**D-Serine Dehydratase from Escherichia coli**

IV. COMPARATIVE SEQUENCES OF PYRIDOXYLPEPTIDES DERIVED FROM THE ACTIVE SITE AND FROM AN INHIBITORY SITE OF THE ENZYME*

(Received for publication, July 24, 1972)

YEH ZEN HUANG AND ESMOND E. SNELL

From the Department of Biochemistry, University of California, Berkeley, California 94720

**SUMMARY**

D-Serine dehydratase from *Escherichia coli* has two distinct binding sites for pyridoxal 5'-phosphate; a high affinity site ($K_{diss} = 0.03 \mu M$) which binds the catalytically essential coenzyme and a low affinity site ($K_{diss}$ near 1000 $\mu M$) which binds pyridoxal-P, pyridoxal, or 5-deoxypyridoxal approximately equally, with resultant inhibition of enzymatic activity.

Following borohydride reduction, carboxymethylation and chymotryptic digestion of the holoenzyme, a single nonapeptide containing the N6-pyridoxyllysine residue, and thus corresponding to a portion of the high affinity coenzyme binding site was isolated by two different chromatographic procedures and shown to have the sequence: Ser-(Pxy)Lys-Gly-Arg-Ile-Asn-Lys-Ala-Thr. The peptide differs from the corresponding coenzyme-binding site peptides isolated from other pyridoxal-P-dependent enzymes in its highly basic and relatively hydrophilic character. When borohydride reduction of D-serine holodehydratase was carried out in the presence of an inhibitory concentration (7.5 mM) of pyridoxal-P, one additional major pyridoxylpeptide, corresponding to a portion of the low affinity pyridoxal-binding site was isolated. The sequence of this inhibitory site peptide was determined to be: Val-(Pxy)Lys-Ala-Gly-Ala-Phe.

Crystalline D-serine dehydratase (EC 4.2.1.14) from *Escherichia coli* contains a single peptide chain (molecular weight 45,500) and one catalytically essential pyridoxal-P per molecule (1). The coenzyme can be covalently attached to the epsilon amine group of a lysine residue by reduction with sodium borohydride (1), as in all other pyridoxal-dependent enzymes so far examined. Amino acid sequences near the lysine residue that participates in binding of pyridoxal-P have recently been summarized for several pyridoxal-P enzymes (2). Comparison of these sequences might reveal certain structural similarities in the immediate vicinity of the cofactor. As part of such a study, the isolation and determination of the sequence of a nonapeptide containing the lysine residue which interacts with pyridoxal-P in D-serine dehydratase are described here.

At concentrations far higher than those required for holoenzyme formation pyridoxal-P inhibits D-serine dehydratase, indicating the presence of one or more binding sites with low affinity for pyridoxal-P (3). Reinvestigation of this inhibition shows that it results from binding of pyridoxal-P mostly at one site on the protein. The sequence around this pyridoxal-P-binding inhibitory site is also reported here.

**EXPERIMENTAL PROCEDURE**

**Materials**

D-Serine dehydratase was prepared by a procedure described previously (1). 1-Chloro-3-tosylamido-7-amino-2-heptanone-treated chymotrypsin was the gift of Dr. F. H. Carpenter of this department. *E. coli* alkaline phosphatase, L (1 tosylamido 2phenyl)ethyl chloromethyl ketene-treated trypsin, diisopropyl fluorophosphatetreated carboxypeptidase A, and leucine aminopeptidase were obtained from Worthington. N4-Pyridoxalysine (4) and 5-deoxypyridoxal (5) were synthesized according to the cited methods. N,N-Dimethylallylamine, phenylisothiocyanate, 1,2-dichloroethane, ethyl acetate, trifluoroacetic acid, pyridine, and benzene were all redistilled before use. Iodoacetic acid was recrystallized twice from hot chloroform. "Ultrapure" guanidine hydrochloride was from Mann Research Laboratories. All other chemicals were reagent grade and were used without further purification.

**Methods**

**Preparation of Borohydride-reduced and Carboxymethylated D-Serine Dehydratase**—For study of the high affinity binding site for pyridoxal-P, 300 mg of crystalline native D-serine holodehydratase (specific activity 310) were dissolved in 15 ml of 0.1 M potassium phosphate buffer, pH 7.2, containing 1.0 mM diethiothreitol and 5 $\mu M$ pyridoxal-P. Then, 0.9 ml of a 3% solution of NaBH4 in distilled water was added dropwise over a period of 10 min, while the enzyme solution was gently stirred then allowed to stand at 0° with stirring for 30 min. The reduced holoenzyme preparation was dialyzed 24 hours against two 5-liter changes of distilled water in the dark at 4°.
For study of the low affinity pyridoxal-P binding site, 300 mg of the pure dehydratase were dissolved in 150 ml of 0.1 M potassium phosphate-7.5 mM pyridoxal-P buffer, pH 7.8. After 30 min at 25°C the solution was brought to 0°C and 33 ml of a 3% solution of NaBH₄ in distilled water was added slowly, with gentle stirring, while the pH was controlled at 7.8 by the slow solution of NaBHa in distilled water was added slowly, with 30 min at 25°C the solution was brought to 0°C and 33 ml of a 3% solution of NaBH₄ in distilled water was added slowly, with gentle stirring, while the pH was controlled at 7.8 by the slow addition of NaBH₄ and the temperature was kept at 0°C. The solution was then allowed to stand at 0°C with stirring for 30 min, then concentrated to about 30 ml by rotary evaporation under vacuum and dialyzed for 36 hours against three 10-liter changes of distilled water in the dark at 4°C.

These reduced and dialyzed samples were carboxymethylated at pH 8.5 by the procedure of Crestfield et al. (6), then dialyzed in the dark at 4°C against several changes of distilled water for a total of about 40 hours.

Chymotryptic Digestion—The borohydride-reduced and carboxymethylated preparations of d-serine dehydratase were suspended in 35 ml of 1% NH₄HCO₃ solution and 3 mg of lyophilized 1-chloro-3-tosylamido-7-amino-2-heptanone-treated chymotrypsin were added. The digestions were carried out with gentle stirring at 25°C in the dark for 15 hours with the further addition of 3 mg of the chymotrypsin preparation after 3 hours. The digests were then adjusted to pH 3 with 2 N HCl and lyophilized.

Amino Acid Analysis—Samples were deaerated, hydrolyzed in sealed tubes with 6 N HCl for 20 to 50 hours at 110°C, then analyzed for amino acids by the method of Spackman et al. (7) with an automatic amino acid analyzer (Beckman model 120C). N°-Pyridoxyllysine does not separate from histidine under the standard conditions used for amino acid analysis, but was completely separated from other amino acids by replacing the sodium citrate buffer (pH 5.28) usually used for the short column of the amino acid analyzer by the same buffer adjusted to pH 4.55 with concentrated HCl (1). N°-Pyridoxyllysine was eluted from the column between 100 and 110 min at a flow rate of 68 ml per hour.

Sequential Edman Degradation of Peptides—The phenylisothiocyanate procedure in its three-stage form was used (8, 9). The liberated phenylthiohydantoin derivatives were identified by comparison with authentic standards after chromatography on Eastman chromatogram sheets No. 6060 (silica gel with fluorescent indicator) in Solvent Systems II and III of Brenner et al. (10). Phenylthiohydantoin-amino acids were located by their fluorescence under ultraviolet light. The phenylthiohydantoin derived from arginine is soluble in water and was identified by use of the Sakaguchi reagent (11) following paper electrophoresis at pH 6.5 and 2000 volts for 1 hour with authentic standards. Subtractive analysis for amino acids was also carried out at each degradation step (11).

Enzymatic Digestions of Peptides—Approximately 0.1 μmole of pyridoxylpeptide was treated at 37°C with either 10 μg of leucine aminopeptidase (disopropyl fluorophosphate-treated) in 0.2 ml of 0.05 M Tris-HCl buffer (pH 8.5) containing 5 mM MgCl₂, or with 50 μg of disopropyl fluorophosphate-treated carboxypeptidase A in 0.2 ml of 0.05 M Tris-HCl buffer (pH 7.5). The latter enzyme (1 mg per ml) was dissolved in 10% (w/v) LiCl before use (13). These digestions were terminated by adding acetic acid until the pH was below 3; the mixtures were then lyophilized. Amino acids released were determined quantitatively on the amino acid analyzer. Control experiments were identical except that the peptide was omitted.

RESULTS

Isolation and Composition of High Affinity Coenzyme binding Site Peptide—All of the pyridoxal-P present in d-serine holodehydratase appears in chymotryptic digests of the reduced carboxymethylated enzyme as a single fluorescent zone, well separated from other peptides, following chromatography of the digest over Dowex 1 and peptide mapping of the combined fluorescent fractions (Fig. 1). The fluorescent peptide was first isolated on a preparative scale by the same procedure described in Fig. 1 by applying the peptide fraction (equivalent to 5 μmoles of pyridoxylpeptide as measured by absorbance at 325 nm) as a band on Whatman No 3MM paper for electrophoresis, then eluting the material in the fluorescent band, lyophilizing, and reapplying it as a band on a second paper for the chromatographic separation. Elution from the paper in each case was with 30% acetic acid; the yield of purified peptide was about 16% of that present in the chymotryptic digest.

A simpler procedure, applicable for preparation of larger amounts of the pyridoxylpeptide, was that developed by Fischer et al. (2, 14), in which the P-pyridoxylpeptide fraction from a given column is modified by removing the phosphate residue by phosphatase digestion, and is then rechromatographed over the same column to separate contaminating peptides from the

![Chromatography](http://www.jbc.org/)

*Fig. 1. Peptide map of the fluorescent fractions isolated from a chymotryptic digest of reduced carboxymethylated n-serine holodehydratase by chromatography over Dowex 1. The lyophilized chymotryptic digest from 300 mg of reduced and carboxymethylated n-serine holodehydratase was dissolved in 5 ml of 2% pyridine and 0.01% acetic acid (w/v), pH 7.3, centrifuged to remove traces of insoluble material, and applied to a column (1 X 115 cm) of Dowex AGI-X2 (200 to 400 mesh, acetate form) previously equilibrated with the same buffer. The column was eluted with this same buffer at 25°C and a flow rate of 20 ml per hour. Fractions (5 ml) were collected and monitored for ninhydrin color values at 570 nm (26) and for the covalently bound pyridoxyl group by their absorbance at 325 nm. The pyridoxylpeptides were not retained by this column and appeared in the first major peptide fraction to be eluted. A sample of this fraction (containing about 0.04 μmole of the pyridoxylpeptide) was chromatographed in the first dimension (solvent butanol-1-pyridine-glacial acetic acid-water, 90:10:18:72) followed by electrophoresis in the second. Hatched zones are fluorescent when viewed in ultraviolet light prior to ninhydrin treatment.*
newly formed pyridoxylpeptide. This procedure, described in Fig. 2, gives pure peptide (as indicated by peptide mapping and analysis) in 40% yield in only two steps, and is clearly the isolation method of choice.

The amino acid composition of these independently prepared samples of the pyridoxylpeptide was identical within experimental error; a representative analysis is shown in Column 2 of Table I and corresponds to that of a nonpeptide. The presence of N₆-pyridoxyllysine and the absence of histidine was verified by paper electrophoresis against appropriate standards.

Sequence Analysis of High Affinity Coenzyme Binding Site Peptide—Extensive digestion of the fluorescent peptide with leucine aminopeptidase (15 hours, 37°) liberated all of its component amino acids except aspartic acid. The amount of serine indicated by amino acid analysis was twice that obtained on acid hydrolysis, indicating the presence of asparagine (which elutes with serine under the standard conditions of analysis) instead of aspartic acid in the peptide. Digestion of the peptide with leucine aminopeptidase for a limited time (10 min, 37°) liberated serine (0.15 residue), N₆-pyridoxyllysine (0.05 residue), and glycine (0.02 residue), corresponding to the probable NH₂-terminal sequence Ser-(Pxy)Lys,Gly-. Carboxypeptidase A (15 hours, 37°) released threonine (0.2 residue), alanine (0.13 residue), and lysine (0.06 residue) indicating that the COOH-terminal amino acid was threonine and the sequence was probably -(Lys,Ala)-Thr. Table I shows the results of amino acid analysis of the peptide remaining after each of five sequential Edman degradations. Subtractive analysis following further degradation was ambiguous, but the results clearly indicate the sequence of the first 5 residues to be Ser-(Pxy)Lys,Gly-Arg-Ile.-

To determine the sequence of the last four amino acids, the fluorescent peptide was subjected to trypsin digestion. On paper electrophoresis at pH 3.6 and 2000 volts for 60 min, the trypsin digest showed three ninhydrin-reactive spots, only one of which exhibited fluorescence (Fig. 3). The peptides from the main

| Residue | Composition after each degradative cycle |
|---------|----------------------------------------|
|         | 0  | 1st | 2nd | 3rd | 4th | 5th |
| Lys.    | 1.12 | 1.17 | 1.13 | 1.05 | 1.09 | 1.05 |
| Pxy)Lys. | 0.86 | 0.77 | 0.18 | 0.3 | 0.06 | 0.05 |
| Arg.    | 1.05 | 1.06 | 1.00 | 0.95 | 0.34 | 0.24 |
| Asx.    | 1.09 | 0.90 | 1.00 | 0.90 | 0.92 | 0.92 |
| Thr.    | 1.00 | 0.92 | 0.95 | 0.90 | 0.92 | 0.92 |
| Ser.    | 0.95 | 0.19 | 0.48 | 0.00 | 0.00 | 0.00 |
| Gly.    | 1.03 | 1.08 | 1.09 | 0.30 | 0.25 | 0.21 |
| Ala.    | 1.00 | 1.00 | 1.05 | 1.1 | 1.00 | 1.02 |
| Ile.    | 0.91 | 0.90 | 0.91 | 1.00 | 0.20 | 0.50 |

* Experimental values which decrease significantly during a given reaction cycle are underlined.

The trace of serine present (<0.19) was not estimated quantitatively.

Fig. 2. Isolation of the high affinity pyridoxal-P-binding site peptide by the differential technique. Lyophilized chymotryptic digest from 300 mg of reduced and carboxymethylated d-serine holodehydratase was dissolved in 5 ml of 0.1 M pyridine-acetate, pH 6.05, centrifuged to remove insoluble material, and applied to a column (2 X 30 cm) of Bio-Rex 70 (a polycarboxylic acid resin, minus 400 mesh, sodium form) previously equilibrated with the same buffer. The column was developed at 25° and a flow rate of 10 ml per hour first with 300 ml of the pH 6.05 buffer (A, in 4), then with 450 ml of 0.5 N acetic acid (B, in 4). Fractions (3 ml) were collected and monitored for ninhydrin color values at 570 nm and for pyridoxylpeptides at 325 nm. The latter fractions were pooled, lyophilized, dissolved in 2 ml of 0.05 M NaHCO₃ (pH 8.2), and treated with 0.3 mg of crystalline Escherichia coli alkaline phosphatase for 3 hours at room temperature (14). The phosphatase digest was then lyophilized, dissolved in 1 ml of 0.1 M pyridine-acetate buffer (pH 6.05), and rechromatographed on the same column of Bio-Rex 70 (B).

Fig. 3. Comparative migration on electrophoresis of N₆-pyridoxyllysine (N₆-Pxy-lysine) and of peptides released by tryptic digestion of the high affinity coenzyme-binding site peptide. For tryptic digestion, approximately 0.5 μmole of the parent peptide was treated with 0.2 mg of r.1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin in 0.05 M Tris-HCl buffer, pH 8.0, at 37° for 15 hours. The reaction was terminated by the addition of acetic acid and the mixture was lyophilized. A sample (about 0.03 μmole) of the peptide mixture and of N₆-pyridoxyllysine were then spotted on filter paper and subjected to electrophoresis at pH 3.6 and 2000 volts for 1 hour. Hatched zones are fluorescent; all zones are ninhydrin-positive.
portion of the digest were then purified by preparative paper electrophoresis and analyzed after acid hydrolysis. Peptide T-1 contained N'-pyridoxyllysine, 0.74 (I); arginine, 1.00 (I); serine, 0.93 (I); glycine, 1.05 (I); and lysine, 0.17 (θ); its composition corresponds to that expected for the NH2-terminal tetrapeptide arising from cleavage of the -Arg-Ile- bond in the parent nonapeptide, the small amount of lysine present arising as a result of partial decomposition of N'-pyridoxyllysine during acid hydrolysis (15). Peptide T-2 contained lysine, 1.03 (I); aspartic acid, 0.86 (I); and isoleucine, 1.14 (I), indicating that the sequence is probably -Ile-Asn-Lys- in terms of the results of Edman degradation (Table I) and the specificity of trypsin. Peptide T-3 contained alanine, 1.03 (I) and threonine, 0.97 (I); and indicates that the COOH-terminal sequence is -Ala-Thr in terms of the results of carboxypeptidase A digestion. Peptide T-2 released the phenylthiohydantoin derivatives of isoleucine and asparagine, respectively, in the first and second Edman degradation cycle; subtractive analysis following the first cycle (lysine, 1.05 (I); aspartic acid, 0.96 (I); isoleucine, 0.13) also showed that isoleucine had been removed, and only free threonine was present in the unhydrolyzed solution after the second degradative cycle. Peptide T-3 released phenylthiohydantoin-alanine after one reaction cycle and only free threonine was present in the unhydrolyzed solution.

These sequence studies are summarized in Fig. 4; there appear to be no ambiguities in assigning the sequence shown for the high affinity coenzyme-binding site of D-serine dehydratase.

### Inhibition of D-Serine Holodehydratase by High Concentrations of Pyridoxal Analogs

The inhibition data previously reported (3) were obtained by first inactivating the enzyme with pyridoxal P and then diluting 50-fold for immediate assay of enzymatic activity. Since inhibition was slowly reversible on dilution, this procedure underestimated its actual extent. To avoid the high blanks produced by high concentrations of pyridoxal analogues and thus permit assay of serine dehydratase at the same concentration of pyridoxal analogues used for its inactivation, the assay procedure was modified as described in Fig. 5, which shows about 90% inhibition of D-serine holodehydratase by 9 mM pyridoxal-P. Pyridoxal and 5-deoxy-pyridoxal inhibit almost as effectively as pyridoxal-P; thus the 5'-phosphate residue does not facilitate binding at the inhibitory site and is not necessary for inhibition.

#### Stoichiometry of Binding of Pyridoxal-P with D-Serine Holodehydratase in Presence of High Concentrations of Pyridoxal Analogue

Following reaction with NaBH4 and acid hydrolysis, D-serine holodehydratase yielded 1 mole of N'-pyridoxyllysine per mole of enzyme (Table II), corresponding to fixation of the coenzyme to the high affinity binding site. Reduction in the presence of sufficient pyridoxal-P to inhibit enzymatic activity by 90%, yields slightly over 2 moles of N'-pyridoxyllysine per mole of enzyme (Table II) with a corresponding decrease in the amount of lysine. Inhibition of the holoenzyme by pyridoxal-P is thus accompanied by the binding of 1 eq of pyridoxal-P per mole of enzyme as a Schiff's base to a lysine residue at one or more sites distinct from that occupied by the catalytically essential pyridoxal-P residue.

### Isolation and Sequence Analysis of Low Affinity Inhibitory Site Peptide

Chymotryptic digests of preparations of D-serine dehydratase that had been reduced with borohydride in the presence of inhibitory concentrations of pyridoxal-P then carboxymethylated, contained two pyridoxylpeptides in large amounts (Fig. 6). The second of these (Fractions 136 to 150, Fig. 6A)
TABLE II

LySine and N^e-pyridoxyllysine content of o-serine holodehydratase after reduction with NaBH₄ in presence and absence of inhibitory concentrations of pyridoxal-P

| Pyridoxal-P added to reaction mixture | LySine residues/mole enzyme | N^e-Pyridoxyllysine |
|-------------------------------------|-----------------------------|---------------------|
| 0                                   | 18                          | 1.0                 |
| 7.5                                 | 16.5                        | 2.1                 |
| 9.0                                 | 15.8                        | 2.3                 |

elutes from the Bio-Rex column at the same position as the high affinity coenzyme binding site peptide (cf. Fig. 2A) and is identical with that peptide. The new pyridoxylpeptides in Fractions 10 to 35 arise as the result of the presence of inhibitory concentrations of pyridoxal-P during borohydride reduction, and will be referred to as the inhibitory site peptides. Fractions 10 to 22 were collected, lyophilized, and applied to a Dowex AG 1 column (Fig. 6B). One major and two minor peptides absorbing at 325 nm appeared. It is not known whether the three pyridoxylpeptides result from combination of pyridoxal-P at independent low affinity sites, or whether they represent different stages in the chymotryptic digestion of a single sequence carrying the pyridoxyl group. The major inhibitory site peptide, after digestion with phosphatase and reapplication to the same column (Fig. 6C) was pure as indicated by peptide mapping; approximately 1 µmole of the peptide was isolated from 6 µmoles of o-serine dehydratase. Its analysis (Column 2, Table III) corresponds to a hexapeptide.

Valine was identified as the NH₂-terminal amino acid by thin layer chromatography of the phenylthiohydantoin derivative and also by subtractive analysis after Edman degradation (Table III). Short term (10 min, 37°) carboxypeptidase A digestion liberated phenylalanine (0.75 residue), alanine (0.5 residue), and glycine (0.08 residue) indicating a probable COOH-terminal sequence, -Ala-Phe. From these results and those of subtractive Edman degradation (Table III) there appears no ambiguity in assigning the sequence Val-(Pxy)Lys-Ala-Gly-Ala-Phe to the principal inhibitory site peptide (Fig. 4B).

DISCUSSION

The structure of pyridoxylpeptides from the coenzyme-binding sites of various pyridoxal-P proteins is compared in Table IV. The active site peptide from o-serine dehydratase is easily the most polar and basic of the group, basic amino acids comprising one-third of its total residues, a frequency over 3 times that expected from the average amino acid composition of the enzyme (1). It is also unique among the pyridoxylpeptides from E. coli enzymes so far studied (but resembles the mammalian transaminases and phosphorylase) in that the amino acid residue on the NH₂-terminal side of the pyridoxyllysine residue is not basic. In particular, it differs markedly from the corresponding sequence in tryptophanase, which catalyzes α,β-elimination reactions of several L-amino acids (including L-serine (16)) formally similar to the reaction catalyzed by o-serine dehydratase (17). All of the enzymes of Table IV except phosphorylase catalyze reactions which, either in the forward or reverse sense, require labilization of a hydrogen atom on the carbon atom corresponding to the α-carbon atom of the substrate amino acid. If these active site peptides contain any common catalytic residues which assist in this act, the only likely candidate is the lysine residue which in these peptides carries the pyridoxyl group, since this is the only...
residue they share in common. In the parent enzymes this lysine residue is present as an azomethine of pyridoxal-P, but according to current concepts (18) its ε-amino group is freed when the enzyme-substrate complex is formed, and if it remains in close proximity to the amino acid (cf. 19, 20) would be free to act as a proton acceptor or donor. In all of the decarboxylases, as well as in tryptophanase and tryptophan synthetase, a histidine or a lysine residue immediately precedes the pyridoxyllysine residue of these peptides, and it has been suggested that this residue could play a catalytic role (14, 21). This possibility is not, of course, eliminated by the present results. In space-filling models, however, these residues are so placed as to readily undergo ion pair formation with the phosphate group of pyridoxal-P during formation of holoenzyme from apoenzyme; they may thus contribute primarily to coenzyme binding, a possibility also recognized previously (2, 21). In the apoenzyme, and in the holoenzyme-substrate complex, such closely adjacent base groupings may also aid in lowering the pK value of the ε-amino group of the lysine residue that undergoes azomethine formation with pyridoxal-P. Such a lowered pK value by increasing the concentration of the nucleophile amino group would contribute both to apoenzyme-coenzyme interaction at low pH, and to the putative catalytic function of this group near the pH optimum of these enzymes. Such speculations at present are useful primarily in providing concepts for further experimental testing, since it is not yet certain that any of the catalytic residues of the enzymes are present in these peptides.

**Table III**

| Residue | Composition after each degradative cycle |
|---------|----------------------------------------|
| (Pxy)Lys | 0.95b 0.89 0.09 (Not tested) |
| Gly     | 1.03 1.07 1.03 1.03 0.33 |
| Ala     | 2.02 2.06 2.00 1.24 1.07 |
| Val     | 0.95 0.00 0.00 0.00 0.00 |
| Phe     | 1.00 0.93 0.97 1.00 1.00 Phe |

*Experimental values which decrease significantly during a given reaction cycle are underlined.*

*The values listed for (Pxy)Lys are the sum of those obtained for Nε-pyridoxyllysine and for free lysine (15). Approximately 10 to 20% destruction of (Pxy)lys occurs during hydrolysis (see text).*

*In the last degradation cycle, the residue peptide was put on the amino acid analyzer column directly without acid hydrolysis.*

**Table IV**

| Enzyme & Reference | Source | Sequence |
|--------------------|--------|---------|
| D-Serine dehydratase | E. coli | Ser-(Pxy)Lys-Gly-Arg-Ile-Asn-Lys-Ala-Thr |
| Arginine decarboxylase (2) | E. coli | Ala-Thr-His-Ser-Thr-His-(Pxy)Lys-Leu-Leu-Asn-Ala-Leu-Ser-Gln-Ala-Ser-Tyr |
| Glutamate decarboxylase (14) | E. coli | Ser-Ile-Ser-Ala-Ser-Gly-His-(Pxy)Lys-Phe |
| Tryptophanase (21) | E. coli | Ser-Ala-Lys-(Pxy)Lys-Asp-Ala-Met-Val-Pro-Met |
| Tryptophan synthetase (22) | E. coli | Leu-Leu-His-Gly-Ala-His-(Pxy)Lys-1hr-Asn-Gln-Val-Leu-Gly-Gln-Ala-Leu-Leu |
| Tryptophan synthetase (23) | Ps. putida | Leu-Asn-His-Thr-Gly-Ala-His-(Pxy)Lys-Val-Asn-Asn-Cys-Ile-Gly-Gln-Val-Leu-Leu |
| Aspartate aminotransferase (24) | | |
| Extramitochondrial | Fjg heart | Ser-(Pxy)Lys-Asp-Phe |
| Mitochondrial | Fjg heart | Ala-(Pxy)Lys-Asp-Met |
| Pyridoxamine-pyruvate aminotransferase | Ps. MA-1 | Val-Thr-Gly-Pro-Asp-(Pxy)Lys-Cys-Leu |
| Phosphorylase (25) | Rabbit muscle | Ala-Ser-Gly-Thr-Gly-Asp-Met-(Pxy)Lys-Phe-Met-Gly-Arg-Thr-Leu |

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Hodsdon, J. M., Kolb, H., Snell, E. E., and Cole, R. D. Unpublished data
tive site is unknown, as is the reason for inhibition. The peptide fragments from the two sites (Fig. 4) are markedly different, the active site peptide being highly basic and hydrophilic, the inhibitory site peptide being hydrophobic and containing only a single basic amino acid residue.

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*J. Biol. Chem.* 1972, 247:7358-7364.

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