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THE CALCIUM BINDING SITES OF THE BAKERS' YEAST TRANSKETOLASE

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Abstract

The calcium binding sites of Bakers' Yeast Transketolase (TK) was elucidated by estimating the pKa values of the functional groups that bind to calcium. These pKa's were found to be 6.25 and 7.2 relating to the pKa's of the two immidazol moieties of histidine residues on the enzyme. The rate of the binding of calcium to the enzyme was obtained separately as a function of pH. Maximum values were obtained at the pKa's of the immidazol rings of histidine correlating closely with the values obtained by direct titration. The activity rate studies as a function of pH showed that the enzyme, when bound to its cofactors, becomes further activated over and above the regular increasing trend of the pH rate profile at similar pH's which are optimal for calcium binding.

Introduction

Transketolase (TK) (EC 2.2.1.1.) (Seduheptulose-7-'hosphate-:D-glyceraldehyde-3-phosphate ;lycoaldehydetransferase.) obtained from Baker's yeast, onsists of two subunits with a total molecular weight if 158-159 kDa [1-4]. Each subunit contains an active enter [5]. The function of the enzyme is to transfer a etal group from a donor molecule (a ketose phosphate) o an acceptor molecule, (an aldose phosphate) and equires thiamine diphosphate (TDP) and a divalent ation for activity [6]. Native holo-TK from Baker's 'east is known to contain 2 moles of TDP and two ram atoms of metal ion per mole of protein [7-9].

The binding of calcium to one of the subunits gives lower association constant [10]. It has also been eported that the enzyme shows allosteric characteristics 11]. While the chemistry of enzyme has been the subject of intensive studies, the mechanism of binding of TDP and the divalent ion to the active site of the enzyme has not been fully understood. Several reports indicate that some functional groups such as arginine [12-14] cysteine [15] and histidine [16] as well as tyrosine [17] on the active sites of enzyme are responsible for the catalytic activity. These functional groups are reported to be involved on substrate binding and/or catalysis, but no reports have been presented to date on the possible binding sites of either the metal ions or the coenzyme TDP. It is the purpose of this study to provide a further understanding about the sites of binding of the metal ions on the active site of the enzyme transketolase. We have employed a previously tested method to identify the functional groups that are involved in the binding of metal ions to the active sites of transketolase [18].

Keywords: Calcium binding; Transketolase

Experimental Section

Baker's yeast transketolase was isolated by the method of Racker et al. [19] with some modifications [20]. CN-Br agarose gel covalently linked to the antibody was additionally used as an immunoabsorbent [21]. The preparation contained mostly apotransketolase, Enzyme assay was carried out using the coupled enzyme procedure [22]. The assay mixture contained glyceraldehyde-3- phosphate dehydrogenase and triose phosphate isomerase isolated from rabbit muscle [22] and a mixture of phosphopentoses, (10mM). Phosphopentoses were used as substrates and obtained by the method described by Gubler C.G. et al. [24]. They were in the form of barium salts and were converted to sodium salts before use by Dowex 50w-x8 cation exchange chromatography. The coupled enzyme and phosphopentoses were all used in excess. The final concentration of other reagents were NADH. 20mM $CaCl_2$ and TDP, 1-2 mM. The reaction was initiated by the addition of TK. at 25°C pH=7.6. The optical density changes due to the conversion of NADH to NAD were monitored at 340 and used for activity determinations. The quantity of proteins were evaluated for the TK preparations by the optical density values of TK solutions at 280, ($E^{1\%}_{280}$ =14.5), thus estimating the protein concentration from the extinction coefficient and the activity obtained, the specific activity of the original preparation was measured to be 16 U/mg. The enzyme was stored at - 20°C in glycerol. The enzyme used for the studies was first passed through a column of sephadex G_{50} equilibrated with .05 M tris buffer pH=7.1 and was concentrated by ultrafiltration. The enzyme thus prepared was used for Ca^{2+} binding studies according to the method previously described [18]. The reaction mixture contained Ca^{2+}, 10mM; TK, 10U in .05M tris buffer, the difference spectra were taken at 0.1-0.2 pH unit intervals and the A% changes at 280 nm were calculated and plotted as a function of pH (Figure 1). The

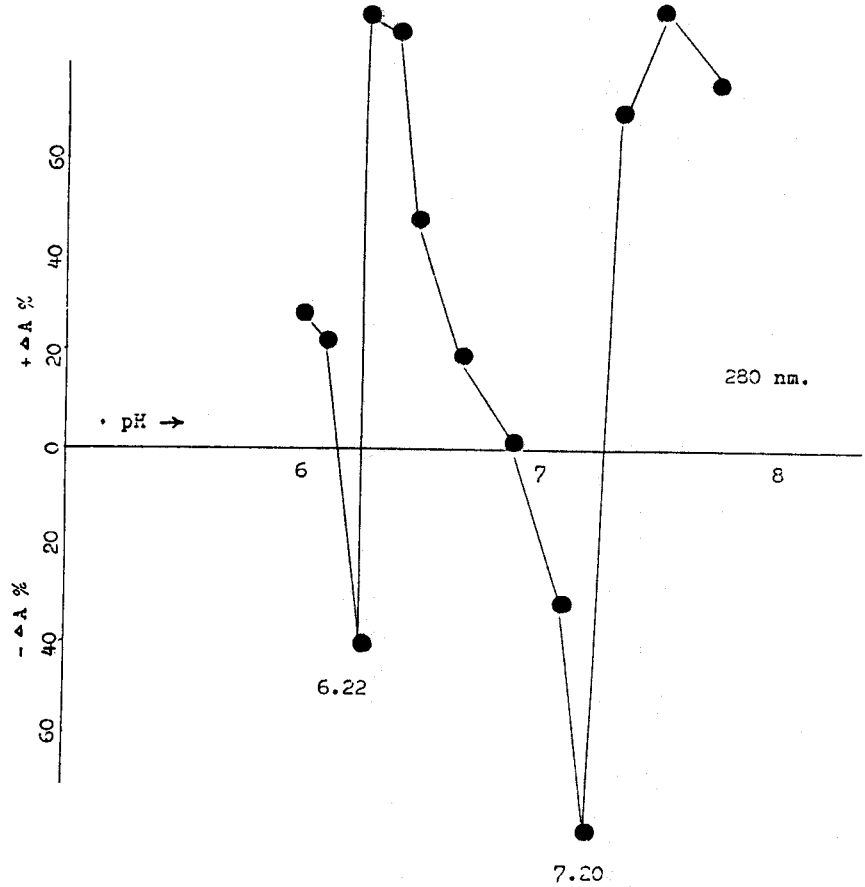

Figure 1. The plot of A% vs. pH at 280 nm. for Apo-TK reacted with Ca^{2+}, reaction condition; Ca^{2+}, 10mM.; TK 10 U Tris, 0.05M; T=25°C

first order rate of binding of apo-TK and Ca^{2+} was estimated as a function of pH at 280 nm. The final reaction mixture contained, Ca^{2+} 10 mM. TK.10U, tris. .05 M. All the reactions were carried out at 25°C. The first order rate constants were calculated at each 0.1-0.2 pH unit intervals and were plotted against pH (Figure 2).

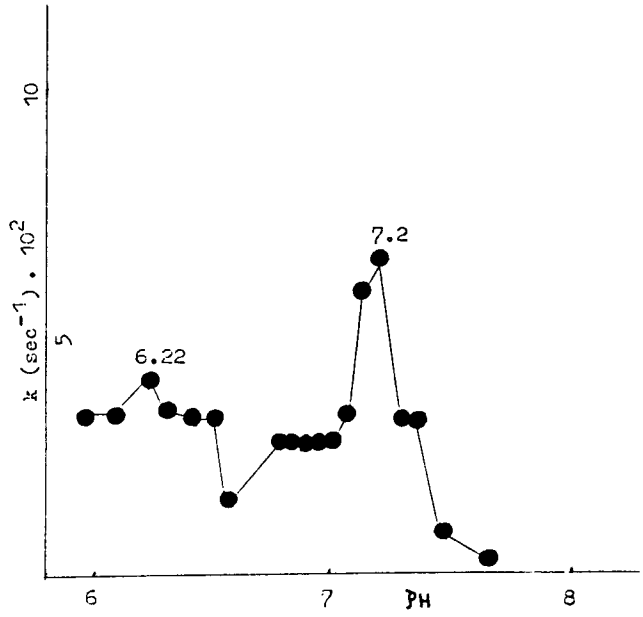

Figure 2. Ca^{2+} recombination reaction with TK. First order rate constants are plotted against pH; reaction condition; Ca^{2+}, 10mM.; TK., 10 U.; Tris. 05 M., T=25°C.

The activity-pH profile was studied using the solutions of enzyme and calcium ion at equilibrium.

Coupled enzyme assay was carried out on each solution at each 0.1-0.2 unit of pH intervals at strict ionic strength. Next, the enzyme was reacted with diethyl pyrocarbonate (DPC), a modifier of immidazolium group in the enzyme [25]. To 1 ml. of enzyme solution containing 1.6 U of TK, and in the presence of all the other components of the reaction, 20 ul of 10mM solution of (DPC) was added and the activity studies were performed with the same condition as before.

Results and Discussion

Figure 1 shows the difference spectroscopic measurements of the per cent changes in absorption due to the addition of calcium ion to the enzyme solution as a function of pH. These changes signified the effect of Ca^{2+} binding in solutions at equilibrium with Ca^{2+} compared to those that void Ca^{2+}.

Our previous results indicate that the minimums obtained correspond to the pkas' of the functional groups that bind specifically to the metal ions in proteins [18]. The results obtained from transketolase (Figure 1). indicate that the two minimums at 6.25 and 7.20 (280 nm) may correspond to the two pkas' of immidazolium groups of histidine in transketolase that bind to Ca^{2+}. It is suggested that if an ionizing group in a protein binds to a metal ion, it will do so by proportionally ionizing as it binds to a metal ion, therefore the charge on the metal ion bound to a functional group will be changed as a function of ionization of the group under the study. This effect can be transmitted to an adjacent aromatic group, thus we see similar changes at the ultraviolet range of spectra similar to the changes due to the direct titration of the group. This effect is more pronounced when a group is located at the active center of the enzyme. We have tested this effect with metal ions such as Mg^{2+} that are normally unreactive with the functional groups. In pyruvate decarboxylase where the Mg^{2+} is the cofactor, this effect was examined and led to the identification of the immizolium group in the active site bound to the Mg^{2+} ion. This effect was further tested with other enzymes such as chymotrypsin, trypsin, subtilisin [18] and model compounds with success. It is the purpose of this paper to identify the calcium binding loci in transketolase by this method and substantiate that by the use of other independent procedures. It has been shown that a quantity such as "L" that is a property of a molecule, i.e. absorption coefficient or the rate constant may correspond to the properties of the bulk solution by a factor of concentration with the dependencies on pH variations. This may be portrayed in the following equation specified for a single species [26].

$$L_H(A_O) = \frac{L_{HA}(A_O)(H^+)}{K_a+(H^+)} + \frac{L_A^-(A_O)K_a}{K_a+(H^+)}$$

or

$$L_H = \frac{L_{HA}(H^+) + L_A\text{-}K_a}{K_a+(H^+)}$$

Where L_{HA} and L_A^- are the properties under study for the molecular species HA and A^- respectively and L_H

constitutes their total quantity. The plot of per cent change for the above equation produces a sharp peak as shown in Figure 1. These points should correspond to the pKas' of the groups that are bound to calcium ion. The above evidence was further substantiated by using another "L" property such as the rate constant, the first order rate for the direct binding of the calcium ion and TK was studied as a function of pH (Figure 2). Two maximums at pH=6.22 and 7.20 are indicative of the enhancement of the rate of binding of Ca^{2+} to the enzyme at these two pH's and may be representative of two immidazol groups that bind to calcium ion. The k_{obs}. at pH=6.22 and 7.2 were 4.2×10^{-2} and 6.2×10^{-2}, respectively. It is worthwhile to note that the minimum value of k_{obs}. at 6.65 coincides with the arithmatic means of the two pKa's or the pI of the immidazolium rings and correspond to a pH point that both immidazol rings are collectively unreactive to Ca^{2+} ion. Figure 3 represents the variation of relative activity versus pH (the relative activity signifies the activit measured for 8 units of TK) with all the components c the reaction present. The two maximums are at pH=6.2 and 7.2 correlating closely with the values obtained fror the direct interaction of Ca^{2+} and the enzyme and i consistant with the pKa values obtained by our differenc spectroscopic method. Comparing the rate constant an activity profile, a close similarity in their trends i evident i.e. the sharp decrease in activity at the pH=6.6 where the two immidazole rings are least reactive witl calcium ion and the two maximums at pH=6.22 an pH=7.2. An increasing trend of activity with pH toward the optimal pH of 7.6 is indicative of other factor involved in catalysis. The modifications of th immidazol moieties by specific modifie diethylpyrocarbonate abolished the activity increase du to the Ca^{2+} binding. This effect could be used as furthe proof for such bindings and their effects in the catalysi of enzyme reaction.

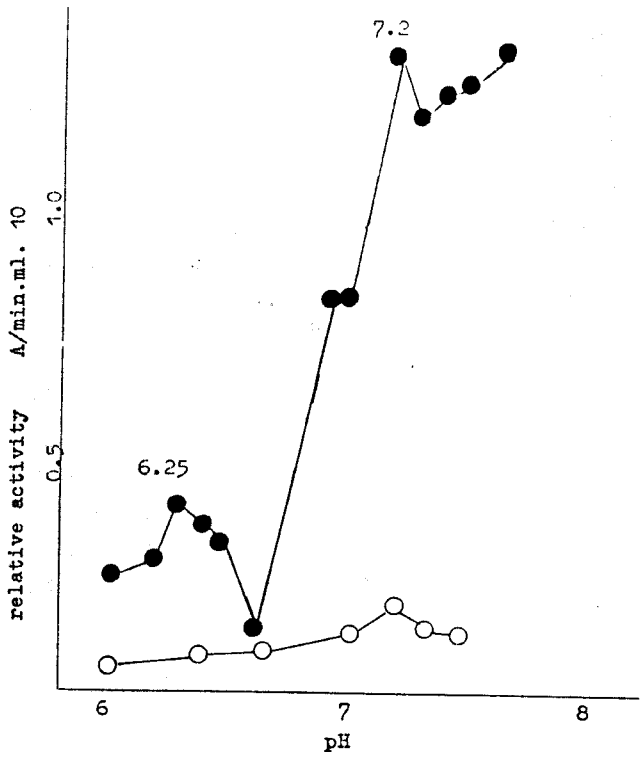

Figure 3. Plots of relative activity of TK. as a function of pH.; assayed by coupled enzyme method. No modifier used, 20 ul. of 10 mM. solution of diethylpyrocarbonate added to 1 ml. solution of 0.8 U of TK.

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