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A CONFORMATIONAL CHANGE IN PHOSPHOGLYCERATE DEHYDROGENASE INDUCED BY A SHIFT IN pH

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Summary

The fluorescence of NADH bound to phosphoglycerate dehydrogenase (3-phosphoglycerate:NAD^+ oxidoreductase, EC 1.1.1.95) decreased by 42% between pH 8.5 and 7.0. Serine, an allosteric inhibitor, quenched the fluorescence of enzyme-bound NADH by 29% at pH 8.5, but not at all at pH 7.0. The kinetics of the fluorescence change which occurred when the pH of an enzyme-NADH solution was rapidly shifted from 8.5 to 7.0 was measured using stopped-flow fluorimetry. The kinetics were first order, with a rate constant of 2.83 s^{-1}. This rate constant was similar in magnitude to the rate constants for fluorescence quenching at pH 8.5 by saturating concentrations of serine and glycine, another allosteric inhibitor (Dubrow, R. and Pizer, L.I. (1977) J. Biol. Chem. 252, 1527—1538). These results indicate that the conformation of phosphoglycerate dehydrogenase at pH 7.0 is similar to, but not identical with, the serine-induced conformation at pH 8.5.

Phosphoglycerate dehydrogenase (3-phosphoglycerate:NAD^+ oxidoreductase, EC 1.1.1.95) from *Escherichia coli*, the first enzyme in the serine biosynthetic pathway, catalyzes the reaction:

3-phosphoglycerate + NAD^+ $\rightleftharpoons$ hydroxypyruvate phosphate + NADH + H^+

Phosphoglycerate dehydrogenase, a tetramer [1] containing four coenzyme-binding sites [2], is inhibited in an allosteric manner by serine and, at higher concentrations, by glycine [2—4].

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Several properties of phosphoglycerate dehydrogenase have been found to be strongly pH dependent: (1) the steady state reaction velocity [3—5]; (2) the rates of 3 isomerization steps which occur during the ternary complex interconversion [6]; (3) the magnitude of the rapid quenching of the fluorescence of the enzyme·NADH complex by hydroxypyruvate-*P*, which was shown to be the result of hydroxypyruvate-*P* binding [6]; (4) the magnitude of the ultraviolet difference spectrum between enzyme and enzyme plus serine [4]; (5) enzyme stability (ref. 1 and Dubrow, R., unpublished).

In going from pH 8.5 to 7.0, properties 1—4 decrease significantly, and enzyme stability increases. These changes are similar to the effects of serine on the enzyme at pH 8.5 (refs. 3,4 and 6 and Dubrow, R., unpublished), and suggest that the conformation of phosphoglycerate dehydrogenase at pH 7.0 may be similar to the inactive conformation induced by serine at pH 8.5.

The fluorescence of phosphoglycerate dehydrogenase-bound NADH is enhanced over that of free NADH in solution, and is quenched by serine

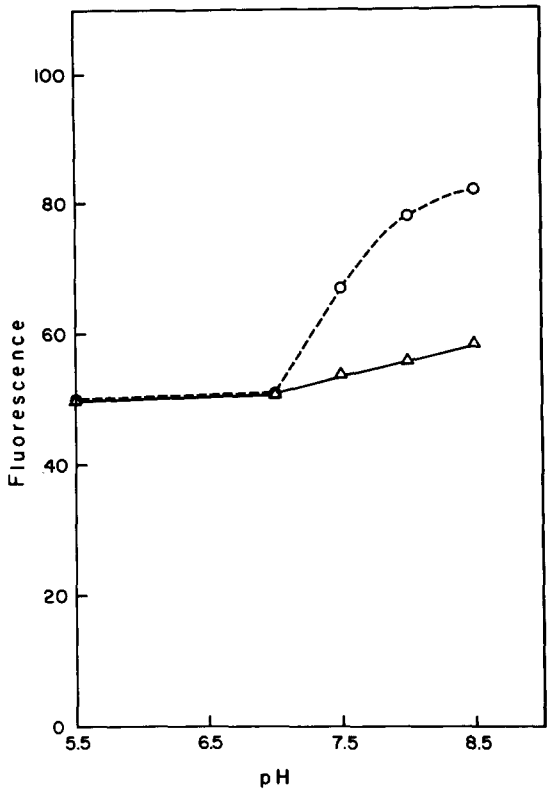

Fig.1. Fluorescence of the enzyme·NADH complex in the presence and absence of serine as a function of pH. The fluorescence of 1 ml of 0.5 μM phosphoglycerate dehydrogenase with 3 bound NADH molecules ($E\cdot(NADH)_3$) [2,6] was measured (○). Serine was then added to the cuvette in μl amounts to 1 mM, and the fluorescence was remeasured (△). This was done as a function of pH. The measurements were performed in an Aminco-Bowman spectrophotofluorometer. Excitation wavelength: 330 nm. Emission wavelength: 430 nm. Fluorescence is expressed in arbitrary units. 50 mM Tris·HCl buffer containing 1 mM dithiothreitol and 1 mM EDTA was used for the pH 7.0—8.5 measurements. 100 mM sodium acetate buffer containing 1 mM EDTA and 1 mM dithiothreitol was used for the pH 5.5 determination.

[2]. This fluorescence quenching is a manifestation of the structural change in the enzyme which is responsible for the inhibition of enzyme activity [4]. In this report, in order to further clarify the relationship between the effects of serine and pH on phosphoglycerate dehydrogenase, we describe the effect of pH on enzyme-bound NADH fluorescence in the presence and absence of serine. In addition, a stopped-flow pH-shift experiment which measures the kinetics of a pH dependent conformational change is described.

The purification of phosphoglycerate dehydrogenase [6] and the instrumentation used for stopped-flow fluorimetry [4] have been presented.

Fig. 1 shows that the fluorescence of enzyme-bound NADH decreased by 42% between pH 8.5 and 7.0. (This change was not due to dissociation of NADH from the enzyme. Previous studies have indicated that the binding of NADH to the enzyme is in fact tighter at low pH values (refs. 1 and 2 and Dubrow, R., unpublished)). There was a corresponding decrease in the magnitude of fluorescence quenching by 1 mM serine, a concentration which completely inhibits enzyme activity, from 29% of the total fluorescence at pH 8.5 to no detectable quenching at pH 7.0. Thus, as with the properties of phosphoglycerate dehydrogenase referred to previously, the change in enzyme-bound NADH fluorescence between pH 8.5 and 7.0 was similar to the change induced by serine at pH 8.5. However, the data indicate that the conformation of the enzyme at pH 7.0 is not identical with the serine-induced conformation at pH 8.5. The fluorescence of the enzyme·NADH complex at pH 7.0 was lower than the fluorescence of the enzyme·NADH·serine complex at pH 8.5 (Fig. 1). In addition, phosphoglycerate dehydrogenase retains 15% of its activity at pH 7.0 compared with pH 8.5, where activity is at its maximum [6], whereas 1 mM serine completely inhibits enzyme activity at pH 8.5. If there were a strict correlation between the magnitude of quenchable fluorescence and the degree of enzyme activity, one would have expected a magnitude of fluorescence quenching by serine at pH 7.0 which was 15% of that at pH 8.5. In fact, no fluorescence quenching by serine was observed at pH 7.0.

The data also indicate that the change in enzyme structure caused by serine must be much more subtle at pH 7.0 than at pH 8.5. The structural change was readily detected at pH 8.5 but was undetectable at pH 7.0 by monitoring enzyme-bound NADH fluorescence. (A similar phenomenon was observed with hydroxypyruvate phosphate binding to the enzyme, which was also readily detectable at pH 8.5, but not at pH 7.0, by monitoring enzyme-bound NADH fluorescence [6]). Thus the conformation of phosphoglycerate dehydrogenase at pH 7.0 is probably similar to, but not identical with, the serine-induced conformation at pH 8.5.

The kinetics of the fluorescence change which occurred when the pH of an enzyme-NADH solution was rapidly shifted from 8.5 to 7.0 was measured using stopped-flow fluorimetry. Phosphoglycerate dehydrogenase and NADH at pH 8.5 were mixed with a buffer solution to which HCl had been added so that when equal volumes to the enzyme and buffer solutions were mixed, a solution with a pH of 7.0 resulted. The decrease in enzyme-bound NADH fluorescence was monitored by exciting at 340 nm. The photograph of the oscilloscope trace from this experiment is shown in

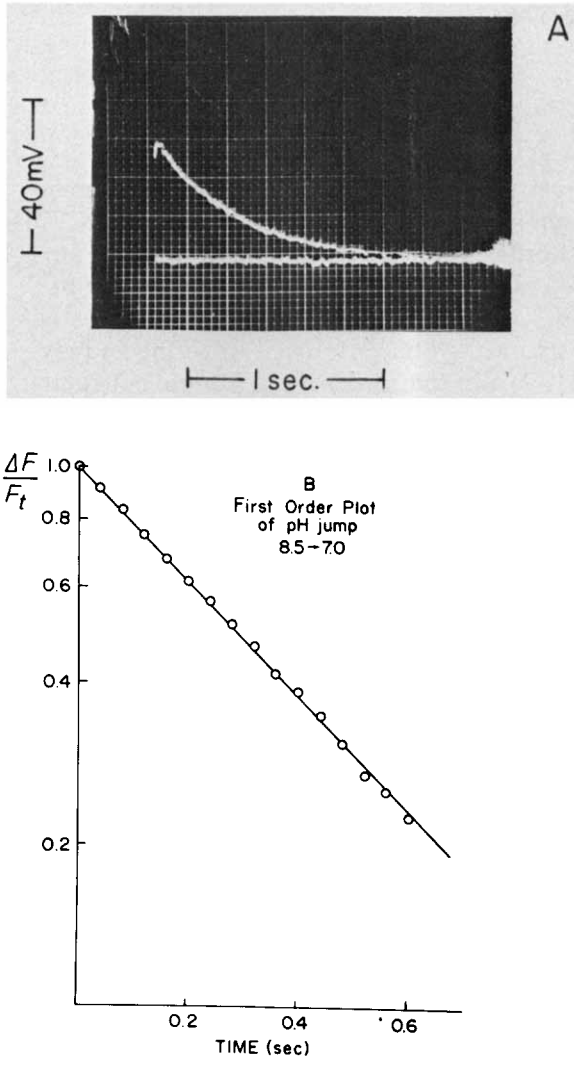

Fig.2. Kinetics of the pH shift from pH 8.5 to 7.0. A. Photograph of the oscilloscope trace from a stopped-flow experiment designed to measure the kinetics of the decrease in enzyme-bound NADH fluorescence caused by a pH shift from 8.5 to 7.0. Phosphoglycerate dehydrogenase and NADH at pH 8.5 were mixed with buffer and a sufficient amount of HCl so that the final pH after mixing was 7.0. The excitation wavelength was 340 nm. Fluorescence was observed through a 400 nm cut-off filter. Reagent concentrations after mixing: 50 mM Tris·HCl buffer, pH 7.0, 40 mM Na_2SO_4, 1 mM EDTA, 1 mM dithiothreitol, 5 μM NADH, 1 μM phosphoglycerate dehydrogenase. The bottom horizontal trace was the end-point. B. Semilog plot of the fraction of quenchable fluorescence remaining ($(\Delta F)/F_T$) vs. time. The plot was derived from the above trace.

Fig. 2A. The semilog plot of the fraction of quenchable fluorescence remaining vs. time is shown in Fig. 2B. The kinetics of the fluorescence change could be described by a single observed first order rate constant equal to 2.83 s^{-1}.

This experiment can be conceptualized as follows:

$$E\text{-}B + H^+ \underset{\text{rapid}}{\overset{\text{I}}{\rightleftharpoons}} E\text{-}BH^+ \underset{\text{slow}}{\overset{\text{II}}{\rightleftharpoons}} E'\text{-}BH^+$$

E represents the enzyme species with high enzyme-bound NADH fluorescence and E′ represents the enzyme species with low enzyme-bound NADH fluorescence. The observed first order process corresponds to Step II, an isomerization step. The observed rate constant of 2.83 s^{-1} was similar in magnitude to the values of 1.1 s^{-1} and 2 s^{-1} obtained for the rate constants for fluorescence quenching by saturating concentrations of serine and glycine, respectively, at pH 8.5 [4]. Again, the similarity in kinetic constants indicates that the pH shift most likely represents a conformational change in phosphoglycerate dehydrogenase which is similar to, but not identical with, the allosteric transition induced by the amino acid inhibitor.

The kinetics of pH dependent conformational changes has been measured in other systems [7—10]. However, a pH dependent conformational change in horse liver alcohol dehydrogenase was too rapid to be observed in a stopped-flow fluorimeter [11].

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