TERMINAL AMINO ACIDS OF ASPARTATE AMINOTRANSFERASE ISOZYMES

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INTRODUCTION

Two types of aspartate aminotransferase were obtained in crystalline form from the soluble and mitochondrial fractions of mammalian tissues, and the differences in their physicochemical, kinetic and immunochemical properties have been reported (Wada and Morino, 1964; Morino, Itoh and Wada, 1963; Wada, Kagamiyama and Watanabe, 1968). The purpose of this communication is to report that N-terminal amino acids of s-GOT and m-GOT are alanine and serine, respectively, and the C-terminal amino acids of s- and m-GOT's are glutamine and lysine, respectively. In the latter experiment, selective tritium labeling of the C-terminal amino acid (Matsuo, Fujimoto and Tatsuno, 1966) was used.

MATERIALS AND METHODS

Crystalline preparations of the GOT isozymes were obtained from pig heart as described previously (Kagamiyama, 1966). Each preparation showed a single band on electrophoresis on polyacrylamide gel, but s-GOT could be

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The abbreviations used are: s-GOT and m-GOT, the soluble fraction and mitochondrial aspartate aminotransferase (EC 2.6.1.1. L-aspartate: 2-oxoglutarate aminotransferase), respectively; CPase, carboxypeptidase; FDNB, fluorodinitrobenzene; PTH, phenylthiohydantoin derivative of amino acid.

separated into three components on starch gel or carrier Ampholite-electrophoresis, indicating that s-GOT consists of α, β and γ conformers (Martinez-Carrion et al., 1967). Molecular weights were about 100,000 for both enzymes as determined by the Archibald method (Archibald, 1947).

CPase A was purchased from Worthington and recrystallized three times. CPase B used was the DFP-treated preparation of Worthington. Other chemicals were obtained from commercial sources.

N-Terminal amino acid analyses were carried out by Sanger's FDNB method (Sanger, 1945). The ether-soluble DNP-derivatives were identified by two-dimensional paper chromatography in Levy's system (Levy, 1954), and the aqueous fraction was examined by paper chromatography using tert-amyl-alcohol phthalate buffer, pH 6.0, as the solvent (Blackburn and Lowther, 1951).

CPase digestion was carried out as follows: GOT's were denatured by heating at 100° for three minutes at pH 3.0, since the native enzymes were not attacked by either CPase A or B. Denatured GOT was incubated with CPase A (one-twentieth by weight) or CPase B (one-fiftieth) in 1% sodium bicarbonate at 30°. During the incubation, aliquots containing 0.1 μmole of GOT were removed and analyzed for amino acids released by use of an automatic amino acid analyzer. The identification of glutamine was performed by two-dimensional paper chromatography (pyridine, 50:acetone, 30:ammonia, 50: water, 20 and isopropanol, 80; formic acid, 10; water, 10 (Armstrong, unpublished)).

Hydrazinolysis was performed essentially as described by Akabori et al. (1952). Tritium labeling of C-terminal amino acid was performed in the following way. GOT (5 mg) was dissolved in the mixture of 0.3 ml of pyridine, 0.1 ml of tritiated water (ca. 50 mc) and 0.05 ml of acetic anhydride and incubated at 37° for 20 hours. The mixture was then dialyzed against running water for 24 hours, and the dialyzed preparation was taken to dryness under reduced pressure. The residue was hydrolyzed in 6 N HCl at 110° for 48 hours. The hydrolysate was subjected to high voltage paper electrophore-

sis (60 volts per cm, pH 3.6) and paper chromatography to identify the radioactive amino acid. For the measurement of radioactivity, the chromatogram was cut into small pieces and each piece was counted directly in 10 ml of a phosphor solution (0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-[2-(5-phenyloxazolyl)]benzene in toluene) with a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION

(A) N-Terminal Amino Acid.—The N-terminal amino acid of s-GOT was found to be alanine, confirming the result obtained by Turano et al. (1963). Similar analysis with m-GOT revealed serine as the N-terminal amino acid. Since DNP-serine is not distinguished from DNP-methionine sulfoxide and sulfone in Levy's solvent system, paper chromatography in tert-amylalcohol phthalate buffer, pH 5.0 (Blackburn and Lowther, 1951), was used to exclude the possibility of methionine. A trace of PTH-serine was also found by Edman's method. No PTH-methionine was detected.

Table I shows the results of the quantitative determination of terminal serine. These results indicate that m-GOT has two moles of N-terminal serine per mole of the protein and also that it is composed of two peptide chains like s-GOT. The occurrence of serine at the N-terminus of m-GOT was also reported by Martinez-Carrion et al. (1967).

(B) C-Terminal Amino Acid. 1. Digestion by CPase A and B.—The rate of appearance of free amino acids from s-GOT during digestion with CPase A is shown in Figure 1. Predominant amino acids released at the initial stage of digestion were isoleucine and glutamine. Valine, threonine and lysine also appeared to a lesser extent. From this result it was difficult to decide which was the terminal amino acid. With CPase B no significant amount of any amino acid was released.

In the case of m-GOT, only lysine was liberated upon digestion by CPase B (Fig. 2). About 1.45 moles of lysine was liberated per mole of m-GOT. The

Table I

Quantitative Determination of N-Terminal Serine of m-GOT

Sample	Hours of hydrolysis	DNP-Serine found	Recovery of synthetic DNP-serine	N-Terminal serine calculated
		μmole	%	μmole
m-GOT	11	0.071	40	0.18
(0.1 μmole)	12	0.069	41	0.18
	18	0.080	46	0.17

For quantitative determination of DNP-serine the yellow spot of DNP-serine on paper chromatogram was eluated with 1% sodium bicarbonate and the optical density of the eluate was measured at 360 mμ. In order to determine the recovery factor for DNP-serine, 0.1 μmole of authentic DNP-serine was mixed with equimolar amounts of DNP-m-GOT and treated in the same manner.

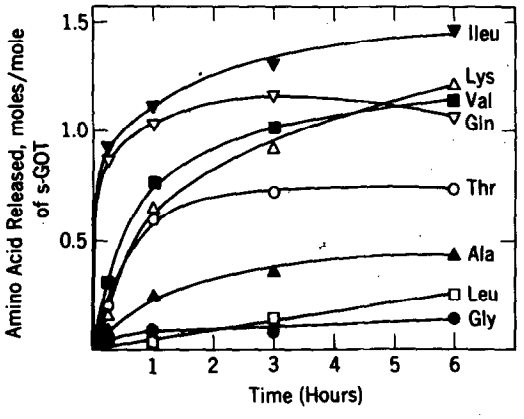

Figure 1. Digestion of s-GOT with CPase A. Reaction conditions are described in the text. Aliquots were removed at intervals and deproteinized by addition of an equal volume of 10% trichloroacetic acid. The supernatant, after salt had been removed by chromatography over Dowex-50, was evaporated to dryness, and the amino acid liberated was measured with the amino acid analyzer. Glutamine was calculated by the increase of glutamic acid after hydrolysis in 2 N HCl for three hours at 110° C.

lower yield of lysine than the expected value of 2 moles might be due to the fact that the denatured protein was insoluble during digestion. By the CPase A digestion, however, valine, alanine and threonine were also found in addition to lysine. This result would be expected if the CPase A used was contaminated with small amounts of CPase B.

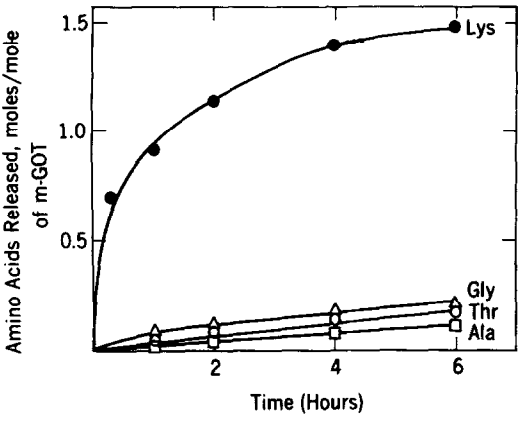

Figure 2. Digestion of m-GOT with CPase B. Reaction conditions are the same as in Fig. 1.

2. Hydrazinolysis.—Hydrazinolysis was carried out under various conditions, including variations in reaction time, the addition of hydrazine sulfate as a catalyst, etc. However, in spite of repeated trials, no significant amount of amino acid was found with either GOT. Since hydrazinolysis is usually not applicable to the analysis of C-terminal glutamine, asparagine or basic amino acids, these results strongly suggest that the C-terminal amino acid of s-GOT is glutamine and that of m-GOT is lysine. The improved hydrazinolytic procedure of Braun and Schroeder (1967) offered no advantages for either GOT.

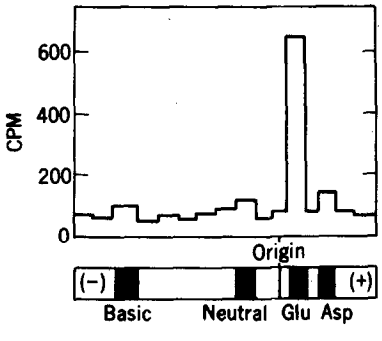

Figure 3. Tritium labeling of C-terminal amino acid of s-GOT. Reaction conditions are described in the text. The figure shows the radioactivity in the region of glutamic acid after high voltage paper electrophoresis.

3. Selective tritium labeling of C-terminal amino acid.—To permit a more definite conclusion this novel method, developed for the analysis of C-terminal amino acids, was employed (Matsuo, Fujimoto and Tatsuno, 1966). The result obtained with s-GOT is shown in Figure 3. Radioactivity was found only in glutamic acid. This result and that of CPase A digestion indicate that the C-terminal amino acid residue of s-GOT is glutamine.*

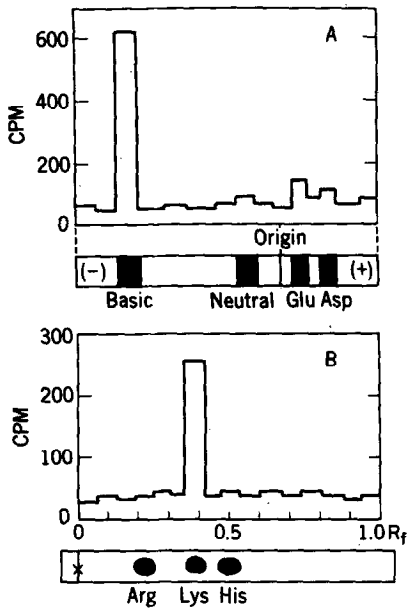

Figure 4. Tritium labeling of the C-terminal amino acid of m-GOT. Reaction conditions are described in the text. The upper figure shows the radioactivity in lysine on the paper chromatogram of the eluate from the basic region of the upper electrophoretogram. The solvent system was pyridine:acetone:ammonia:water (50:20:5:50).

In the case of m-GOT, the radioactivity was found only in the basic amino acid region on the paper electropherogram (Fig. 4). Paper chromatography of the eluate in pyridine:acetone:ammonia:water (50:20:5:20) revealed lysine as the radioactive compound. This confirms the result of CPase B digestion that the C-terminal amino acid is lysine.

*When sodium acetate was used as a catalyst instead of pyridine, radioactivity was recognized in histidine in addition to glutamic acid (Wada, Kagamiyama and Watanabe, 1968). This might be due to the splitting of the peptide chain in the presence of sodium acetate, thus giving rise to a peptide fragment with histidine at the C-terminus.

From these results it is concluded that s- and m-GOT have different terminal amino acids. Recently, Martinez-Carrion et al. (1967) reported that peptide maps obtained by tryptic digestion of these GOT's are also quite different. These facts suggest that the two isozymes have quite different amino acid sequences. Preliminary experiments in this laboratory indicate that the peptides containing pyridoxal phosphate obtained from s- and m-GOT also differ in amino acid composition. Investigations of the amino acid sequence of these peptides are now in progress. A partial report of this work has been made (Wada, Kagamiyama and Watanabe, 1968; Wada, Kagamiyama and Watanabe, in press).

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