase from rat erythrocytes.

2. Material and methods

The phosphofructokinase was prepared from rat erythrocytes as described before [3, 6]. Its specific activity was 40 μmoles/mg protein. The details of the method used for the determination of enzyme activity have been reported [3, 6]. Care was taken to remove all ammonium and sulfate ions from all enzymes used by means of Sephadex G_{25} [3]. The reaction was started with the phosphofructokinase. The activities are given in μmoles of Fru-6-P/ml·min.

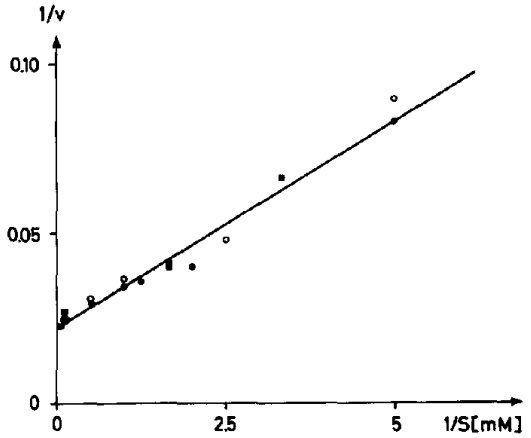

Fig. 1. Activation of the phosphofructokinase in KCl and NH_4Cl system by sulfate and phosphate ions. Conditions: pH 7.2, 1 mM Fru-6-P, 0.2 mM ATP, 2.0 mM $MgCl_2$, temperature 37°C; (•—•—•) 100 mM KCl and Na_2SO_4; (○—○—○) 100 mM KCl and Na_2HPO_4/NaH_2PO_4; (▪—▪—▪) 100 mM NH_4Cl and Na_2SO_4.

3. Results and discussion

In fig. 1 is shown a comparison of the effects of sulfate and phosphate ions on the K^+ and the NH_4^+ activated phosphofructokinase. It may be seen that there is no significant difference between the two ions in either system. The K_D value being about 0.5 mM.

So far a close comparison between the cation-activated and non-activated enzyme preparation with respect to the effects of the anions has not been carried out. It may be seen from fig. 2 that the maximal

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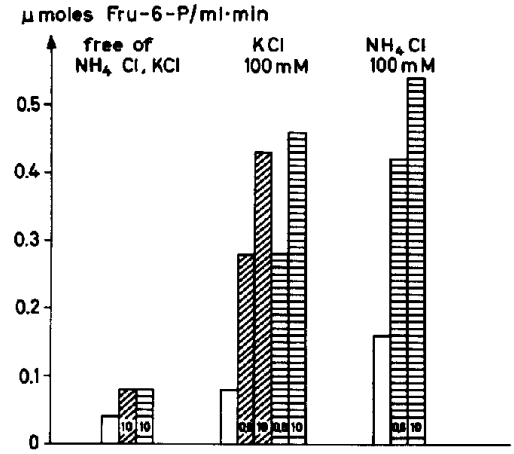

Fig. 2. The influence of sulfate and phosphate ions on the phosphofructokinase as a function of cation-activation. Conditions: pH 7.2, 1 mM Fru-6-P, 0.2 mM ATP, 2.0 mM $MgCl_2$, temperature 37°C; (▩) Na_2SO_4; (▨) Na_2HPO_4/NaH_2PO_4 pH 7.2. Values are given in mM.

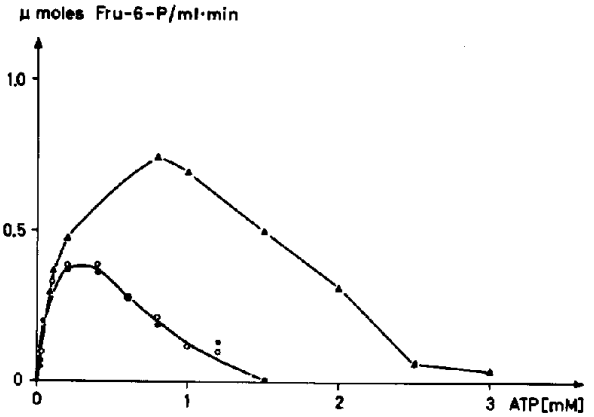

Fig. 3. The influence of sulfate and phosphate ions on the ATP dependence of the phosphofructokinase. Conditions: pH 7.2, 1 mM Fru-6-P, 100 mM, KCl 2.0 mM $MgCl_2$, temperature 37°C; (●—●—●) 1 mM Na_2SO_4, (▲—▲—▲) 10 mM Na_2SO_4; (○—○—○) 1 mM Na_2HPO_4/NaH_2PO_4 pH 7.2.

activity of the enzyme without any activators amounts to 7% of that in presence of NH_4^+ ions and either phosphate or sulfate ions. The addition of either one of the anions doubles this basic activity as does that of K^+ ions, while ammonium ions quadruple it. Combined addition of cations and anions results in three- to five-fold activation respectively in the NH_4^+ and K^+ system. The activating effects of the ions varied somewhat between different enzyme preparations; they appear to be the greater the more sensitive the enzyme is to the inhibition by ATP.

In fig. 3 are shown the effects of sulfate and phosphate ions on the ATP dependence of the phosphofructokinase at pH 7.2. It is evident that an elevation of sulfate ions to 10 mM results both in an increased maximal activity of the enzyme as well as in a reduced inhibition by ATP. There is no difference between phosphate and sulfate ions at the 1 mM concentration investigated. It has been demonstrated previously [6] that the ATP inhibition is related to the Fru-6-P dependence of the phosphofructokinase. The similarity of the effects of phosphate and sulfate ions expresses itself in the fact that the $S_{0.5}$ values for Fru-6-P were 0.68 mM in 1.0 mM sulfate and 0.8 mM in 1 mM phosphate, values which do not differ significantly. The Hill coefficients were 1.80 and 1.76 respectively. As has been described for phosphate ions [3], elevation of the sulfate ion concentration weakens the cooperative behaviour of the enzyme. In 10 mM sulfate the $S_{0.5}$ value for Fru-6-P declined to 0.24 mM and the Hill coefficient to 1.13.

The results presented show that sulfate ions are an activator of the phosphofructokinase. From the identity of the effects of sulfate and phosphate ions on the non-activated as well as on the K^+- and the NH_4^+-activated enzyme, it may be assumed that both anions act in the same manner on a common site. It appears likely that all enzymes of animal orgin are affected in the same manner [1, 8]. In keeping with this a decrease of the $S_{0.5}$ for Fru-6-P to one-third was found with the phosphofructokinase from Schistosoma Mansoni [8].

It may be assumed that sulfate ions as the other anions act by way of a weakening of the ATP inhibition of the phosphofructokinase [9]. Since there is competition between ATP and Fru-6-P [6, 7] a decrease of the $S_{0.5}$ for Fru-6-P is thereby produced. With non-saturating Fru-6-P concentrations, there results an increased enzyme activity.

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