BBA 66708

FUNCTIONAL PROPERTIES OF ORNITHINE–KETOACID AMINOTRANSFERASE FROM RAT LIVER

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(Received May 4th, 1972)

SUMMARY

Functional properties of rat liver ornithine–ketoacid aminotransferase (L-ornithine:2-oxoacid aminotransferase, EC 2.6.1.13) were examined by two procedures: (A) The overall enzyme reaction was separated into its component half-reactions and the capacity of the enzyme to react with each of its substrates individually was investigated under different conditions. (B) The enzyme was exposed to pyridoxal phosphate, and resultant effects on enzyme activity and pyridoxal phosphate binding were analysed.

The following properties of the enzyme were disclosed by these studies.

1. The reaction of the enzyme with ornithine alone is maximal between pH 8 and 10 whereas its reaction with α-ketoglutarate is maximal between pH 6 and 8. The pH optimum for the overall reaction (pH 8.15) coincides with the point of intersection of the curves for the half reactions.

2. The pyridoxamine form of the enzyme, produced by the reaction of the pyridoxal enzyme with ornithine, reverts to the pyridoxal form in the absence of the second substrate. The rate of this reversion is lower in Tris than in phosphate buffer, although the catalytic activity of the enzyme is identical in both buffers. The lability of pyridoxamine at the active site is a consequence of its attachment to the apoenzyme, since free pyridoxamine phosphate does not release its amino group under the same conditions. It is suggested that this destabilizing effect of the apoenzyme is important to its role in the catalysis of the amino-transfer reaction.

3. Exposure of the enzyme to excess pyridoxal phosphate is accompanied by the partial inactivation of the enzyme. This partial inactivation coincides with the binding of a small amount of pyridoxal phosphate to the enzyme. The further binding of pyridoxal phosphate produces no additional inactivation. Since pyridoxal phosphate reacts specifically with lysine residues in proteins the results suggest that certain of the lysine residues are involved in maintaining the enzyme at full catalytic capacity while others are functionally inert.

INTRODUCTION

Ornithine–ketoacid aminotransferase (L-ornithine:2-oxoacid aminotransferase, EC 2.6.1.13) of rat liver is a highly adaptive enzyme that shows a variety of responses to dietary and hormonal stimuli[1]. The enzyme has been crystallized, and several of its physical and chemical properties have been described[2,3].

Ornithine–ketoacid aminotransferase catalyses the reaction:

L-Ornithine + α-ketoglutarate ⇋ glutamic semialdehyde + glutamate

This reaction is rendered almost irreversible by the spontaneous cyclization of glutamic semialdehyde to form Δ'-pyrroline-5-carboxylate[4–6]. Tsai and Jenkins[7] have shown that the enzyme reaction follows ping-pong kinetics[8]. This means that the overall amino-transfer reaction is the sum of the following half-reactions.

L-Ornithine + pyridoxal–enzyme ⇋ glutamic semialdehyde + pyridoxamine–enzyme (1)

α-Ketoglutarate + pyridoxamine–enzyme ⇌ glutamate + pyridoxal–enzyme (2)

The present communication describes functional characteristics of the ornithine–ketoacid aminotransferase molecule as revealed through two types of studies. In the first of these, experiments were designed to test the capacity of the enzyme to participate in each half-reaction under different conditions. In the second, functional consequences of exposing the enzyme to excess pyridoxal phosphate (pyridoxal-*P*) were investigated.

METHODS

Preparation of enzyme

Ornithine–ketoacid aminotransferase was purified as described previously[3]. Each purified preparation was stored at −85 °C.

Rapid equilibration procedure

In several of the experiments to be described it was necessary to change the molecular environment of a given enzyme preparation as rapidly as possible. This was accomplished by centrifuging the enzyme through Sephadex G-25 equilibrated with the appropriate medium.

The procedure was as follows. A slurry of Sephadex was poured into plastic disposable 10-ml syringe barrels which had previously been plugged with glass wool and placed in 50-ml cellulose nitrate tubes. The tops of the syringes were covered with Parafilm and the tube–syringe assemblies were centrifuged at 1000 rev./min for 5 min in an International PR-2. The liquid in the tubes was poured off and 2-ml aliquots of enzyme solution were pipetted into the syringes containing the packed Sephadex (which filled the barrels to within 2 cm of the tops), after which the assemblies were centrifuged for 2 min. The tubes then contained the equilibrated enzyme.

Pyridoxal phosphate determination

A solution of enzyme was mixed with an equal volume of 1 M $HClO_4$, and the denatured protein was removed by centrifugation. The pyridoxal-*P* content of the supernatant was determined by using phenylhydrazine[9].

Protein determination

Each sample for protein determination was mixed with 10 vol. of 10% (w/v) trichloroacetic acid. The resulting precipitates were isolated by centrifugation and analyzed by the procedure of Lowry *et al.*[10] with bovine serum albumin as the standard. The accuracy of this procedure was verified by estimating the protein content of ornithine–ketoacid aminotransferase solutions of known concentration. These were prepared from weighed samples of lyophilized ornithine–ketoacid aminotransferase.

Enzyme assay

Ornithine–ketoacid aminotransferase activity was assayed by a modification of earlier methods[11,12]. To 0.7 ml of a solution of 0.07 M L-ornithine·HCl in 0.1 M phosphate buffer (pH 8.15) was added 0.1 ml of enzyme solution. 0.1 ml of a solution of 1% (w/v) *o*-aminobenzaldehyde in 10% (v/v) ethanol was then added (after warming the solution to 40 °C to dissolve *o*-aminobenzaldehyde) followed by 0.1 ml of 0.25 M α-ketoglutarate in water (pH previously adjusted to 6.8). Each addition was accompanied by mixing. The final mixture was incubated at 37 °C for 10–30 min depending on the activity of the sample. 1 ml of 10% (w/v) trichloroacetic acid was then added and the denatured protein was removed by centrifugation. The absorbance at 440 nm was then determined. One unit of activity equals an A_{440} of 1.000 (1-cm light path) in 30 min under the assay conditions described above.

Assay of half-reactions

L-[U-^{14}C]ornithine·HCl (spec. act. 6.5 mCi/mmole, New England Nuclear), L-[U-^{14}C]glutamic acid (spec. act. 260 mCi/mmole, Amersham) or [U^{14}-C]α-ketoglutarate (spec. act. 17.1 mCi/mmole, Amersham) was mixed with its unlabeled counterpart in distilled water to give the desired molar concentrations and specific activities. These preparations either were used immediately or divided into 1-ml portions and stored at −85 °C. Such portions were thawed just prior to use and unused remnants were discarded.

To assay each half-reaction, approximately 2.5 mg of enzyme (exact amount in each sample determined by protein analysis) in either the pyridoxal or pyridoxamine form were mixed with 10 μmoles (except in Fig. 1, see Results) of the appropriate labeled substrate and 300 μmoles of phosphate buffer (potassium salt), pH 8.15 (except in the pH experiments) in a final volume of 3 ml. [The concentration of enzyme in this mixture was approximately 1000 times that present in the assay of catalytic activity, which means that the velocity of each half-reaction was elevated by a factor of approximately 2000. Preliminary experiments involving the measurement of absorbance changes in the enzyme solution at 412 and 330 nm (the absorbance maxima of the pyridoxal and pyridoxamine prosthetic groups, respectively[3]) after adding ornithine or α-ketoglutarate, verified that at this high enzyme concentration each half-reaction occurred at a sufficiently high velocity as to appear instantaneous under the experimental conditions used. In addition, as will be demonstrated below, the concentrations of substrates used in the forward half-reactions was 100 fold greater than that required to transform all the functional reactant–enzyme present into product–enzyme.] After the reaction mixture had been allowed to stand at 25 °C for a standard time of 1 min, (except for the data shown in Fig. 5, see Results) 1 ml of 1 M $HClO_4$ was added and the sample was centrifuged at 40 000 × g for 10 min. An aliquot of

the supernatant was then applied to the top of a 50 cm × 1.5 cm column of Dowex 50-X8, 100–200 mesh cation exchange resin, washed previously with 1 M HCl. The column was then eluted with 1 M HCl and 10-ml fractions were collected. When ornithine was the substrate (first half-reaction), a single 150-ml fraction was collected prior to the collection of the 10-ml fractions; pyrroline carboxylate was eluted after approximately 15 of the latter fractions. The location of the pyrroline carboxylate peak was determined in trial runs by the addition of *o*-aminobenzaldehyde to the fractions. After elution of the pyrroline carboxylate peak, 4 M HCl was added to the top of the column and the remainder of the radioactivity (unreacted ornithine) was removed in an additional 150 ml. When α-ketoglutarate was the substrate (second half-reaction), only 1 M HCl was used as the eluting agent, and the first peak of radioactivity (unreacted α-ketoglutarate) emerged from the column approximately between Fractions 2 and 13, while the second peak (glutamate) emerged approximately between Fractions 15 and 30. An aliquot of each fraction (1.0 ml for samples containing 1 M HCl, and 0.20 ml *plus* 0.8 ml of water for samples containing 4 M HCl) was placed in a scintillation vial, 20 ml of scintillation fluid [naphthalene, 10% (w/v), PPO, 0.5% (w/v) in dioxane] were added, and the samples were counted in a Beckman Liquid Scintillation Spectrometer. For each half-reaction, the proportion of the total counts eluted from each column that appeared in the reaction product was used to calculate the moles of product formed per mole of enzyme under the experimental conditions.

The accuracy of this procedure, and the purity and stability of the labeled ornithine and α-ketoglutarate were tested in the following manner. The enzyme was incubated at 37 °C in the presence of both substrates, one of which was taken from the labeled stocks prepared as described above. The reaction was stopped with 1 M $HClO_4$ and the precipitate was removed by centrifugation. An aliquot of the supernate was reacted with *o*-aminobenzaldehyde and the A_{440} was measured. The concentration of pyrroline carboxylate was calculated, using a millimolar ε_{440} of 2.71[6]. Another aliquot of the supernate was applied to an ion-exchange column and treated as described above; the elution procedure depended on the labeled substrate used. An exact correspondence was obtained between the concentration of product, as assayed colorimetrically, and that determined by radioactivity measurements using either substrate. Periodic checking revealed no deviation from this correspondence. The labeled substrates therefore were sufficiently pure as received, and storage under the conditions described above did not produce measurable degradation.

In order to study the second half-reaction the enzyme was converted to the pyridoxamine form by adding an excess of labeled ornithine to an enzyme preparation that had been treated with Sephadex G-25 to remove extraneous pyridoxal-*P*. The converted enzyme was treated again with Sephadex to remove pyrroline carboxylate and unreacted ornithine. Completeness of removal was verified by testing aliquots of the enzyme solution for residual radioactivity.

The enzyme was protected from light in all experiments in which it had to stand for extended periods. Additional procedures specific to each experiment are explained in the figure legends.

RESULTS

Effects of substrate concentration on half-reactions

As the concentration of substrates was raised, both forward half-reactions at first showed a similar positive response (Fig. 1), but more product was finally formed in the first half-reaction than in the second. In the reaction of glutamate with the pyridoxal enzyme (reverse of the second half-reaction) α-ketoglutarate did not appear in significant amounts until the concentration of glutamate was well beyond that

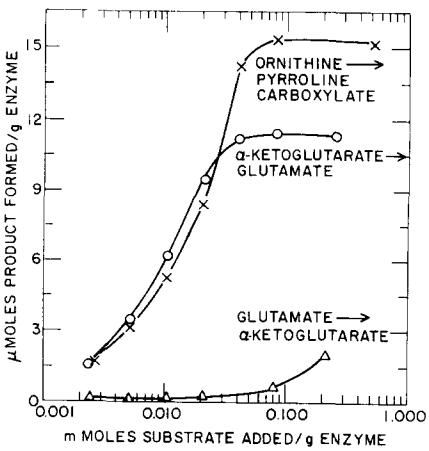


Fig. 1. Effects of substrate concentration on the ornithine–ketoacid aminotransferase half-reactions. ×, first half-reaction; ○, second half-reaction; △, reverse of second half-reaction. Each sample contained enzyme, varying amounts of substrate, as indicated along the abscissa, and 100 μmoles of phosphate buffer, pH 8.15. 30 min elapsed between the conversion of the enzyme to the pyridoxamine form and its use in the second half-reaction. The pyridoxal form of the enzyme was used in the reverse of the second half-reaction.

produced in the forward version of the second half-reaction. Both forward half-reactions reached a plateau when 0.02–0.04 mmole of substrate had been added per g of enzyme. Therefore, the concentration of substrates used in the other half-reaction experiments (4 mmoles per g enzyme) ensured that all the functional reactant–enzyme in a given reaction mixture was transformed into product–enzyme.

pH effects on half-reactions

Fig. 2 shows the effect of changes in pH on the capacity of ornithine–ketoacid aminotransferase to participate in each half-reaction. As the pH was lowered from 8 to 6 the first half-reaction declined and the second half-reaction remained maximal. Increasing the pH from 8 to 10 produced a decline in the second half-reaction, while the first half-reaction remained maximal. The point of intersection of the curves for the half-reactions coincides with the pH optimum for the overall reaction (pH 8.15). When the pyridoxal enzyme was kept at pH 6.25 or 9.75 for 30 min and was then returned to pH 8.15 neither the ability to perform in each half-reaction, nor the catalytic activity, was impaired.

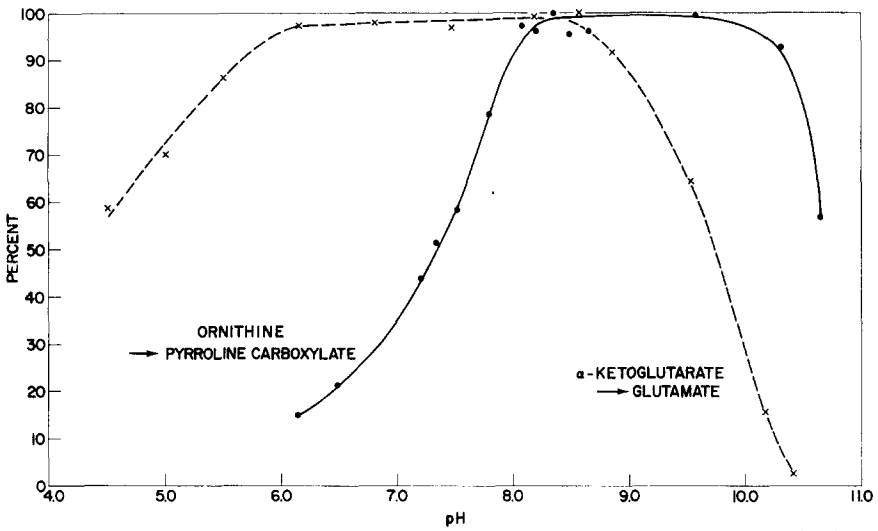


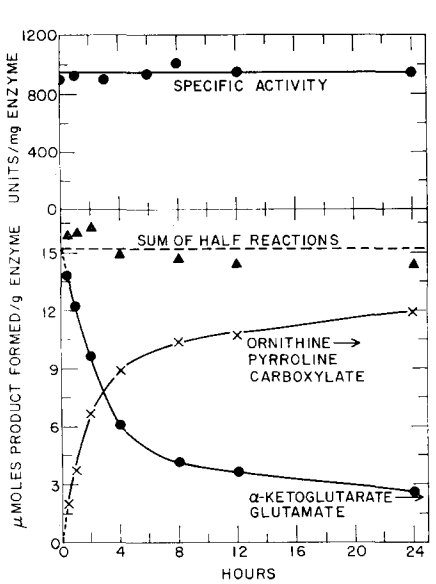
Fig. 2. Differential effects of pH on the ornithine–ketoacid aminotransferase half-reaction. ●, first half-reaction; ×, second half-reaction. Each sample contained enzyme, labeled substrate (ornithine for the first half-reaction or α-ketoglutarate for the second half-reaction), and 100 μmoles of the appropriate zwitterionic buffer[13] at the desired pH. The enzyme (in the pyridoxal form for the first half-reaction and in the pyridoxamine form for the second half-reaction) was added to each sample in 0.5 ml of 0.01 M phosphate buffer (pH 8.15). The substrate was added last and the pH of the sample was measured just before the addition of $HClO_4$. 30 min elapsed between the conversion of the enzyme to the pyridoxamine form and its use in the second half-reaction. The results are expressed as a percentage of the maximum value obtained for each half-reaction. For the first half-reaction this value was 15 μmoles of pyrroline carboxylate formed per g of enzyme; for the second half-reaction the value was 12 moles of glutamate formed per g of enzyme.

Reversion of pyridoxamine enzyme to pyridoxal form

On standing, the pyridoxamine enzyme lost its capacity to perform the second half-reaction, while simultaneously regaining an equivalent capacity to perform the first half-reaction (Fig. 3). This indicates that the pyridoxamine enzyme reverted to the pyridoxal form. The catalytic capacity of the enzyme remained constant during the reversion process. Extrapolation of the curve for the second half-reaction back to the ordinate indicates that the pyridoxamine enzyme could react with α-ketoglutarate as efficiently as the pyridoxal enzyme reacted with ornithine. The lower value for the second half-reaction obtained in Fig. 1, therefore, resulted from the partial reversion of the pyridoxamine enzyme to the pyridoxal form prior to assay. When enzyme that had reverted to the pyridoxal form was again converted to the pyridoxamine form, reversion occurred as before; a third such cycle produced the same results.

Partial inhibition of ornithine–ketoacid aminotransferase reversion

The rate at which the pyridoxamine enzyme reverted to the pyridoxal form, as indicated by changes in the half-reactions, was reduced when the medium contained Tris rather than phosphate buffer (Fig. 4, right side). The catalytic activity of the enzyme was the same in phosphate or Tris. The inhibitory effect of Tris on the reversion is also shown in Fig. 5. In this figure the A_{412} of the pyridoxal enzyme, at a concentration of 1 mg/ml in Tris or phosphate, is indicated by the heavy arrow at 0.115



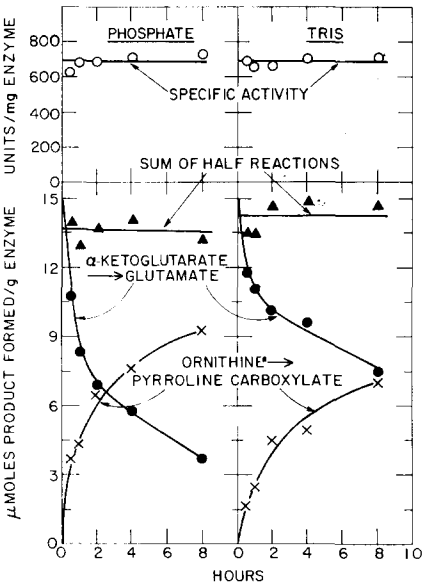


Fig. 3. Reversion of the pyridoxamine form of ornithine–ketoacid aminotransferase to the pyridoxal form. ×, first half-reaction; ●, (lower curve), second half-reaction; ▲, sum of half-reactions; ●, (upper curve), catalytic activity. The enzyme (in 0.1 M phosphate buffer, pH 8.15) was converted to the pyridoxamine form by the addition of ornithine at zero time, and freed of pyrroline carboxylate and unreacted ornithine after 1 min by centrifugation through Sephadex G-25 in the same buffer. The enzyme was then allowed to stand at 25 °C and aliquots were taken at intervals, beginning as soon as possible after zero time, for the measurement of catalytic activity and the ability to participate in each half-reaction.

Fig. 4. Effect of Tris on the reversion of the pyridoxamine form of ornithine–ketoacid aminotransferase to the pyridoxal form. Conditions are as described in Fig. 3, except that on the right side of Fig. 4 the enzyme was in 0.1 M Tris (pH 8.15) before conversion to the pyridoxamine enzyme, and remained in this buffer throughout the experiment.

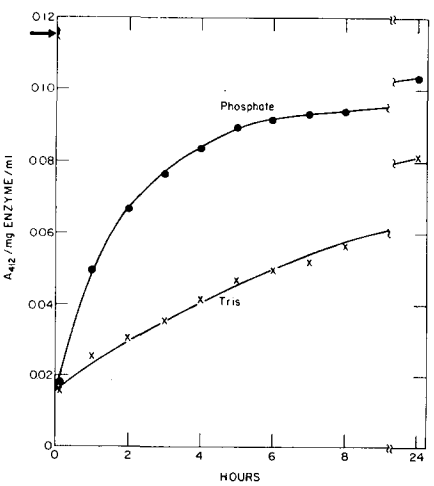


Fig. 5. Changes in A_{412} during reversion of the pyridoxamine form of ornithine–ketoacid aminotransferase to the pyridoxal form in phosphate or Tris. Conditions were as described in Fig. 4. The heavy arrow at 0.115 on the ordinate shows the A_{412} of the enzyme in phosphate or Tris prior to conversion to the pyridoxamine form. Absorbance readings were taken at the intervals shown in the figure.

on the ordinate. Converting the enzyme to the pyridoxamine form decreased the A_{412} to less than 0.02, owing to the lack of absorbance of pyridoxamine at 412 nm, after which the A_{412} spontaneously increased as the enzyme reverted. The rate of increase in the A_{412} was lower in Tris than in phosphate. No significant change in the rate of reversion was observed when nitrogen or oxygen was bubbled through a solution of the pyridoxamine enzyme in 0.1 M phosphate buffer pH 8.15 or when EDTA was present at a concentration of 1 mM. Under the same conditions used in the experiment shown in Fig. 5, free pyridoxamine phosphate (pyridoxamine-*P*) dissolved in phosphate buffer did not show an increase in A_{412} or a decrease in A_{330} (the absorbance maximum for pyridoxamine-*P*).

Enzyme inactivation by added pyridoxal-P

The addition of pyridoxal-*P* to a solution of ornithine–ketoacid aminotransferase in phosphate buffer resulted in partial inactivation of the enzyme (Fig. 6). The degree and rate of inactivation increased with increasing amounts of added pyridoxal-*P*, but pyridoxal-*P* additions could not bring the enzyme activity below approximately 30% of its original level. Increasing the pyridoxal-*P* level from 0.75 to 7.5 mmoles per g of enzyme only increased the rate at which this plateau at 30% activity was reached.

The inactivation produced by the addition of pyridoxal-*P* was accompanied by the binding of additional pyridoxal-*P* to the enzyme beyond that normally bound (Fig. 7). (In the absence of added pyridoxal-*P*, 18 μmoles of pyridoxal-*P* were bound per g enzyme.) The kinetics of binding and inactivation were dissimilar, with the

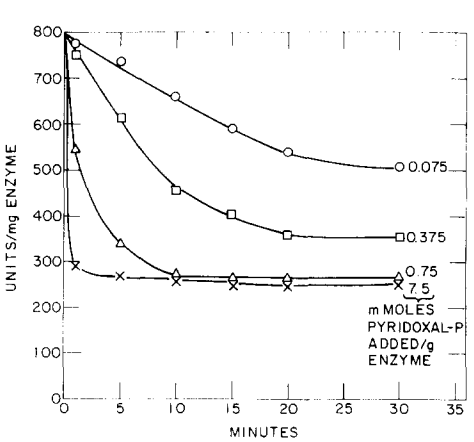


Fig. 6. Partial inactivation of ornithine–ketoacid aminotransferase by different levels of pyridoxal-*P*. ○, 0.075; □, 0.375; △, 0.75; ×, 7.5 mmoles of pyridoxal-*P* added per g of enzyme in 0.1 M phosphate buffer (pH 8.15). The mixture was incubated at 25 °C, and catalytic activity was measured at the intervals shown.

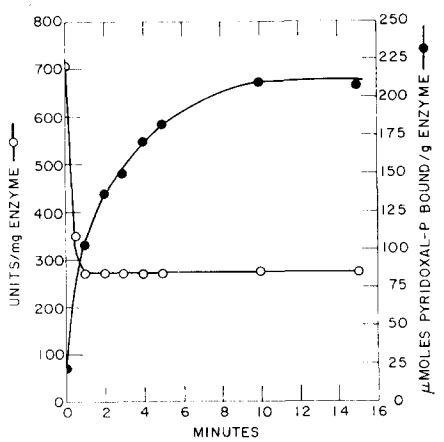


Fig. 7. Partial inactivation of ornithine–ketoacid aminotransferase and concomitant binding of pyridoxal-*P* to the enzyme after the addition of 7.5 mmoles of pyridoxal-*P* per g enzyme. Pyridoxal-*P* was added to the enzyme (in 0.1 M phosphate buffer, pH 8.15) at zero time, and the mixture was incubated at 25 °C. At the intervals shown a sample was taken for the assay of activity and another sample was freed of unbound pyridoxal-*P* by centrifugation through Sephadex G-25 in the same buffer. The pyridoxal-*P* remaining bound to the enzyme was then determined. ○—○, enzyme activity; ●—●, pyridoxal-*P* binding.

plateau in activity being reached within 2 min, whereas additional pyridoxal-*P* binding continued to occur for 10 min. Thus, no further inactivation occurred after approximately 80 μmoles of pyridoxal-*P* had become bound per g of enzyme, although further pyridoxal-*P* binding occurred to a level of approximately 210 μmoles of pyridoxal-*P* per g of enzyme.

DISCUSSION

The observation that the optimum pH range for the first half-reaction was substantially higher (pH 8–10) than that for the second half-reaction (pH 6–8) indicates that the ionic properties of the reactive sites on the enzyme for ornithine and α-ketoglutarate are dissimilar, that for ornithine being relatively acidic and that for α-ketoglutarate, being relatively basic. The reactivity of these sites toward their respective substrates can be altered reversibly between pH 6 and 10, as indicated by the reversible changes in the capacity of the enzyme to participate in each half-reaction within this pH range. The point of overlap (pH 8.15) of the pH curves for the half-reactions represents the only pH at which the sites on the enzyme for ornithine and α-ketoglutarate retain the ionic characteristics necessary for fully productive interaction with both substrates. Consideration of both half-reactions together indicates that the overall functional integrity of the enzyme is retained between pH 6 and 10. On either side of this region both half-reactions decrease similarly, probably because of non-specific enzyme denaturation coupled with changes in the ionic properties of the substrates.

One of the determinants of the ionic dissimilarity in reactive sites may be the presence, at the active site of the pyridoxamine enzyme, of the amino group acquired from ornithine. It is likely, however, that additional ionic differences exist between the binding sites for ornithine and α-ketoglutarate in order to accomodate those ionic groups which differ between the two substrates but which do not enter directly into the aminotransferase reaction (*i.e.* the α-amino group of ornithine and the γ-carboxyl group of α-ketoglutarate).

The data in Fig. 2 (as is the case with the other half-reaction experiments included in this report) describe conditions at equilibrium—not during the approach to equilibrium—and indicate, therefore, the ability of pH changes to alter the proportion of the total amount of enzyme present that can interact with the substrate regardless of the duration of exposure. Measurement of the rates of each half-reaction (*i.e.* the approach to equilibrium), using rapid assay techniques, would probably yield more detailed information regarding the ionic properties of the substrate-binding sites. Although the enzyme can react to completion with each substrate within the appropriate pH range, the rate at which equilibrium is attained is probably not constant throughout each range. Such differences would reflect subtle changes in the ionic properties of the sites, which might affect the efficiency of the enzyme–substrate interaction, whereas the data in Fig. 2 describe the limiting conditions within which these interactions can occur.

In the experiments shown in Fig. 1 the lower plateau for the second half-reaction indicates that the pyridoxamine enzyme is less stable than the pyridoxal form. This instability is characterized by the reversion of the pyridoxamine enzyme to the pyridoxal form (Fig. 3), with the loss of the amino group from a bound pyridox-

amine prosthetic group. The contrasting stability of free pyridoxamine-*P* under similar conditions indicates that in the pyridoxamine prosthetic group the binding of the substrate-donated amino group is destabilized by the presence of the apoenzyme. The destabilization may result in part from the competetive tendency of the Schiff-base linkage to be reestablished between the prosthetic group and the ε-amino group of a lysine residue on the apoenzyme[14]. Since such destabilization would facilitate the transfer of amino groups to the second substrate it is probably an important aspect of the role of the apoenzyme in the catalysis of the amino-transfer reaction.

A complete explanation of the mechanism of reversion must await the determination of the tertiary structure of ornithine–ketoacid aminotransferase, since only then can the influence of the apoenzyme configuration on the strength of the bond between the substrate-donated amino group and the prosthetic group be determined. While the manifestation of reversion is fortuitous in its revelation of a hitherto unknown facet of enzyme-catalyzed transamination, the reason for this manifestation under the experimental conditions used is not yet clear.

The observation that, although no α-ketoglutarate is present, the enzyme can undergo repeated reversions indicates that the pyridoxamine-*P* at the active site releases amino groups to an alternative acceptor in the medium. This acceptor is approximately 5 orders of magnitude less efficient than α-ketoglutarate, indicating a relatively poor affinity for the active site. Since the loss of the amino group from the pyridoxamine enzyme is an oxidative process, the most likely candidate for this amino acceptor is dissolved molecular oxygen. Attempts to demonstrate the requirement for molecular oxygen during reversion have thus far been unsuccessful, however, because it has not yet been possible to decrease the concentration of oxygen in the medium to a limiting level in the face of the various required experimental manipulations.

A similar degree of difficulty is encountered in obtaining reliable measurements of the minute quantity of ammonia generated during the reversion, and render this approach not feasible at present. Indirect evidence for the release of amino groups from the pyridoxamine enzyme is provided both by the phenomenon of repeated reversion described above and by the observation that the reversion is retarded in the presence of Tris. It is suggested that the latter effect arises from the contribution of amino groups to the medium by Tris, with a consequent shift in the equilibrium for the two forms of the enzyme toward the pyridoxamine form. The possibility that Tris retards reversion by interacting directly with the enzyme molecule, thereby changing its functional properties, is rendered unlikely by the observation that Tris has no effect on the catalytic capacity of the enzyme (Fig. 4). The additional possibility that Tris retards reversion by chelating trace metals that might catalyze the interaction with molecular oxygen is also unlikely in view of the failure of millimolar EDTA to retard reversion. In any event, the identification of the amino acceptor during reversion is of secondary importance in comparison to the analysis of those features of the ornithine–ketoacid aminotransferase molecule that are responsible for rendering pyridoxamine at the active site less stable than free pyridoxamine.

The data for the inactivation of ornithine–ketoacid aminotransferase by pyridoxal-*P* indicates that the interaction of the enzyme with a certain level of excess pyridoxal-*P* causes a partial reduction in the efficiency with which the enzyme interacts with its substrates. Once the critical pyridoxal-*P*–ornithine–ketoacid aminotransferase interaction has occurred the further addition of pyridoxal-*P* is ineffective.

Correlation of pyridoxal-*P* binding and enzyme inactivation (Fig. 7) shows that approximately 1/3 of the sites on ornithine–ketoacid aminotransferase that bind excess pyridoxal-*P* are important for maximal enzyme efficiency, though not essential for enzymatic function. The remaining pyridoxal-*P* binding sites, however, appear to have no functional significance.

Studies on the binding of excess pyridoxal-*P* to several other enzymes[15–23] indicate that such binding invariably involves Schiff base formation between pyridoxal-*P* and the ε-amino groups of the lysine residues in the protein; it is now used as a specific reagent for such residues[18]. It is likely, therefore that all binding sites for pyridoxal-*P* on the ornithine–ketoacid aminotransferase molecule contain lysine residues, and certain of these are involved in maintaining the enzyme at maximal catalytic efficiency while the remainder are functionally inert.

ADDENDUM

The kinetic studies of Südi and Dénes[24] also suggest that ornithine-ketoacid aminotransferase has different binding sites for ornithine and α-ketoglutarate.

ACKNOWLEDGEMENT

Excellent technical assistance was provided by Miss Aldona Prapuolenis. This work was supported by the U.S. Atomic Energy Commission.

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