AMINO ACID SEQUENCE OF 37 RESIDUES SURROUNDING N^{ε}-PYRIDOXYLLYSINE IN MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE

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SUMMARY

Following reduction with $NaBH_4$, carboxymethylation and cleavage with cyanogen bromide, a peptide of thirty-seven amino acid residues containing N^{ε}-pyridoxyllysine (coenzyme binding lysine) was isolated from the mitochondrial aspartate aminotransferase of pig heart by Sephadex G-75 column chromatography and then preparative polyacrylamide gel electrophoresis. The primary structure of this peptide was determined to be *Ala-Tyr-Gln-Gly-Phe-Ala-Ser-Gly-Asp-Gly-Asn-Lys-Asp-Ala-Trp-Ala-Val-Arg-His-Phe-Ile-Glu-Gln-Gly-Ile-Asn-Val-Cys-Leu-Cys-Gln-Ser-Tyr-Ala-(Pxy)Lys-Asn-Met*. Its structure showed a high degree of homology with the corresponding part of the cytoplasmic isozyme.

There are two distinct forms of aspartate aminotransferase (Glutamate-Oxaloacetate Transaminase:GOT): one localized in the cytoplasmic fraction (s-GOT) and the other in the mitochondrial fraction (m-GOT)(1,2). These two isozymes are known to differ distinctly in physicochemical, immunochemical and enzymological properties (3-12). Thus, to elucidate their origins and mechanisms of action, it would be of considerable interest and importance to compare their primary structures.

The complete amino acid sequence of s-GOT has been determined by Ovchinnikov et al. (13). Studies on the primary structure of m-GOT are now in progress in our laboratories, and the sequence of fifty-two residues from the N-terminus of this isozyme was presented in the preceding report (14). This paper describes the isolation and determination of the sequence of

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Abbrevations: s-GOT and m-GOT, soluble and mitochondrial glutamate-oxaloacetate transaminase (E.C.2.6.1.1., L-Aspartate:2-Oxoglutarate Aminotransferase), respectively; (Pxy)peptide, a peptide which contains the N^{ε}-pyridoxyl lysine residue; (Pxy)Lys, N^{ε}-pyridoxyl-L-lysine residue.

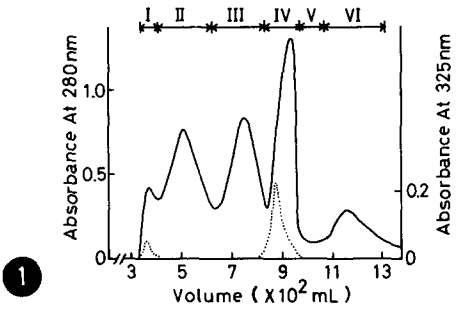
thirty seven amino acid residues containing the specific lysine residue which combines with pyridoxal-5'-phosphate in m-GOT.

METHODS

The preparation of m-GOT and its carboxymethylation were carried out as described previously (14). About 200 mg of m-GOT were reduced with $NaBH_4$ before carboxymethylation to convert the linkage between pyridoxal-5'phosphate and the apoprotein to a stable form, as described earlier (10). The reduced carboxymethylated m-GOT was then treated with cyanogen bromide under the conditions described by Watanabe et al. (15). The peptides obtained were chromatographed on a Sephadex G-75 column (5 x 120cm) with 30% acetic acid. The absorption of fractions of the eluate was measured at 280 nm and 325 nm. The latter shows the presence of the N -pyridoxyllysine residue (16). The peptide fractions eluted were purified by preparative polyacrylamide gel electrophoresis, following the method of Wada et al. (17,18) in the presence of 10% acetic acid containing 7 M urea. Paper chromatography was carried out using n-butanol:pyridine:acetic acid:water,(15:10:3:12, by volume) as solvent (19). High voltage paper electrophoresis was performed at pH 3.7 in pyridine: acetic acid:water (1:10:289, by volume) (20) or at pH 1.9 in 1% formic acid and 8% acetic acid (21). Amino acids were analyzed in a Hitachi amino acid analyzer. The amino acid sequence of the peptide was determined by Edman degradation following the method of Blombäck et al. (22). The residues cleaved were identified by thin layer chromatography or gas chromatography after conversion to their phenylthiohydantoine derivatives, as described in the previous report (14).

RESULTS

The peptides obtained by cyanogen bromide cleavage of m-GOT were subjected to Sephadex G-75 column chromatography (Fig. 1) and the (Pxy)peptides (exhibiting absorption at 325 nm) were eluted in fraction IV. This fraction was lyophilized and dissolved in 4 ml of 10% acetic acid containing 7 M urea and then subjected to preparative polyacrylamide gel electrophoresis.



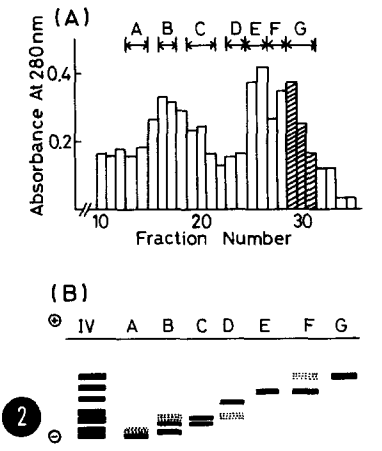


Fig 1. Chromatography of the cyanogen bromide fragments on Sephadex G-75. ——, fractions exhibiting absorption at 280 nm; ···· fractions exhibiting absorption at 325 nm.

Fig 2. (A), Isolation of the pyridoxylpeptide by preparative polyacrylamide gel electrophoresis. Conditions are described in the text. Hatched zones showed absorption at 325 nm. (B), Analytical electrophoresis of the seven fractions from (A). Gels were stained with amido black. Dotted zones stained weakly.

Figure 2(A) shows the elution pattern obtained on electrophoresis in the presence of 10% acetic acid containing 7 M urea for 17 hours. Urea was removed from each fraction by dialysis against 30% acetic acid, and the purities of the fractions were then examined by thin layer polyacrylamide gel electrophoresis under the same conditions as described above. The results are shown in Fig.2(B). (Pxy)peptide was eluted from the gel in about 11 hours as a slowest fraction (Fig. 2(A)), and gave a single band on analytical electrophoresis (Fig. 2(B)).

The amino acid composition of this peptide is shown in table I. Its structure was determined by a combination of Edman degradation and tryptic digestion. The N-terminal sequence was deduced from 14 cycles of Edman degradation (Fig. 3). The peptide was then digested with trypsin to establish its complete sequence. The resulting digest was separated by a combination of paper chromatography and high voltage paper electrophoresis. Three

Table I Amino acid composition of the (Pxy)peptide

Lysine	1.44 (1)[a]	Alanine	4.98 (5)
Histidine	1.48 (1)[a]	Valine	1.65 (2)
Arginine	1.21 (1)	Methionine	(+)[c]
CM-Cysteine[b]	2.16 (2)	Isoleucine	2.28 (2)
Aspartic acid	5.31 (5)	Leucine	0.92 (1)
Serine	1.54 (2)	Tyrosine	1.92 (2)
Glutamic acid	3.96 (4)	Phenylalanine	2.32 (2)
Glycine	3.75 (4)	Tryptophan	+[d]
		(Pxy)Lysine	+[e]

Numbers in parentheses show integral numbers of residues

a): These values are partly due to (pxy)lysine, because (Pxy)lysine was not separated from histidine under our standard conditions of amino acid analysis (15 cm column, pH 5.28) and partial breakdown of (Pxy)lysine during acid hydrolysis has been found to result in lysine release (26).
b): Carboxymethyl cysteine
c): Homoserine lactone and homoserine were detected, but they were not measured quantitatively.
d): Tryptophan was detected by the Ehrlich reaction on peptide spot on paper.
e): The presence of (Pxy)lysine is deduced from the absorbance at 325nm and the results of sequence studies. See also footnote a) of this table.

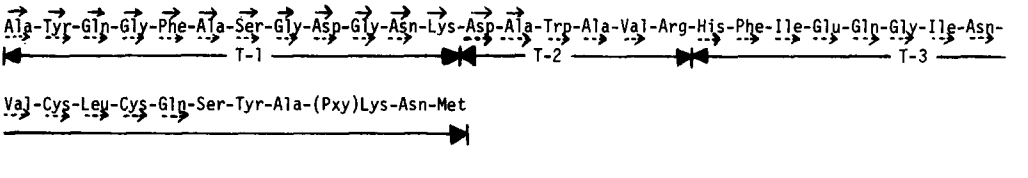


Fig 3. Sequence and fragmentation products of the pyridoxylpeptide. ⟶ and ---→, indicate the results of Edman degradation of the (Pxy)peptide and the tryptic fragments, respectively; T-1~3, see text.

peptides (T-1 to T-3) were isolated and their sequences were determined by Edman degradation. The results are summarized in Fig. 3. From its sequence, T-1 must be the N-terminal peptide. T-3 must be the C-terminal peptide because it contains a methionine residue. The sequence of two N-terminal

residues of T-2 (Asp-Ala) provides an overlap with the 13th and 14th residues and thus T-2 could be placed between T-1 and T-3. The methionine residue must be adjacent to the N-terminal alanine.

DISCUSSION

The structure of the (Pxy)peptide obtained from m-GOT was reported previously by Morino et al.(10, 23). The results shown in Fig. 3 extend the known sequence on the N-terminal side by 18 residues. Results on the sequence in the other direction were recently reported by Doonan et al. (24). From the results thus far reported on the structure of (Pxy)peptide of m-GOT, a total of fifty three residues can be arranged as shown in Fig. 4. In this

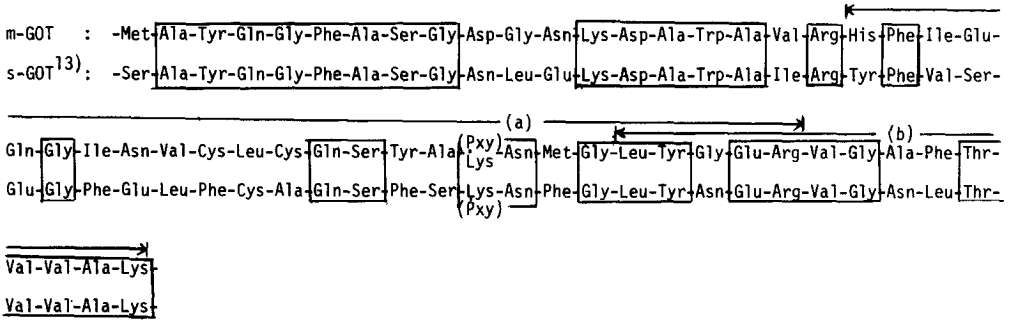


Fig 4. Comparison of the extended sequences of the (Pxy)peptides of m-GOT and s-GOT. Homologous sequences are enclosed in frames. (a), from the results obtained by Morino (23); (b), from the results reported by Doonan et. al. (24).

figure the sequence of s-GOT is also shown for comparison. It is interesting to note the absence of proline residues and the presence in the same positions of the same numbers of lysine (three) and arginine (two) residues, differences in the numbers of serine plus threonine (m-GOT:3, s-GOT:6) and cysteine (m-GOT:2, s-GOT:1) residues and considerable resemblance in the C-terminal site from (Pxy)lysine.

The sequences of amino acids surrounding the (Pxy)lysine residue differ markedly in many of the pyridoxal phosphate enzymes so far studied. Figure 4

shows, however, a high degree of homology between the structures of the peptides from m-GOT and s-GOT, inspite of their different immunochemical and enzymological properties. To elucidate the significance of the structure of this portion in the transamination reaction, the structures of the corresponding parts of other types of transaminases and of aspartate aminotransferases from other sources must be examined. Our studies show remarkable similarities in the total primary structures of s- and m-GOT*. Doonan et al., independently, have also reported the highly homologous structures of GOT isozymes (24). Completion of these sequence studies may contribute much to our knowledge on the basic enzymology, biology and evolution of these isozymes.

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