Vol. 156, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1988 Pages 445-450

Partial Amino Acid Sequence Analysis of Human Placenta Monoamine Oxidase A and Bovine Liver Monoamine Oxidase B

Shiuan Chen[1]+ and Walter Weyler[2]

[1]Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA 91010

[2]Molecular Biology Division, VA Medical Center San Francisco, CA 94121

Received August 15, 1988

We have prepared peptide maps from human placenta monoamine oxidase type A (MAO-A) and bovine monoamine oxidase type B (MAO-B) and determined the amino acid sequences of 21of these peptides. These sequences have been compared to the cDNA deduced amino acid sequences of human MAO-A and -B. A result of special interest is the identification of two sets of MAO-A peptides which have sequences different from those deduced from cDNA sequences. This observation is consistent with the notion that MAO-A may be composed of at two subunits which are similar but not identical in primary amino acid sequence.

Monoamine oxidase (MA0) [monoamine: oxygen oxidoreductase (deaminating), EC 1.4.3.4.], a covalent FAD flavoenzyme, is of considerable pharmacological interest because of its central position in the metabolism of monoamine neurotransmitters. Based on their specificity for substrates and inhibitors, particularly the selective suicide inhibitors clorgyline and deprenyl, two types of MAO have been described, MAO-A and B [1-3]. Although the human brain contains both types of MAO, MAO-B, but not MAO-A, has recently been found to be directly involved in the induction of Parkinsonism by the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [4-6].

Recently, we have prepared tryptic peptides of both purified human placenta MAO-A and bovine liver MAO-B. A significant number of peptides from these two preparations have been purified and sequenced. This is the first time that amino acid sequences of two types of MAO other than that of the FAD penta peptide have been determined. These sequences are

+Correspondence should be addressed to this author.

0006-291X/88 $1.50

important not only in illustrating the structural difference between two types of MAO, but also in designing oligonucleotide probes for the identification of MAO clones. These sequences are also very important in confirming cDNA sequences, especially frame shift errors [7], and for the detection of a frame shift in cDNA clones [8].

Materials and Methods

Enzyme Isolation. Bovine liver MAO-B was purified from bovine liver mitochrondia according to the procedure described by Salach [9] and further purified by sucrose density centrifugation as previously reported [10]. Human placenta MAO-A was purified as previously reported [11].

Amino Acid Composition. Amino acid composition was determined on a Beckman 121 MB amino acid analyzer after hydrolysis in 5.7N HCl at 110^0 C for 24 hours and cysteine content was determined as cysteic acid after performic acid oxidation.

Carboxymethylation and Trypsin Digestion of MAO. Enzyme (1 mg) in 1 ml of 100 mM phosphate buffer, pH 7.4, containing 6 M guanidine hydrochloride, was incubated with 0.96 mM 2-mercaptoethanol for 6 hours at room temperature, followed by an overnight incubation in the presence of 60 mg of iodoacetic acid. The sample was dialyzed for 24 hr against 4 liters of water and for 24 hr against 4 liters of 100 mM ammonium bicarbonate. The dialyzed sample was digested overnight in the presence of trypsin (40 µl) at 37^0 C.

Separation of peptides by reverse-phase HPLC. The peptides were separated on a Vydac C-18 (Western Analytical Labs) analytical column and rechromatographed with an Altex ultrasphere ODS (Beckman Instruments) analytical column. Chromatographic conditions were similar to those described by Yuan et al. [12].

Analysis of isolated peptides. The isolated peptides were characterized by amino acid analysis, sequence analysis and fast atom bombardment mass spectral (FAB/MS) analysis. Automated sequence analysis was performed on a gas phase sequencer built at the City of Hope [13]. Positive ion FAB/MS was used to confirm our results as previously described [14].

Results and Discussion

The tryptic maps of the human placenta MAO-A and bovine liver MAO-B generated by reverse phase HPLC are shown in Figure 1 and are clearly not the same,indicating that the primary structures of these two MAOs are substantially different from each other. The cysteine-containing peptides (identified by [^{14}C] iodoacetic acid labeling) are also different for the two MAOs. The amino acid sequences of seven tryptic peptides of human placental MAO-A were determined and used to identify a cDNA clone encoding for human MAO-A [15]. The amino acid sequences of fourteen tryptic peptides of bovine liver MAO-B were also determined. These bovine MAO-B sequences are compared to the sequences deduced from human MAO-A and -B cDNA [15, 16]. It has been reported that the amino acid sequences of

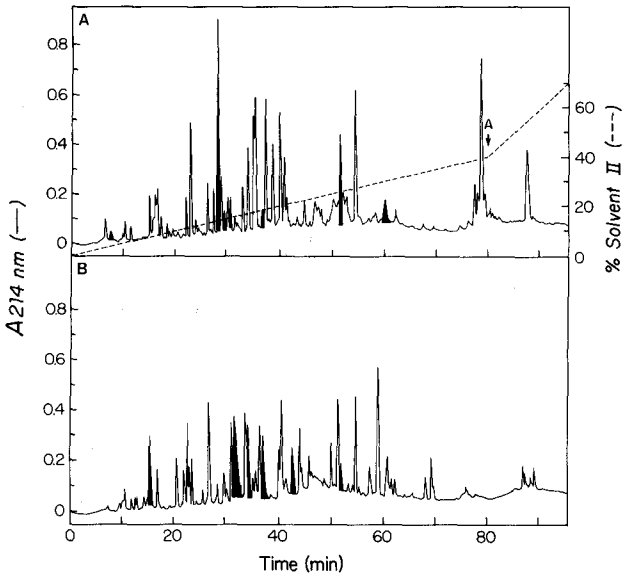

Fig.1. Fractionation of tryptic digest of [^{14}C]-carboxymethylated human placenta MAO-A (A) and bovine liver MAO-B (B) by reverse phase HPLC.

The peptides were separated on a Vydac C-18 column (25 cm x 5 mm) with an 80-min linear gradient from 100% solvent I (0.1% TFA) to 40% solvent II (TFA-water-acetonitrile = 0.1:9.9:90, V/V/V). The flow rate was 0.8 ml/min. One ml of 50% 1-propanol was injected to the column at the end of 80 min. gradient program, indicated as "A" on the chromatogram, to elute the core peptides. Fractions were manually collected and 10µl of aliquot from each peak fraction was counted for [^{14}C] radioactivity. The shaded peaks represent the radioactive fractions.

human MAO-A and -B are ~ 70% identical [16]. Figure 2 shows that those amino acid residues that are identical between human MAO-A and -B are usually conserved in bovine MAO-B sequences as well. More interestingly, among those amino acid residues that differ between human MAO-A and -B, the corresponding residues in bovine MAO-B peptides are identical to those of human MAO-B in most cases. These results show that the primary structures among the same type of MAO from different species are more conserved than those of the two types of MAO from the same species. These results suggest that the genes for MAO-A and -B are derived from a common progenitor gene that probably diverged very early during the revolution.

During amino acid sequence analysis of peptides isolated from the placenta MAO-A, we found that two peptides have eight residues identical to each other over 10 residues (Figure 3). They contain one conservative substitution from Asn to Gln and one substitution from Asp to Tyr. These sequences are different from the amino acid sequence deduced from

Fig. 2. Comparison of amino acid sequences of bovine liver MAO-B peptides with the sequences deduced from human MAO-A and -B.

In each set, the deduced sequence of human MAO-A, the deduced sequence of human MAO-B, and the determined amino acid sequences of bovine MAO-B are shown in lanes 1, 2, and 3, respectively. The numbering of amino acids of human MAO-A peptides is shown according to Hsu et al. [15]and used to indicate the positions of these peptides. The asterisks indicate positions occupied by identical amino acids.

cDNA ^{230}I M D L L G D Q V K^{239}. Furthermore, we determined the sequence of one peptide from human placenta MAO-A as E V L H A V G K. This sequence has strong homology to that of one peptide from bovine liver MAO-B, E I L H A M G K. As shown in Figure 2, the

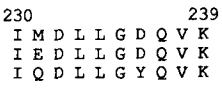

Fig. 3. Comparison of amino aicd sequences of two human placenta MAO-A peptides (lanes 2 and 3) with a sequence deduced from human MAO-A cDNA (lane 1).

sequence of this bovine liver MAO-B peptide is completely identical to that of the corresponding peptide in human MAO-B. However, the sequence of the mentioned human placenta MAO-A peptide is different from that of the corresponding peptide region deduced from the cDNA sequence, ^{458}E V L N G L G K^{465}. These results are consistent with the notion that human placenta MAO-A consists of two subunits which are very similar, and possibly arose by gene duplication. Our results can also be explained by DNA polymorphism as suggested by Ozelius et al. (17).

In summary, this communication we have presented data including peptide mapping and amino acid sequences information of human placenta MAO-A and bovine liver MAO-B. We have compared the sequences of bovine liver MAO-B peptides with those of human MAO-A and -B. We have also provided results indicating sequence heterogeneity for human placenta MAO-A.

Acknowledgments: This work was supported by the Veterans Administration and NIH grants (MH42462, NS25786 and CA33572) to Shiuan Chen. The authors would like to thank Dr. Terry D. Lee and Mr. Kassu Legesse for assistance with FAB Mass Spectral analysis of the isolated peptides.

References

1. Fuller, R.W., (1968) Biochem. Pharmac., 17, 2097.

2. Johnston, J.P. (1968) Biochem. Pharmac., 17, 1285.

3. Knoll, J.P. and Magyar, K., (1972) Adv. Biochem. Pyschopharmac., 5, 393.

4. Chiba, K., Trevor, A., and Castagnoli, Jr., N., (1984) Biochem. Biophys. Res. Commun., 120, 574-578.

5. Langston, J.W., Irwin, J., Langston, E.B., and Bomo, L.S. (1984) Science, 225, 1480-1482.

6. Markey, S.P., Johannessen, J.N., Chiueh, C.L., Burns, R.S., and Herkenham, M.A., (1984) Nature, 311, 464-467.

7. Bienkowski, M.J., Haniu, M., Nakajin, S., Shinoda, M., Yanagibashi, K., Hall, P.F., and Shively, J.E., Biochem. Biophys Res. Commun., (1985) 25, 734-740.

8. Chen, S., Besman, M.J., Sparkes, R.S., Zollman, S., Klisak, I., Mohandas, T., Hall, P.F., and Shively, J.E. (1988), DNA, 7, 27-38.

9. Salach, J.I., (1979) Arch. Biochem. Biophys., 192, 128-137.

10. Weyler, W. and Salach, J.I., (1981) Arch. Biochem. Biophys., 212, 147-153.

11. Weyler, W. and Salach, J.I., (1985) J. Biol. Chem., 260, 13199-13207.

12. Yuan, P.-M., Pande, H., Clark, B.R., and Shively, J.E., (1986) Anal. Biochem., 120, 289-320.

13. Hawke, D.H., Harris, D.C., and Shively, J.E. (1985) Anal. Biochem., 147, 315-320.

14. Chen, S., Lee, T.D., Legesse, K., and Shively, J.E. (1986) Biochemistry, 25, 5391-5395.

15. Hsu, Y.-P.P., Weyler, W., Chen, S., Sims, K.B., Rinehart, W.B., Utterback, M., Powell, J.F., and Breakefield, X.O. (1988), J. Neurochem., in press.

16. Bach, A.W.J., Lan, N.C., Johnson, D.L., Abell, C.W., Bembenek, M.E., Kwan, S.-W., Seeburg, P.H., and Shih, J.C. (1988) Proc. Natl. Acad. Sci. USA, 85, 4934-4938.

17. Ozelius, L., Hsu Y.-P.P., Bruns, G., Powell, J.F., Chen, S., Weyler, W., Uttenback, M., Zucker, D., Haines, J., Trofatter, J.A., Conneally, P.M., Gusella, J.F., and Breakefield, X.O. (1988), Genomics, in press.