ACTIVATION OF YEAST PHOSPHOGLYCERATE KINASE BY SALTS OF MONOVALENT CATIONS

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1. Introduction

Certain phosphotransferases require, in addition to a divalent metal ion, monovalent cations such as K^+, NH_4^+, or Rb^+ for their catalytic activity (cf. muscle pyruvate kinase [1, 2]) and others are stimulated by these ions (see [3, 4] for reviews). Requirement for, or stimulation by monovalent cations has never been demonstrated for phosphoglycerate kinase (EC 2.7.2.3). As nucleotides and other reagents are introduced to the reaction mixture in form of their Na^+ or K^+ salts, such a demand can easily be overlooked. In the present work, measures were taken to eliminate questions whether the residual activity is due to counterions of positive or negative charge that cannot fully be excluded in the assay mixture. Both activation and inhibition by monovalent cation salts are found.

2. Materials and methods

2.1. Enzymes

Crystalline phosphoglycerate kinase from yeast and glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12) from rabbit muscle were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). The kinase was further purified by column electrophoresis, and the main electrophoretic component (II)B [5] was used.

2.2. Reagents

ATP·$(Tris)_2$, NADH·Na_2, and the barium salt of 3-phospho-D-glycerate were from Sigma Chemical Co. 3-Phospho-D-glycerate was liberated from the water-insoluble barium salt by treatment with an equivalent amount of H_2SO_4, and was then brought to a pH of about 7.8 with Tris, after removal of the $BaSO_4$ precipitate (cf. [6]). The concentration of 3-phospho-D-glycerate was determined as described earlier [6]. Only analytical grade reagents were used. Methods for removing contaminating metal ions were as described earlier [7].

2.3. Activity measurements

The activity of phosphoglycerate kinase was determined by the spectrophotometric method of Bücher [8], and was expressed as the initial velocity, $v = (dA_{366}/dt)_{t=0}$ (in min^{-1}), cf. [7]. If not otherwise stated the experiments were performed in 50 mM Tris-HCl buffer (pH 7.8, 25°). The assay mixture contained 1.0 mM ATP·$(Tris)_2$, 1.0 mM $MgCl_2$, 2.0 mM 3-phospho-D-glycerate, and 0.50 mM NADH·Na_2. About 0.3 μg of phosphoglycerate kinase and 300 μg of glyceraldehyde phosphate dehydrogenase were used per ml of substrate.

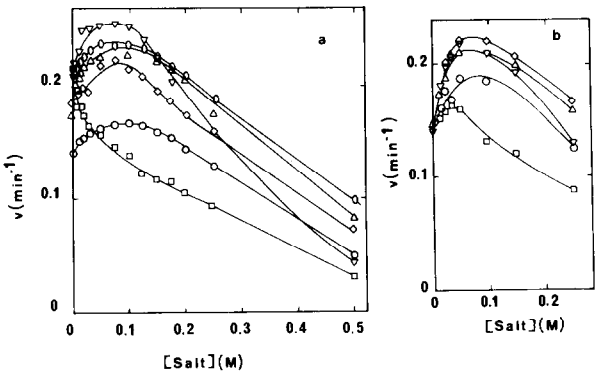

Fig. 1. Influence of monovalent cation chlorides on the activity of phosphoglycerate kinase. (a) Varying salt concentrations in the substrate solution, the enzyme stored in "salt-free" buffer. (b) Salt of the same kind and concentration in the substrate and enzyme solutions: (○—○—○): NaCl; (△—△—△): KCl; (▽—▽—▽): NH_4Cl; (◇—◇—◇): RbCl; (◇—◇—◇): CsCl; (□—□—□): LiCl. Note: in a (▽—▽—▽) represents both KCl and CsCl.

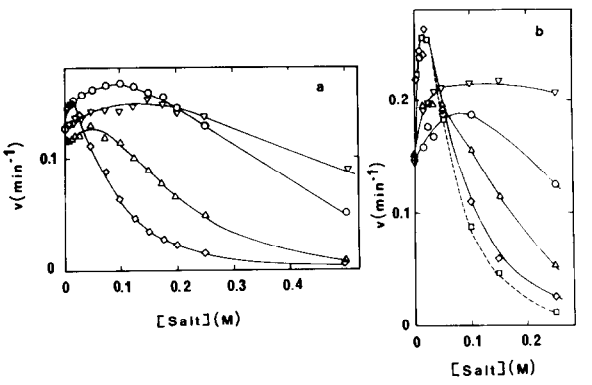

Fig. 2. Influence of different sodium salts on the activity of phosphoglycerate kinase. (a) Varying salt concentrations in the substrate solution, the enzyme stored in "salt-free" buffer. (b) Salt of the same kind and concentration in substrate and enzyme solutions. (○—○—○): NaCl; (△—△—△): $NaNO_3$; (▽—▽—▽): NaAc; (◇—◇—◇): Na_2SO_4. In (b) an experiment with $(NH_4)_2SO_4$ (□—□—□) is also included.

3. Results and discussion

3.1. Influence of different monovalent cation salts on the enzymic activity

a) *Cation varied.* Fig. 1a shows that when Na^+, K^+, NH_4^+, Cs^+, or Rb^+ as their chloride salts are introduced to the assay mixture the rate of the phosphoglycerate kinase catalyzed reaction first increases with an increase in the salt concentration, then reaches an optimum value at a salt concentration around 0.08 M, and finally decreases. In the presence of LiCl, the curve looks different, since only inhibition is seen. Fig. 1b shows results of experiments performed after preincubation of the enzyme with buffer containing salt of the same kind and concentration as was used in the assay mixture. With Na^+, K^+, NH_4^+, Cs^+, and Rb^+ the curves in fig. 1b are very similar to those in fig. 1a. The curve in the presence of LiCl is, after preincubation has been performed, similar in form to those of the other monovalent metal ion chlorides, but the optimum occurs at a lower salt concentration. Thus it appears that the activation by LiCl requires preincubation, indicating that a change in the conformation of the protein is responsible for the activation.

b) *Anion varied.* In fig. 1a and b the cation was varied while the anion was chloride. Results with salts of varying anions and sodium as cation are shown in fig. 2a and b (an experiment in the presence of varying concentration of $(NH_4)_2SO_4$ is included as a comparison in fig. 2b). Both activation and inhibition occur with an increase in the concentration of NaCl, NaAc, $NaNO_3$, or Na_2SO_4. The anion appears to determine the form of the rate curve (note the agreement between the curves in the presence of Na_2SO_4 respective $(NH_4)_2SO_4$), even if the cation effect cannot entirely be excluded (cf. LiCl, fig. 1). Preincubation of the enzyme with the respective salt used in the experiments does not influence the form of the rate curve (fig. 2b) even if the optimum in some cases are obtained at somewhat lower salt concentrations, and the extent of activation is increased if compared with results obtained without preincubation (fig. 2a). These observations support the above suggestion that a change in the conformation of the enzyme is responsible for the activation.

3.2. Are monovalent cations or buffer anions necessary for the catalytic activity?

Sodium ions are introduced to the assay mixture with NADH. Fig. 3 shows the attempt made to clarify

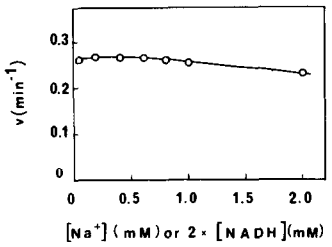

Fig. 3. Influence of NADH·Na_2 on the activity of phosphoglycerate kinase.

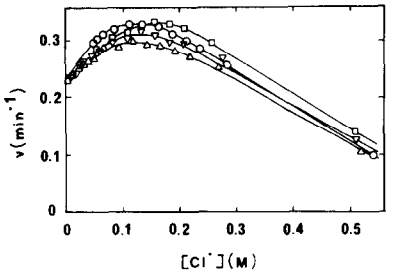

Fig. 4. Influence of NaCl (in terms of the total Cl^- concentration) on the activity of phosphoglycerate kinase at different concentrations of Tris (○—○—○): 50 mM, pH 7.8; (△—△—△): 25 mM, pH 7.7; (▽—▽—▽): 10 mM, pH 7.5; (□—□—□): 5.0 mM, pH 7.3.

whether or not the residual activity without the addition of extra salt as seen in fig. 1 and 2 is due to the sodium ions accompanying NADH. The sodium ion level, which varies between 0.05 and 2 mM, appears to have no positive effect on the enzymic activity. The possible Na^+ binding necessary for the residual activity might, of course, be so strong that ligation is already completed at a Na^+ level of 0.05 mM. In that case the cation binding is much stronger than the corresponding binding in muscle pyruvate kinase [1, 2], for example. Lower levels of NADH·Na_2 should show an apparent decrease in activity since NADH concentration then becomes limiting for glyceraldehydephosphate dehydrogenase catalyzed reaction.

Chloride ions are introduced to the assay mixture as part of the Tris-Cl buffer and as $MgCl_2$. A study on the effect of NaCl in terms of the total Cl^- concentration on the reaction rate at different Tris concentrations is presented in fig. 4. The enzymic activity approaches the same value at zero concentration of Cl^- independent of the level of Tris. That indicates that neither Cl^- nor Tris is responsible for the residual activity. On the other hand Tris appears to influence the activation (cf. fig. 1, LiCl). Part of this effect might be caused by the small shift in pH occurring when the buffer was diluted for the different series of measurement (but see [9]).

4. Conclusions

The present study indicates that neither monovalent cations nor accompanying anions are necessary for the catalytic activity of phosphoglycerate kinase, even if these salts cause activation and are inhibitory at higher concentrations. The activation appears to be accompanied by a change in conformation of the enzyme. The extent of activation is probably not only an effect of the monovalent cation but also of factors due to the ionic strength, the anion, and the extent of inhibition. The inhibition in part is an effect of the ionic strength, but since the extent of inhibition varies with the anion, evidence is obtained for some type of anion specificity (cf. ATP:creatine phosphotransferase [10]).

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[1] J.F. Kachmar and P.D. Boyer, J. Biol. Chem. 200 (1953) 669.
[2] C.H. Suelter, R. Singleton, Jr., F.J. Kayne, S. Arrington, J. Glass and A.S. Mildvan, Biochemistry 5 (1966) 131.
[3] H.J. Evans and G.J. Sorger, Ann. Rev. Plant Physiol. 17 (1966) 47.
[4] C.H. Suelter, Science 168 (1970) 789.
[5] M. Larsson-Raźnikiewicz, European J. Biochem. 15 (1970) 574.
[6] M. Larsson-Raźnikiewicz, European J. Biochem. 30 (1972) 579.
[7] M. Larsson-Raźnikiewicz, Biochim. Biophys. Acta 85 (1964) 60.
[8] T. Bücher, Biochim. Biophys. Acta 1 (1947) 292.
[9] W.K.G. Krietsch and T. Bücher, European J. Biochem. 17 (1970) 568.
[10] E.J. Milner-White and D.C. Watts, Biochem. J. 122 (1971) 727.