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Tryptophan Transaminase from *Clostridium sporogenes*

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Tryptophan transaminase of *C. sporogenes* 175 was partially purified 200-fold and its properties were studied. A continuous spectrophotometric assay was modified to determine the enzyme activity by following the absorption change owing to the formation of enol-indolepyruvate at 305 mμ. Pyridoxal phosphate is required for full activation. The respective K_m values for pyridoxal phosphate, tryptophan and α-ketoglutarate are 2.18×10^{-6} M, 2.68×10^{-3} M, and 1.58×10^{-4} M. The optimal pH for the reaction is 8.4. The sedimentation constant of the enzyme is 5.91 S, estimated by centrifugation through a sucrose gradient, and corresponds to an ideal molecular weight of approximately 97,000. The enzyme appears to catalyze the transamination of tyrosine and phenylalanine in addition to tryptophan.

The presence of a tryptophan transaminase in bacteria has been reported for *Escherichia coli* (Meister and Rudman, 1953), *Rhizobium leguminosarum* (Hartmann and Glombitza, 1967), and *Agrobacterium tumefaciens* (Sukanyo and Vaidanathan, 1964). In an investigation of the pathway that is responsible for the production of indolepropionate in *Clostridium sporogenes*, a tryptophan transaminase has been found which catalyzes the production of indolepyruvate and glutamate from tryptophan and α-ketoglutarate. The enzyme appears to catalyze the first step in the proposed pathway.

We report some studies on the purification and characterization of the transaminase.

MATERIALS AND METHODS

Bacteria and growth. C. sporogenes strain 175 was obtained from L. S. McClung. The medium used for the production of cells contained, per liter of distilled water: tryptone, 10 g; neopeptone, 10 g; and sodium thioglycollate, 1 g (Difco Laboratories). The cells were grown at 37° under nitrogen with agitation in an American Sterilizer Co. Biogen. The pH was maintained at 7.2 throughout growth. After 14-16 hr of growth, the cells were harvested by centrifugation in a Sharples centrifuge and frozen at −20° until preparation of the cell-free extract.

Assays. Protein was determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as the standard. Tryptophan was determined by the method of Frank and DeMoss (1957) with partially purified *E. coli* tryptophanase. α-Ketoglutarate was determined by the method of Friedemann and Haugen (1943) using crystalline α-ketoglutarate as the standard. Indolepyruvate was determined by the method of Gordon and Weber (1951) using crystalline indolepyruvate as the standard. Glutamate was determined by the method of Strecher (1955) using crystalline L-glutamic dehydrogenase (Sigma Chemical Co.). PLP[1] was determined with phenylhydrazine as described by Wada and Snell (1961).

A continuous spectrophotometric assay (Lin *et al.*, 1958) was modified to measure the rate of formation of indolepyruvate. The reaction mixture contained: PLP, 0.5 μmoles; Tris buffer, pH 8.4, 500 μmoles; α-ketoglutarate (the stock solution was at pH 7.0), 12 μmoles; L-tryptophan, 20 μmoles; and enzyme in a total volume of 3.0 ml. The PLP and enzyme were incubated for 5 minutes at room temperature before addition of Tris buffer, α-ketoglutarate and water. The reaction was initiated by the addition of tryptophan. A Gilford 2000 recording spectrophotometer was used to measure the increase in absorbance at 305 mμ of the enol form

[1] Abbreviations used: PLP, pyridoxal-5-phosphate; Tris, tris-(hydroxymethyl)-aminomethane chloride; TEAE-cellulose, triethylaminoethyl-cellulose.

of indolepyruvate. The extinction coefficient of the enol-indolepyruvate is 3.9×10^3 cm^2/mmole. One unit of enzyme is defined as that amount of enzyme which forms 1.0 μmole of indolepyruvate per min at room temperature (approximately 22°). Specific activity is expressed as units of enzyme per mg of protein. The initial reaction velocity is linear with respect to time for at least two minutes and increases linearly with increasing concentrations of the enzyme. Unless otherwise noted, all assays were run using the tris buffer system.

A modification of the method of Lin *et al.* (1958) was also used for assaying tryptophan transaminase activity. A glutathione-dependent indolepyruvate keto-enol tautomerase was partially purified from rat liver according to the method of Spencer and Knox (1962). The assay was in 3 ml of 0.33 M sodium borate buffer, pH 8.1 containing 0.5 μmole PLP, 12 μmoles α-ketoglutarate, 20 μmoles L-tryptophan, 0.88 μmole glutathione, tautomerase and enzyme (transaminase) solution. Assays were at room temperature (about 22°) in the Gilford 2000 spectrophotometer at 328 mμ.

L-phenylalanine-α-ketoglutarate and L-tyrosine-α-ketoglutarate reaction. A modification of the method of Lin *et al.* (1958) was used in these assays. A phenylpyruvate-*p*-OH-phenylpyruvate keto-enol tautomerase was purified from pig kidney according to the method of Knox and Pitt (1957). The assay was in 3 ml of 0.33 M sodium borate, pH 8.1, containing 0.5 μmole PLP, 12 μmoles α-ketoglutarate, 20 μmoles L-tyrosine or L-phenylalanine, tautomerase and enzyme solution. Assays were at room temperature (about 22°) in the Gilford 2000 spectrophotometer at 310 mμ.

Starch block electrophoresis. The starch solution was prepared from hydrolyzed starch (Connaught Medical Research Laboratories) according to the method of Smithies (1955).

RESULTS AND DISCUSSION

Standardization of enzyme assay. The spectrophotometric assay for indolepyruvate formation, developed by Lin *et al.* (1958) was based upon the change in absorbance at 328 mμ exhibited by the reacting system in the presence of borate. The procedure described by Lin was found to be unsatisfactory in the present work, owing to the nonlinear increase in absorbance which was observed with respect to time. Consequently, the transaminase assay procedure was modified in two important respects. Borate was eliminated and the wavelength of observation was changed. The enol-form of indolepyruvate has an absorption maximum at 305 mμ, in contrast to the absorption maximum at 328 which is observed for the borate-indolepyruvate complex. The formation of indolepyruvate was followed at 305 mμ in the presence of different buffers. The results obtained with three buffer systems are shown in Table I. Tris, pH 8.4, is clearly the buffer of choice and was adopted as part of the standard enzyme assay procedure. No increase in rate was observed with higher tris concentrations.

TABLE I

EFFECT OF BUFFER ON THE TRYPTOPHAN TRANSAMINASE ASSAY PROCEDURE

The reaction mixture contained 20 μg pyridoxal-5-phosphate, 16 μmoles L-tryptophan and 12 μmoles α-ketoglutarate. The initial rate was measured at 305 mμ as described.

Buffer	mμ	Final concentration (M)	Initial velocity, Δ OD min^{-1} (mg protein)$^{-1}$
Tris-chloride, pH 8.4	305	0.033	0.411
		0.067	0.463
		0.100	0.476
		0.167	0.578
Potassium phosphate, pH 8.0	305	0.033	0.180
		0.067	0.206
		0.167	0.296
		0.333	0.463
Sodium borate, pH 7.9	328	0.067	No linear rate
		0.333	

PURIFICATION OF TRANSAMINASE

Step 1. *Cell-free extract.* Frozen cells (30 g) were thawed and suspended in 4 volumes (ml/g) of 0.1 M potassium phosphate, pH 7.5. The cells were disrupted with a Branson sonifier in ten 30-second treatments applied at 30-sec intervals. The disrupted cell suspension was centrifuged at 35,000*g* for 30 min using the SS-34 head of the Servall RC-II centrifuge. The supernatant was diluted with 5.3 volumes of 0.1 M potassium phosphate, pH 7.5 and stirred for 5 min at 0°. The supernatant was made 2.5 mM with

respect to PLP by the addition of 0.05 M PLP, pH 7.0. The pH was adjusted to 6.0 by the addition of cold 6 N acetic acid. The pH meter (Metrohm) previously had been calibrated at 22° (room temperature) with standard buffer, pH 7.0.

Step 2. *Heat treatment.* Samples (90–100 ml) of the cell-free extract in 500 ml erlenmeyer flasks were swirled gently in a boiling water bath until the temperature of the contents reached 64°. At this time the flasks were transferred to a water bath held at 67–68°. After 4 min at 64–65° the flasks were rapidly cooled in an ethanol-ice bath to 5°. The heated suspension was centrifuged at 27,000*g* for 20 min to sediment the denatured protein. The pellet was discarded.

Step 3. *Ammonium sulfate fractionation.* The supernatant from the above step was brought to 55% saturation by the addition of solid ammonium sulfate. After 40 min stirring at 0°, the suspension was centrifuged at 27,000*g* for 20 min. The pellet was discarded. Solid ammonium sulfate was added to 70% saturation. After stirring 40 min at 0°, the suspension was centrifuged at 27,000*g* for 20 min. After thoroughly draining the centrifuge tube, the precipitate was dissolved in a volume of 0.1M potassium phosphate, pH 7.5, the volume equal to two-thirds of the weight of the frozen cells (ml/g).

Step 4. *TEAE - cellulose columns.* TEAE-cellulose (Bio-Rad, lot #3258) was equilibrated with 0.1 M potassium phosphate, pH 7.5. The Bio-Rad TEAE-cellulose exchangers were found to vary widely between different lots and thus the same preparation was used for all purification runs. All columns were used at 3°.

Two columns (22 × 16 cm) were poured and each was washed with one bed volume of 0.1 M potassium phosphate, pH 7.5 and one bed volume of 0.01 M potassium phosphate, pH 7.5. The 55–70% ammonium sulfate fraction was diluted 20-fold with cold distilled water and half of the enzyme solution was added to each column. The columns were each washed with one volume of 0.01 M potassium phosphate, pH 7.5 and one volume of 0.05 M potassium phosphate, pH 7.5. Elution was started with a linear gradient formed with 100 ml of 0.05 M potassium phosphate, pH 7.5, in the mixing chamber and 100 ml of 0.3 M KCl in 0.05 M potassium phosphate, pH 7.5, in an identical reservoir. Both chambers were at atmospheric pressure. The flow rate was approximately 24 ml per hr and 4.0–5.0 ml fractions were collected. Each collecting tube contained 0.5 ml of 1 mM PLP, pH 7.5. The enzyme activity peak tubes from each column were pooled. The pool was frozen and stored at −20°.

Step 5. *Second TEAE-cellulose column.* A column (1.2 × 16 cm) was poured and washed with one volume each of 0.1 and 0.001 M potassium phosphate, pH 7.5. The pool from the previous step was diluted 20-fold in cold distilled water and

TABLE II

SUMMARY OF PURIFICATION PROCEDURE FOR TRYPTOPHAN TRANSAMINASE

One unit of enzyme catalyzes the formation of 1.0 μmole of indolepyruvate per min at room temperature (about 22°). Specific activity is units of enzyme per milligram of protein. Assays were in 0.33 M borate, pH 8.1, as already described. The extinction coefficient of the borate-indolepyruvate complex at 328 mμ is 1.4×10^4 cm²/mmole (Lin *et al.*, 1958).

Purification step	Volume (ml)	Protein (mg)	Total units	Specific activity	Purification factor	Recovery (%)
(1) Cell-free extract	288	1956	235	0.12	—	100
(2) Heated supernatant	285	812	195	0.24	2	83
(3) 55–70% ammonium sulfate	20.8	190	201	1.06	9	86
(4) TEAE I eluate	30.5	26.7	155	5.82	49	66
(5) TEAE II eluate	19.0	2.7	64	23.6	197	27

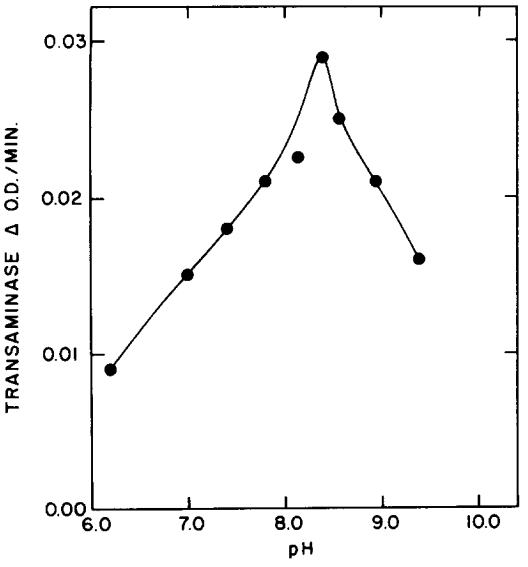

FIG. 1. Effect of pH on transaminase activity.

applied to the column. Elution was started with a linear gradient formed with 100 ml of 0.05 M potassium phosphate, pH 7.5 in the mixing chamber and 100 ml of 0.1 M KCl in 0.05 M potassium phosphate, pH 7.5, in the reservoir chamber. The flow rate was approximately 28 ml per hr and 4.5–5.0 ml fractions were collected. Each collecting tube contained 0.5 ml of 1 mM PLP, pH 7.5. The enzyme activity peak tubes were pooled and stored overnight at 0°.

The enzyme is stable for at least 48 hr at 0° and loses activity upon freezing and thawing. Thus, it is unnecessary, and perhaps undesirable, to freeze the pool from step 4 between columns.

Low buffer concentration results in the loss of activity of the transaminase. The enzyme was partially purified using 0.10 M potassium phosphate, pH 7.5, containing 0.01 M mercaptoethanol and 0.04 mM PLP; the purification was repeated using 0.01 M potassium phosphate, pH 7.5, containing 0.01 M mercaptoethanol and 0.04 mM PLP. There was a 2.5-fold increase in the final specific activity and in the total activity recovered using the high salt buffer. A summary of a typical purification is shown in Table II.

Optimum pH for transaminase activity. The transaminase shows maximal activity at pH 8.4 using a partially purified preparation of enzyme, as shown in Fig. 1. This value was obtained by measuring the initial velocity of the transaminase at different pH values, which had been established by varying the relative concentrations of mono- and di-basic potassium phosphate.

Stoichiometry of the transaminase reaction. The results of several determinations of the stoichiometry of the reaction are shown in Table III.

Affinity of transaminase for substrates. The Michaelis-Menten constants for the substrates of the reaction were estimated from initial velocities in the presence of nonlimiting amounts of the second substrate. The apparent K_m for L-tryptophan is 2.68 mM and that for α-ketoglutarate is 0.158 mM.

Affinity of transaminase for pyridoxal phosphate. The enzyme was freed of PLP by dialysis for 48 hr at 5° against five changes of 400 volumes each of 0.1 M potassium phosphate, pH 7.5, containing

TABLE III

STOICHIOMETRY OF THE TRYPTOPHAN TRANSAMINASE REACTION

Conditions: 0.22 units of transaminase in Expts. 1 and 2, 0.67 units in expt. 3, 100 μg of PLP, 48 μmoles L-tryptophan, 36 μmoles α-ketoglutarate, 1500 μmoles Tris, pH 8.4, in a total volume of 10 ml. The reaction mixture was incubated for 20 min at room temperature and terminated by addition of 1 ml of 100% TCA. After 15 min at 0° the mixture was centrifuged at 8700*g* for 5 min and the products were determined in the supernatant as described in Methods. In Expts. 1 and 2, the transaminase had been dialyzed for 18 hr against 0.1 M KPO_4, pH 7.5 containing 10^{-2} M mercaptoethanol; in Expt. 3 the transaminase had been dialyzed for 10 hr against the same buffer.

Expt. No.	μmoles/ml			
	Tryptophan	α-keto-glutarate	Indole-pyruvate	Glutamate
1	−0.40	−0.41	+0.40	+0.41
2	−0.33	−0.32	+0.26	+0.31
3	−1.09	−0.76	+1.00	+1.07

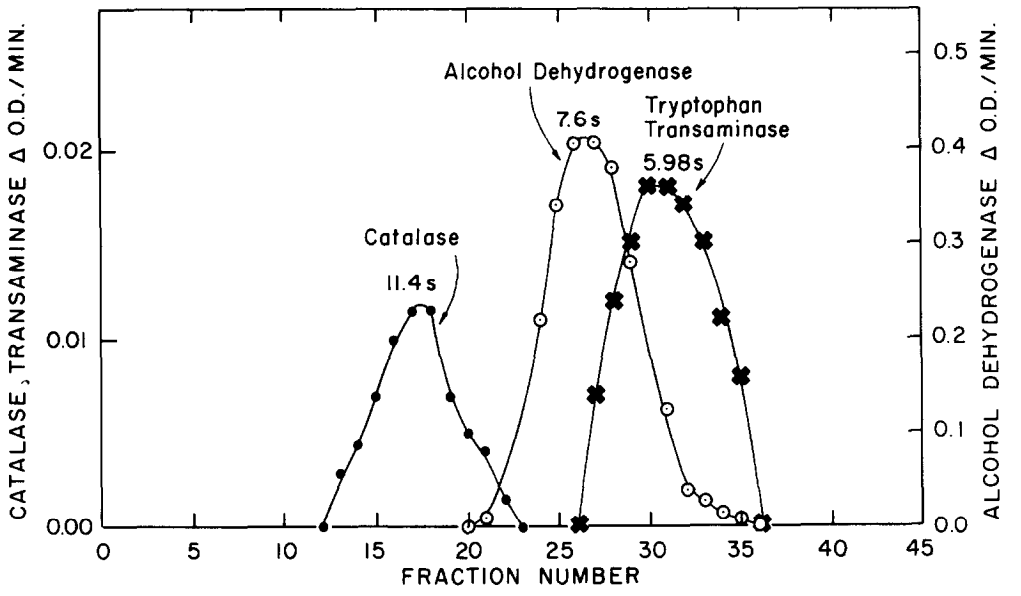

FIG. 2. Sedimentation behavior of tryptophan transaminase. The sucrose gradient (5–20%) was in 0.1 M potassium phosphate, pH 7.5. The sedimentation time was 16 hrs at 33,000 rpm. Symbols: ● , catalase; ⊙ , alcohol dehydrogenase; ×, tryptophan transaminase.

TABLE IV

RELATIVE RECOVERY OF THREE TRANSAMINASE ACTIVITIES DURING PURIFICATION OF TRYPTOPHAN TRANSAMINASE

Assays were in 0.33 M borate, pH 8.1, as described. The extinction coefficient of the borate-phenylpyruvate complex is 5.8×10^3 cm²/mmole and that of the borate-*p*-hydroxy-phenylpyruvate complex is 9.85×10^3 cm²/mmole (Lin *et al.*, 1958).

Purification step	Tryptophan transaminase			Phenylalanine transaminase		Tyrosine transaminase	
	Protein (mg)	Total activity	Specific activity	Specific activity	Activity relative to tryptophan (%)	Specific activity	Activity relative to tryptophan (%)
(1) Crude extract	1000	122	0.122	0.036	29.5	0.017	13.9
(2) Heated supernatant	421	111	0.264	0.076	28.8	0.032	12.2
(3) 55–70% ammonium sulfate	115	128	1.11	0.163	14.6	0.084	7.5
(4) TEAE I eluate	10.5	79	7.50	1.50	20.0	0.618	8.2
(5) TEAE II eluate	2.04	32	15.6	3.09	19.7	1.42	9.1
(6) Hydroxylapatite eluate	0.75	25	33.3	6.81	20.4	2.42	7.3

0.01 M mercaptoethanol. No residual PLP could be detected by the colorimetric assay for this compound as described in the Methods and no enzymatic activity was detectable in the absence of added PLP. The K_m for PLP was estimated to be 2.18×10^{-6} M.

Maximum activation of the enzyme is attained after incubation of the enzyme and cofactor for 5 minutes. Pyridoxamine-5-phosphate will also activate the enzyme but requires an incubation period twice that of PLP to obtain the same activity. Pyridoxine, pyridoxal and pyridoxamine do not activate the enxyme.

Sedimentation constant. The sedimen-

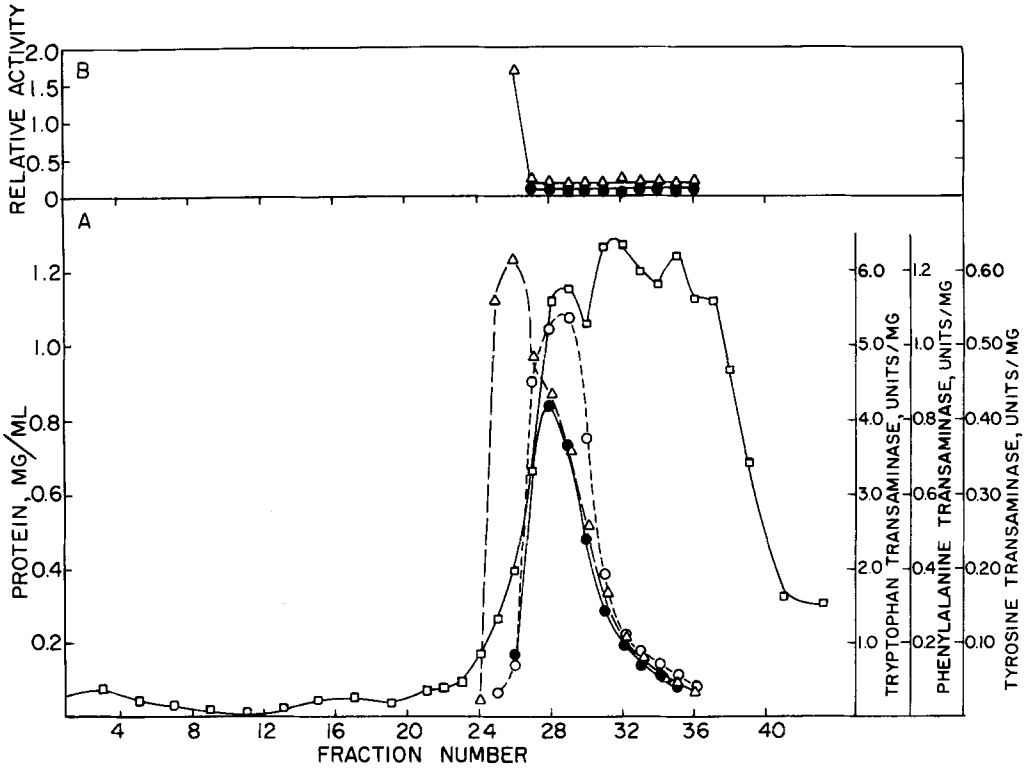

FIG. 3. Elution of transaminase from TEAE column using 0–0.3 M KCl gradient in 0.05 M potassium phosphate, pH 7.5. A, profile of protein and activity; B, activity relative to tryptophan transaminase activity. Symbols: □, protein; ○, tryptophan transaminase; △, phenylalanine transaminase; ●, tyrosine transaminase.

tation constant of the enzyme was determined by sucrose density-gradient centrifugation using catalase and alcohol dehydrogenase as standards, essentially as described by Martin and Ames (1961). The mean of a series of gradient analyses gave a value of 5.91 S corresponding to an approximate ideal molecular weight of 97,000. A typical gradient is shown in Fig. 2.

Specificity. The tryptophan transaminase exhibited activity with regard to the other aromatic amino acids, phenylalanine and tyrosine, throughout purification, as shown in Table IV. This purification procedure included, in addition to steps 1–5 described above, an hydroxylapatite column (1.1 × 1.7 cm) prepared according to the method of Tiselius *et al.* (1956). Elution was achieved using four steps, each consisting of 1.5 volumes (bed volume) of potassium phosphate, pH 7.0 in 0.03 M increments from 0.05 M to 0.17 M and a final elution with 0.4 M potassium phosphate, pH 7.0.

The results indicate that there are at least two separate aromatic transaminases in *C. sporogenes*, and that the tryptophan-phenylalanine-tyrosine transaminase may be distinct from the phenylalanine transaminase.

There is a separate and distinct peak of phenylalanine transaminase activity upon elution from the first TEAE column as shown in Fig. 3. This peak also contains a shoulder, the elution pattern of which corresponds to that of the tryptophan-tyrosine activities. The fractions in the shoulder region, although varying in amount of tryptophan transaminase activity, exhibit nearly constant ratios of activity for all three amino acids. From each TEAE column, the four fractions which contained maximum tryptophan

transaminase activity were combined and chromatographed on a column of hydroxylapatite. The more basic phenylalanine transaminase which emerged from the TEAE column just ahead of the tryptophan transaminase was apparently not present in the fractions obtained by elution of the hydroxylapatite column. Furthermore, the activity ratios observed in each fraction eluted from the hydroxylapatite column corresponded to those of the TEAE columns.

Tyrosine and phenylalanine cannot be assayed using the Tris buffer procedure. Therefore we decided to use the borate assay in this experiment so that the estimations of activity would be of comparable sensitivity for each of the three amino acids. The tautomerases were essential in measuring the rate of formation of the aromatic keto acids. Without the addition of indolepyruvate tautomerase, the tryptophan transaminase exhibited an increasing rate of reaction for the first 5–10 minutes. The transaminase activities for tyrosine and phenylalanine were assayable without tautomerase but the rate of nonenzymatic formation of keto acid-borate complexes was low, and thus also yielded incorrect estimates of transaminase activity. For valid determinations of the transamination rates with each of the three amino acids, it was necessary that the amount of added tautomerase should permit a constant initial transamination rate for at least one minute. In the presence of phenylpyruvate tautomerase, the duration of the initial rates of phenylalanine and tyrosine transamination was too short at high concentrations of tautomerase. Therefore phenylpyruvate tautomerase was added at a concentration which was less than that necessary to achieve maximum phenylalanine and tyrosine transamination rates, but which permitted measurement of a linear initial transamination rate for at least one minute. Consequently, the true ratios of the three activities is not apparent from Table IV.

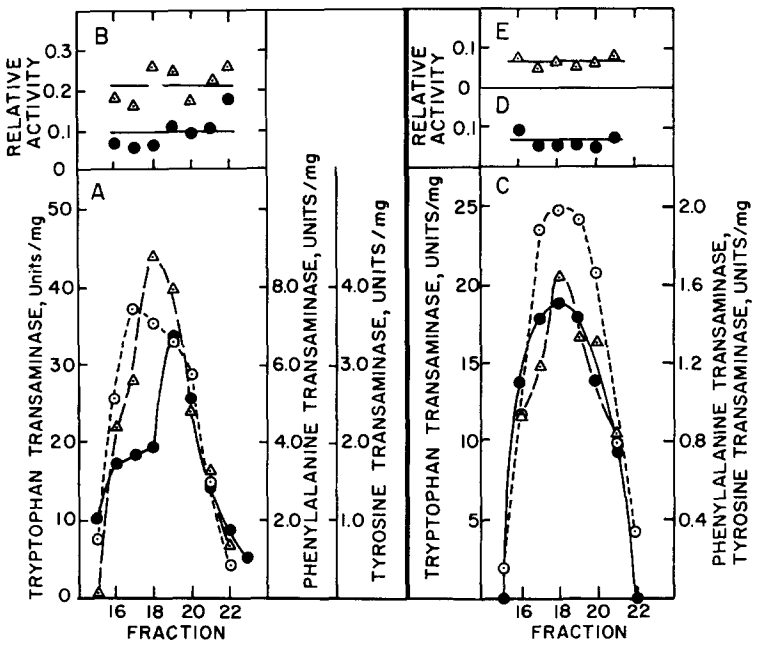

FIG. 4. Elution of transaminase from hydroxylapatite column. A, B, The appropriate tautomerase was added to each assay mixture. C, D, E, No tautomerases were used in the assays. A, C, profiles of activity; B, D, E, activity relative to tryptophan transaminase activity. Symbols: O, tryptophan transaminase; △, phenylalanine transaminase; ●, tyrosine transaminase.

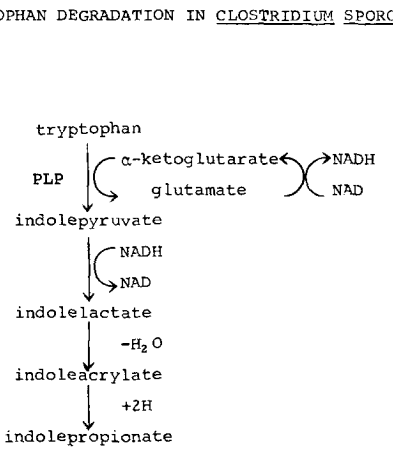

FIG. 5. Proposed metabolic path for the conversion of L-tryptophan to indolepropionate by *C. sporogenes*.

To ascertain the amount of phenylpyruvate tautomerase necessary for maximum activity, increasing amounts of tautomerase were added to replicate samples of transaminase. The initial velocities for both the phenylalanine and tyrosine reactions increased hyperbolically as a function of increasing amounts of tautomerase. Reciprocal plots of tautomerase concentration as a function of initial reaction rates indicated first order kinetics; the ordinate intercepts were taken to be the maximum phenylalanine and tyrosine transaminase rates possible at saturating amounts of tautomerase. Such a plot can be made only with the assumption that the tautomerase in no way inhibits the actual transaminase reactions. Under these conditions, and subject to the assumptions stated, the actual ratio of activities appears to be 1:0.60:0.42 for tryptophan, tyrosine and phenylalanine respectively.

Because of the difficulties associated with the use of the tautomerase, the fractions from the hydroxylapatite column were also assayed in the absence of tautomerases to determine if there were any differences in the elution pattern which could be attributed to tautomerases in the transaminase preparation. The results are shown in Fig. 4 and are consistent with the hypothesis that the tryptophan transaminase also contains phenylalanine activity and tyrosine activity.

Since the tryptophan transaminase could not be dissociated from tyrosine-phenylalanine activity on the three columns, an attempt was made to separate it using starch gel block electrophoresis. A 10% starch solution was prepared in 0.05 M potassium phosphate, pH 7.5; the electrode compartments contained 0.1 M potassium phosphate, pH 7.5. A sample of the 55–70% ammonium sulfate fraction was applied, and in each of two experiments, four distinct protein bands were obtained. Each block was divided in half and each segment eluted into 0.10 M potassium phosphate, pH 7.5, containing 0.1 mM PLP. The fractions were assayed without tautomerase. In the fractions containing the maximum tryptophan activity, the ratios of activities found upon assaying the hydroxylapatite column fractions were again maintained; that of phenylalanine-tryptophan was 0.08–0.09; tyrosine-tryptophan, 0.06–0.08.

We conclude that at least two distinct aromatic transaminases exist in *C. sporogenes* 175, one active with tryptophan, tyrosine and phenylalanine, and the other active with phenylalanine.

Physiological role. The preferential use of four amino acids, arginine, tyrosine, phenylalanine and tryptophan, by *C. sporogenes* was reported by Shull, Thoma and Peterson (1948) although the manner of their utilization was not known. Indolepropionate has been reported by Hoogerheide and Kocholaty (1938) as a product formed during growth of *C. sporogenes* on complex medium. It is possible that a metabolic pathway for the conversion of tryptophan to indolepropionate represents a physiologically important means for the removal of electrons in the growth of this anaerobe. The proposed sequence is shown in Fig. 5. The indolelactate dehydrogenase has been demonstrated and partially characterized in cell-free extracts of *C. sporogenes* by Jean and DeMoss (*Can. J. Microbiol.*, 1968). Boezi and DeMoss (*Bact. Proc.*, 1958) demonstrated that dried cell preparations and

Hughes press preparations of *C. sporogenes* convert indolelactate or indoleacrylate to indolepropionate.

If the tryptophan transaminase catalyzes the first step in this pathway, the enzyme might be expected to be inducible. To test the hypothesis, *C. sporogenes* was grown in a medium containing, per liter, 20 g casein hydrolysate (Nutritional Biochem. Corp), 5 g yeast extract and 1 g sodium thioglycollate (Difco Laboratories). L-Tryptophan (Mann Research Laboratories) was added to a final concentration of 5 or 500 μg per ml. The specific activity of the tryptophan transaminase increased 2.5-fold when grown on the higher level of tryptophan. The indolelactate dehydrogenase did not increase in activity with the higher level of tryptophan. The two results need not be considered incompatible; the transaminase may have other metabolic functions which are not coupled to indolelactate dehydrogenase. The physiological importance of the tryptophan transaminase has not been investigated further but its apparent inducibility by tryptophan suggests at least a partial reason for the preferential use of this amino acid by *C. sporogenes*.

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REFERENCES

1. FRANK, L. H. AND R. D. DEMOSS. *Arch. Biochem. Biophys.* **67,** 387-397 (1957).
2. FRIEDEMANN, T. E. AND G. E. HAUGEN. *J. Biol. Chem.* **147,** 415-442 (1943).
3. GORDON, S. A. AND R. P. WEBER. *Plant Physiol.* **26,** 192-195 (1951).
4. HARTMANN, T. AND K. GLOMBITZA. *Arch. Mikrobiol.* **56,** 1-8 (1967).
5. HOOGERHEIDE, J. C. AND W. KOCHOLATY. *Biochem. J.* **32,** 949-957 (1938); JEAN, M. AND R. D. DEMOSS. *Can. J. Microbiol.* **14,** 429-435 (1968).
6. KNOX, W. E. AND B. M. PITT. *J. Biol. Chem.* **225,** 675-688 (1957).
7. LIN, E. C., B. M. PITT, M. CIVEN, AND W. E. KNOX. *J. Biol. Chem.* **233,** 668-673 (1958).
8. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. *J. Biol. Chem.* **193,** 265-275 (1951).
9. MARTIN, R. G. AND B. N. AMES. *J. Biol. Chem.* **236,** 1372-1379 (1961).
10. RUDMAN, D. AND A. MEISTER. *J. Biol. Chem.* **200,** 591-604 (1953).
11. SHULL, G. M., R. W. THOMA AND W. H. PETERSON. *Arch. Biochem.* **20,** 227-241 (1949).
12. SMITHIES, O. *Biochem. J.* **61,** 629-641 (1955).
13. SPENCER, R. P. AND W. E. KNOX. *Arch. Biochem. Biophys.* **96,** 115-124 (1962).
14. STRECHER, H., *in* "Methods in Enzymology" Vol. II, pp. 220-225 (1955).
15. SUKANYA, N. K. AND C. S. VAIDYANATHAN. *Biochem. J.* **92,** 594-598 (1964).
16. TISELIUS, A., S. HJERTEN AND Ö. LEVIN. *Arch. Biochem. Biophys.* **65,** 132-155 (1956).
17. WADA, H. AND E. E. SNELL. *J. Biol. Chem.* **236,** 2089-2095 (1961).