AMINO ACID SEQUENCE OF FIFTY TWO RESIDUES FROM THE N-TERMINUS OF MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE

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SUMMARY

The amino acid sequence of mitochondrial aspartate aminotransferase was determined up to fifty two residues from the N-terminus with the use of an automatic amino acid sequence analyzer and by a manual determination of a peptide isolated from a tryptic digest of the enzyme. The results were compared with the corresponding amino acid sequence of cytoplasmic aspartate aminotransferase. Although little similarities were found up to twenty nine residues from N-terminus, the sequence from the residues 30 to 52 is nearly identical.

Two distinct forms of aspartate aminotransferase (Glutamate-Oxaloacetate Transaminase: GOT) are present in mammalian tissues, one localized in the cytoplasmic fraction (s-GOT) and the other in the mitochondrial fraction (m-GOT)(1,2). Although they catalyze the same reaction, their structure seems to be significantly different (3-12).

Ovchinnikov et al.(13) have recently determined the complete amino acid sequence of s-GOT of bovine heart. Work on the complete sequence of m-GOT is now in progress in our laboratories to compare the primary structure of these two isozymes, since it is considered to be one of the approaches in understanding the enzymatic mechanism of transamination reaction.

This paper describes N-terminal sequence determination of m-GOT performed mainly by a sequence analyzer.

Abbrevations: s-GOT and m-GOT, soluble and mitochondrial glutamate oxaloacetate transaminase (E.C.2.6.L.L., l-Aspartate : 2-Oxoglutarate Aminotransferase). respectively; PTH, phenylthiohydantoin

MATERIALS AND METHODS

m-GOT was purified from pig heart by a modification of the procedures described for the beef enzymes (3). Carboxymethylation of the enzyme was performed essentially same as the method of Crestfield et al. (14), except guanidine hydrochloride as the denaturant was used in the present procedure. Carboxymethylated m-GOT was lyophilized after dialysis against distilled water and used for the Edman degradation.

Automatic Edman Degradation: The Edman degradation was carried out with a JEOL sequencer JAS-47K (Tokyo) essentially as described in JEOL sequencer manual with the sequencer reagents of Wako Pure Chemical Industries, LTD. (Osaka). The lyophilized enzyme (10-15 mg) was dissolved in 2 ml of 0.1N formic acid and placed in the reaction cell of the sequencer for the determination of the sequence. Thiazolinone derivatives of amino acid were extracted with ethylacetate containing 0.1% ethanthiol.

Analyses of Sequencer Products: The extracted thiazolinone derivatives of amino acids were converted to their PTH-derivatives by the method of Edman et al.(15). PTH-amino acids were identified by gas chromatography using a JEOL JGC-20K gas chromatograph under the constant column temperature of 210°. Thin layer chromatography of PTH-amino acids was performed as described by Jeppson et al.(16). Hydriodic acid hydrolysis of thiazolinone derivatives was employed to generate amino acids as described by Smithies et al.(17) and the regenerated amino acids were analyzed on a JEOL JLC-6AH automatic amino acid analyzer.

Tryptic Digestion: Tryptic digestion of carboxymethylated m-GOT was performed as described by Morino et al.(18). Sephadex G-50 column chromatography, DE-52 ion exchange column chromatography and the combination of paper chromatography and high voltage

paper electrophoresis were used for the fractionation of the digested peptides. More detailed procedures will be published elsewhere.

RESULTS AND DISCUSSION

Forty-eight cycles of Edman degradation were performed by the sequence analyzer and the results were given in Table I. Definitive identification of PTH-amino acids after twenty cycles by gas chromatography and thin layer chromatography was difficult because of the appearance of many subpeaks or subspots. Hydrolysis with hydriodic acid of thiazolinone derivatives was more useful for this purpose, since quantitative comparison of small changes in the amount of amino acid was made possible by this method. Forty-eight of the cycles yielded forty-one identifiable amino acid residues with the average recovery of 92% at each step.

Residues 28 and 31 were determined as serine and methionine, respectively, as follows. Carboxymethylated m-GOT was cleaved by cyanogen bromide and was chromatographed on a sephadex G-75 column (5 x 120 cm), followed by the preparative acrylamide gel electrophoresis which was designed by Wada et al. (19). One of the fractions which contained a mixture of peptides was subjected to tryptic digestion after blocking the ε-amino groups of lysine residues by citraconic anhydride. The digest was purified by the combination of paper chromatography and high voltage paper electrophoresis. One of the isolated peptides was shown to have a sequence of Asp-Thr-Asn(Ser, Lys, Lys) Met, which shows an overlap with the residues 25 to 31. Thus we concluded that the residues 28 and 31 are serine and methionine, respectively.

The remainder of the sequence was established from tryptic digestion of the carboxymethylated m-GOT. Among the tryptic peptides thus far isolated, the sequences of six peptides have

TABLE I

RESULTS OF SEQUENCER ANALYSES

STEP	GC	TLC	HI	DEDUCED RECIDUE	YIELD (NMOLE)	STEP	GC	TLC	HI	DEDUCED RECIDUE	YIELD (NMOLE)
1	—	—	ALA	SER	70	25			ASP	ASX	20
2	—	—	ALA	SER	80	26			α-ABA	THR	24
3	—	TRP	ALA,GLY	TRP	128	27			ASP	ASX	25
4	—	TRP	ALA,GLY	TRP	88	28			ALA	ALA/SER	5
5	ALA	ALA	ALA	ALA	100	29			LYS	LYS	12
6	—	—	HIS	HIS	83	30			LYS	LYS	13
7	VAL	VAL	VAL	VAL	100	31			X	X	
8	—	GLU	GLU	GLU	100	32			ASP	ASX	9
9	MET	MET	—	MET		33			LEU	LEU	11
10	GLY	GLY	GLY	GLY	85	34			GLY	GLY	8
11	PRO	PRO	PRO	PRO	38	35			VAL	VAL	10
12	PRO	PRO	PRO	PRO	38	36			GLY	GLY	10
13	—	ASP	ASP	ASP	78	37			ALA	ALA	13
14	PRO	PRO	PRO	PRO	33	38			TYR	TYR	4
15	ILE	ILE/LEU	ILE	ILE	43	39			ARG	ARG	7
16	LEU	ILE/LEU	LEU	LEU	53	40			ASP	ASX	10
17	GLY	GLY	GLY	GLY	68	41			ASP	ASX	9
18	VAL	VAL	VAL	VAL	30	42			X	X	
19	—	—	α-ABA	THR	33	43			X	X	
20	—	GLU	GLU	GLU	34	44			LYS	LYS	(4)
21			ALA	ALA	17	45			X	X	
22			PHE	PHE	22	46			X	X	
23			LYS	LYS	20	47			X	X	
24			ARG	ARG	21	48			LEU	LEU	(2)

The sequence analyses were performed with 250 nmole of m-GOT. Yield was calculated from the data of amino acid analyses after hydriodic acid hydrolysis. The residues from 21 to 48 were not determined by GC and TLC. - : The residues were not detectable by these methods, α-ABA : α-aminobutyric acid, X : Could not be decided, GC : Gas chromatography, TLC : Thin layer chromatography, HI : Hydrolysis with hydriodic acid

been determined. They include a peptide with a sequence of Asp-Asp-Asp-Gly-Lys-Pro-Tyr-Val-Leu-Pro-Ser-Val-Arg. Based on the results obtained from the sequencer (Table I)(Asx_{40}, Asx_{41}, Lys_{44}, and Leu_{48}) this peptides was assigned to the residues between 40-52.

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                  1                                   10
m-GOT             Ser-Ser-Trp-Trp-Ala-His-Val-Glu-Met-Gly-Pro-Pro-Asp-Pro-Ile-

s-GOT   Ala-Pro-Pro-Ser-Val-Phe-Ala-Glu-Val-Pro-Gln-Ala-Gln-Pro-Val-Leu-Val-
          1                                   10

                  20                                  30
Leu-Gly-Val-Thr-Glu-Ala-Phe-Lys-Arg-Asp-Thr-Asn-Ser-Lys-Lys-Met-Asx-Leu-Gly-

Phe-Lys-Leu-Ile-Ala-Asp-Phe-Arg-Glu-Asp-Pro-Asp-Pro-Arg-Lys-Val-Asn-Leu-Gly-
          20                                  30

                      40                                  50
Val-Gly-Ala-Tyr-Arg-Asp-Asp-Asp-Gly-Lys-Pro-Tyr-Val-Leu-Pro-Ser-Val-Arg

Val-Gly-Ala-Tyr-Arg-Thr-Asp-Asp-Cys-Gln-Pro-Trp-Val-Leu-Pro-Val-Val-Arg
              40                                  50
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Fig 1. Comparative N-terminal sequences of aspartate aminotransferase isozymes from pig heart. The regions which show homologous sequence are underlined.

Based on these results fifty-two amino acid residues from the N-terminus of m-GOT have been determined and presented in Fig. 1. The figure also compares the sequence with that of s-GOT. The most homology was obtained by matching the N-terminus of m-GOT (Ser) with the third residue of s-GOT (Pro). Wada et al. (20) previously described the sequence of nine residues from the N-terminus of these two isozymes and have provided clear dissimilarities between the two. The present results not only confirmed their earlier data, but the sequence has been extended to fifty-two residues. In contrast to the lack of similarities in the first twenty-nine residues from the N-terminus, high degree of sequence homology was found in the region of latter half. It may be tentatively concluded that these homologous region of the proteins are essential for the GOT activity, if not the active site itself, since they catalyze the same reaction.

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REFERENCES

1. Boyd, J. W. (1961) Biochem. J. 81, 434-441.
2. Katsunuma, N., Matsuzawa, T., and Fujino, A. (1962) J. Vitaminol. (Kyoto) 8, 74-79.
3. Morino, Y., Itoh, W., and Wada, H. (1963) Biochem. Biophys. Res. Commun. 13, 348-352.
4. Morino, Y., Kagamiyama, H., and Wada, H. (1964) J. Biol. Chem. 239, PC 943-944.
5. Wada, H., and Morino, Y. (1964) Vitamines and Hormones 22, 411-444.
6. Wada, H., Kagamiyama, H., and Watanabe, T. (1966) Pyridoxal Catalysis: Enzymes and Model Systems, Snell, E. E. et al., Ed., pp. 111-129. Wiley-Interscience, New York.
7. Martinez-Carrion, M. and Tiemeier, D. C. (1967) Biochemistry 6, 1715-1722.
8. Kagamiyama, H., Watanabe, T., and Wada, H. (1968) Biochem. Biophys. Res. Commun. 32, 678-684.
9. Michuda, C. M., and Martinez-Carrion, M. (1969) J. Biol. Chem. 244, 5920-5927.
10. Morino, Y., and Watanabe, T. (1969) Biochemistry 8, 3412-3417.
11. Michuda, C. M., and Martinez-Carrion, M. (1970) J. Biol. Chem. 245, 262-269.
12. Morino, Y., and Okamoto, M. (1970) Biochem. Biophys. Res. Commun. 40, 600-605.
13. Ovchinnikov, Yu, A., Egorov, C. A., Aldanova, N. A., Feigina, M. Yu., Lipkin, V. M., Abdulaev, N. G., Grishin, E. V., Kiselev, A. P., Modyanov, N. N., Braunstein, A. E., Polyanovsky, D. L., and Nosikov, V. V. (1972) FEBS Lett. 29, 31-33.
14. Crestfield, A. M., Moore, S., and Stein, W. H. (1963) J. Biol. Chem. 238, 622-627.
15. Edman, P., and Begg, G. (1967) Eur. J. Biochem. 1, 80-91.
16. Jeppson, J. D., and Sjöquist, J. (1967) Anal. Biochem. 18, 264-269.
17. Smithies, O., Gibson, D., Fanning, E. M., Goodfliesh, R. M., Gilman, J. G., and Ballantyne, D. L. (1971) Biochemistry 10, 4912-4921.
18. Morino, Y., and Okamoto, M. (1973) Biochem. Biophys. Res. Commun. 50, 1061-1067.
19. Wada, H., and Kagamiyama, H. (1973) Abstract of 9th International Congress of Biochemistry (Stockholm) p. 29
20. Wada, H., Watanabe, T., and Miyatake, A. (1971) Biochem. Biophys. Res. Commun. 43, 1318-1323.