STABILIZATION OF DIFFERENT CONFORMATIONAL STATES OF LIVER PYRUVATE KINASE TYPE L BY THE ALLOSTERIC ACTIVATORS

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Received 3 September 1973

1. Introduction

Pyruvate kinase type L from liver is a regulatory enzyme capable of mediating complex allosteric interactions among a considerable number of effectors [1]. The enzyme exhibits positive homotropic cooperative effects with respect to phosphoenol pyruvate (PEP) [2–5]. This effect is strengthened by the inhibitors ATP and alanine [5, 6] and is abolished by the heterotropic effector FDP [1–8]. All these allosteric interactions and the influence of pH on them [7, 8] were compatible with a model involving an equilibrium between two conformational states, as proposed by Monod et al. [9]. Similar kinetic properties have been found with the pyruvate kinase type I isolated from kidney cortex [10]. Some discrepancies in this interpretation became apparent when the activation kinetics by K^+ was studied [11, 12]. Although several criteria indicated that this activation is of allosteric nature, K^+ exhibited no heterotropic effect on the homotropic cooperativity of PEP and ATP [11, 12]. Other discrepancies were noticed when the K^+ cooperativity was studied as a function of pH [11, 12]. These results, which differ from those obtained with FDP, suggested that the allosteric activators K^+ and FDP may stabilize different conformational states rather than a unique state as it would be predicted by the model of Monod et al. [9]. In order to discriminate between these alternatives and to obtain evidence that these allosteric effectors produce conformational changes of the enzyme we have studied the susceptibility of the liver pyruvate kinase type L to proteolytic attack and to thermal inactivation in the presence of saturating concentrations of the allosteric activators K^+, Mg^{2+} and FDP.

2. Materials and methods

Pyruvate kinase type L was isolated from rat liver and purified by $(NH_4)_2SO_4$ fractionation, chromatography on DEAE- and CM-cellulose columns as previously described [11, 12]. The overall purification ranged from 400- to 600-fold and the specific activity of the CM-cellulose eluate was 90 to 120 units per mg of protein. Enzyme activity was measured spectrophotometrically by coupling the system with excess lactate dehydrogenase [7]. Pronase and trypsin were from Sigma (U.S.A.). All other materials and methods were as previously described.

3. Results and discussion

The enzymatic activity of liver pyruvate kinase type L as function of time of incubation at 30°C in the absence or presence of the allosteric activators K^+, Mg^{2+} and FDP, is shown in fig. 1. The inactivation process follows a first order kinetics under all these conditions. The enzyme activity is markedly stabilized by Mg^{2+} and K^+ while no effect was observed in the presence of FDP. The PEP and ADP tested under similar conditions did not protect the enzyme activity (results not shown). In other experiments carried out at 50°C, FDP increased the rate of inactivation while K^+ had the opposite effect.

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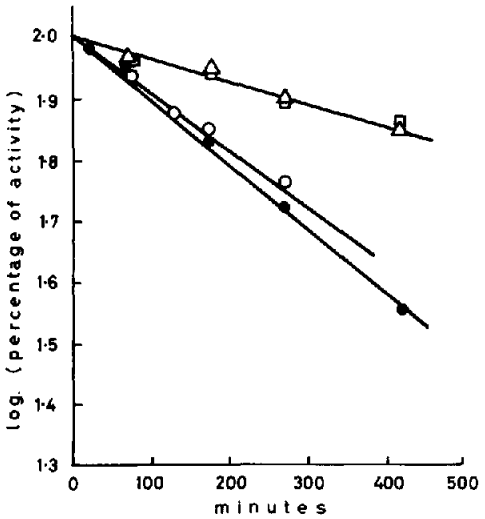

Fig. 1. Stability of the liver pyruvate kinase type L at 30°C in the absence (•) or presence of 100 mM KCl (△), 8 mM Mg^{2+} (□) or 0.1 mM FDP (○). The enzyme preparation (150 μg/ml) was in a medium containing 50 mM Tris–maleate buffer, pH 7.5, 1 mM EDTA and 1 mM dithrothreitol. At different times as indicated, aliquots of the enzyme solution were assayed for pyruvate kinase activity by the colorimetric procedure described previously [2] in the presence of saturating concentrations of substrates and activators. Other experimental details were as described under Materials and methods.

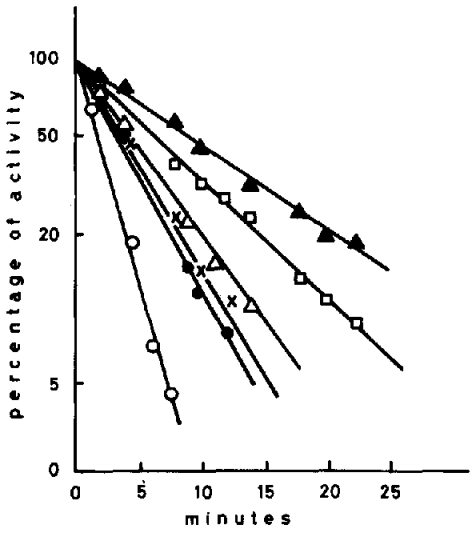

Fig. 2. Inactivation of liver pyruvate kinase type L by pronase in the absence (control •) or presence of different additions. The digestion mixture containing 32 μg/ml of the pyruvate kinase preparation, 10 μg/ml of pronase, 20 mM Tris-maleate buffer, pH 7.5, 0.5 mM EDTA and 1 mM dithrothreitol was incubated at 30°C. The additions were as follows: 100 mM KCl (▲); 25 mM KCl (△); 8 mM $MgCl_2$ (□); 50 mM NaCl (X) and 0.1 mM FDP (○). At different times, aliquots of the digestion mixtures were assayed for pyruvate kinase activity using the spectrophotometric procedure described previously [2] in the presence of saturating concentrations of substrates and activators. Other experimental details were as described under Materials and methods.

The effect of the allosteric activators on the inactivation of pyruvate kinase by proteolytic enzymes was also determined to further evaluate the possibility that these ligands stabilize different conformational states. Fig. 2 shows that K^+ and Mg^{2+} protected the enzyme against loss of catalytic activity produced by pronase; while Na^+, a non-activating ion [2], had no appreciable effect.

Opposite effects of K^+ and FDP on the rate of inactivation of liver pyruvate kinase were also observed when the enzyme was exposed to trypsin (fig. 3). The effect of Mg^{2+} was similar to that of K^+. Conversely, these ligands (K^+, Mg^{2+}, and FDP) had no appreciable effect on the rate of casein digestion by either pronase or trypsin.

Susceptibility to proteolysis and to thermal inactivation are well recognized criteria for detecting a change in the conformation of a protein [13]. The results presented in this communication show that the liver pyruvate kinase type L undergoes conformational changes in the presence of the allosteric activators. The three conformational probes used agree in

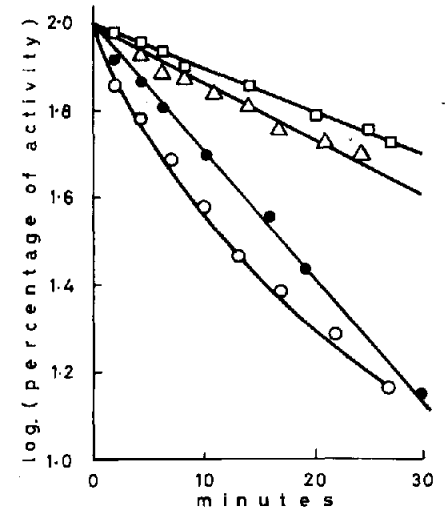

Fig. 3. Inactivation of liver pyruvate kinase type L by trypsin in the absence (control •) or presence of 8 mM $MgCl_2$ (□), 100 KCl (△) and 0.1 mM FDP (○). The digestion mixture and other experimental details were similar to those described in the legend of fig. 2 except that pronase was replaced by trypsin.

showing that the allosteric activators K^+ and FDP stabilize or induce different conformational states of the enzyme. These results and the kinetic data previously reported with K^+ [11, 12] are difficult to reconcile with a two-state model and suggest that sequential conformational changes are involved [14, 15] in the allosteric transitions of this enzyme.

Acknowledgements

We are grateful to Dr. Lius F. Leloir for helpful advice. We also wish to express our appreciation to other members of the Instituto de Investigaciones Bioquimicas for their interest and comments. This investigation was supported in part by grants from the Consejo Nacional de Investigaciones Cientificas y Tecnicas (Argentine) and the Universidad de Buenos Aires. L.J. de A. and H. C. are career investigators of the Consejo Nacional de Investigaciones Cientificas y Tecnicas (Argentine).

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