Kinetic and Equilibrium Studies on the Activation of Escherichia coli K12 Tryptophanase by Pyridoxal 5'-Phosphate and Monovalent Cations

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SUMMARY

An improved purification of Escherichia coli K12 tryptophanase is presented. It is shown that the apoenzyme crystals, oxidized by exposure to air, can be reactivated by treatment with a reducing agent. The titration of sulfhydryl groups shows that four -SH groups are exposed and two are masked per protomer. The influence of two effectors, monovalent cations and the coenzyme pyridoxal 5'-phosphate, on the reactivity of -SH groups and the enzymatic activity was investigated. The -SH groups react more slowly in holo- than in apoenzyme in the presence of potassium ions. If these ions are replaced by sodium ions, the reactivity becomes the same. Potassium and ammonium ions, both activators, give sigmoidal activation curves. The sodium ion is a Michaelian inhibitor of potassium activation. The binding of pyridoxal 5'-phosphate was examined by kinetics and at equilibrium. The kinetics are shown to be very slow; the rate constants of the forward and reverse reactions have been measured. The binding equilibrium, examined with 3H-labeled pyridoxal 5'-phosphate, gives one site per protomer with a KD value of (3.2 ± 0.8) × 10^{-7} M. The KD for pyridoxal-P was determined by activity measurements. The binding equilibrium is attained after several hours, giving a value of 4.2 × 10^{-7} M, being nearly identical with the dissociation constant and 5 times smaller than previously reported.

It has been known for a long time that pyridoxal-P and certain monovalent cations are able to activate tryptophanase of Escherichia coli (a tetramer of 4×55,000 g, carrying 4 molecules of pyridoxal-P). As early as 1947, Wood et al. (1) demonstrated that apotryptophanase was active only in the presence of pyridoxal-P and potassium ions. K12 tryptophanase becomes the same. Potassium and ammonium ions, both activators, give sigmoidal activation curves. The sodium ion is a Michaelian inhibitor of potassium activation. The binding of pyridoxal 5'-phosphate was examined by kinetics and at equilibrium. The kinetics are shown to be very slow; the rate constants of the forward and reverse reactions have been measured. The binding equilibrium, examined with 3H-labeled pyridoxal 5'-phosphate, gives one site per protomer with a KD value of (3.2 ± 0.8) × 10^{-7} M. The KD for pyridoxal-P was determined by activity measurements. The binding equilibrium is attained after several hours, giving a value of 4.2 × 10^{-7} M, being nearly identical with the dissociation constant and 5 times smaller than previously reported.

MATERIALS AND METHODS

Tryptophanase Preparation

Step 1: Growth of Cells—Escherichia coli K12, wild type 3000, was induced for tryptophanase as previously described by London and Goldberg (10), except for the following modifications. The growth took place in a Biofermentor which contained 18 liters of culture medium inoculated with 300 ml of cells in the resting phase. The bacteria, grown under vigorous aeration at 37°, were harvested when the optical density at 600 nm had reached...
4.0 to 4.5 (after about 6 hours). The bacteria could be stored for months at −20° without any loss of tryptophanase activity.

**Step 2: Purification of Tryptophanase**—The buffer used throughout the purification was 0.1 M potassium phosphate buffer, pH 7.8, containing 5 × 10⁻⁴ M pyridoxal-P (Sigma), 10⁻³ M β-mercaptoethanol, and 2 × 10⁻³ M EDTA. The purification was performed according to the method of Newton et al. (6), with one critical step before the crystallization. This step consists of a zonal centrifugation. The active precipitate from the ammonium sulfate fractionation was dissolved and dialyzed against the buffer containing 2% ammonium sulfate as a stabilizing agent. The dialysate was then layered on a 10 to 30% sucrose gradient in the same buffer and centrifuged for 22 hours at 45,000 rpm and 20° in a zonal rotor, as described by London and Goldberg (10). The fractions retained for further purification were pooled and precipitated with 70% ammonium sulfate.

**Step 3: Crystallization of Apotryptophanase**—The whole procedure was performed, unless otherwise stated, at 4° in 0.1 M potassium phosphate buffer, pH 7.0, containing 10⁻² M β-mercaptoethanol and 2 × 10⁻³ M EDTA. All the solutions of ammonium sulfate were prepared in this buffer and the pH was adjusted to 7.0 with ammonium hydroxide. The precipitate from the zonal centrifugation step was collected by centrifugation and redisolved in 0.8 M ammonium sulfate to a final concentration of about 20 mg/ml. The solution was then treated with 10⁻² M DL-penicillamine for 15 min at 37° to remove pyridoxal-P from the holotryptophanase. After precipitation with ammonium sulfate and redissolution in 1.2 M ammonium sulfate, the procedure was repeated to assure a complete resolution of the holoenzyme. The apoenzyme thus obtained was precipitated, centrifuged, and redisolved in 1.2 M ammonium sulfate to a final concentration of about 10 mg of apotryptophanase per ml. After 2 hours of dialysis against 1.8 M ammonium sulfate, the solution was inoculated with a small amount of crystals from a previous preparation. The dialysis was continued against a gradient of ammonium sulfate to bring the concentration from 1.8 to 2.3 M overnight. For the second crystallization, the crystals were centrifuged and resuspended in 1.2 M ammonium sulfate. Most of the crystals were redisolved, but the solution remained opalescent. The recrystallization was performed as the first one, but without inoculation. The needle-like crystals so formed were collected by centrifugation and resuspended in 2.5 M ammonium sulfate. The ammonium sulfate was overdosed 1.0 M potassium phosphate buffer, pH 7.0, containing 10⁻² M β-mercaptoethanol and 2 × 10⁻³ M EDTA. The crystals were sealed under nitrogen and stored at 4°. The tryptophanase kept in this way is very stable, but once exposed to air it rapidly loses its activity.

**Reactivation of Apeoenzyme**

The enzyme, when inactivated by oxidation, could be reactivated in the following way. The required amount of crystals was centrifuged, dissolved in 0.1 M potassium phosphate buffer, pH 8.0, containing 5% ammonium sulfate and 2 × 10⁻³ M dithiothreitol, and incubated 1 hour at 50°. To remove ammonium sulfate and dithiothreitol the enzyme was filtered on a Sephadex G-25 column equilibrated with 0.1 M potassium phosphate buffer, pH 7.8, containing 2 × 10⁻³ M β-mercaptoethanol and 2 × 10⁻³ M EDTA. The holoenzyme was reconstituted by a 1-hour incubation of apotryptophanase in Buffer A containing 10⁻³ M pyridoxal-P at 37°.

**Characterization of Oxidized Apoenzyme**

S-Alkylation of Tryptophanase

The reaction was stopped by addition of 10⁻³ M iodoacetate in the presence of 1% sodium dodecyl sulfate. After dialysis against 0.02 M Tris-HCl buffer, pH 8.1, containing 0.1% sodium dodecyl sulfate, the enzyme was layered on a polyacrylamide gel in the presence of sodium dodecyl sulfate. The electrophoresis was performed according to the method of Shapiro et al. (15).
Analytical Centrifugation—The centrifugations were performed in a Centriscan 75 (M.S.E. England) using the ultraviolet absorption scanning system equipped with a 280 nm interference filter. Protein solutions (about 0.4 mg/ml in 0.1 M Tricine (N-tris(hydroxymethyl)methylglycine)-KOH buffer, pH 7.8, and 0.1 M KCl) were centrifuged in 20-mm thick single sector cells at 35,000 rpm and 4°. Resolution of the dimer and tetramer was achieved 70 to 80 min after full speed had been reached.

Acetylation of Bovine Serum Albumin

Because of the high affinity of bovine serum albumin for pyridoxal P (the dissociation constant is about $10^{-8}$ M (16)), we have taken the precaution of acetylating the bovine serum albumin used in all reconstitution experiments. The acetylation was performed as described by Oppenheimer et al. (17).

RESULTS

Purification of Tryptophanase—After the final crystallization the specific activity of S-alkyl-L-cysteine lyase was 17.5 e.u. per mg. The tryptophanase specific activity was 24 e.u. per mg, compared with 24 to 27 e.u. per mg reported by Watanabe and Snell (18).

Reactivation of Enzyme Stock Solutions—As reported earlier (14) the reduction state of certain cysteine residues is critical for the enzyme activity. Upon exposure to air, the tryptophanase crystals rapidly lose their activity, about 10% remaining after 4 weeks; this effect can be reversed by treatment with reducing agents (9). For the storage of the apotryptophanase two solutions are possible: sealing the enzyme under nitrogen or vigorous treatment with a reducing agent. Using this latter method a 90% inactivated preparation of apotryptophanase regained all of its initial activity upon treatment with diithiothreitol as described under "Materials and Methods."

Structural Properties of Oxidized Enzyme—As reported by London and Goldberg (10), the apoenzyme undergoes a reversible dissociation to dimers at low temperature. We observed that this cryosensitivity was not reproducible in old tryptophanase preparations. Ultracentrifugal experiments showed that the freshly purified apoenzyme sedimented at 4° as a dimer whereas an old preparation of apoenzyme, having lost 90% of its initial enzymatic activity, sedimented as the tetramer. The inactivated apoenzyme completely regained its cryosensitivity after reactivation with dithiothreitol.

One possible explanation for this phenomenon is that the oxidation results in a disulfide linkage between the two dimers. This possibility was tested by studying the electrophoretic behavior of the oxidized preparation under denaturing conditions. The oxidized enzyme migrated as a monomer (MW 55,000). A parallel experiment with rabbit immunoglobulin G indicated under saturating conditions of the other factor, the pyridoxal P.

The activation by potassium and ammonium ions both gave sigmoidal curves as a function of the added cation (Fig. 2A) with a Hill coefficient of 2.7 for the potassium and 1.5 for the ammonium ion (inset, Fig. 2A). At higher potassium concentrations, however, a slight inactivation could be observed (about 20% at 0.4 M K+). The sigmoidal shape of the curves might indicate a concerted transition in the tetramer from an inactive form T not fixing the cation, to an active form R with affinity for the same ion. In this optic it seemed interesting to examine

![Fig. 1. Rate and extent of reaction of —SH groups. A, titration in potassium phosphate. The reaction mixture contained 0.55 mg per ml of apotryptophanase (Apo-E) or holotryptophanase (Holo-E). The titration was performed in 0.1 M potassium phosphate buffer, pH 7.5. At the time indicated by the arrow an equal volume of 9 m guanidinium-HCl containing 2 × 10^{-4} M 5,5'-dithiobis(2-nitrobenzoic acid) was added. B, effect of the cations. The reaction mixture contained 1.1 mg per ml of protein. The titration was made in 0.1 m triethanolamine-HCl, pH 7.8, at 25°, containing 0.1 M NaCl (Holo-E-Na+, 0—0) or 0.1 M KCl (Holo-E-K+, •—•, and Apo-E-K+, ○—○).](http://www.jbc.org/)

The activation by potassium and ammonium ions both gave sigmoidal curves as a function of the added cation (Fig. 2A) with a Hill coefficient of 2.7 for the potassium and 1.5 for the ammonium ion (inset, Fig. 2A). At higher potassium concentrations, however, a slight inactivation could be observed (about 20% at 0.4 M K+). The sigmoidal shape of the curves might indicate a concerted transition in the tetramer from an inactive form T not fixing the cation, to an active form R with affinity for the same ion. In this optic it seemed interesting to examine
Fig. 2. The effect of monovalent cations on the enzymatic activity. A, direct plot (inset: Hill plot) of velocity against the cation concentration. The activities were standardized, taking as 100% the velocity obtained with 50 mM K+ (14 e.u. per mg). B, double reciprocal plot of velocity against the substrate concentration at 6 mM K+. C, reciprocal plot of velocity against the concentration of Na+ at fixed concentrations of K+ as indicated on each line.

Fig. 3. Kinetics of binding of pyridoxal-P to apotryptophanase. Apoenzyme (0.25 mg per ml), prepared in Buffer A as indicated under "Materials and Methods," was mixed at time zero with pyridoxal-P to a final concentration of 5 × 10^-3 M and incubated in the dark at 37°C. Samples were withdrawn from the solution at various times, diluted 1000 fold in the absence of pyridoxal P, and assayed immediately for enzymatic activity. The results are plotted as percentage of activity versus time, taking as 100% the value measured after 21 hours. Inset: semilog plot of the difference in per cent between the maximum value and each measurement. The decomposition in exponentials is shown in dashed lines.

Fig. 4. K_m measurements of the tryptophanase for pyridoxal-P. Double reciprocal plots of activity versus pyridoxal-P concentrations. Apotryptophanase (0.7 µg per ml) was incubated in the dark at 37°C in the assay mixture (i.e. without substrate) containing different concentrations of pyridoxal-P. At the times indicated, the substrate was added and the enzymatic activity was measured. Inset: time dependence of the "apparent K_m."

The effect of the variation of substrate concentration on the activity at low potassium concentrations. As shown in Fig. 2B the behavior is Michaelian; the observed Michaelis constant for the S-methyl-L-cysteine was 14 × 10^-2 M, the same as in 0.1 M potassium phosphate buffer, pH 7.8.

As opposed to the two cations mentioned, sodium ions do not activate the enzyme, and even reverses the effect of these ions. The inhibition was of the Michaelian type (see Fig. 2C).

Kinetics of Pyridoxal-P Binding—The preceding results showed that the K+ concentration necessary to saturate the holoenzyme is 0.1 M. It is therefore at this concentration that the effect of the second factor, the pyridoxal-P, was examined. During this study we observed that fully reconstituted holotryptophanase had the same enzymatic activity, whether or not the pyridoxal-P was included in the assay mixture. The second observation was that the interaction of apoenzyme and pyridoxal-P is a rather slow phenomenon. A typical reconstitution experiment is shown in Fig. 3. As can be seen, the reconstitution is not a simple first order reaction, but consists of at least two kinetically distinct steps. The first one has a constant of 0.49 min^-1 (half-life of 1.4 min). The second step is slower with a constant of 0.095 min^-1 (half-life of 7.3 min).

It should be pointed out that in all of the reconstitution experiments, the enzyme solution was protected from light because of the high photosensitivity of the holoenzyme.

K_P of Tryptophanase for Pyridoxal-P—The binding of pyridoxal-P to the apotryptophanase thus appeared as an unusually slow process. At 37°C and 5 × 10^-5 M pyridoxal-P it was essentially complete in about 35 min; at lower pyridoxal-P concentrations, the binding was likely to be even slower. Newton et al. (6) reported a K_m value for the pyridoxal-P of 2 × 10^-6 M, measured after a preliminary incubation of only 10 min at 37°C. It thus seemed unlikely that the binding equilibrium was really reached, and that this K_m value represents a true K_m. We therefore wanted to determine the K_m after a time long enough to ensure a real equilibrium. Fig. 4 shows the double reciprocal plots of enzymatic activity versus the concentration of pyridoxal-P, measured after various times of incubation at 37°C. As can be seen, a stable curve is obtained after an incubation of at least 16 hours at 37°C, giving a true K_m value of 5 × 10^-7 M.

Since it was previously reported (19) that the reconstitution of apo-aspartate aminotransferase strongly depends on the presence of phosphate ions, the effect of this ion on the reconstitution of tryptophanase has been investigated. Experiments, similar to those described in Figs. 3 and 4, have been
The apoenzyme was dialyzed for 24 hours at 37°C as described under "Materials and Methods." After dialysis, the different samples had a specific activity of 16.0 ± 0.5 e.u. per mg. A, inversion Scatchard plot for the binding of [3H]pyridoxal-P. n is the number of bound ligand per mol of apoenzyme protomer. The points indicated were averaged from three independent experiments. The experimental dispersion is shown by the bar across each point. B, Woolf-Hofseth plot of enzyme activity against pyridoxal-P concentration. The activity of each sample after one of the dialysis experiments was measured after dilution in either the corresponding dialysis buffer with the addition of acetylated bovine serum albumin and DPNH (0) or in the assay mixture containing no pyridoxal-P (●). The enzyme was assayed for 10 min.

performed using, instead of potassium phosphate, Tricine-KOH buffer containing 0.1 M KCl. The results were quantitatively identical with those obtained in the presence of potassium ions.

In order to verify that the $K_m$ obtained by activity measurements corresponds to the real dissociation constant, the fixation of [3H]pyridoxal-P was followed by equilibrium dialysis. The results are shown in Fig. 5. To show that the equilibrium was attained after 24 hours, a dialysis was performed during 40 hours; the same result was obtained. Fig. 5A might indicate a certain heterogeneity of the sites. However, the sigmoidal distribution of the points does not appear significant because of the large dispersion of the points from one experiment to another. Furthermore, the activity measurements (Fig. 5B) indicate a good site homogeneity. For these reasons the results in Fig. 5A were fitted with a straight line by the least square method. The line thus obtained shows the existence of 0.93 site for pyridoxal-P per protomer with a dissociation constant of $(3.2 ± 0.8) \times 10^{-4}$ M. Fig. 5B yields a $K_m$ for pyridoxal-P of $4.2 \times 10^{-4}$ M, in good agreement with the former value. The presence or absence of pyridoxal-P in the assay mixture for the samples recovered after the dialysis did not change the activity, indicating that at any degree of saturation the enzyme loses its pyridoxal-P very slowly.

Dissociation of Coenzyme from Holoenzyme—From the preceding observations it could be inferred that the dissociation of the enzyme-coenzyme complex should be very slow. The following experiments were aimed at verifying this point and permitted us to measure the rate constant of the dissociation reaction. A, chromatography of the holoenzyme; 0.5 ml of apotryptophanase (1 mg per ml) was reconstituted with $10^{-6}$ M of [3H]pyridoxal-P for 21 hours at 20°C in the dark. It was then passed (at room temperature) through a Sephadex G-25 column equilibrated with Buffer A. Fractions of 0.3 ml were collected and tested for protein content and radioactivity without precipitation with trichloroacetic acid. Activity (●) was added $10^{-4}$ M (○) or $2 \times 10^{-4}$ M (●) of nonradioactive pyridoxal-P, and the solution was incubated at 37°C in the dark. At different times 100-μl aliquots were withdrawn, reduced with sodium borohydride (○), and precipitated with 3% trichloroacetic acid (TCA). The radioactivity of the supernatant was measured, and the number of counts per min precipitated with trichloroacetic acid was calculated by difference with a control experiment performed without reduction. At time zero, the radioactivity found in the supernatant represented 12% of the total radioactivity of the sample. This value is considered as a background for the calculations.

The radioactivity measured after deproteinization with trichloroacetic acid as described under "Materials and Methods" gave by five independent measurements a total number of 1 ± 0.1 molecules of pyridoxal-P bound per tryptophanase monomer.

The pool of [3H]pyridoxal-P labeled holoenzyme thus obtained was then incubated with a high concentration of nonradioactive pyridoxal-P, and the release of radioactivity was followed. (For experimental conditions see Fig. 6A for experimental conditions). The specific activity was constant (18.6 e.u. per mg) throughout the elution peak, and the activity obtained without added pyridoxal-P (final pyridoxal-P concentration in the assay mixture less than $1.5 \times 10^{-4}$ M) was 98% of that obtained in the presence of pyridoxal-P.

The radioactivity measured after deproteinization with trichloroacetic acid and deproteinization with trichloroacetic acid as described under "Materials and Methods" gave by five independent measurements a total number of 1 ± 0.1 molecules of pyridoxal-P bound per tryptophanase monomer.

The pool of [3H]pyridoxal-P labeled holoenzyme thus obtained was then incubated with a high concentration of nonradioactive pyridoxal-P, and the release of radioactivity was followed. (For experimental conditions see Fig. 6B) The kinetics of the chase of [3H]pyridoxal-P by nonradioactive pyridoxal-P permitted us to calculate the rate constant of pyridoxal-P dissociation. The value obtained was $8.3 \times 10^{-4}$ min$^{-1}$ giving a half-life of 14 hours for this reaction.
**DISCUSSION**

In the present paper we have reported some basic data dealing with the effect of monovalent cations and pyridoxal-P on the transformation of apotryptophanase into the functional holoenzyme. In this study, the variations of enzymatic activity with the concentration of either of these factors were followed at saturation of the other one. The activation of tryptophanase by monovalent cations had already been reported (2, 4, 5) and is not very surprising in view of the fact that many other enzymes show the same effect (20). However, for a large majority of these enzymes the response to cations is Michaelian. A few regulatory enzymes show a sigmoidal response to potassium and ammonium ions; these enzymes, however, also show cooperativity toward their other effectors (21), which does not seem to be the case for the tryptophanase (22). However, the activation curves of holotryptophanase by potassium or ammonium ions have a typical sigmoidal aspect (Fig. 2). The set of results we obtained can be interpreted according to the two following simple models.

(a) At least three sites per protomer (the Hill coefficient = 2.7) need to be saturated by potassium ions in order to obtain the active holoenzyme. In this model there are no interactions involved between protomers. (b) Only one site per protomer needs to be saturated to activate the holotryptophanase. To explain the observed cooperativity, the binding of one potassium ion to a protomer has to induce a change leading to an increase in affinity of the other protomers. This can be achieved either according to a sequential (23) or to a concerted (24) model. In the latter case the allosteric system should be of the V type to account for the absence of cooperativity in the response of the enzyme to its substrate at low potassium ion concentration (Fig. 2B). The data obtained in this work do not permit us to discriminate between these two models. However, the existence of several activator sites per protomer seems rather unlikely, since it has never been reported in the numerous descriptions of the activation of enzymes by the potassium ion.

In addition we have determined the kinetic and equilibrium constants of the binding of pyridoxal-P to the apoenzyme; this has been done at a potassium ion concentration sufficient to saturate the holoenzyme. The reconstitution of holoenzyme, shown in Fig. 3, can be decomposed into two kinetics, first order in enzyme, indicating a heterogeneity in sites. In contrast, when the binding equilibrium is reached, all the sites appear homogeneous ($K_D = 4 \times 10^{-7}$ M). It should first be emphasized that, under the experimental conditions used, the apo- as well as holoenzyme remains tetrameric (3). The results obtained on the kinetics of binding of pyridoxal-P can be accounted for by the following simple models, postulating, respectively, (a) the existence of two different conformations of apotryptophanase in very slow equilibrium, only one of them with affinity for pyridoxal-P; (b) the existence of two isoenzymes, not interconvertible, binding the coenzyme with different velocities; (c) the existence of a kinetically anticooperative binding of pyridoxal-P in the tetramer.

It seemed important to verify the compatibility of these models with the kinetics of release and the equilibrium constant observed experimentally. For this purpose, one can use the experimental results to calculate the forward rate constants and the equilibrium constant. From these values one can then compute the rate constant of the reverse reaction and compare it to the value obtained from the chase experiment. The forward rate constants can be determined from the data reported in Fig. 3, assuming that the binding kinetics are first order in pyridoxal P. That the appearance of activity with the time, shown in this figure, is parallel with the binding kinetics of pyridoxal-P, was justified by the results of Fig. 5. At any saturation state of the enzyme, the activity measured in the absence of free pyridoxal-P is directly proportional to the number of bound pyridoxal-P molecules.

According to the first model the Scatchard plot should not be linear. This was not the case, allowing us to discard this model. For model b to be consistent with the homogeneity in sites, observed at equilibrium, the following relation must hold: $k_1/k_{-1} = k_3/k_{-3} = K_D$, where $k_1$ and $k_3$ are the rate constants for the forward and reverse reactions for each isoenzyme. From the slopes of the lines in Fig. 3 one can compute the rate constants: $k_1 = 9.8 \times 10^4$ min$^{-1}$ M$^{-1}$ and $k_{-1} = k_3 = 40 \times 10^4$ min$^{-1}$ for the isoenzyme binding the pyridoxal-P more rapidly, and $k_2 = 1.9 \times 10^4$ min$^{-1}$ giving $k_{-2} = 7.6 \times 10^4$ min$^{-1}$ for the other isoenzyme. The value thus obtained for the rapid phase of the reverse reaction ($40 \times 10^4$ min$^{-1}$) is 5 times higher than the experimental value ($8.3 \times 10^4$ min$^{-1}$) obtained from Fig. 6B.

The last model could be visualized by the following scheme:

$$E + P \xrightarrow{k_1} EP + P \xrightarrow{k_3} EP_1$$

(in this scheme $P$ represents the pyridoxal-P and the enzyme, $E$, has been represented as a dimer. The anticooperativity allowed us to distinguish only two types of sites, and for further calculations the tetrameric protein will be considered as a double dimer.) To account for the anticooperativity in pyridoxal-P binding $2k_1$ should be superior to $k_3$ and the observation of the homogeneity in affinity of the sites at equilibrium requires that $k_1/k_{-1} = k_3/k_{-3} = K_D$. (To account for this property one might for instance assume that the saturation of the first coenzyme site results only in a decrease in accessibility of the second pyridoxal-P.) Proceeding as before, we can calculate the rate constants: $k_1 = 4.9 \times 10^4$ min$^{-1}$ M$^{-1}$, $k_{-1} = 2 \times 10^4$ min$^{-1}$, $k_3 = 1.9 \times 10^4$ min$^{-1}$ M$^{-1}$, and $k_{-3} = 7.6 \times 10^4$ min$^{-1}$. The rate constant found by the chase experiment (Fig. 6B) should be equal to $2k_{-3}$, i.e. $15.2 \times 10^4$ min$^{-1}$, which is close to the experimentally obtained value of $8.3 \times 10^4$ min$^{-1}$.

Similar studies have been performed, which deal with the binding of pyridoxal-P to other enzymes. In at least two cases (25, 26) an important discrepancy exists between the very slow kinetics observed for the dissociation on one hand, and the dissociation constant predicted from the equilibrium and the association rate constants on the other hand. This discrepancy led O'Leary to postulate a mechanism, by which a rapid binding of the coenzyme is followed by a slow, rate-limiting change in conformation of the protein. This type of mechanism has been verified (27, 28) by studying the changes in optical properties of pyridoxal-P upon reconstitution of the enzyme. As regards tryptophanase, the models proposed above do not require such a complex mechanism, even though our results do not disprove it. Further investigations should be carried on to gain more insight into the details of the reconstitution of holotryptophanase.
Table I

Effect of monovalent cations and pyridoxal-P on tryptophanase

The following was used: apotryptophanase and holotryptophanase, in a buffer adjusted to pH 7.8, containing neither potassium, ammonium, nor sodium ions. When Na⁺ or K⁺ is indicated, the buffer was supplemented with 0.1 M NaCl or KCl. The enzymatic activity of S-alkyl-L-cysteine lyase was indicated as percentage of the activity obtained in the presence of 0.1 M potassium ions. The sedimentation coefficients (s₂₀,w) were obtained at 25°C by Morino and Snell (3). The times, necessary to titrate half of the exposed cysteines (t₁₁), are calculated from Fig. 1B.

<table>
<thead>
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<th>Activity</th>
<th>s₂₀,w, Δ</th>
<th>t₁₁</th>
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<tr>
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</tr>
<tr>
<td>Apotryptophanase-Na</td>
<td>0</td>
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<tr>
<td>Apotryptophanase-K</td>
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<tr>
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<tr>
<td>Holotryptophanase-K</td>
<td>100</td>
<td>10.5</td>
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</table>

Conclusions can be drawn from this table. (a) The three observed properties vary together with respect to monovalent cations and the cofactor, leading to an appearance of activity, a tightening of the molecule, and the burying of the cysteines in the holoenzyme. (b) Neither pyridoxal-P nor potassium alone is able to promote this transition of the molecule toward its functional shape. (c) The influence of the potassium ion is not an effect of ionic strength, since this ion cannot be replaced by the sodium ion.

These results are of interest for future studies on the physical chemical aspects of the apo- to holotryptophanase transition.

References

Kinetic and equilibrium studies on the activation of Escherichia coli K12 tryptophanase by pyridoxal 5'-phosphate and monovalent cations.
A Högberg-Raibaud, O Raibaud and M E Goldberg


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