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THE COMPLETE AMINO ACID SEQUENCE OF ASPARTATE AMINOTRANSFERASE FROM ESCHERICHIA COLI : SEQUENCE COMPARISON WITH PIG ISOENZYMES

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SUMMARY: The amino acid sequence of aspartate aminotransferase from E. coli B was determined by the alignment of seven cyanogen bromide peptides. The established sequence of the subunit was composed of 396 amino acid residues, and the molecular weight was calculated to be 43,573. The sequence was compared with those of the pig cytoplasmic and mitochondrial isoenzymes, showing that nearly 30% of all residues were invariant and that the E. coli enzyme exhibited the same degree of homology (about 40%) with either of them. Although majority of the residues were substituted, the functional residues constituting the active site structure were conserved.

Aspartate aminotransferase (EC 2.6.1.1, AST) exists as distinct cytosolic and mitochondrial forms in vertebrates (1), while in microorganisms only one molecular form is found (2-5). These enzymes are dimeric proteins, consisting of two identical subunits of about 400 amino acid residues, and the essential mechanisms of their catalytic actions are identical. Although the complete or partial amino acid sequences of the cytosolic and mitochondrial isoenzymes from several kinds of vertebrates have been reported (6-14), little is known about the molecular structures of the microbial enzymes. Elucidation of the molecular structure of microbial ASTs should provide important information on the structure-function relationship and on the evolutionary process of diversion into the two isoenzymes in the higher animals.

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This paper is dedicated to Dr. Esmond E. Snell on the occasion of his 70th birthday.

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We have previously reported the isolation and characterization of AST from E. coli B (15,16). Although the E. coli enzyme was similar in molecular size and catalytic properties to the pig heart isoenzymes, its primary structure seems to be different, because its isoelectric point and immunological properties differed distinctly from either of the pig isoenzymes (15,17).

In this preliminary communication, we report the entire amino acid sequence of E. coli AST, comparing it with the well-known sequences of pig heart isoenzymes.

MATERIALS AND METHODS

Materials. The following materials were purchased commercially: Toyopearl HW-50 (Toyo Soda); Sephadex G-50 and SP-Sephadex C-25 (Pharmacia); DEAE-cellulose DE-32 (Whatman); sodium boro[^{3}H]hydride (New England Nuclear); L-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin and carboxypeptidase A (Worthington); Staphylococcus aureus V8 protease (Miles); cyanogen bromide (Nakarai Chemicals). Chemicals of special grade for sequence analyses were obtained from Wako Pure Chemicals Co. Other chemicals were of analytical grade.

Preparation of AST. E. coli B AST was purified as described previously (15). The enzyme was reduced with sodium boro[^{3}H]hydride to convert the linkage between pyridoxal-5'-phosphate (PLP) and the apoenzyme to a stable form (18), and then carboxymethylated (19).

Isolation of Peptides. The reduced and carboxymethylated enzyme (Cm-enzyme) was cleaved with cyanogen bromide by incubation at 4°C for 16 hours (20). The peptide mixture was fractionated on a Toyopearl HW-50 column with 0.1 M ammonium bicarbonate, and then by SP-Sephadex C-25 or DEAE-cellulose column chromatography. Some peptides were purified by high voltage paper electrophoresis (pH 3.5 or pH 6.5) or by high performance liquid chromatography on a Cosmosil C-18 column (Nakarai Chemicals) with 0-50% gradient of acetonitrile in 0.1% trifluoroacetic acid. The Cm-AST was also digested with trypsin for 6 hours at 37°C. The digest was fractionated on a Sephadex G-50 column with 0.1 M ammonium bicarbonate. Peptides were further purified as described above.

Sequence Analysis. The amino-terminal 33 residues of the Cm-protein was determined with a JEOL 47K sequenator (16). The peptides isolated were sequenced by automated Edman degradation with LKB 4030 solid-phase sequenator (21-23) or by the manual Edman method (24), with a combination of carboxypeptidase digestions. 2-Anilinothiazolinones of amino acids were converted to phenylthiohydantoin (PTH) derivatives by treatment in 1 M HCl at 80°C for 10 min (24). PTH-amino acids were identified by high performance liquid chromatography on a LS-410K C-18 column (Toyo Soda)(25).

Amino Acid Analysis. Amino acid analysis was performed with a JEOL JLC-200A automated amino acid analyzer after the hydrolysis of proteins and peptides in 6 M HCl containing 1% (v/v) phenol or 4 M methanesulfonic acid containing 0.2% (w/v) 3-(2-aminoethyl)indole at 110°C (26).

RESULTS AND DISCUSSION

Seven cyanogen bromide peptides (CN-peptides) were recovered: CN-1 (residues 1-234); CN-2 (235-275); CN-3 (276-314); CN-4 (315-321); CN-5 (322-

347); CN-6 (348-385) and CN-7 (386-396). CN-1 was found to be the amino-terminal peptide on the basis of the results obtained from the sequenator analysis of the whole protein (16). CN-1 was started with heterogeneous amino-terminal residues: methionine (30%); homoserine/homoserine lactone (50%); and phenylalanine (20%), showing the existence of more than one peptide unseparable through the isolation procedure. As the amino-terminal residue of the whole AST was methionine (16), incomplete cleavage of the amino-terminal methionylphenylalanine bond with cyanogen bromide may have resulted in the two peptides. Resistance of an amino-terminal methionyl bond to cyanogen bromide cleavage has been reported previously (27). CN-6 contained two homoserine /homoserine lactone residues, and one of them resulted from incomplete cleavage of a methionylthreonine bond in the peptide. All CN-peptides, except CN-7, were terminated with homoserine/homoserine lactone residues. CN-7 was located at the carboxyl-terminus of the enzyme, because it was a sole peptide lacking a methionine residue. This was confirmed by carboxypeptidase A digestion of the whole Cm-protein: leucine and valine were released rapidly in this order (unpublished data).

Thus, the number of methionine residues in the CN-peptides reaches a total of eight. Since the AST subunit contained eight methionine residues (15), we conclude that all CN-peptides produced were recovered. The sum of amino acid compositions of the seven CN-peptides agreed well with that obtained by hydrolysis of the intact enzyme.

The remaining five CN-peptides (CN-2 to CN-6) were aligned on the basis of sequence information obtained from six overlapping tryptic peptides: T-VI-B333 (230-236); T-VI-B332 (271-276); T-VI-M22 (305-315); T-VII-412 (321-322); T-VI-H21 (344-355) and T-III-IV-G (375-396).

The entire amino acid sequence of E. coli AST is shown in Fig. 1. The subunit contains 396 amino acids and the amino acid composition is Asp_{20}, Asn_{23}, Thr_{22}, Ser_{21}, Glu_{27}, Gln_{16}, Pro_{15}, Gly_{30}, Ala_{46}, Cys_{5}, Val_{26}, Met_{8}, Ile_{17}, Leu_{38}, Tyr_{11}, Phe_{20}, Lys_{18}, His_{6}, Arg_{22}, Trp_{5}. The subunit molecular weight of the apoenzyme is calculated to be 43,573, which is in good agreement

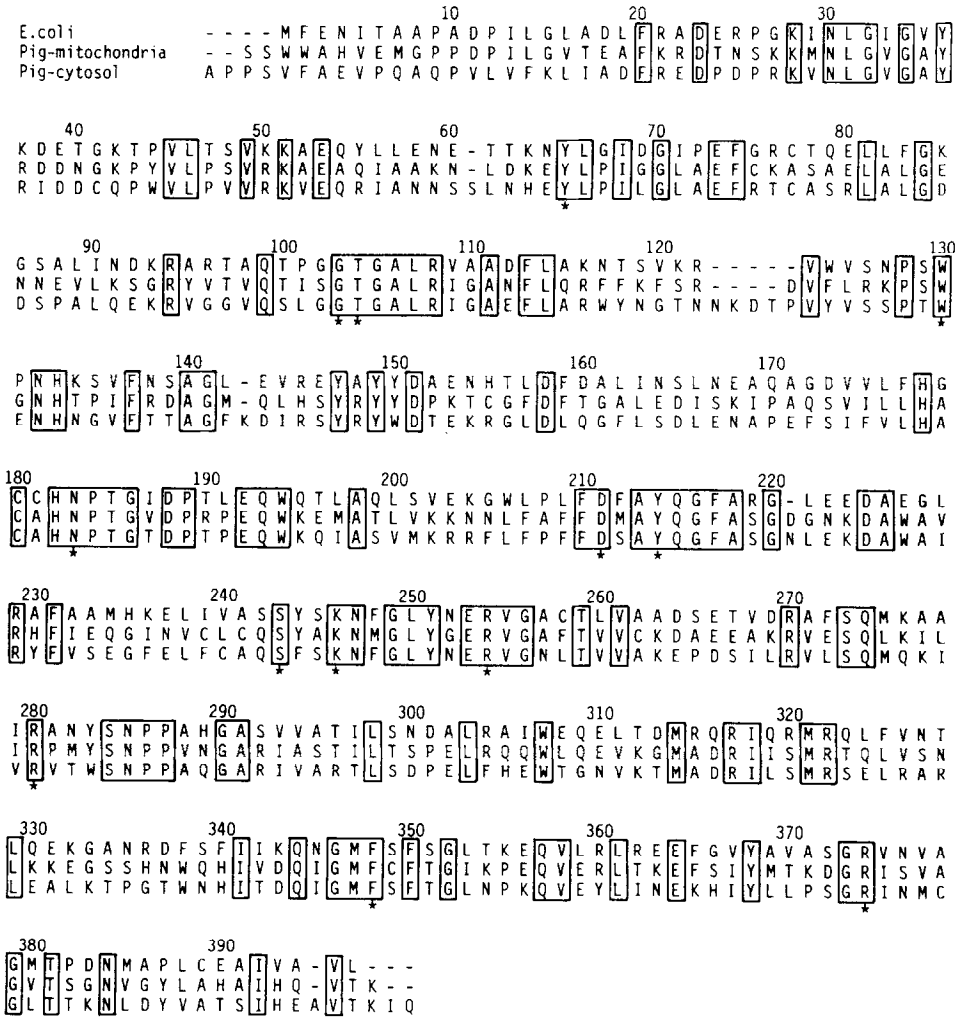

Figure 1. Comparison of amino acid sequences of aspartate aminotransferases from E. coli B and pig heart (6,7,10,11). Deletions are indicated by dashes. Residues identical in three enzymes are boxed. Symbols (*) designate the functional residues participating the active site structure.

with that reported previously (15). The cofactor (PLP)-binding residue was identified as lysine 246 (according to the numbering in E. coli AST) by tritium incorporated into the stabilized almidine linkage.

Fig. 1 shows the comparison of amino acid sequences of E. coli AST and two pig isoenzymes. The E. coli enzyme is the shortest of the three, i.e., 16 and 5 residues less than the cytosolic and mitochondrial enzymes, respectively. In order to obtain maximum sequence homology, we introduce four and five internal gaps in the amino acid sequence of mitochondrial AST and E. coli AST,

respectively, together with some residue deletions at the amino- and carboxyl-termini. The comparison of sequences of the three enzymes shows that there is 29% homology: 120 amino acids are invariant in the three sequences. X-Ray diffraction studies of the isoenzymes from chicken and pig indicated that their three dimensional structures are essentially the same, and the active site residues were proposed in these studies (28-30). Fig. 1 indicates that all these active site residues are conserved during the long evolution, even though numerous residues are substituted. The E. coli enzyme shows a similar degree of identity to either the cytosolic or mitochondrial isoenzyme, i.e., 39% and 40% of identity, respectively. However, it is interesting to note that four of the five internal gap positions in the amino acid sequence of the E. coli enzyme are identical, except an additional deletion in the second gap, with those of the mitochondrial isoenzymes. Closer relatedness between the E. coli enzyme and the mitochondrial isoenzymes may be predicted.

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