Physiological Comparison of L-Serine Dehydratase and Tryptophanase from *Bacillus alvei*[1]

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Tryptophanase from *Bacillus alvei* also possesses serine dehydratase activity. A comparison of this enzyme with L-serine dehydratase [L-serine hydro-lyase (deaminating), EC 4.2.1.13] in toluene-treated whole cell preparations of the organism was undertaken. Tryptophanase is a constitutive enzyme in *B. alvei*. The dehydratase undergoes a repression-derepression-repression sequence as the L-serine level in the growth medium is increased from 0 to 0.1 M. Tryptophanase activity is decreased in organisms grown in medium containing glucose. Both enzymes are repressed in organisms grown in glycerol-containing medium. L-Serine dehydratase has a pH optimum of 7.5 in potassium phosphate buffer; tryptophanase functions optimally in this buffer at pH 8.2. Both enzymes lose activity in the presence of tris(hydroxymethyl)aminomethane buffer. Either K^+ or NH_4^+ is required for full tryptophanase activity, but Na^+ is markedly inhibitory. These three cations are stimulatory to L-serine dehydratase activity. Both enzymes are subject to apparent substrate inhibition at high concentrations of their respective amino acids, but the inhibition of tryptophanase activity can be completely overcome by the removal of indole as it is formed. The dehydratase does not catalyze cleavage of D-serine, L-threonine, or α-substituted serine analogues at the concentrations tested. However, activity of the enzyme in cleaving L-serine is competitively inhibited by D-serine, indicating that the D-isomer can occupy an active site on the enzyme. The enzyme catalyzes cleavage of some β-substituted serine analogues.

Tryptophanases from *Escherichia coli* (13) and from *Bacillus alvei* (S. R. O'Neil, Ph.D. Thesis, Univ. of Illinois, Urbana, 1969) have been shown to catalyze both α,β-elimination reactions and β-replacement reactions. The major activity of the enzyme, that of degrading tryptophan to indole, pyruvate, and ammonia, is an α,β-elimination reaction. The deamination of serine to pyruvate and ammonia is also an α,β-elimination reaction, but, in the case of tryptophanase from *B. alvei*, this reaction is catalyzed only 15% as efficiently as the tryptophan degradation reaction. Since *B. alvei* possesses an active L-serine dehydratase [L-serine hydro-lyase (deaminating), EC 4.2.1.13] which catalyzes the same serine-deaminating reaction as tryptophanase, it is of interest to ask why the organism possesses two enzymes which can perform the same function. It is also of interest to determine whether the genes specifying both enzymes might have arisen from a common ancestral

gene. The initial approach to these questions has been the comparison of physiological properties of the two enzymes in toluene-treated whole cell preparations of *B. alvei*. The results of this investigation are reported here.

MATERIALS AND METHODS

Bacterial strain. A nonmucoid colony variant of *B. alvei* (ATCC 6348), isolated by Hoch (8) and designated strain F, was used in the experiments reported here.

Growth media and culture conditions. For most experiments, the bacteria were grown overnight in 2% (w/v) Trypticase (BBL) broth which had been supplemented with thiamine hydrochloride (10 μg/ml) and adjusted to pH 7.1 before autoclaving.

Cells used in catabolite repression experiments were transferred from the supplemented Trypticase medium to the minimal salts medium of Vogel and Bonner (16) supplemented with thiamine hydrochloride (10 μg/ml) and 1% vitamin-free, salt-free casein hydrolysate (Nutritional Biochemicals Corp., Cleveland, Ohio). During the exponential growth phase, the cells were transferred to fresh supplemented minimal salts medium without further addi-

tions (control) or to which had been added either 1% glucose or 1% glycerol.

For studying the inducibility of enzymes, cells were transferred from supplemented Trypticase medium to the minimal salts medium supplemented this time with thiamine hydrochloride (10 μg/ml) and one of the following: 1% glucose alone, 1% glucose and a single amino acid (20 μg/ml), or a mixture of 21 L-amino acids (each 20 μg/ml) excluding L-serine. The single amino acids were glycine, L-threonine, L-serine, and L-homoserine. The mixture of 21 amino acids included glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-threonine, L-homoserine, L-methionine, L-tryptophan, L-cystine, L-glutamic acid, L-glutamine, L-aspartic acid, L-asparagine, L-arginine, L-histidine, L-phenylalanine, L-lysine, L-tyrosine, L-cysteine, and L-proline. During the exponential growth phase, the cells were transferred to fresh supplemented minimal salts medium to which had been added glucose, glucose and the single amino acid, or the amino acid mixture plus various concentrations of L-serine. Cultures were harvested at approximately the same level of growth during late log growth phase. The specific activity of tryptophanase in *B. alvei* is maximal, and the specific activity of L-serine dehydratase is still relatively high at this stage of growth. The absorbancy reading at which late log growth phase occurred was dependent upon the degree of supplementation of the culture medium.

The ratio of culture volume to flask volume was maintained at 1:4. All culture flasks were shaken on a New Brunswick gyratory shaker at 37 C. The absorbancy of cultures was measured at 660 nm in a Zeiss PMQII spectrophotometer. If the absorbancy reading was greater than 0.6, the sample was diluted to give a more accurate turbidity measurement. Throughout the course of this investigation and under all growth conditions used, the ratio of absorbancy (turbidity) of cultures to cell protein content was constant. One unit of absorbancy is equivalent to 0.240 mg of cell protein per ml.

Toluene-treated whole cell preparation. All preparatory and assay procedures were at 0 C, unless otherwise indicated. Samples of cultures were centrifuged at 10,000 $\times$ g for 10 min; the cells were washed with 1 sample volume of 0.05 M potassium phosphate or other indicated buffer (pH 7.5) and suspended to an absorbancy reading of approximately 5.0 at 660 nm. Samples (0.1 ml) of washed cells were then incubated with 0.05 ml of toluene at 37 C for 5 min to destroy the permeability barrier of the cells before addition of the assay mixture.

Tryptophanase assay. For most experiments, the following modification of the assay procedure of Pardee and Prestidge (15) was utilized. Duplicate reaction mixtures containing, unless otherwise stated, 25 μmoles of potassium phosphate or other indicated buffer (pH 8.2), 16 nmoles of pyridoxal phosphate, 4 μmoles of L-tryptophan, and toluene-treated cell suspension in a final volume of 0.5 ml were incubated at 37 C for 0 or 10 min. Zero-time reaction mixtures provided blank values which were later subtracted from the experimental (10-min) values. The reactions

were terminated by the addition of 3 ml of color reagent (14.7 g of *p*-diaminobenzaldehyde, 52 ml of concentrated H_2SO_4, 948 ml of 95% ethyl alcohol). After a 20-min color development period at room temperature, the tubes were centrifuged to sediment cellular debris and the absorbancies of the supernatant fluids were measured at 568 nm in a Gilford 300 spectrophotometer. Indole was used as the standard.

In substrate affinity experiments, it was desirable to remove indole as it was formed. The following modification of the assay procedure described by Boezi and DeMoss (1) was utilized. Duplicate reaction mixtures containing 210 μmoles of potassium phosphate buffer (pH 8.2), 200 nmoles of pyridoxal phosphate, and toluene-treated cell suspension in a final volume of 1.5 ml were overlaid with 4 ml of toluene in 50-ml Erlenmeyer flasks and shaken slowly at 37 C for 5 min. The reactions were initiated by the addition of 0.5 ml of various concentrations of tryptophan, and the incubation was continued at 37 C with shaking for 10 min. The reactions were terminated by the addition of 0.2 ml of 2.5 N NaOH, and the shaking was continued for 5 min. Samples (1.0 ml) of the toluene layers were assayed for indole by adding 9 ml of color reagent (5.4 g of *p*-diaminobenzaldehyde, 64 ml of concentrated H_2SO_4, 908 ml of 95% ethyl alcohol), incubating at room temperature for 20 min, and measuring color intensities at 568 nm.

L-Serine dehydratase assay. Duplicate reaction mixtures containing, unless otherwise stated, 55 μmoles of potassium phosphate or other indicated buffer (pH 7.5), 40 nmoles of pyridoxal phosphate, 100 μmoles of L-serine, and toluene-treated cell suspension in a final volume of 1.0 ml were incubated at 37 C for 0 or 10 min. The reactions were terminated by the addition of 1.0 ml of 10% trichloroacetic acid. Precipitated protein was removed by centrifugation. The supernatant fluids were assayed for keto acid (pyruvic, β-phenylpyruvic, or α-ketobutyric) by the direct method of Friedemann and Haugen (3), and the resultant color intensities were measured at 515 nm in a Gilford 300 spectrophotometer. Crystalline sodium pyruvate, sodium β-phenylpyruvate, and α-ketobutyric acid were used as standards.

Determination of intracellular serine and pyruvate concentrations. Cultures (150 ml), grown as for inducibility experiments in minimal salts medium supplemented with thiamine hydrochloride (10 μg/ml), the mixture of 21 amino acids (each 20 μg/ml), and various concentrations of L-serine, were harvested at approximately the same growth level during late log growth phase. Cultures were centrifuged at 10,000 $\times$ g for 10 min; the cells were washed with 1 culture volume of 0.05 M potassium phosphate buffer (pH 7.5) and suspended to an absorbancy reading of approximately 20.0 at 660 nm (volume of approximately 5.0 ml). Suspensions were incubated with 0.1 ml of toluene at 37 C for 5 min and then centrifuged at 35,000 $\times$ g for 10 min. Assay of 0.5-ml samples of supernatant fluids for pyruvate was as described above. Samples (2.5 ml) of supernatant fluids were deproteinized by the addition of 0.5 ml of 30% trichloroacetic acid; after standing for 10 min in an ice

bath, samples were centrifuged at 35,000 × g for 10 min. Resultant supernatant fluids were freed from trichloroacetic acid by five successive extractions, each with 2 volumes of ether. Residual ether was removed by evaporation into an airstream directed over the sample surface. Samples were diluted with an equal volume of sodium citrate buffer (0.4 M with respect to sodium and 0.2 M with respect to citrate), pH 2.2; 1.0-ml sample volumes were analyzed for serine content on the 150-cm column of a Beckman/Spinco model 120 amino acid analyzer. The instrument was calibrated with Beckman amino acid calibration mixture (type I).

Protein determination. After overnight digestion of 0.05-ml samples of washed whole cell suspensions in the presence of 0.1 ml of 2.5 N NaOH at room temperature, protein content was determined by the method of Lowry et al. (11). Crystalline bovine serum albumin, similarly treated with NaOH overnight, was used as the standard.

Enzyme units and specific activity. One enzyme unit is defined as the amount of enzyme producing 1.0 μmole of product (keto acid or indole) per min at 37 C. Specific activity is expressed as units of enzyme per milligram of protein.

RESULTS AND DISCUSSION

Effect of substrate on enzyme level. In contrast to the tryptophanase of *E. coli* which is inducible at high levels of tryptophan (7, 12), tryptophanase of *B. alvei* is a constitutive enzyme, not influenced by the exogenous level of tryptophan or related compounds (8). In experiments reported here, tryptophanase was repressed in *B. alvei* cells grown in the presence of moderate to high levels of L-serine (Table 1). Large amounts of indole were excreted by cells grown in the presence of low exogenous serine levels, but indole excretion diminished markedly as the serine concentration was increased. Diminished indole excretion would be the expected consequence of repression of the enzyme responsible for its formation.

L-Serine dehydratase has been shown to be readily inducible in *E. coli* by L-leucine and glycine, but only to a small extent by L-serine (14). In the present investigation, the level of L-serine dehydratase in *B. alvei* was not influenced by the absence of amino acids or by the presence of L-serine, glycine, L-threonine, or L-homoserine at levels of 20 μg/ml (Table 2).

The enzyme, however, although appearing to be constitutive in cells grown in the absence of L-serine and in the presence of low and high levels of the amino acid, was derepressed at least twofold in the presence of intermediate levels of L-serine. Figure 1 shows the repression-derepression-repression sequence as the L-serine level in the growth medium was increased from 0 to 0.1 M. Also note that pyruvate excretion into the

TABLE 1. *Effect of exogenous serine concentration on tryptophanase synthesis and indole accumulation[a]*

Serine concn	Growth	Specific activity	Indole excreted[b]
M			
0	0.91	0.0090	0.0434
0.0005	1.08	0.0081	0.0326
0.001	0.93	0.0088	0.0206
0.005	1.07	0.0090	0.0166
0.01	1.00	0.0087	0.0080
0.02	1.07	0.0047	0.0012
0.04	1.12	0.0040	0.0006
0.10	1.08	0.0040	0.0006

[a] Cells were grown in minimal salts medium supplemented with thiamine hydrochloride (10 μg/ml), a mixture of 21 L-amino acids (each 20 μg/ml), and the indicated concentration of L-serine. Growth is presented as absorbancy at 660 nm; one unit of absorbancy is equivalent to 0.240 mg of cell protein per ml.

[b] Amount (μmoles) of indole excreted into medium per milliliter of culture at corresponding growth level.

TABLE 2. *Effect of single amino acids on L-serine dehydratase synthesis[a]*

Amino acid	Growth	Specific activity
None	.347	.144
Glycine	.343	.146
L-Serine	.336	.149
L-Threonine	.378	.132
L-Homoserine	.359	.139

[a] The data were compiled from three separate experiments. Cells were grown in minimal salts medium supplemented with thiamine hydrochloride (10 μg/ml), 1% glucose, and the indicated amino acid (20 μg/ml). Growth is presented as absorbancy at 660 nm; one unit of absorbancy is equivalent to 0.240 mg of cell protein per ml.

medium did not commence until derepression was maximal.

A tentative explanation for the repression-derepression-repression phenomenon involved speculation concerning internal pyruvate and serine concentrations. It was suggested that, at low serine concentrations, pyruvate is formed predominantly by other metabolic pathways and might serve to repress the dehydratase. Then a gradual increase in serine concentration could override the repression of the enzyme by pyruvate. However, the increased dehydratase activity would result in the formation of larger amounts of pyruvate which could again repress the enzyme.
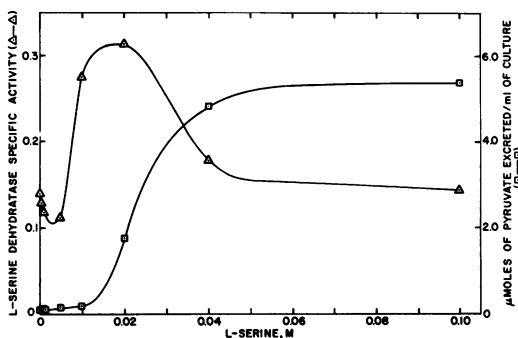
FIG. 1. *Effect of exogenous serine concentration on L-serine dehydratase synthesis and pyruvate excretion. Cells were grown in minimal salts medium supplemented with thiamine hydrochloride (10 μg/ml), a mixture of 21 L-amino acids (each 20 μg/ml), and various concentrations of L-serine. All cultures were harvested at approximately the same level of growth (late exponential growth phase). Samples were centrifuged. Supernatant fluids were assayed for pyruvate as described in Materials and Methods. Cells were washed, resuspended, toluene-treated, and assayed for L-serine dehydratase activity.*

TABLE 3. *Relationship of intracellular serine and pyruvate concentrations to L-serine dehydratase synthesis*[a]

External [Serine]	Growth	Enzyme specific activity	Internal [Serine]	Internal [Pyruvate]	Internal [Pyruvate] /Internal [Serine]
M					
0	1.05	0.091	0.41[b]	1.58[b]	3.85
0.0001	1.12	0.124	2.07	2.41	1.16
0.0002	1.01	0.076	0.57	1.89	3.32
0.0005	1.17	0.132	6.19	3.56	0.58
0.001	1.08	0.127	3.67	3.17	0.86
0.005	1.12	0.179	10.60	3.80	0.36
0.01	1.06	0.302	18.49	3.32	0.18
0.02	1.08	0.206	9.67	3.68	0.38
0.04	1.12	0.097	5.00	1.80	0.36
0.10	1.14	0.100	1.75	0.65	0.37

[a] Growth conditions were as described in Table 1.

[b] Expressed as nanomoles of compound within organisms present in 1.0 ml of culture at indicated growth level.

On the basis of this explanation, one might postulate that only at a certain narrow range of serine concentrations is the internal pyruvate to serine ratio such that the enzyme is maximally derepressed.

However, determination of the intracellular serine and pyruvate concentrations negated the portion of the above theory explaining the second repression phase of the phenomenon. The data in Table 3 indicate that both the internal serine level and the internal pyruvate level varied directly with the specific activity of the enzyme. At exogenous serine concentrations varying from 0.005 to 0.1 M, the ratio of internal pyruvate to internal serine remained constant. If increased internal pyruvate concentration had been responsible for the repression of the enzyme at high exogenous serine concentrations, then the ratio of internal pyruvate to internal serine would have increased at these high exogenous concentrations.

Since the enzyme was again repressed at exogenous serine concentrations above 0.01 M, it would seem likely that, although sufficient exogenous serine was present, the transport system across the cell membrane was not rapid enough to supply the additional serine necessary to both keep the enzyme derepressed and at the same time serve as substrate for the enzyme. Thus, with the serine transport system operating at its maximal rate, enzyme synthesis would decrease to a level at which it would remain

constant regardless of any further increase in exogenous serine concentration.

This explanation is supported by the recent work of Kay and Gronlund (10). They demonstrated that serine is in the group of seven amino acids exhibiting the lowest rates of transport into whole cells of *Pseudomonas aeruginosa* and that the amino acid transport system of the organism becomes saturated at high external amino acid concentrations. Serine was shown to have a 19-fold lower rate of transport than, for example, leucine.

Effect of catabolites on enzyme synthesis and product excretion. It had been reported previously (J. A. Hoch, Ph.D. Thesis, Univ. of Illinois, Urbana, 1965) that tryptophanase from *B. alvei* is not subject to catabolite repression. The current investigation has shown that the enzyme is definitely subject to repression by glucose and to an even greater extent by glycerol (Fig. 2). Although during the middle period of exponential growth (4th to 8th hr) the absorbancy of the control culture lagged approximately 1 hr behind that of either the glucose-containing or the glycerol-containing culture, differences in enzyme activity did not appear to be related to differences in growth of the cultures. The tryptophanase of *E. coli* is known to be repressible by glucose (2, 6).

Another manifestation of catabolite repression is evident in Fig. 3. *B. alvei* excretes indole at two stages in its growth cycle (8). "Early" indole is excreted as a result of indole-3-glycerol phosphate fission and is then reutilized in the trypto-
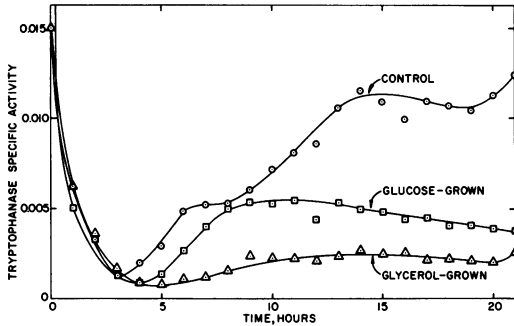
FIG. 2. *Effects of glucose and glycerol on trypto-phanase synthesis. Growth and assay conditions were as described in Materials and Methods. Cultures were sampled hourly and the growth was measured. Samples were centrifuged, and the supernatant fluids were retained for indole determination (see Fig. 3) and pyruvate determination (see Fig. 5). The cells were washed, resuspended, toluene-treated, and assayed for trypto-phanase activity (above figure) and L-serine dehydratase activity (see Fig. 4).*
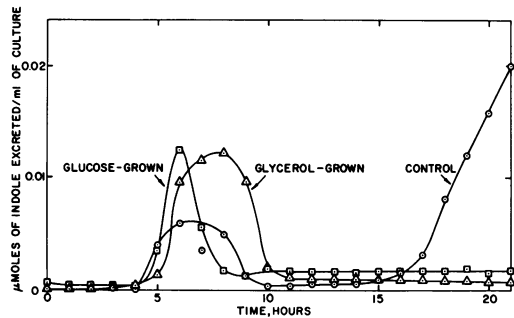

FIG. 3. *Effects of glucose and glycerol on excretion of indole during growth. Conditions are as described in Fig. 2. Samples (0.5 ml) of culture supernatant fluids were assayed for indole by the addition of 3 ml of color reagent, incubation at room temperature for 20 min, and measurement of color intensities at 568 nm.*

phan synthetase-catalyzed biosynthesis of trypto-phan. The excretion of indole late in the growth cycle is a consequence of tryptophanase activity. "Late" indole excretion was completely eliminated when the organisms were grown in the presence of 1% concentrations of either glucose or glycerol.

In all three cases, "early" indole was excreted and then reutilized, suggesting that tryptophan synthetase is probably not repressible by either glucose or glycerol. This possibility was substantiated in C. W. Roth's studies on tryptophan synthetase in *B. alvei* (Ph.D. Thesis, Univ. of Illinois, Urbana, 1969). The 1% glucose present in media used in his experiments did not appreciably affect the production and reutilization of

"early" indole or the level of tryptophan synthetase in the organisms. Furthermore, Freundlich and Lichstein (2) have found not only that glucose fails to repress tryptophan synthetase in *E. coli*, but also that high glucose levels result in an increased enzyme level.

The effect of glucose on L-serine dehydratase synthesis differed markedly from the effect of the same compound on the synthesis of tryptophanase. The specific activity curves of the control culture and the glucose-grown culture are quite different in shape (Fig. 4). However, the areas under the curves are similar, indicating that the total amounts of enzyme formed during the growth cycle of the organism with and without glucose were the same. Again, differences in enzyme activity did not appear to be related to differences in growth of the cultures (4th to 8th hr). The mechanism responsible for the displacement of L-serine dehydratase synthesis by glucose is not clear at the present time. It may be noted that glycerol exerted a repressive effect on this enzyme also.

Pyruvate excretion in the absence of added glucose or glycerol and in the presence of glucose showed a slight increase early in the exponential growth phase and then dropped to a relatively low constant level (Fig. 5). In the case of glycerol, however, pyruvate was excreted in copious amounts during exponential growth. The excreted pyruvate is probably derived from glycerol. From the repression of the dehydratase by glycerol and the excretion of pyruvate, it may be suggested that the conversion of glycerol to pyruvate is largely unregulated in comparison to the conversion of glucose to pyruvate.

Optimal pH for enzyme activity. It has been concluded by Hoch et al. (9) that tryptophanase in crude extracts of *B. alvei* possesses maximal activity at pH 9.0 in glycine-phosphate buffer, but that this optimum shifts to pH 7.5 to 8.4 in phosphate-acetate-borate buffer upon purifica-
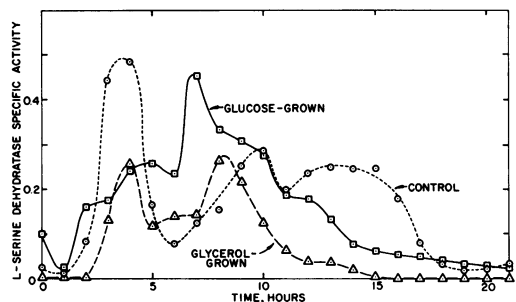

FIG. 4. *Effects of glucose and glycerol on L-serine dehydratase synthesis. Conditions are as described in Fig. 2.*
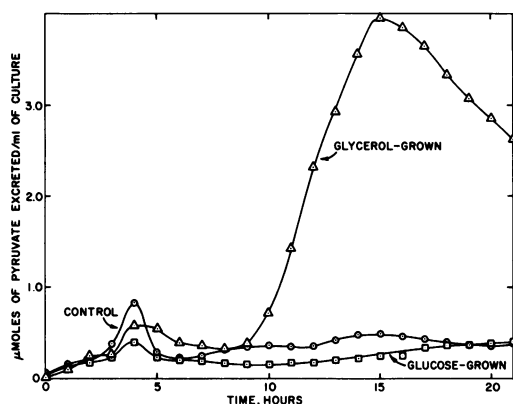
FIG. 5. Effects of glucose and glycerol on excretion of pyruvate during growth. Conditions are as described in Fig. 2. Samples (0.25 ml) of control and glucose-grown culture supernatant fluids and 0.1-ml samples of glycerol-grown culture supernatant fluids were assayed for pyruvate as described in Materials and Methods.

tion of the enzyme. Analysis of additional experiments (Hoch, unpublished data) showed that the purified enzyme has a pH optimum of 8.2 in potassium phosphate buffer. The present investigation yielded a pH optimum of approximately 8.2 for tryptophanase in toluene-treated whole cell preparations in potassium phosphate buffer (Fig. 6A).

L-Serine dehydratase activity in toluene-treated whole cell preparations in potassium phosphate buffer was optimal at pH 7.5 (Fig. 6B).

Effect of monovalent cations and tris(hydroxymethyl)aminomethane (Tris) on enzyme activity. Activation of tryptophanase by K^+ and NH_4^+ and inhibition of activity by Na^+ have been demonstrated by Hoch et al. (9). Furthermore, inhibition of enzyme activity in the presence of Tris has been suggested by Gopinathan and DeMoss (4, 5) to be the result of Schiff-base formation between Tris and the coenzyme pyridoxal phosphate, followed by dissociation of the enzyme into inactive subunits. The same activation by K^+ and NH_4^+ and inhibition by Na^+ and Tris were observed in the current investigation (Table 4). All cations were present in the form of phosphate buffer (pH 7.5) at a final concentration of 0.09 M with respect to cation and 0.05 M with respect to phosphate. Tris, present at a final concentration of 0.05 M, was adjusted to pH 7.5 with hydrochloric acid.

Examination of L-serine dehydratase activity in the presence of the same monovalent cations yielded somewhat different results. All three cations stimulated enzyme activity (Table 4). As in the case of tryptophanase, Tris was inhibitory

to dehydratase activity, though not to the same degree. Whether the observed inhibition was a result of partial dissociation of the enzyme into inactive or partially active subunits has not been determined.

Affinity of tryptophanase and L-serine dehydratase for their respective substrates. The saturation curve for the L-serine dehydratase-L-serine reaction (Fig. 7) and the double reciprocal plot of the data (Fig. 8) indicate that inhibition of enzyme activity occurred at high substrate concentrations. To test whether this inhibition was due to product accumulation, enzyme activity was examined in the presence of 0.1 M L-serine (the usual assay level) and increasing
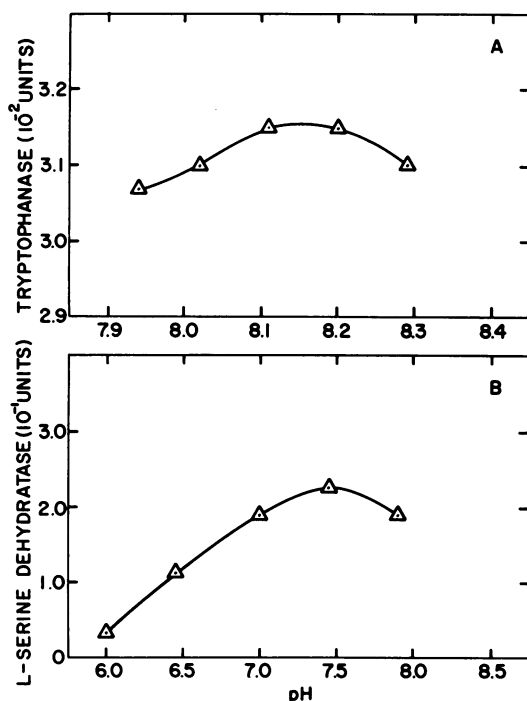

FIG. 6. Effect of the pH of potassium phosphate buffer on enzyme activity. (A) Tryptophanase. Reaction mixtures contained 25 μmoles of buffer of appropriate pH, 4 μmoles of L-tryptophan, 32 nmoles of pyridoxal phosphate, and toluene-treated cell suspension in a final volume of 0.5 ml. After 10 min of incubation at 37 C, reactions were terminated by the addition of 3 ml of color reagent. The pH values were determined separately on fourfold quantities of the reaction mixtures after incubation for 10 min at 37 C (without termination of reactions). (B) L-Serine dehydratase. Conditions are as described for (A), except that 50 μmoles of L-serine was substituted for L-tryptophan, reactions were terminated by the addition of 0.5 ml of 10% trichloroacetic acid, and supernatant fluids were assayed for pyruvate.

concentrations of pyruvate. At initial pyruvate levels ranging from 0 to 0.1 M, enzyme activity remained constant. Thus, the apparent substrate inhibition was actual inhibition.

In the case of tryptophanase, inhibition of activity also occurred at high tryptophan levels when the modified version of the assay procedure of Pardee and Prestidge was used. This method prevents removal of indole as it is formed. Use of the modification of the assay procedure of Boezi and DeMoss, whereby indole is removed

TABLE 4. *Effect of monovalent cations and Tris on tryptophanase and L-serine dehydratase activities[a]*

Enzyme	Cation or Tris	Reaction final pH	Enzyme units	Per cent of K^+ activity
Tryptophanase	K^+	7.63	0.0083	100
	NH_4^+	7.45	0.0081	98
	Na^+	7.49	0.0023	28
	Tris	7.77	0.0015	18
	Water	8.22	0.0032	38
L-Serine dehydratase	K^+	7.59	0.272	100
	NH_4^+	7.53	0.289	106
	Na^+	7.59	0.221	81
	Tris	7.71	0.127	47
	Water	7.72	0.174	64

[a] All cations were present in the form of phosphate buffer (pH 7.5) at a final concentration of 0.09 M with respect to cation and 0.05 M with respect to phosphate. Tris, at a final concentration of 0.05 M, was adjusted to pH 7.5 with hydrochloric acid. The pH of cell suspensions in distilled water was 7.5, and the pH of the distilled water reaction mixtures was 7.3 at the onset of incubation.
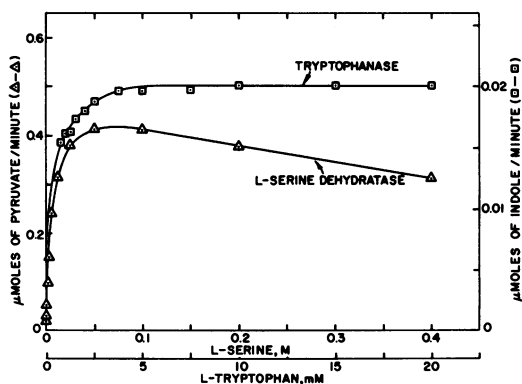

FIG. 7. *Affinities of tryptophanase for L-tryptophan and L-serine dehydratase for L-serine. Assay conditions were as described in Materials and Methods, except that various levels of the substrates were incorporated into the reaction mixtures.*
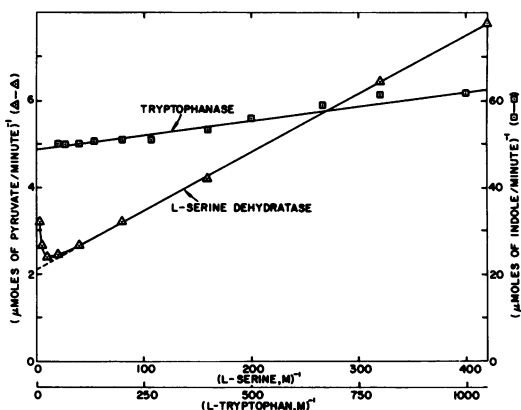

FIG. 8. *Affinities of tryptophanase for L-tryptophan and L-serine dehydratase for L-serine. Double reciprocal (Lineweaver-Burk) plots of the data from Fig. 7.*

by dissolution in the toluene overlay, eliminated all traces of substrate inhibition at high tryptophan concentrations.

Note (Fig. 7) that the range of L-serine concentrations for maximal L-serine dehydratase activity is very narrow as compared to that of L-tryptophan for maximal tryptophanase activity.

The apparent K_m (L-tryptophan) for tryptophanase, calculated from the data in Fig. 8, is 0.267 mM. K_m values of 0.272 mM and 0.273 mM for L-tryptophan were obtained by Hoch et al. (9) and by S. R. O'Neil (Ph.D. Thesis, Univ. of Illinois, Urbana, 1969), respectively, by using purified tryptophanase preparations. The value obtained with toluene-treated whole cell preparations is in good agreement with those obtained for the purified enzyme.

The apparent K_m (L-serine) for L-serine dehydratase, calculated from the data in Fig. 8, is 6.30 mM. O'Neil (Ph.D. Thesis, Univ. of Illinois, Urbana, 1969) obtained a K_m value of 0.129 M for the tryptophanase-catalyzed deamination of L-serine. In view of the 20-fold difference in affinities of the two enzymes for the same substrate and keeping in mind that the concentration of L-serine during enzyme assays was optimal (0.1 M) for the L-serine dehydratase-L-serine reaction, it seems reasonable to assume that the total L-serine deamination obtained in the current investigation was a result of L-serine dehydratase activity and not of tryptophanase activity.

Specificity of L-serine dehydratase. Table 5 shows the effect of L-serine dehydratase on various amino acid substrates. L-Threonine and L-tryptophan were not cleaved in the presence of the enzyme. The efficiency of the enzyme in catalyzing the degradation of D-serine was less than 1% of that for the L-isomer. However, when subjected

TABLE 5. *Effect of L-serine dehydratase on amino acid substrates*

Substrate	Final concn	Keto acid formed	Per cent of L-serine activity
	M	*μmoles/ min*	
Expt A			
L-Serine	0.05	0.340	100
D-Serine	0.10	0.000	0
DL-α-Methylserine	0.08	0.002	0.6
DL-β-Phenylserine	0.06	0.184	54
DL-O-methylserine	0.08	0.000	0
Cycloserine	0.10	0.000	0
L-Threonine	0.08	0.004	1.2
L-Tryptophan	0.02	0.000	0
Expt B			
L-Serine	0.10	0.456	100
D-Serine	0.10	0.004	1
L-Threonine	0.08	0.000	0
L-Serine	0.10	0.214	47
D-Serine	0.10		
L-Serine	0.10	0.464	102
L-Threonine	0.08		

to equimolar concentrations of the two isomers, the enzyme was only one-half as effective in catalyzing the degradation of L-serine, suggesting that D-serine is able to occupy an active site on the enzyme. D-Serine has, in fact, been shown to be a competitive inhibitor of the L-serine dehydratase-L-serine reaction. In this capacity, D-serine exhibited a K_i of 4.56 mM (*unpublished data*).

Of the serine analogues tested (Table 5), only the β-phenyl derivative was cleaved in the presence of the enzyme and then only 54% as effectively as L-serine. Since the β-phenylserine used as the substrate was a racemic mixture, the reduced efficiency of the enzyme in catalyzing cleavage of the β-phenyl compound might be explained as in the preceding paragraph; that is, the D-isomer may serve as a competitive inhibitor of the reaction.

We conclude that the two enzymes in *B. alvei* which catalyze the deamination of L-serine are quite dissimilar in the physiological properties

investigated. The dehydratase is currently being purified in our laboratory, so that a more accurate physical comparison of the two enzymes can be achieved.

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