Comparative Properties of Yeast and Muscle Aldolase*

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Aldolases derived from various sources may be conveniently segregated into two types on the basis of the effect of metal-chelating agents on their catalytic activity (1). For example, the activity of highly purified yeast aldolase (type II) is inhibited by chelating agents, and contains zinc apparently as an integral part of the enzyme. It has also been shown that this enzyme is specifically activated by potassium ions (2). On the other hand, the activity of muscle aldolase (type I) is indifferent to chelating agents, does not contain significant quantities of divalent metal ions, and is not activated by potassium ions (2). It is conceivable that the differences noted are reflections of distinctive properties of yeast and muscle aldolase should resolve this question.

In the present report it is shown that the muscle and yeast enzyme have characteristically different specificities, pH profiles, temperature dependences, and response to the action of carboxypeptidase.

EXPERIMENTAL PROCEDURE

Chemicals—The crystalline cyclohexylamine sulfate salt of fructose-di-P was prepared (3) from a sample of dibarium fructose-di-P procured from Schwarz BioResearch Inc. Sorbose-1-P and sorbose 1,6-diphosphate were kindly supplied by Dr. Henry A. Lardy. Fructose-1-P was cordially supplied by Dr. Carl S. Vestling; in addition, this compound was obtained from Sigma Chemical Company. Fructose-6-P was obtained from Schwarz BioResearch, Inc. The barium salts of the above compounds were converted to the sodium salts by treatment with Dowex 50-H+, followed by neutralization with NaHCO₃.

In the experiments reported, the D isomer of glyceraldehyde-3-P was used exclusively. Samples of n-glyceraldehyde-3-P as well as dihydroxyacetone-P were graciously donated by Dr. Clinton Ballou; in addition, these compounds were synthesized according to the procedures of Ballou and Fischer (4, 5). The products obtained compared favorably with the published analysis; in particular, there was no detectable contamination of glyceraldehyde-3-P with dihydroxyacetone-P or vice versa. Acetol phosphate was synthesized by an independently developed procedure resembling that recently reported by Sellinger and Miller (6). The sources of other compounds employed has been cited (2).

Enzyme Assays—The assays for aldolase activity were performed at pH 7.5, 25°, according to procedures previously described (2). One unit of aldolase activity was defined as that amount of enzyme catalyzing the aldol synthesis or cleavage of 1 μmole of fructose-di-P per minute under the conditions of the assay.

The aldolase-catalyzed hydrogen exchange between dihydroxyacetone-P and water was detected according to the convenient method with the tritium isotope developed by Rose and Rieder (7).

A stock supply of stoichiometrically tritiated dihydroxyaceto-P was obtained by incubation of 55 μmoles of dihydroxyacetone-P with 4.5 mg of triosephosphate isomerase-free muscle aldolase at pH 7.5 in a medium containing tritiated water (6 X 10⁶ c.p.m. per μmole of water), 1 μM EDTA, and glycolytic components, 50 μmoles, in a total volume of 1 ml. After 10 minutes incubation at 25°, the solution was deproteinized by the addition of 5 ml of ethanol, and the supernatant solution placed on Dowex 1 chloride column (200 to 400 mesh, 3.5 X 2.4 cm). The column was washed with 0.001 N HCl until the eluate was essentially free of radioactivity. The tritiated dihydroxyacetone-P was then eluted with 0.03 N HCl. Fractions were assayed for dihydroxyacetone-P quantitatively in a system containing excess α-glycerophosphate dehydrogenase and DPNH. Tritium analyses were performed directly on 0.5 ml aqueous aliquots with the Kinard scintillator mixture (8) and a Packard Tri-Carb liquid scintillation spectrometer. The counting efficiency with this procedure is about 10%. The dihydroxyacetone-P obtained (3 X 10⁵ c.p.m. per μmole) was diluted with cold dihydroxyacetone-P to about 2 X 10⁴ c.p.m. per μmole for use in the exchange experiments. The detrinitiation of dihydroxyacetone-P was measured both by the change in specific activity of dihydroxyacetone-P on incubation, and by the accumulation of tritiated water during the reaction. The rates of detrinitiation actually follow first order kinetics; the rates are, however, corrected to initial velocities (micromoles per minute).

At the high dilutions (0.05 to 0.09 unit of aldolase) employed there was marked loss of yeast aldolase and significant loss of muscle aldolase exchange activity during the course of the experiments. Addition of bovine serum albumin at a final concentration of 1 mg per ml prevented the loss of activity, and was routinely added to experimental systems in which it was efficacious.

Triosephosphate isomerase activity was assayed by the rate of conversion of glyceraldehyde-3-P to α-glycerol-P in the presence of excess α-glycero-P dehydrogenase and DPNH. The system contained 1.1 μmole of glyceraldehyde-3-P, 0.5 μmole of DPNH, 5 μmole of cysteine, 150 μmole of glycoaldehyde, pH 7.5, 20 μg of crystalline α-glycero-P dehydrogenase, solution...
containing 0.01 to 0.1 unit of isomerase, and water to 3.0 ml.
One unit of triosephosphate isomerase activity was defined as that amount of enzyme facilitating the conversion of sufficient glyceraldehyde-3-P to dihydroxyacetone-P to allow oxidation of 1 μmole of DPNH per minute under the assay conditions stated at pH 7.5, 25°.

Enzymes—Highly purified yeast aldolase was isolated as previously described (2). The specific activity of the preparations employed varied from 85 to 115; triosephosphate isomerase activity could not be detected (aldolase activity)/isomerase activity > 104).

Muscle aldolase was isolated by the method of Taylor et al. (9). Triosephosphate isomerase activity was present even after several recrystallizations. It was found that the isomerase activity could be effectively removed on a DEAE-cellulose column, as shown in Fig. 1. The aldolase passes directly through the column under these conditions while the triose isomerase is retained. In practice, it was found most convenient to use recrystallized muscle aldolase that had been twice washed according to the procedure of Beisenherz et al. (11) for DEAE-cellulose chromatography. Under these conditions, the DEAE eluate representing 50% of the added aldolase contained no detectable isomerase activity (aldolase activity)/isomerase activity > 104). The specific activity of the muscle enzyme under conditions defined previously was 20. Crystalline α-glycero-P dehydrogenase was purchased from Sigma Chemical Company.

Carboxypeptidase recrystallized from a solution containing diisopropyl fluorophosphate and containing little if any trypsin or chymotrypsin was procured from Worthington Biochemicals Corporation. The concentration of this enzyme was determined, as described in “Experimental Procedure.” Nine milliliter fractions were collected and assayed for aldol cleavage activity, ---, (hydrazine assay (2)), triosephosphate isomerase activity, ---, and protein, ---, (2). In the fractions measured, 88% of the initial protein and 88% of the initial activity were recovered. In Fractions 9 to 11, which are essentially triosephosphate isomerase-free, 40% of the initial protein and 47% of the initial activity were recovered.

RESULTS

Effect of Temperature on Aldol Cleavage Reaction—The effect of temperature on the aldol cleavage activity of yeast and muscle aldolase was measured with the hydrazine assay system. As shown in Table I, there is a marked difference in the heat stability of the enzymes; yeast aldolase is unstable at temperatures above 35°, whereas the muscle enzyme is apparently unaffected at 45°. Even in the range of temperature stability, the Arrhenius plots for both enzymes are concave downwards, indicating a degree of dependence of the energy of activation on the temperature. An approximate fit of the data for the region from 20° to 35° yields values for the energy of activation of 14,250 and 17,300 calories per mole for yeast and muscle aldolase, respectively. Using the same assay system, Drechsler et al. (10) have reported an energy of activation close to 16,000 calories per mole for both native and carboxypeptidase-degraded muscle aldolase. This is considered acceptable agreement considering the curvature of the Arrhenius plots. Beisenherz et al. (11), who employed the α-glycero-P dehydrogenase assay, present data corresponding to an energy of activation of approximately 15,000 calories for the muscle aldolase system. Jagannathan et al. (12) report data for Aspergillus niger aldolase (glyceraldehyde-3-P dehydrogenase assay) which suggest an energy of activation of about 14,500 calories. It is interesting that the A. niger enzyme, like the yeast enzyme, is stable to temperatures higher than 35°. The absolute values for the energy of activation vary considerably with the various conditions employed in the assay, but when com-
parisons are made of similar systems, the muscle enzyme appears to have a significantly higher energy of activation than the yeast enzymes.

According to the absolute reaction rate theory, the rate of the reaction is directly related to the free energy of activation, $\Delta F^\ddagger$, of the rate-limiting transition state. The calculated $\Delta F^\ddagger$ is of the order of 15,000 calories for the aldolases, the $\Delta F^\ddagger$ of the yeast enzyme being about 250 calories lower than the muscle aldolase. The difference in the enthalpy of activation $\Delta H^\ddagger$ of the two enzyme-catalyzed reaction would be the same as the difference between the energies of activation of the two systems (2,750 calories) and considerably larger than the discrepancy of $\Delta F^\ddagger$ in the two processes. This implies that the entropy of activation, $\Delta S^\ddagger$ of the rate-limiting transition step of the muscle aldolase-catalyzed reaction is significantly more positive than that of the yeast aldolase-catalyzed process.

Effect of pH on Aldol Cleavage and Hydrogen Exchange Reactions—In addition to the aldol cleavage of fructose-di-P, both yeast and muscle aldolase catalyze a stereospecific hydrogen exchange between dihydroxyacetone-P and water of the medium (13-15). The influence of pH on both activities was determined in this study. The exchange reaction was measured by the detritiation of specifically labeled dihydroxyacetone-P (see “Experimental Procedure”) and the over-all activity by the synthesis of fructose-di-P from dihydroxyacetone-P and glyceraldehyde-3-P (2). The latter assay was chosen in order to exclude the possibility of an effect on the coupled detection system, since the pH optima previously reported in the literature have varied with comparisons made of similar systems. The muscle enzyme appears to have a significantly higher energy of activation than the yeast enzymes.

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As shown in Fig. 2, the pH profiles of the reactions catalyzed by yeast and muscle aldolase differ markedly. The aldol synthetic activity of muscle aldolase is practically constant in the pH range 9 to 6, then tails off somewhat below pH 6. The hydrogen exchange activity also has a broad optimum in the region of pH 9 to 7 decreasing to about half this activity from pH 7 to 6, and then remaining essentially constant from pH 6 to 5. In the higher pH regions, the exchange activity is distinctly greater than the aldol cleavage activity, but the reverse is true in the pH 6 to 5.5 region.

The aldol synthetic activity of the yeast enzyme is optimal at pH 7.0 to 7.5 with pronounced loss of activity on either side of this region; the exchange activity on the other hand, shows a rather more sharp optimum at pH 6.0. In marked contrast to the muscle enzyme system, the exchange activity of yeast enzyme is greater than the over-all synthetic activity at pH values below 6.0 and less at pH values above 7.0; at pH 8.0 for example, the exchange activity is only 20% of the rate of the over-all reaction.

It is emphasized that these experiments were performed at substrate concentrations approximating the $K_m$ values. This was essential in the assay of the over-all reaction since there was marked substrate inhibition at higher concentrations of triose phosphates (that was not overcome by manipulation of the ratios of dihydroxyacetone-P and glyceraldehyde-3-P). The pH profile in each case could, therefore, be a reflection of the $K_m$ or $V_{max}$ of the system. However, at least above pH 6.5, similar profiles to those presented in Fig. 2 were obtained for the aldol cleavage reaction with both yeast and muscle aldolases, by use of extrapolated $V_{max}$ values obtained with both the hydrazine

### Table I

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Turnover No.</th>
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<tbody>
<tr>
<td></td>
<td>Yeast aldolase</td>
</tr>
<tr>
<td>20°</td>
<td>4,600</td>
</tr>
<tr>
<td>24.5°</td>
<td>6,900</td>
</tr>
<tr>
<td>30°</td>
<td>10,500</td>
</tr>
<tr>
<td>35°</td>
<td>13,300</td>
</tr>
<tr>
<td>38°</td>
<td>13,300</td>
</tr>
<tr>
<td>39.5°</td>
<td>10,600</td>
</tr>
<tr>
<td>44°</td>
<td>5,100</td>
</tr>
</tbody>
</table>

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and α-glycero-P dehydrogenase assays. The differences in the pH profiles, therefore, imply different dissociable groups at the catalytic site, or in a sensitive structural region producing conformational changes at the catalytic site. The exchange and aldol synthetic activities were carried out at similar dihydroxyacetone-P concentrations. The dissimilarity of the pH profiles (for both reactions) therefore clearly reflect difference in the two processes and suggest an important interaction of glyceraldehyde-3-P with other dissociable groups in the system.

The pronounced influence of pH on the yeast-catalyzed reactions prompted a study of the pH dependence of the potassium activation of the yeast system. The experiments were performed with a yeast aldolase which had been previously exposed to 0.01 M EDTA. As shown in Fig. 3, the exchange activity under these circumstances is completely dependent on the addition of zinc, at all pH values; this confirms and extends the initial observations of Rose and Rieder (7). In addition, potassium ion stimulates the exchange at all pH values, the magnitude of the stimulation is 2.5- to 6-fold; maximal stimulation being obtained at pH values less than 6. The pH optimum of the exchange system is not markedly altered by potassium although it may be lowered a few tenths of a pH unit. The pH optimum for over-all activity also remains at 7 to 7.5 in the absence of K⁺ ions. Thus, the observed stimulation by potassium cannot be ascribed to an adventitious alteration of the pH profile. It is perhaps significant that the degree of stimulation by potassium ion is considerably lower for the exchange than for the over-all reaction.

**Substrate Specificity**—Although there is considerable qualitative data on the specificity of muscle aldolase, quantitative data, especially in terms of Michaelis constants and maximal velocities are available for only a few compounds. No data concerning the specificity of the yeast enzyme have been previously reported. The present studies were confined to a limited number of compounds that differ from the natural substrates in the following aspects: position and number of phosphate groups, the presence and stereochemical position of certain of the hydroxyl groups, and the conformation of the molecules.

Where possible, Michaelis constants and maximal velocities were determined, but when the reactions were too slow for an evaluation of these parameters, inhibitor constants were usually determined and limits placed on the velocities relative to fructose-di-P. A summary of the data obtained is presented in Table II. Some of the data for the muscle enzyme may be compared with previously reported values. The relative rate with fructose-1-P (rate fructose-1-P/rate fructose-di-P = 0.013) is in satisfactory agreement with the relative rate of 0.02 reported by Dreschel et al. (10), especially since somewhat different assays were employed. However, the single rate measurement reported by Tung et al. (16) for fructose-1-P as well as sorbose-1-P and sorbose-1,6-diphosphate are all about 10 times higher than the maximal velocities reported here. This discrepancy has not been resolved. The evaluation of maximal velocities and Michaelis constants for dihydroxyacetone-P and glyceraldehyde-3-P is difficult due to the previously mentioned substrate inhibition. The same maximal velocities were obtained by independent variation of dihydroxyacetone-P and glyceraldehyde-3-P concentrations, and by maintaining the ratio constant of dihydroxyacetone-P to glyceraldehyde-3-P and varying the total triose phosphate concentration in the systems. The substrate inhibition caused at higher concentrations of dihydroxyacetone-P or glyceraldehyde-3-P is not affected markedly by varying the ratio of the two substrates. It is also emphasized that the data for D-glyceraldehyde no doubt are composite values, since both isomers are substrates (16).

Inspection of the data shows at once that the yeast enzyme has stringent substrate specificity. The most favorable substrate analogue (sorbose-di-P) supported a rate about three orders of magnitude less than fructose-di-P. Sorbose-di-P behaved as a typical competitive inhibitor with a $K_i$ of the same order of magnitude as the $K_m$ of fructose-di-P. This fact suggests that the large discrepancy in catalytic rates is not caused by a failure of interaction of the compound with the enzyme but rather to the formation of a relatively inactive complex. The same general argument probably applies to the other compounds tested.

As might be expected muscle aldolase has considerably less rigorous structural requirements than the yeast enzyme. Even so, the removal of the phosphate group in C-6 as in fructose-1-P, or the change of configuration of C5 hydroxyl as in sorbose phosphates lowers the activity by one to two orders of magnitude.

The $K_m$ and $K_i$ values for sorbose-1-P are about one-tenth those for fructose-1-P in both the muscle and yeast systems. This suggestion of stronger binding may really be a reflection of different conformational forms of these substrates present in

![Fig. 3. Influence of pH and cofactor requirements on exchange activity of yeast aldolase. An alumina eluate, specific activity 56 (hydrazine assay), was used as a source of yeast aldolase. The stock enzyme solution was prepared in 0.01 M EDTA and 6.5 × 10⁻³ M phosphate, pH 7.0. Exchange reactions (see "Experimental Procedure") were performed as described in Fig. 2. In each case, enough yeast aldolase was added to provide a rate of 0.14 μmole of fructose-di-P cleaved per minute (hydrazine assay).](image-url)
solution, a point which will be considered in more detail in the discussion.

With both enzymes the dihydroxyacetone-P moiety seems a fairly rigid requirement. No detectable reaction was observed with either glucose 6-P, or free dihydroxyacetone in the presence of optimal concentrations of glyceraldehyde-3-P. The very slow reaction of acetol phosphate in both enzyme systems is a point which will be considered in more detail in the discussion. With both enzymes the dihydroxyacetone-P moiety seems a fairly rigid requirement. No detectable reaction was observed with either glucose 6-P, or free dihydroxyacetone in the presence of optimal concentrations of glyceraldehyde-3-P. The very slow reaction of acetol phosphate in both enzyme systems is a point which will be considered in more detail in the discussion.

Effect of Carboxypeptidase on Aldol Cleavage and Exchange Activities—Drechsler et al. (10, 17) have shown that carboxypeptidase treatment of muscle aldolase yields a product with approximately 7% the aldol cleavage activity of the untreated control. The effect of carboxypeptidase on both the aldol cleavage and exchange activities of muscle and yeast aldolases have been investigated. The results of a typical experiment are presented in Table III. In confirmation of Drechsler et al. carboxypeptidase treatment of muscle aldolase yielded a product with about 6% the original aldol cleavage activity. In contrast, the exchange activity is almost completely destroyed; there is only 0.1% residual exchange activity after carboxypeptidase treatment. Thus, the two activities have been dissociated by carboxypeptidase treatment of muscle aldolase. In confirmation of Drechsler et al. carboxypeptidase treatment of muscle aldolase yielded a product with about 6% the original aldol cleavage activity. In contrast, the exchange activity is almost completely destroyed; there is only 0.1% residual exchange activity after carboxypeptidase treatment. Thus, the two activities have been dissociated by carboxypeptidase treatment of muscle aldolase.
bovinepeptidase treatment in this enzyme. Other experiments have been performed with molar (muscle aldolase to carboxypeptidase) ratios varying from 43 to 10,000, with essentially similar results. At a molar ratio of 2,000 the dissociation of activities was complete in 3 hours, but at a ratio of 50, or less, the dissociation of activities was too rapid to measure in 5 minutes. A more detailed study (18) of the kinetics of the action of carboxypeptidase on muscle aldolase shows that the dissociation of activities occurs subsequent to the removal of the tyrosine residues, during the removal of two alanine residues. Neither the aldol cleavage or exchange activity is altered by incubation of yeast aldolase with carboxypeptidase. At yeast aldolase to carboxypeptidase ratios ranging from 8 to 114, there is no significant change in either activity relative to the control.

**DISCUSSION**

The data in a previous report have indicated significant differences in the molecular properties of yeast and muscle aldolase (2). The present study allows a further comparison of the catalytic properties of these enzymes. Since both catalyze the hydrogen exchange as well as the reversible aldol cleavage, a statement of the enzyme-catalyzed reactions must include both activities. Two general formulations are visualized. The first, illustrated by Equation 1, places the exchange reaction as an obligatory step in the over-all reaction.

1. CH₂OH-CO-CH₂OPO₃H₂ + aldolase ⇄
   aldolase-CH₂OH-CO-CH₂OPO₃H₂ + H⁺

2. H⁺ + glyceraldehyde-3-P
   + aldolase-CH₂OH-CO-CH₂OPO₃H₂ ⇄
   aldolase + fructose-di-P

The second formulation, illustrated by Equation 2, does not place the hydrogen exchange as an obligatory step in the over-all reaction and hence does not demand any relationship of the rates 2b and 2ac.

a. CH₂OH-CO-CH₂OPO₃H₂ + aldolase ⇄
   aldolase-CH₂OH-CO-CH₂OPO₃H₂

b. Aldolase-CH₂OH-CO-CH₂OPO₃H₂ + H⁺ ⇄
   aldolase-CH⁺OH-CO-CH₂OPO₃H₂ + H⁺

c. Aldolase-CH₂OH-CO-CH₂OPO₃H₂ + glyceraldehyde-3-P ⇄ fructose-di-P | aldolase

In this instance, the aldolase-dihydroxyacetone-P complex may result in the polarization of the specific C-π bond so that Exchange 2b or condensation with the aldehyde moiety 2c may occur. Both Reactions 1 and 2 must conform to the known steric configurations of the products. Rose (19) has shown that the position of tritium in the labeled dihydroxyacetone-P is sterically equivalent to that of the carbon in the aldol adduct; therefore, the labeling process may proceed with retention of configuration or with double inversion. This constraint applies to both yeast and muscle aldolase.

Rose and Rieder (7) have shown that the rate of exchange was greater than the rate of aldol cleavage, hence the data were in apparent accord with formulation (1). In a more rigorous test of this case, the present studies have compared the rate of exchange with the rate of aldol synthesis of fructose-di-P. Even in this instance the rate of exchange under optimal conditions is equal to or greater than the rate of fructose-di-P synthesis. However, under other conditions, the rate of exchange for both muscle and yeast aldolase does not conform to the limiting condition of rates imposed by the formulation in Equation 1. The exchange rate is slower than the aldol cleavage rate for the yeast enzyme, at pH values greater than 7. The evidence is not so compelling for the muscle enzyme; nevertheless, there is a significant discrepancy at pH 6.0 to 6.5. In addition, for the muscle enzyme, there is a dramatic dissociation of the exchange and over-all reaction rates by carboxypeptidase treatment. If it is assumed that the basic mechanism of the carboxypeptidase-treated and the native aldolases are similar, this is decisive evidence against all mechanisms that require correspondence of the two activities. Equation 1 as written, therefore, may be excluded for both yeast and muscle aldolase.

Equations 2 are consistent with all available data. It is emphasized that the mechanisms (Equations 1a and 2) presume a preferred order of addition of substrates; in part this is consistent with the considerably slower rate of exchange of glyceraldehyde-3-P into fructose-di-P as compared to dihydroxyacetone-P into fructose-di-P. However, the possibility that the presence of glyceraldehyde-3-P could affect the velocity constants of the partial reactions is not excluded. Equation 3 illustrates one of the variants of Equation 1 that are still formally allowed by the data, provided only that 3b is fast compared with 3d.

a. Aldolase + glyceraldehyde-3-P
   + CH₂OH-CO-CH₂OPO₃H₂ ⇄
   glyceraldehyde-3-P . aldolase-CH₂OH-CO-CH₂OPO₃H₂

b. Aldolase-CH₂OH-CO-CH₂OPO₃H₂
   + H⁺ + glyceraldehyde-3-P . aldolase-CH₂OH-CO-CH₂OPO₃H₂
   ⇄ glyceraldehyde-3-P . aldolase-CH₂OH-CO-CH₂OPO₃H₂
   + H⁺

c. H⁺ +
   glyceraldehyde-3-P . aldolase-CH₂OH-CO-CH₂OPO₃H₂
   ⇄ glyceraldehyde-3-P . aldolase-CH₂OH-CO-CH₂OPO₃H₂

Apart from the question of whether glyceraldehyde-3-P may participate functionally in the catalytic process, the type of chemical interactions which would be required to form an intermediate as predicted in Equations 1 or 3 are considered unattractive for a catalytic process, but the predictions of such
mechanisms, eis stoichiometric production of protons on interaction of dihydroxyacetone-P with aldolases, and formation of a stable dihydroxyacetone-P aldolase compound involving the appropriate carbon atom of dihydroxyacetone-P seem amenable to experimental testing. The type of intermediate visualized in Equation 2 is favored for both yeast and muscle aldolase. The specific chemical nature of the interaction between the aldolases and substrates remains, of course, undefined, but the data obtained in this work emphasize differences rather than similarities in the two enzymes. In addition to significant differences in the energy of activation of the two processes, the pH profile, the specificity pattern, and the susceptibility to the action of carboxypeptidase are distinctive.

The pH profiles of reactions catalyzed by yeast aldolase suggest the participation of groups with pK values 5.5 to 6.0 and 8.0 to 8.5 in the over-all process, and groups with pK values of 5.0 to 5.5 and 0.5 to 7.0 in the exchange reaction. On the other hand, the muscle enzyme appears to have no groups dissociating in the region 5 to 9 which participate decisively in the catalytic process, only one influential group with a pK of approximately 5 to 5.5 appears possible for the over-all reaction and one at 6.5 to 7.0 for the exchange reaction. The dissimilarities in the pH profiles between the exchange and over-all reactions for both enzyme systems could conceivably be a result of one or more of the following conditions: the action of different groups in the two processes; direct participation of glyceraldehyde-3-P in the catalytic process; the shielding of an inhibitory group by glyceraldehyde-3-P; the alteration of the ionization of a group by the presence of glyceraldehyde-3-P. The last three possibilities emphasize alteration by glyceraldehyde-3-P of the active site.

The specificity patterns of both enzymes suggest a prominent role of both phosphate groups in the enzyme-substrate interaction. Whether this is simply a role of phosphate in orienting the substrates, or whether the phosphate of the dihydroxyacetone-P moiety may be used purposefully in the catalytic process is undecided. A ketone or incipient ketone on C-2, and an hydroxyl group on C-3 appears to be a requirement for both enzymes.

The specificity pattern of the two enzymes differs considerably; the yeast aldolase exhibits activities with the examined substrate analogues at least two orders of magnitude lower than the corresponding rates with muscle aldolase. Nevertheless, the inhibitor constants indicate these compounds react with the yeast enzyme. Jagannathan et al. (12) have reported that the A. niger aldolase does not catalyze the aldol cleavage of fructose-1-P, and hence this enzyme may show the kind of stringent specificity shown by the yeast enzyme.

Perhaps the most significant deterrent to the analysis and comparison of the specificities of the aldolases is the lack of information concerning the conformation of the substrate molecules preferred by the enzymes. The ketobenose diposphates can exist in the α- and β-furanose and linear forms, while the ketobenose-1-P can exist in the α- and β-furanose and pyranose, as well as the linear forms. The possible relationship of reaction mechanism to the substrate conformation has been discussed elsewhere (1).

The differences in stability to heat and especially to carboxypeptidase, although not unexpected in themselves, may provide a means for the further delineation of structural differences between the enzymes. The dissociation of the aldol cleavage and exchange activities of muscle aldolase by carboxypeptidase, and especially the role of tyrosine in the enzyme will be discussed subsequently (18).

The results presented suggest significant differences, not only in the over-all structure but at the catalytically active site of these two aldolases. Some of these may be reflections of general dissimilarities between Group I and Group II aldolases. In addition to their intrinsic interest, these facts are relevant to an evaluation of the processes involved in phylogeny (20).

**SUMMARY**

1. The catalytic properties of the aldolase from yeast and muscle have been compared. The data emphasize significant differences in the two enzymes, probably in the catalytically active-center.
2. Arrhenius plots of the velocity constants are slightly concave downwards for both enzymes. The energy of activation of the muscle enzyme is considerably greater than for the yeast enzyme studied under comparable conditions. Yeast aldolase is considerably more heat-labile than the muscle enzyme.
3. The pH profile for the hydrogen exchange and over-all reactions of both enzymes differ. The pH optimum (yeast aldolase) is 7.0 to 7.5 for aldol synthesis. The hydrogen exchange rate exceeds the aldol cleavage rate at pH values below 6.5; at higher pH values, the reverse is true. For muscle aldolase, on the other hand, the aldol synthetic rate is pH-independent from pH 6.5 to 9.0, and the exchange activity is relatively independent of pH from 7 to 9. The exchange rate is greater than the aldol synthetic rate at pH values above 7.
4. The yeast aldolase-catalyzed hydrogen exchange reaction is stimulated specifically by potassium ions (as is the over-all reaction). The pH optimum of the exchange reaction is similar in the presence or absence of potassium ions. The activity of muscle aldolase is not influenced by potassium ions.
5. The action of muscle and yeast aldolase on several substrate analogues have been defined in terms of maximal velocities and Michaelis and inhibitor constants. The yeast enzyme has stringent structural requirements for effective catalysis. Even though the yeast enzyme is more active than the muscle enzyme with respect to fructose-1,6-diphosphate synthesis and cleavage, it is at least two orders of magnitude less active toward various substrate analogues tested.
6. Carboxypeptidase treatment of muscle aldolase decreases the aldol cleavage activity to 5 to 7% and the hydrogen exchange activity to 0.1% of the unincubated control. Similar treatment of the yeast enzyme has no effect on the exchange or aldol cleavage activity.
7. All mechanisms which require the rate of the hydrogen exchange reaction with dihydroxyacetone phosphate to be as great as the rate of the over-all reaction are eliminated by the data.

**REFERENCES**


Comparative Properties of Yeast and Muscle Aldolase
Oliver C. Richards and William J. Rutter


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