CONFORMATIONAL CHANGES OF NEUROSPORA PYRUVATE KINASE INDUCED BY TRIS BUFFER

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1. Introduction

Pyruvate kinase of a wild-type strain of *Neuraspora crassa* (St. Lawrence Standard; FGSC #533) has been shown to undergo inactivation on storage in Tris buffer [1]. In enzyme assays performed in the presence of Tris the progress curve showed an initial lag, following which linearity was attained. In the presence of potassium phosphate buffer, the progress curve was completely linear. The reaction rate in Tris – as determined by the slope of the progress curve during the linear phase – was also observed to be considerably lower than that in phosphate.

The so-called 'Tris effect' was observed at all pH values between 7.5 to 9.5, the lag becoming more prolonged at higher pH. The introduction of the lag could be interpreted as a conformational change resulting from an interaction of the enzyme with Tris. The subsequent appearance of linearity is due to a partial reversal of the conformational change on addition of substrates to the assay system.

In this communication, evidence is presented demonstrating a Tris-induced conformational change in pyruvate kinase. The influence of some compounds on the reversal of this effect is also presented.

2. Experimental

Pyruvate kinase was isolated from *N. crassa* and purified by a slight modification of the procedure described in a previous publication [1]. The purified enzyme was stored in 0.2 M potassium phosphate–10^{-4} M EDTA–2×10^{-3} M dithiothreitol, pH 7.5, at a concentration of 10.0 mg/ml. Enzyme activity was determined by measuring the pyruvate produced from PEP and ADP in the presence of Mg^{2+}, by a coupled reaction with LDH. The reaction system was as described previously, except that either 33.3 mM potassium or Tris was used as a buffer in the assay.

Circular dichroism measurements were made with a Jasco ORD/UV-5 spectropolarimeter with a CD attachment. For CD spectra, 100 μl of pyruvate kinase was diluted with 0.1 M potassium phosphate or Tris to a final volume of 1.0 ml. The diluted sample was permitted to stand for 1 hr at 2–4° and measurements were made in 0.5 mm light path cells, at room temp. Molar ellipticity values, $[\theta]_\lambda$, were calculated from the following relationship:

$$[\theta]_\lambda = \frac{\theta_\lambda \times \mathrm{MRW}}{100 \times c \times l}$$

where θ_λ is the observed ellipticity; MRW, the mean residue weight; l the path length in decimeters; and c, the concentration of the enzyme in g/cm^3.

3. Results and discussion

The assay for pyruvate kinase activity was normally conducted in the presence of phosphate buffer. In this buffer the progress curve is linear (figs. 1 and 2; curve A); the same is true of the reaction in hepes buffer (fig. 1, curve B). If Tris was used instead of phosphate the progress curve showed an initial lag, which became more pronounced with increasing concentrations (fig. 1, curves C, D, E, F, and G). To determine whether the inhibitory effect was confined to Tris alone, a variety of other buffers, in-

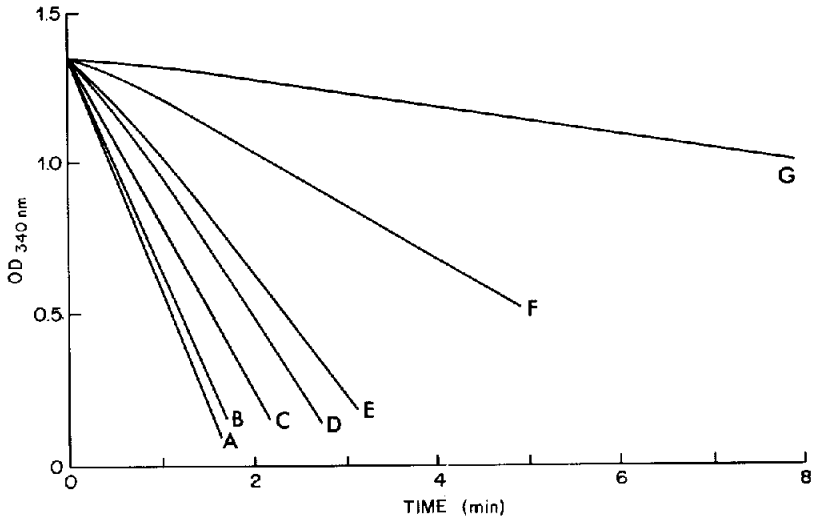

Fig. 1. Effect of varying concentrations of Tris on the pyruvate kinase reaction. A) Phosphate assay, 33.3 mM phosphate buffer, pH 7.5; B) 33.3 mM Hepes; C, D, E, F, and G) Tris buffer, 16.6 mM, 25 mM, 33.3 mM, 50 mM and 66.6 mM, respectively.

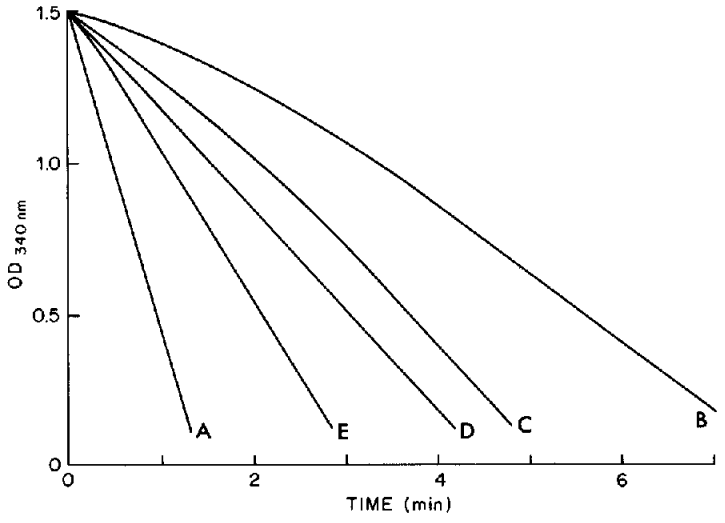

Fig. 2. Partial reversal of the 'Tris effect'. A) Assay conducted in 33.3 mM phosphate buffer, pH 7.5; B) 33.3 mM Tris; C) 33.3 mM Tris + 3.33 mM KCl; D) Tris + 3.33 mM Na_2HPO_4; and E) Tris + 3.33 mM K_2HPO_4.

cluding Mes, Tes, Tricine, Bicine, Bis-Tris, Taps and Mops were used as substitutes for it. At pH 7.5, only with Tes and Tricine was a slight lag evident, none of the others produced any inhibition or a detectable lag.

Next, the effect of some compounds was tested vis-a-vis their capacity to reverse the 'Tris effect', by including them in the assay system containing Tris buffer. Addition of 3.33 mM KCl or Na_2HPO_4 or Na_2HPO_4 caused a partial reversal of the lag and inhibition (fig. 2, curves C and D); Na_2HPO_4 appeared

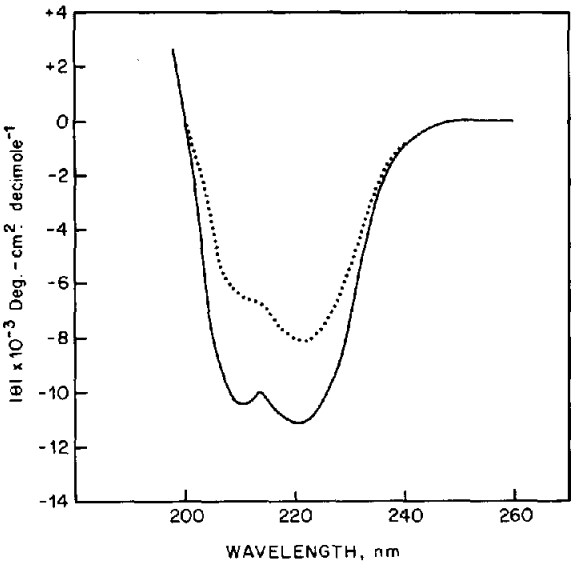

Fig. 3. CD spectra of pyruvate kinase in 0.1 M potassium phosphate (——) and Tris (.....) buffers.

to be more effective than KCl. The most efficient reversal was brought about by K_2HPO_4 (fig. 2, curve E) – attesting to the fact that both K^+ and phosphate are necessary for reversal. Several other compounds were also tested for ability to overcome the 'Tris effect' – ATP, ADP, AMP, glutamate and α-keto-glutarate. ADP alone was active in this regard. Pre-incubation with substrates, PEP and ADP, has been reported to bring about a partial reversal [1].

It was concluded that the introduction of a lag in the reaction was probably due to a conformational change in the protein which was induced specifically by Tris. Since CD spectra of proteins are dependent upon conformations [2], evidence for a conformational change in pyruvate kinase was sought by employing this technique. The CD spectrum of pyruvate kinase did not reveal any optical activity in the spectral region 250 nm–400 nm. Transitions occurring above 250 nm are ascribed to optical activity resulting from aromatic residues [3, 4] and disulphide bonds [5]. As shown in fig. 3 the spectrum of pyruvate kinase is typical of an α-helical protein [6]. Two bands with ellipticity minima at 221 nm and 210 nm, characteristic of $n-\pi^*$ and $\pi-\pi^*$ peptide transitions are evident in the spectrum. In the presence of phosphate these two bands are clearly distinguishable, but in Tris, there is a decrease in $[\theta]_{221\,nm}$. The 210 band is also considerably less intense.

Assuming a mean residue weight of 110, the molar ellipticity of the enzyme at 221 nm, $[\theta]_{221\,nm}$, and $[\theta]_{210\,nm}$ in phosphate can be calculated to be about $-11{,}110$ deg-cm^2·decimole^{-1} and $-10{,}450$ deg-cm^2·decimole^{-1}; the corresponding values for pyruvate kinase in Tris are, $[\theta]_{221\,nm} = -7{,}900$ deg-cm^2·decimole^{-1}. If the $[\theta]_{222\,nm}$ value of $-32{,}000$ deg-cm^2·decimole^{-1} for poly-α-L-glutaminic acid at pH 4.5 is considered to correspond to a 100% helical polypeptide, the helix content of pyruvate kinase in phosphate and Tris buffer is approx. 34% and 25%, respectively. It is interesting that the secondary structure of the enzyme is different in the two buffers. The actual mechanism by which Tris brings about this alteration in the secondary structure of pyruvate kinase is not clear.

CD measurements have also been used to explore the mechanism of interaction of rabbit muscle pyruvate kinase with univalent cations, and alterations in the ellipticities in the 250 nm–290 nm region have been reported [7]. The use of CD as a tool in investigations of conformational changes related to the binding of substrates and allosteric effectors should yield fruitful results with Neurospora pyruvate kinase.

Acknowledgements

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