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PRIMARY STRUCTURE OF MITOCHONDRIAL GLUTAMIC OXALOACETIC TRANSAMINASE FROM RAT LIVER : COMPARISON WITH THAT OF THE PIG HEART ISOZYME

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SUMMARY

The complete amino acid sequence of the mitochondrial glutamic oxaloacetic transaminase isozyme from rat liver is presented. The sequence contained 401 amino acid residues, 10 of which are methionine. Cyanogen bromide cleavage of mitochondrial glutamic oxaloacetic transaminase produced 12 peptides, one of which contained an internal homoserine residue resulting from incomplete cleavage by cyanogen bromide. The calculated molecular weight was 44,358. The sequence showed 94% homology with that of the corresponding isozyme from pig heart. These findings support the conclusion that the rate of evolution of the mitochondrial isozymes is lower than that of their cytosolic isozymes.

Glutamic oxaloacetic transaminase [L-aspartate : 2-oxoglutarate aminotransferase (EC, 2.6.1.1.)] exists in eukaryotic cells as two different isozymes, one located in the cytoplasm and the other in the mitochondria (1). Both isozymes are composed of two identical polypeptide chains of about 400 amino acid residues, coded for by nuclear DNA (2) and synthesized by cytosolic ribosomes (3). One of the isozymes is then translocated into the mitochondria where a 2000 - 3000 dalton piece is cleaved off to yield mature m-GOT (4,5). The reactions catalyzed by the two isozymes are essentially the same (1). However, the isozymes differ from each other in various

Abbreviations : GOT, glutamic oxaloacetic transaminase ; s-GOT, cytosolic isozyme ; m-GOT, mitochondrial isozyme ; CB, peptides obtained by cleavage with cyanogen bromide

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properties (1) and in their amino acid sequences (6-11). We are interested in comparing the reaction mechanisms and evolutionary rates of the s- and m-GOT isozymes. Comparison of the amino acid sequences of GOT isozymes in various animals is a useful approach to these problems. Previously, we reported differences in the amino acid compositions and NH_2-terminal amino acid sequences of GOT isozymes from rat liver, and pig and human heart (11). This paper describes the complete amino acid sequence of m-GOT from rat liver and its comparison with that of the corresponding isozyme from pig heart.

MATERIALS AND METHODS

Mitochondrial GOT was purified from rat liver as described previously (11). The enzyme was reduced with sodium borohydride (12) and carboxymethylated (13). All chemicals were of analytical reagent grade.

Isolation of CNBr-peptides : Reduced and carboxylmethylated m-GOT was treated with cyanogen bromide (14) and the peptides obtained were chromatographed on a column of Sephadex G-75 equilibrated with 30 % acetic acid. For further purification, the CNBr-peptides were chromatographed on a column of Bio-Gel P-10 equilibrated with 0.05M formic acid or on a column of phosphocellulose equilibrated with 1 mM sodium formate (pH 3.3) containing 8 M urea in a linear gradient of 0 to 0.4 M NaCl. Small CNBr-peptides were separated by paper chromatography or high voltage paper electrophoresis.

Amino Acid Compositions and Sequence Analysis : Amino acid compositions of the peptides were determined in the presence of 4 % thioglycolic acid (15). The hydrolysates of all CNBr-peptides were incubated at 105°C for 1 h in 0.2 N pyridine adjusted to pH 6.5 with acetic acid to convert homoserine lactone to homoserine before analysis (16). Cysteine was determined as S-carboxymethyl cysteine (17). The largest CNBr-peptides were hydrolyzed with trypsin after maleylation (18). The sequences of isolated peptides were determined by a combination of tryptic (or chymotryptic) digestion, carboxypeptidase digestion and manual Edman degradation (19). Phenylthiohydantoin derivatives were identified by thin layer chromatography (20). In some cases, the subtractive procedure was employed for identification of cleaved residues.

Details of this work will be reported elsewhere.

RESULTS AND DISCUSSION

The aligment of CNBr-peptides from rat liver m-GOT was deduced by homology along the polypeptide chain with the sequence of the pig heart isozyme. The complete amino acid sequence of the rat

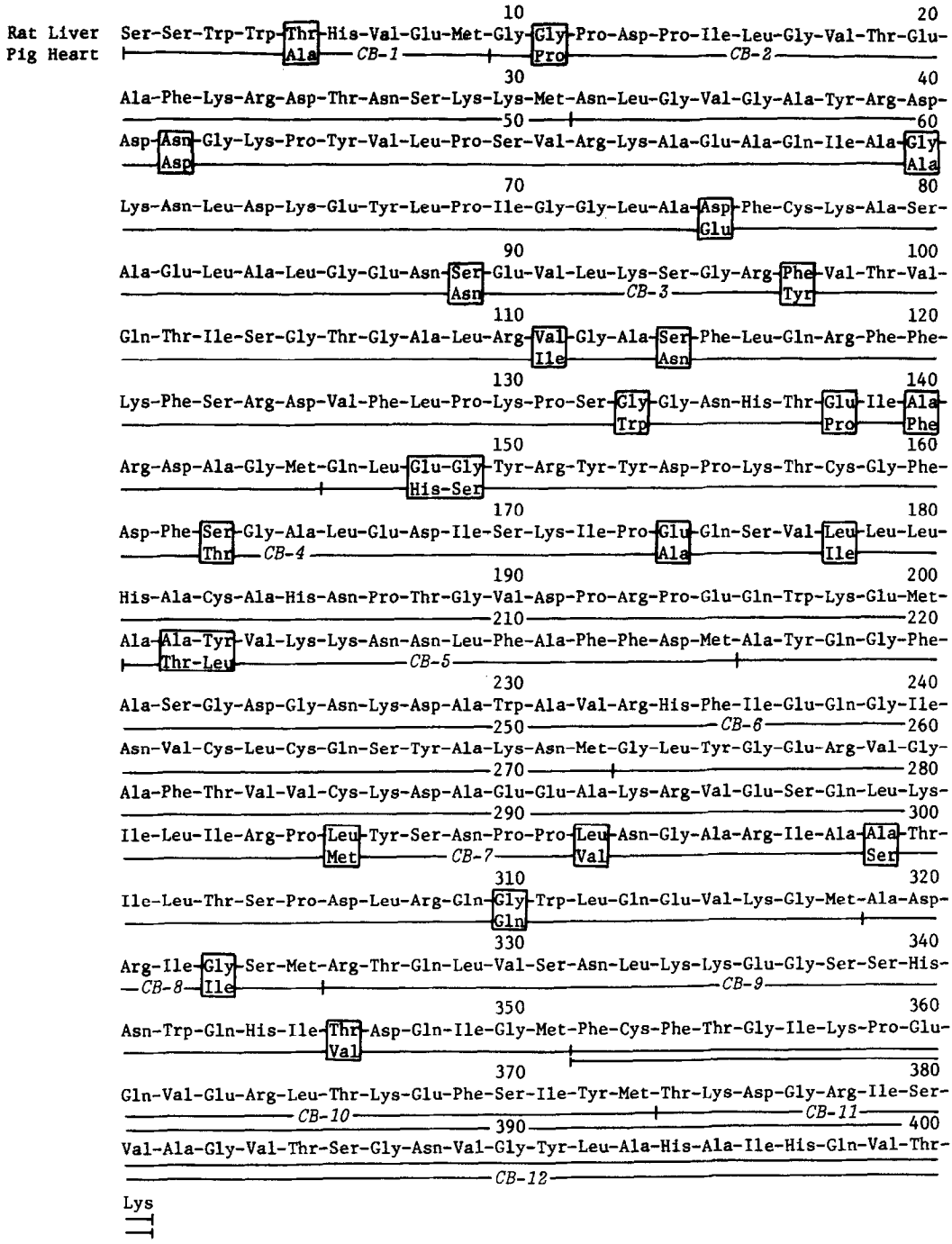

Fig. 1 Primary Structure of m-GOT from Rat Liver in Comparison with That of the Corresponding Isozyme from Pig Heart (7,8). The continuous sequence is that of rat liver m-GOT. Frames indicate differences between the two isozymes.

liver enzyme is shown in Fig. 1, indicating that the subunit consists of 401 residues. The amino acid composition of the subunit was Trp_6, Lys_{28}, His_9, Arg_{20}, Asp_{20}, Asn_{17}, Thr_{19}, Ser_{26}, Glu_{22}, Gln_{18}, Pro_{17}, Gly_{39}, Ala_{34}, Cys_7, Val_{26}, Met_{10}, Ile_{20}, Leu_{31}, Tyr_{13}, and Phe_{19}. The calculated molecular weight was 44,358, which is similar to that reported previously (11). Cyanogen bromide peptide -12 (CB-12) contained an internal homoserine residue resulting from incomplete cleavage by cyanogen bromide. This finding explains the isolation of 12, rather than 11, CNBr-peptides from rat liver m-GOT containing 10 methionine residues. Cyanogen bromide peptide-7 (CB -7) in rat liver m-GOT corresponds with CB-7 and CB-8 in pig heart m-GOT. This difference results from the fact that Met_{286} of pig heart m-GOT is replaced by Leu_{286} in rat liver m-GOT. The sequence is compared with that of m-GOT from pig heart in Fig. 1. There is a high homology between the two isozymes and about 94 % of the total amino acids are identical. The homology between the two isozymes varies in different regions of the molecules : it is lower in the N-terminal portion (17 of the first 180 residues are substituted), but this portion is followed by a region of perfect homology (residues 205 - 285, including Lys_{250} which forms the aldimine bond with the cofactor). The C-terminus part is a region of high similarity (6 of the last 116 residues are substituted). A high degree of positional subtitution of glycine is noticed. No functional role of glycine in m-GOT has been reported, but glycine residues have a small side chain and are known to be strong helix breakers, so this difference may indicate a significant difference in the structures of the two isozymes.

Further comparison showed that only 25 of 401 residues of rat liver m-GOT differed from those of the pig heart isozyme. These results support the conclusion that the rate of evolution of

the m-GOT isozymes in various animals has been slower than that of their s-GOT isozymes (21,22). It will be interesting to determine the complete amino acid sequence of s-GOT from rat liver to obtain information on the differences in the rates of evolution of GOT isozymes.

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