Pyridoxal 5′-Phosphate Binding Site of Pig Heart Alanine Aminotransferase†

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ABSTRACT: After borohydride reduction, carboxymethylation, and tryptic digestion of the holoenzyme of pig heart alanine aminotransferase, a single icosapeptide containing the N^6-(phosphopyridoxyl)lysine residue was isolated by a combination of gel filtration and ion-exchange chromatography. Its primary structure was determined as Gln-Glu-Leu-Ala-Ser-Phe-His-Ser-Val-Ser-Lys(Pxy)-Gly-Phe-Met-Gly-Glu-Cys-Gly-Phe-Arg.

The kinetic and physicochemical properties of alanine aminotransferase have been described in a number of reports (Hopper & Segal, 1962; Bulos & Handler, 1965; Saier & Jenkins, 1967a,b; Gatehouse et al., 1967; Matsuzawa & Segal, 1968; Golichowski et al., 1977). However, little is known about the chemical structure of the active site of this enzyme. This is in striking contrast to extensive similar studies on aspartate aminotransferase (Braunstein, 1973; Ovchinnikov et al., 1973; Doonan et al., 1975; Kagamiyama et al., 1977; Barra et al., 1977). This difference in understanding between the two species of aminotransferase may be ascribed to the fact that the cellular concentration and the molecular stability of alanine aminotransferase are far inferior to those of aspartate aminotransferases (Saier & Jenkins, 1967a; DeRosa & Swick, 1975; Bosron et al., 1978). Recently, however, we have described a large-scale procedure by which a crystalline preparation of pig heart alanine aminotransferase could be obtained.[1] This has allowed us to investigate in some detail the chemical structure of the active site of this enzyme. Like the other pyridoxal enzymes previously examined, alanine aminotransferase from pig heart binds pyridoxal 5′-phosphate to the lysyl residue via an aldimine bond (Pfleiderer et al., 1968). As described first by Fischer et al. (1958), this Schiff base can be converted to a stable secondary amine bond by reduction with sodium borohydride. The treatment permits the isolation of a peptide containing the (phosphopyridoxyl)lysine residue and, hence, the chemical analysis of its primary structure. Similar procedures have been applied to a variety of pyridoxal enzymes.

One of the aims of this study was to establish the structural basis for the difference in substrate specificity between aspartate and alanine aminotransferase. As a first step toward this goal, the present communication describes the isolation and the amino acid sequence of a 20-residue peptide containing the coenzyme binding site of pig heart alanine aminotransferase.

Experimental Procedures

Materials. Alanine aminotransferase (EC 2.6.1.2) was purified from pig heart by a modification[1] of the procedure described by Saier & Jenkins (1967a). The enzyme was crystallized upon dialysis against 20 mM sodium phosphate buffer, pH 5.5. The specific activity of the crystalline preparation was 500 units/mg at 25 °C. The enzyme assay was performed as described (Morino et al., 1979). One unit of the enzyme activity was defined as the amount of the enzyme giving rise to 1 μmol of pyruvate per min under the standard assay conditions. Based on a value of 98 000 for the molecular weight of this enzyme,[1] the molar absorption coefficient of the holoenzyme at 427 nm at pH 5.5 was 9060 M^{-1} cm^{-1}, and a value of $A_{279nm}^{1\%}$ of 7.5 was employed for the calculation of the concentration of the enzyme. The A_{279nm}/A_{427nm} and A_{427nm}/A_{327nm} absorbance ratios at pH 5.5 were 4.06 and 3.50, respectively. Trypsin (Type IX), α-chymotrypsin (Type II), thermolysin, and TPCK[2] were purchased from Sigma. TPCK–trypsin was prepared according to Carpenter (1967). Carboxypeptidase Y was from Oriental Yeast Co., Bio-Gels P-10, P-6, and P-2 were obtained from Bio-Rad Laboratory, SP-Sephadex C-25 was from Pharmacia, and sodium boro[^{3}H]hydride (6 Ci/mmol) was from New England Nuclear. Sodium boro[^{3}H]hydride (10 mCi) was diluted with 100 mg of cold $NaBH_4$ in 1 mL of freshly distilled dimethylformamide. Reagents for the Edman degradation were of sequenator grade. All other chemicals were of the best grade commercially available.

Reduction, Carboxymethylation, and Tryptic Digestion of the Enzyme. The pyridoxal 5′-phosphate form of alanine aminotransferase (100 mg in 7 mL of 0.1 M Tris-HCl buffer, pH 7.5) was reduced by the addition of 0.1 mL of the dimethylformamide solution containing 1 mCi of sodium boro[^{3}H]hydride (3.8 mCi/mmol). After incubation for 20 min at 25 °C, the reduced preparation was dialyzed against 0.05 M Tris-HCl buffer (pH 8.0) for 20 h at 5 °C with several changes of buffer. The resulting preparation exhibited two absorption bands at 277 and 325 nm with an absorbance ratio, A_{277nm}/A_{325nm}, of 4.2 in 0.05 M Tris-HCl, pH 7.5. The specific activity was 1.54×10^6 cpm/μmol of enzyme. All procedures for the purification of peptides were performed in the dark to minimize photodestruction of the chromophore (Ritchey et al., 1977). The reduced enzyme preparation was incubated for 2 h at 37 °C under nitrogen in 6 M guanidine hydrochloride containing 0.1 M Tris-HCl buffer (pH 8.0), 1 mM EDTA, and 2 mM dithiothreitol. Carboxymethylation was performed essentially as described by Crestfield et al. (1963); after denaturation, sodium iodoacetate was added to a final concentration of 5 mM, and the solution was incubated at 37 °C for 20 min. The carboxymethylated preparation was extensively dialyzed against distilled water. The resulting heavy precipitate was collected by centrifugation and suspended in 5 mL of 0.1 M NH_4HCO_3, pH 8.2. The turbid solution was then adjusted to a protein concentration of 15 mg/mL, and

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[1] Unpublished experiments.

[2] Abbreviations used: TPCK, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone; dansyl, 5-(dimethylamino)naphthalene-1-sulfonyl; PTH, phenylthiohydantoin; EDTA, ethylenediaminetetraacetate; Lys(Pxy), N^6-(phosphopyridoxyl)-L-lysine.

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1 mg of TPCK treated trypsin was added. After incubation for 3 h at 37 °C under gentle stirring, the solution became clear.

Peptide Mapping. Descending paper chromatography was performed on Whatman 3MM filter papers with 1-butanol–pyridine–acetic acid–water (15:10:3:12) or 1-butanol–acetic acid–water (4:1:5; upper phase) as the solvent. High-voltage paper electrophoresis was carried out either at pH 3.7 (pyridine–acetic acid–water, 1:10:289) or at pH 1.8 (1 N formic acid) at 2500 V, 1–2 mA/cm. Visualization of peptides was carried out by the fluorescamine staining procedure (Mendez & Lai, 1975), and visualization of phosphopyridoxyl peptides was carried out by the blue fluorescence emitted when exposed to UV light. The purity of the peptides was checked by thin-layer peptide mapping on cellulose-coated thin-layer sheets (plastic based; from Tokyo Kasei Industry).

Sequence Determination. Sequence analysis was performed by a manual Edman procedure (Edman, 1950) and the dansyl[2]-monitored Edman method (Gray & Hartley, 1963). PTH amino acids were identified either on silica gel thin-layer glass plates (Merck) or on polyamide sheets (Cheng Chin Trading Co.) as previously described (Niederwieser, 1972; Summers et al., 1973). Dansyl amino acids were identified on polyamide sheets (Woods & Wang, 1967). The phenylthiohydantoin derivative of N^6-(phosphopyridoxyl)lysine remained in the acidic aqueous phase. It was identified by its distinct blue fluorescence on the silica gel thin-layer chromatogram and confirmed by its characteristic absorption band at 325 nm at pH 7 and by its radioactivity which was incorporated upon reduction with sodium boro[^{3}H]hydride. Phenylthiohydantoin derivatives of pyridoxyllysine and histidine appeared as red-orange and red-brown spots, respectively, after staining the filter paper with Pauly's reagent (Stepka, 1957). Carboxypeptidase Y digestion was performed at 25 °C, pH 5.6 (50 mM sodium acetate buffer), at an enzyme to substrate molar ratio of 1:200. The enzymic hydrolysis was monitored for 60 min, and the products were assayed in the amino acid analyzer.

Amino Acid Analysis. Amino acid analysis was performed according to Spackman et al. (1958) in a Hitachi KLA-5A automatic amino acid analyzer equipped with a Hitachi chromato-data processor, Model 834. Hydrolysis was performed in 5.7 N HCl for 24 h at 110 °C in vacuo. N^6-Pyridoxyllysine emerged from the short column between lysine and histidine.

Radioactivity Measurement. Radioactivity was measured in a Packard Model 3385 liquid scintillation spectrometer with Bray's solution as the scintillant (Bray, 1960). On paper, radioactivity was monitored in a Packard radiochromatogram scanner, Model 7201.

Other Methods. Absorption measurements were performed with a Hitachi Model 200-10 and a Union SM-401 high-sensitivity spectrophotometer. For the calculation of the concentration of the phosphopyridoxyl peptides, a value of 7500 M^{-1} cm^{-1} (at 320 nm in 0.1 M NH_4HCO_3) was assumed for the molar absorption coefficient of a pyridoxyl derivative.

Results

Purification of the Phosphopyridoxyl Peptides. The tryptic digest was passed over a 2.6 × 140 cm column of Bio-Gel P-10 (200–400 mesh) equilibrated with 0.1 M ammonium bicarbonate (pH 8.5). The elution profile is shown in Figure 1. One major radioactive peak (TP-I) and one minor peak (TP-II) were observed. Materials in both peaks exhibited an intense blue fluorescence under an ultraviolet lamp (Mineralight 3250 Å) and showed an absorption band at 317 nm

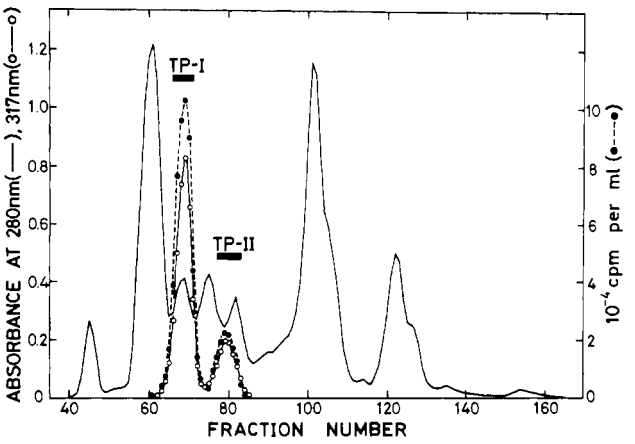

FIGURE 1: Purification of pyridoxyl peptides from tryptic digests of Na[^{3}H]BH_4 reduced enzyme on Bio-Gel P-10. Fractions (3.5 mL) were collected and monitored for absorbance and radioactivity: absorbance at 280 nm (—); absorbance at 317 nm (O); radioactivity (●). The bar indicates the fractions pooled. See the text for details.

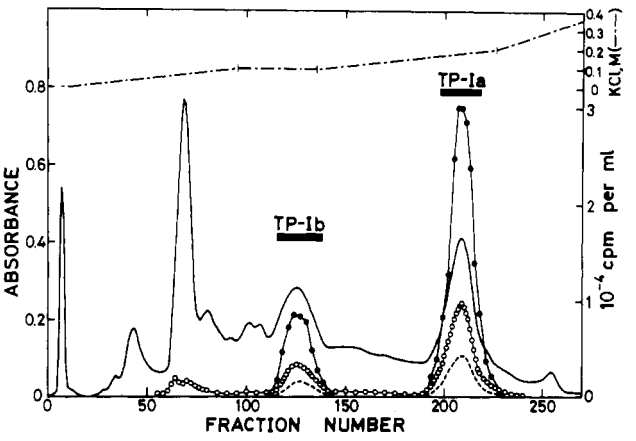

FIGURE 2: Isolation of pyridoxyl peptides on a column of SP-Sephadex C-25. Fractions (1.1 mL) were collected and analyzed for absorbance and radioactivity: absorbance at 230 nm (—); absorbance at 290 nm (O); absorbance at 325 nm (---); radioactivity (●). The bar indicates the fractions pooled. Experimental details are given in the text.

which was attributed to the pyridoxyl derivative. About 90% of the radioactivity in the original digests was recovered in both peak fractions (TP-I and TP-II). Each peak fraction was combined separately and lyophilized. Lyophilized TP-I was dissolved in 0.1 M formic acid and applied to a column of SP-Sephadex C-25 (1 × 15 cm) equilibrated with 30 mM formic acid, pH 2.7. The elution profile is illustrated in Figure 2. As monitored by fluorescence and radioactivity, two peak fractions containing pyridoxyl peptides were obtained: a minor peak (TP-Ib) which appeared at 0.1 M KCl of the salt gradient and a major one (TP-Ia) which emerged in 0.19 M KCl. No additional radioactive fraction was observed upon further increasing the concentration of KCl up to 0.5 M or by washing the column with 0.3 M ammonium acetate (pH 6.3) and then with 1 M ammonium hydroxide. Both radioactive fractions (TP-Ia and TP-Ib) were separately lyophilized and desalted on Bio-Gel P-6 (200–400 mesh) columns equilibrated with 0.1 M NH_4HCO_3. Both radioactive peptides (TP-Ia and TP-Ib) thus obtained exhibited two absorption bands at 250 and 320 nm in 0.1 M NH_4HCO_3 (pH 8.5). The specific radioactivity was 1.25 × 10^6 cpm/μmol of peptide TP-Ia. Under the same chromatographic condition, peptide TP-II was eluted at 0.16 M KCl as a single radioactive peak. Peptide TP-II was further purified by preparative paper chromatography and by electrophoresis as described under Experimental Procedures (data

Table I: Amino Acid Composition of Phosphopyridoxyl Peptides

	μmol (nearest integral no.)		
	TP-Ia	TP-Ib	TP-II
Lys(Pxy)[a]	0.45 (1)	0.14 (1)	0.054 (1)
His	0.45 (1)	0.14 (1)	
Arg	0.53 (1)	0.17 (1)	0.064 (1)
CM-Cys[b]	0.48 (1)	0.14 (1)	0.058 (1)
Ser	1.27 (3)	0.38 (3)	0.096 (2)
Glx	1.52 (3)	0.46 (3)	0.070 (1)
Gly	1.40 (3)	0.42 (3)	0.165 (3)
Ala	0.49 (1)	0.16 (1)	
Val	0.54 (1)	0.15 (1)	0.060 (1)
Met	0.51 (1)	0.15 (1)	0.064 (1)
Leu	0.45 (1)	0.15 (1)	
Phe	1.35 (3)	0.42 (3)	0.110 (2)
yield (%)[c]	25	8	3

[a] N^{ϵ}-(Phosphopyridoxyl)-L-lysine. [b] *S*-(Carboxymethyl)cysteine. [c] Based on 2 μmol of the enzyme as the starting material.

not shown). Amino acid compositions of purified phosphopyridoxyl peptides and overall yields are listed in Table I. The rather low yields obtained for these peptides can be mostly attributed to the fact that marginal fractions of TP-Ia, TP-Ib, and TP-II were discarded. The data indicate that peptide TP-Ia has the same amino acid composition as TP-Ib and these peptides are derived from a single coenzyme-combining site of this enzyme.

Further Fragmentation of the TP-Ia Peptide. Further fragmentation of peptide TP-Ia was performed by two different enzymatic procedures, namely, chymotryptic and thermolytic digestions. Peptide TP-Ia (0.15 μmol) was digested with 0.01 mg of α-chymotrypsin at 30 °C for 20 h in 0.1 M NH_4HCO_3 (pH 8.2). Thermolysin (10 μg) was added to the peptide TP-Ia (0.12 μmol) solution containing 20 mM Tris-HCl buffer (pH 8.0) and 10 mM $CaCl_2$. The solution was incubated at 30 °C for 2 h. Digests were spotted on a Whatman 3MM paper and subjected to chromatography and then high-voltage electrophoresis as described under Experimental Procedures. Three major chymotryptic peptides (CT-1, -2, and -3) were isolated (Figure 3A). Only the peptide CT-2 was fluorescent and radioactive. This peptide was further purified by passage through a Bio-Gel P-2 column equilibrated with 0.1 M NH_4HCO_3. From a thermolytic digest of TP-Ia, seven major peptides (TT-1 to TT-7) were isolated (Figure 3B) by the peptide mapping procedure. Only peptide TT-4 was fluorescent and radioactive. Peptide TT-3 and peptide TT-4 appeared as a red-brown spot and a red-orange spot, respectively, after staining with Pauly's reagent (Stepka, 1957).

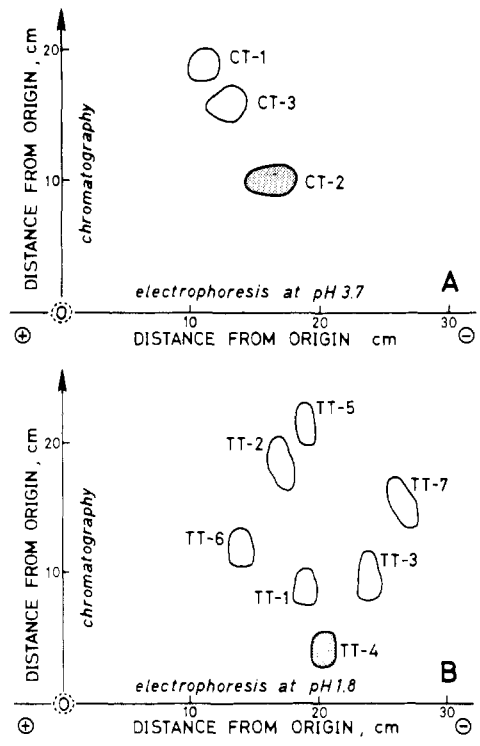

FIGURE 3: Peptide maps of chymotryptic (A) and thermolytic (B) digests of peptide TP-Ia. Electrophoresis was in the horizontal direction with the cathode (−) to the right and the anode (+) on the left. Descending paper chromatography was done in the direction of the arrow. (A) Chromatographed in 1-butanol–pyridine–acetic acid–water (15:10:3:12). (B) Chromatographed in 1-butanol–acetic acid–water (4:1:5; upper phase). Samples were applied at the origin as indicated by the dashed-line circles. Dotted areas (CT-2 and TT-4) show the spots having both fluorescence and radioactivity.

Amino acid compositions of these peptides are given in Table II.

Sequence Analysis of Phosphopyridoxyl Peptides. The Edman degradation of peptide TP-Ia was carried out up to the 19th step. The sequence from the NH_2 terminus to the 14th residue was established by the identification of each residue as its PTH derivative. The remainder of the sequence (residues 15–20) was analyzed by the dansyl–Edman procedure. Major amounts of radioactivity were found to be released into the acidic aqueous phase obtained after the conversion step at the 11th cycle of the degradation. In addition, the aqueous phase exhibited an absorption band at 325 nm (at pH 7), which is characteristic of a pyridoxyl derivative. The product at the seventh cycle of the degradation

Table II: Amino Acid Composition of Peptides Fragmented from Phosphopyridoxyl Peptide TP-Ia

	nmol (nearest integral no.)									
	CT[a]-1	CT-2	CT-3	TT[b]-1	TT-2	TT-3	TT-4	TT-5	TT-6	TT-7
Lys(Pxy)[c]		44 (1)					77 (1)			
His						74 (1)				
Arg			112 (1)							77 (1)
CM-Cys[d]			90 (1)						75 (1)	
Ser	27 (1)	89 (2)			65 (1)	65 (1)	79 (1)			
Glx	65 (2)		110 (1)	150 (2)					88 (1)	
Gly		64 (1)	224 (2)				80 (1)		167 (2)	
Ala	29 (1)				68 (1)					
Val		54 (1)					84 (1)			
Met			92 (1)						70 (1)	
Leu	27 (1)				60 (1)					
Phe	34 (1)	52 (1)	102 (1)			70 (1)		25 (1)		66 (1)
yield (%)	18	35	67	62	54	60	67	21	66	59

[a] CT denotes chymotryptic peptide. [b] TT denotes thermolytic peptide. [c] N^{ϵ}-(Phosphopyridoxyl)-L-lysine. [d] *S*-(Carboxymethyl)cysteine.

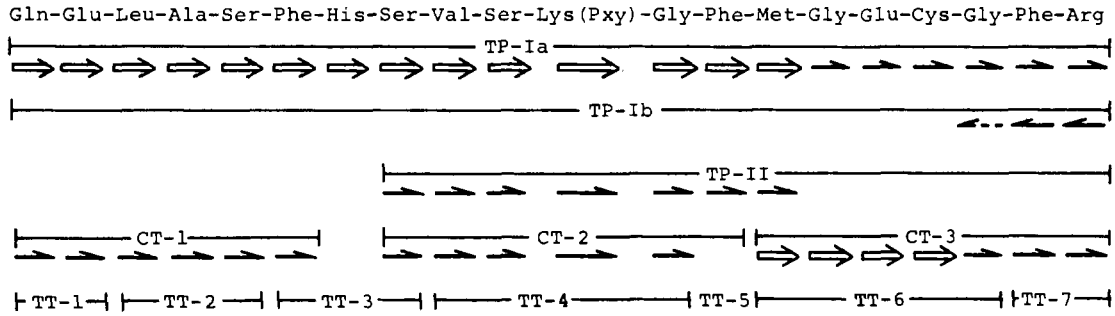

FIGURE 4: Amino acid sequence around the lysyl residue combining pyridoxal 5′-phosphate in alanine aminotransferase. The arrow below the sequence (⟹) indicates the residues identified as PTH derivatives. Arrows (⟶) and (⟵) show the results of dansyl-monitored Edman degradation and of carboxypeptidase Y digestion of peptides, respectively. Cysteine is determined as a *S*-carboxymethyl derivative. Lys(Pxy) denotes the N^{ϵ}-(phosphopyridoxyl)-L-lysine residue.

was mainly in the acidic aqueous phase after the conversion. This PTH derivative cochromatographed (R_f 0.07) with an authentic PTH-histidine on silica gel plates with solvent system II (chloroform–methanol, 90:10 v/v) (Jeppsson & Sjöqvist, 1967) and appeared as a red-brown spot upon staining with Pauly's reagent. Its identification as PTH-histidine was further confirmed by chromatography on a polyamide sheet with 25% acetic acid as the solvent (R_f 0.9). Thus, the following sequence is tentatively proposed: Gln-Glu-Leu-Ala-Ser-Phe-His-Ser-Val-Ser-Lys(Pxy)-Gly-Phe-Met-Gly-Glx-Cys-Gly-Phe-Arg. Assignment of the residue at position 16 was established as Glu by the sequential Edman degradation of the peptide CT-3.

No PTH or dansyl derivative was detected upon the Edman degradation of peptide TP-Ib, consistent with a blocked NH_2 terminus. Carboxypeptidase Y digestion of TP-Ib indicated that the carboxyl-terminal sequence must be Gly-Phe-Arg (data not shown). As expected from the behavior of carboxypeptidase Y (Hayashi et al., 1973), release of the third residue (Gly) from the carboxyl terminus was very slow. Taken together, the result of amino-terminal analysis, the chromatographic behavior, and the amino acid composition of TP-Ib (see Table I) indicate that this peptide must arise from the parent peptide TP-Ia by cyclization of the NH_2-terminal glutaminyl into a pyroglutamyl residue.

Dansyl-monitored Edman degradation of TP-II was carried out up to the seventh step. The bulk of radioactivity was released at the fourth cycle. Its partial sequence was established as Ser-Val-Ser-Lys(Pxy)-Gly-Phe-Met–, which corresponds to the sequence from residues 8 to 14 in the parent peptide TP-Ia. This minor peptide (TP-II) probably arose from the chymotryptic activity of trypsin.

The peptides CT-1, CT-2, and CT-3, obtained upon further fragmentation of the parent peptide TP-Ia by chymotryptic digestion, were analyzed by the dansyl–Edman procedure or by the Edman method. Data obtained (Figure 4) were largely consistent with the sequence established earlier for the parent peptide TP-Ia. However, no peptide containing the histidine at position 7 of TP-Ia was found in the major peptides isolated from the chymotryptic digests. This could be due to the fact that the His–Ser peptide bond was cleaved by chymotrypsin to form free histidine which probably migrated off the paper during prolonged electrophoresis.

To confirm the presence of a histidine at position 7 of TP-Ia, thermolytic peptides were analyzed. Data in Table II unequivocally demonstrated that the amino acid compositions of thermolytic products obtained from TP-Ia are all compatible with the sequence proposed for this peptide. Taken together, these data clearly establish the sequence of the tryptic peptide containing (phosphopyridoxyl)lysine isolated from the coenzyme-combining site of pig heart alanine aminotransferase: Gln-Glu-Leu-Ala-Ser-Phe-His-Ser-Val-Ser-Lys(Pxy)-Gly-Phe-Met-Gly-Glu-Cys-Gly-Phe-Arg.

Discussion

In the present study, an icosapeptide containing a (phosphopyridoxyl)lysine residue was isolated from the tryptic digest of the Na[^{3}H]BH_4 reduced preparation of the pyridoxal form of pig heart alanine aminotransferase. Two peak fractions containing phosphopyridoxyl peptides TP-I and TP-II (see Figure 1) were obtained upon chromatography of the tryptic digest on a Bio-Gel P-10 column. However, the result of chemical analysis on these peptides clearly indicated that peptide TP-II was formed upon a further cleavage of the parent peptide TP-I and that these two peptides were derived from a single site of the enzyme. Total amounts of phosphopyridoxyl peptides (TP-I and TP-II) as calculated from the areas under these two peak fractions fully accounted for the amount of phosphopyridoxyl peptides initially present in the whole tryptic digests. Based on the assumed value of the molar absorption coefficient (7500 M^{-1} cm^{-1} at 320 nm) for a phosphopyridoxyl derivative, 1 mol of the phosphopyridoxyl group was estimated to be bound to approximately 50 000 g of the enzyme protein. The molecular weight of the native preparation of this enzyme was reported to be 115 000 (Saier & Jenkins, 1967a). However, as estimated in sodium dodecyl sulfate–polyacrylamide gel electrophoresis, the molecular weight of its subunit was in the range from 49 000 to 50 000.[1] Spectrophotometric determinations indicate that 50 000 g of enzyme binds 1 mol of pyridoxal 5′-phosphate.[1] These data, taken together, strongly support the contention that pig heart alanine aminotransferase is composed of two identical subunits, each binding 1 equiv of pyridoxal 5′-phosphate. It was previously reported that alanine aminotransferase from rat liver contains 2 mol of pyridoxal 5′-phosphate per 114 000 g of the enzyme (Matsuzawa & Segal, 1968).

In all pyridoxal 5′-phosphate containing enzymes so far analyzed (Table III), the coenzyme binds to the ϵ-amino group of a lysyl residue of apoenzymes. Alanine aminotransferase is no exception. Pfleiderer et al. (1968) demonstrated earlier that pyridoxal phosphate binds to alanine aminotransferase through the ϵ-amino group of a lysyl residue via an aldimine linkage. In addition, these investigators isolated a pyridoxyl peptide from chymotryptic digests of a Na[^{3}H]BH_4 reduced preparation of partially purified alanine aminotransferase from pig heart. However, its amino acid composition does not seem to be consistent with the structure of the phosphopyridoxyl peptide presented herein.

The primary structures so far described for phosphopyridoxyl peptides from a number of pyridoxal enzymes are

Table III: Amino Acid Sequence of Pyridoxyl Peptide Obtained from Various Pyridoxal Enzymes

Enzyme (source)	Sequence	Ref
Alanine Aminotransferase(pig heart)	Gln-Glu-Leu-Ala-Ser-Phe-His-Ser-Val-Ser-Lys*-Gly-Phe-Met-Gly-Glu-Cys-Gly-Phe-Arg	
Aspartate Aminotransferase(cytosolic)		
(pig heart)	Tyr-Phe-Val-Ser-Glu-Gly-Phe-Glu-Leu-Phe-Cys-Ala-Gln-Ser-Phe-Ser-Lys*-Asn-Phe-Gly-Leu-Tyr-Asn-Glu-Arg	a
(human heart)	Tyr-Phe-Val-Ser-Glu-Gly-Phe-Glu-Phe-Phe-Cys-Ala-Gln-Ser-Phe-Ser-Lys*-Asn-Phe-Gly-Leu-Tyr-Asn-Glu-Arg	b
(sheep liver)	Ser-Lys*-Asn-Phe	c
(chicken heart)	Ser-Lys*-Asn-Phe	d
Aspartate Aminotransferase(mitochondrial)		
(pig heart)	His-Phe-Ile-Glu-Gln-Gly-Ile-Asn-Val-Cys-Leu-Cys-Gln-Ser-Tyr-Ala-Lys*-Asn-Met-Gly-Leu-Tyr-Gly-Glu-Arg	e
(human heart)	His-Phe-Ile-Glu-Gln-Gly-Ile-Asn-Val-Cys-Leu-Cys-Gln-Ser-Tyr-Ala-Lys*-Asn-Met-Gly-Leu-Tyr-Gly-Glu-Arg	b
(sheep liver)	Ala-Lys*-Asx-Met-Gly-Leu-Tyr	c
(beef kidney)	Tyr-Ala-Lys*-Asn-Met-Gly	f
D-Serine Dehydratase(E.coli)	Ser-Lys*-Gly-Arg-Ile-Asn-Lys-Ala-Thr	g
Tryptophanase(E.coli)	Tyr-Ala-Asp-Met-Leu-Ala-Met-Ser-Ala-Lys-Lys*-Asp-Ala-Met-Val-Pro-Met-Gly-Gly-Leu-Leu-Cys-Met	h
Tryptophan Synthase(E.coli)	Arg-Glu-Asp-Leu-Leu-His-Gly-Gly-Ala-His-Lys*-Thr-Asn-Gln-Val-Leu-Gly-Gln-Ala-Leu-Leu-Ala-Lys	i
(Ps.putida)	Leu-Asn-His-Thr-Gly-Ala-His-Lys*-Val-Asn-Asn-Cys-Ile-Gly-Gln-Val-Leu-Leu	j
Arginine Decarboxylase(E.coli)	Ala-Thr-His-Ser-Thr-His-Lys*-Leu-Leu-Asn-Ala-Leu-Ser-Gln-Ala-Ser-Tyr	k
Lysine Decarboxylase(E.coli)	Val-Ile-Tyr-Glu-Thr-Glu-Ser-Thr-His-Lys*-Leu-Leu-Ala-Ala-Phe	l
Ornithine Decarboxylase(E.coli)	Val-His-Lys*-Gln-Gln-Ala-Gly-Gln	m
Glutamate Decarboxylase(E.coli)	Ser-Ile-Ser-Ala-Ser-Gly-His-Lys*-Phe	n
L-DOPA Decarboxylase(pig kidney)	Asn-Phe-Asn-Pro-His-Lys*-Trp	o
Serine Transhydroxymethylase(rabbit liver)	Val-Val-Thr-Thr-Thr-His-Lys*-Thr-Leu	p
Pyridoxamine-pyruvate Aminotransferase (Ps.MA-1)	Ala-Asp-Ile-Tyr-Val-Thr-Gly-Pro-Asp-Lys*-Cys-Leu-($Pro_2Gly_2Ala_2Met$)(Thr,Leu_2)-Gly-Val-Ser-Glu-Arg	q
Phosphorylase		
(rabbit muscle)	Gln-Ile-Ser-Thr-Ala-Gly-Thr-Glu-Ala-Ser-Gly-Thr-Gly-Asn-Met-Lys*-Phe-Met-Leu-Asn-Gly-Ala-Leu-Thr-Ile-Thr-Ile-Gly	r
(yeast)	Ile-Ser-Thr-Ala-Gly-Thr-Glu-Ala-Ser-Gly-Thr-Ser-Asn-Met-Lys*-Phe-Val-Met	s
(potato)	His-Ile-Ser-Thr-Ala-Gly-Met-Glu-Ala-Ser-Gly-Thr-Ser-Asn-Met-Lys*-Phe-Ala-Met-Asx-Gly-(Cys,Glx,Thr,Gly,Ile_2)-Leu-Asp-Gly	t

Lys*; N^6-Phosphopyridoxyl-L-Lysine. [a]Ovchinnikov et al.(1973). [b]Teranishi et al.(1978). [c]Campos-Cavieres and Milstein(1975). [d]Torchinsky et al.(1974). [e]Kagamiyama et al.(1977). [f]Bossa et al.(1976a). [g]Huang and Snell(1972). [h]Kagamiyama et al.(1970). [i]Fluri et al.(1971). [j]Maurer and Crawford(1971). [k]Boeker et al.(1971). [l]Sabo and Fischer(1974). [m]Appelbaum et al.(1975). [n]Strausbauch and Fischer(1970). [o]Bossa et al.(1977). [p]Bossa et al.(1976b). [q]Hodsdon et al.(1978). [r]Titani et al.(1977). [s]Lerch and Fischer(1975). [t]Nakano et al.(1978).

listed in Table III. It should be noted that the partial structure -Ser-X-X-Lys(Pxy)- seems to be a structural feature most commonly observed among these peptides, although nothing definite can be predicated about its structural or functional significance at this time.

Among a number of aminotransferases, the primary structure of phosphopyridoxyl peptides has so far been described for both the mitochondrial and the cytosolic isoenzymes of aspartate aminotransferase from several species (see Table III). When compared with the structures of these peptides from two isoenzymes of aspartate aminotransferase, the structure of the peptide from alanine aminotransferase[3] seems to be more homologous with that from the cytosolic isoenzyme than with that from the mitochondrial isoenzyme.

As previously described (Morino & Okamoto, 1972, 1973; Morino et al., 1974; Morino & Tanase, 1978), both isoenzymes of aspartate aminotransferase were affinity-labeled by 3-chloro-L-alanine. The labeled site was located to the ϵ-amino group of the lysyl residue [at position 258 with the cytosolic isoenzyme (Ovchinnikov et al., 1973) and at position 250 with the mitochondrial isoenzyme (Kagamiyama et al., 1977)] which is originally involved in the formation of an aldimine bond with the coenzyme pyridoxal 5'-phosphate. These studies have led to a mechanistic consideration that in both isoenzymes of aspartate aminotransferase an essential lysyl residue not only participates in binding pyridoxal 5'-phosphate but also functions as a base to abstract the α-hydrogen atom of the bound substrate (Morino & Okamoto, 1973; Morino et al., 1974; Morino & Tanase, 1978). Alanine aminotransferase has also been known to be successfully affinity-labeled by 3-chloro-L-alanine (Morino et al., 1979; Silverman & Ables, 1976; Golichowski & Jenkins, 1978). It seems important to determine the structure of the affinity-labeled site in alanine aminotransferase and compare it with that of the coenzyme-combining site. The present investigation would lead to this future work which may be important in inquiring into the catalytic function of the coenzyme-combining lysyl residue of alanine aminotransferase.

[3] This may be related to the fact that most of the activity of alanine aminotransferase in pig heart is of cytosolic origin (Saier & Jenkins, 1967a).

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