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PRIMARY STRUCTURE OF RAT BRAIN PROTEIN CARBOXYL METHYLTRANSFERASE DEDUCED FROM cDNA SEQUENCE

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Two cDNA clones for protein carboxyl methyltransferase were isolated from a rat brain cDNA library in λgt 11 with synthetic oligonucleotides as probes. The two clones differ in size, but the nucleotide sequence including the whole coding region of the shorter cDNA is completely identical with the corresponding sequence of the longer cDNA. The open reading frame encodes a polypeptide of 227 amino acid residues, with a molecular weight of 24,626. This molecular weight is comparable to those reported for other protein carboxyl methyltransferases from several animals, which were determined by gel filtration chromatography or sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Protein carboxyl methyltransferase (E.C. 2.1.1.24) can methylate side-chain carboxyl groups in proteinaceous substrates with S-adenosyl-L-methionine as the methyl donor. In the rat, this enzyme was found in all tissues examined, with the highest activity in the brain (1), where we found that tubulin and high molecular weight microtubule-associated proteins were good substrates (2). At present, this enzyme is believed to methylate abnormal aspartic acid residues such as D-aspartyl and L-isoaspartyl residues, on a wide variety of proteins (cf. ref. 3,4). Considering these circumstances, Clarke (3) and Aswad and Johnson (4) have proposed that protein carboxyl methyltransferase plays a role in the repair or degradation of the D-aspartyl and L-isoaspartyl residues that accumulate in aged proteins.

There are reported to be several isozymes of protein carboxyl methyltransferases. Two major isozymes have been purified from the cytosolic fraction of bovine brain (5,6) and human red blood cells (7) to near homogeneity. These two isozymes have similar catalytic and structural properties, but

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can be separated by anion exchange column chromatography. Recently, Gilbert et al. (8) reported considerable similarities in the amino acid compositions and peptide maps of the two isozymes of human erythrocytes. Furthermore, the analysis of their partial amino acid sequences revealed very high structural homology between them. From these findings, they suggested that the two isozymes have nearly identical structures.

In mammalian brain, this enzyme activity was also detected in membranous fractions (9,10). However, the properties of membrane bound enzyme and its relation to the cytosolic enzymes are unknown.

To obtain further informations about the primary structures of these isozymes, in this work we isolated and sequenced cDNA clones for protein carboxyl methyltransferase from a rat brain cDNA library.

MATERIALS AND METHODS

Screening of a rat brain cDNA library : A λgt 11 library of rat brain cDNA was obtained from Clonetech (Palo Alto). The phage library was subjected to hybridization in 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 1M NaCl, 0.2% Ficol 400, 0.2% polyvinyl-pyrrolidone K-90, 0.2% bovine serum albumin, 0.1% sodium dodecyl sulfate, 100 μg/ml of herring DNA and ^{32}P-labeled probes at 50°C. Two synthetic oligonucleotides (P-48, ATGAAGCCCCTGATGGGC-GTGATCTATGTGCCCCTGACAGACAAGGAG and P-66, CTGATCCTGCCTGTGGGCCCTG-CTGGCGGCAACCAGATGCTGGAGCAGTATGACAAGCTGCAGGAT) were labeled at the 5' end with T4 polynucleotide kinase and used as probes. These nucleotide sequences were designed by the method of Lathe (11) from the reported amino acid sequences of tryptic fragments of the human erythrocyte enzymes (8); P-48 corresponds to the amino acid sequence Met-Lys-Pro-Leu-Met-Gly-Val-Ile-Tyr-Val-Pro-Leu-Thr-Asp-Lys-Glu and P-66 to Leu-Ile-Leu-Pro-Val-Gly-Pro-Ala-Gly-Gly-Asn-Glu-Met-Leu-Glu-Gln-Tyr-Asp-Lys-Leu-Gln-Asp. Phage DNAs were prepared from positive clones and the cDNA inserts were excised from the phage DNA by digestion with EcoRI and subcloned into pBluescriptII vector (Stratagene, La Jolla). DNA was sequenced by the chain termination technique (12).

RNA gel blot analysis : Total RNA was prepared from rat brain by the method of Chirgwin et al. (13). Poly (A^+) RNA, which was separated on an oligo (dT) cellulose column (14), was subjected to electrophoresis on 1.5% agarose/formaldehyde gel and transferred to a nitrocellulose membrane as described in (14). The RNA blot was hybridized with a ^{32}P-labeled probe, which was made by the random primer labeling method described by Feinberg and Vogelstein (15).

RESULTS AND DISCUSSION

Cloning and sequencing of protein carboxyl methyltransferase : A rat brain cDNA library (λgt 11) was screened with the two

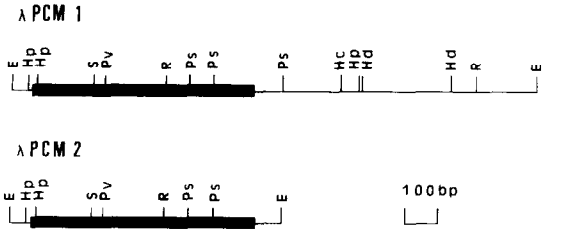

Fig.1 : Restriction Cleavage Maps of cDNAs (λPCM 1 and λPCM 2) for Rat Brain Protein Carboxyl Methyltransferase. The closed column in λPCM 1 and λPCM 2 represents the coding region. Untranslated sequences are shown by a thin lines. The restriction endonucleases used were : E, EcoRI; Hp, HpaII; S, SphI; R, RsaI; Ps, PstI; Pv, PvuII; Hc, HincII; Hd, HindIII.

oligonucleotides (P-48 and P-66). Five positive clones were isolated from the library. Two of these clones have DNA inserts of about 1.6 Kbp (λPCM 1), while the others have inserts of about 0.8 Kbp (λPCM 2). These clones were digested with various restriction endonucleases to make restriction maps of their cDNA (Fig.1). For determination of the nucleotide sequence by the chain termination method, we made several deletion mutants by digesting the subcloned plasmid with KpnI and XbaI, followed by deletion with exonucleaseIII according to the methods recommended by the suppliers. The nucleotide sequence of λPCM 2 is completely identical with the corresponding sequence of λPCM 1. This nucleotide sequence and the deduced amino acid sequence are shown in Fig.2. The open reading frame starts from a putative ATG initiation codon [1-3] preceded by 60 nucleotides of a 5'-untranslated sequence and ends at a TGA codon [682-684]. The nucleotide sequence including initiator AUG, TCGCGAUGG, is quite similar to the consensus sequence of $CC^{A}_{G}CCAUGG$ (16).

The primary structure deduced from the nucleotide sequence of the cloned cDNA is composed of 227 amino acid residues with a molecular weight of 24,626. This molecular weight is consistent with the values of 24,300-28,000 reported for several animal enzymes (5-8), which were determined by gel filtration chromatography or sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The deduced amino acid sequence of the rat brain enzyme coincides almost completely with the sequences of tryptic peptides (T1, T4-T6, T8, T10, T12-T15) obtained from the human erythrocyte enzymes, indicating that our cDNA clone encodes protein carboxyl methyltransferase. This enzyme is

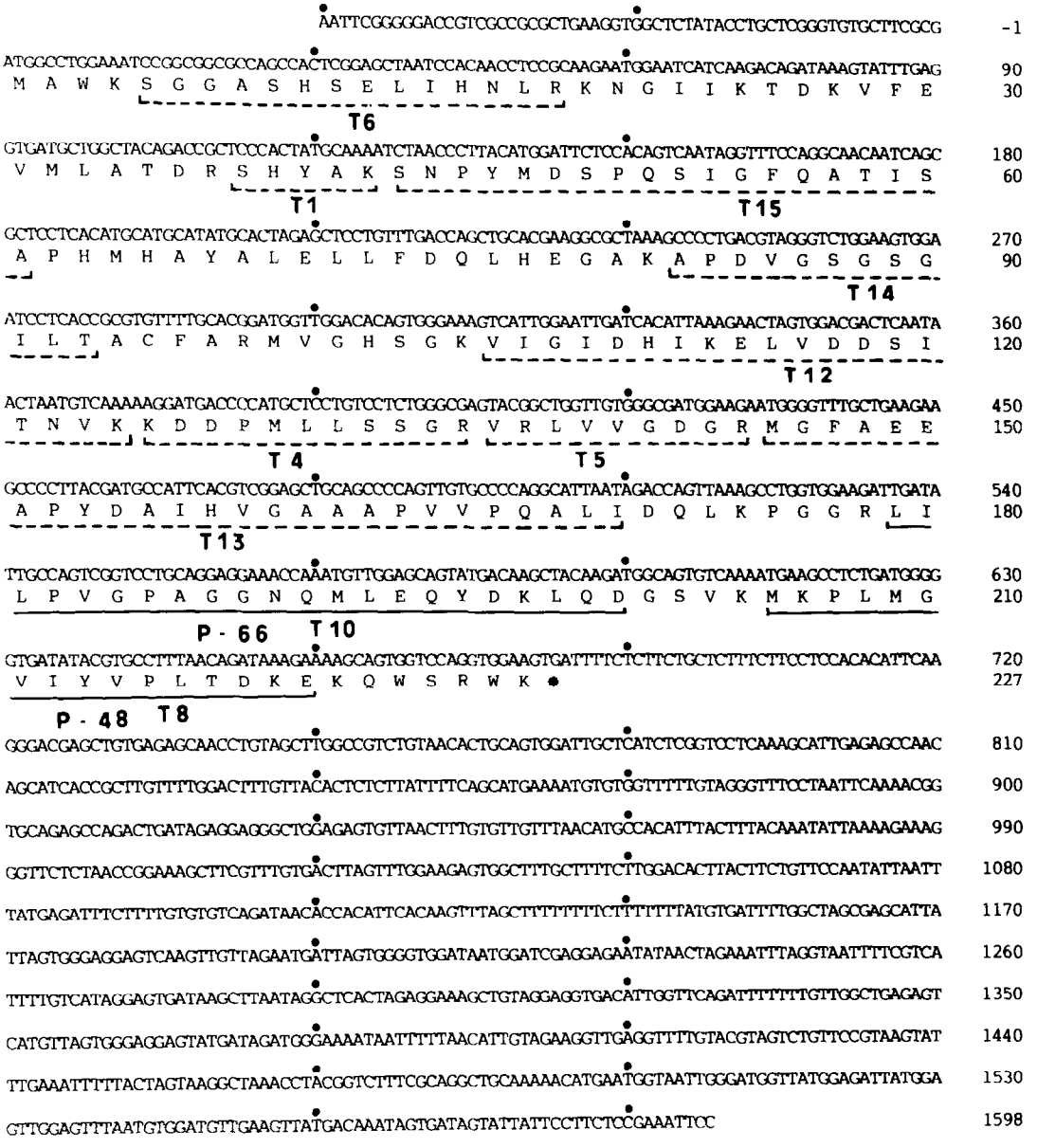

Fig.2 : Nucleotide and Predicted Amino Acid Sequences of the Rat Brain Enzyme. Nucleotides and amino acids are numbered at the right side of the sequence. The terminal codon is indicated by an asterisk. Two amino acid sequences, T8 and T10, were used for design of the synthetic oligonucleotide probes, P-48 and P-66, respectively. Broken underlines (T1, T4-T6, T8, T10, T12-T15) indicate amino acid sequences corresponding to those of erythrocyte enzymes (8).

probably highly conserved, judging from the considerable similarities in the amino acid sequences of the enzymes in rat brain, human erythrocytes and bovine erythrocytes (Fig.3), although the complete sequences of the erythrocyte enzymes are

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rat      MAWKSGGASHSELIHNLRKNGIIKTDKVFEVMLATDRSHY    40
human        --------------                  ---
bovine       --------------                  ---

         AKSNPYMDSPQSIGFQATISAPHMHAYALELLFDQLHEGA    80
         --G------------------
         --A------------------

         KAPDVGSGSGILTACFARMVGHSGKVIGIDHIKELVDDSI   120
          -L----------           --------------V
          -L----------           ---------------

         TNVKKDDPMLLSSGRVRLVVGDGRMGFAEEADYDAIHVGA   160
         N--R----T-------Q---------T-------------
         N--R------------Q---------T-------------

         AAPVVPQALIDQLKPGGRLILPVGPAGGNQMLEQYDKLQD   200
         ----------       ----------------------
         ----------------------------------------

(3)      GSVKMKPLMGVIYVPLTDKEKQWSRWK                227
         --I-----------------
         --- -----------------
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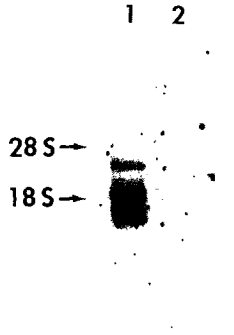

(4)

Fig.3 : Comparison of the Deduced Amino Acid Sequence of the Rat Brain Enzyme with those of Human and Bovine Erythrocyte Enzymes. The numbering of amino acids is shown on the right side. The dashes indicate positions occupied by identical amino acids. The sequences are shown in the single-letter code. Sequences of erythrocyte enzymes are cited from (8).

Fig.4 : Sizes of mRNAs for Rat Brain Enzyme(s) by RNA Blot Hybridization Analysis. Poly (A^+) RNA prepared from rat brain total RNA was electrophoresed on 1.5% agarose/0.66 M formaldehyde gel, then blotted onto a nitrocellulose membrane. The membrane was hybridized with a ^{32}P-labeled DNA fragment (HpaII/EcoRI, nucleotide number 14/762) from the cDNA. The amounts of RNA used were 7.5 µg (lane 1) and 2.5 µg (lane 2). Ribosomal 28S (4.7 Kb, ref.17) and 18S (1.9 Kb, ref.18) RNAs were used as size markers.

not known. The cysteine residue at position 112 may be important for enzymatic activity, because protein carboxyl methyltransferase lost its activity during purification in the absence of thiol compounds (6,7).

We compared the nucleotide sequence of the rat brain enzyme with the nucleotide sequences supplied by GenBank using the FASTA program of Pearson and Lipman (19). We could not find any nucleotide sequences having significant homology with the coding region of the cDNA. However, the computer picked up one nucleotide sequence that showed apparent homology (63.3%) with the 3'-untranslated region (nucleotide residues 1310 to 1580) of our cDNA. This nucleotide sequence is a KpnI repetitive sequence (20) found in 3'-untranslated or 3'-noncoding sequences of primate cDNAs or genes.

RNA blot hybridization analysis : Using the cDNA fragment (HpaII/ EcoRI, 14/762) from λPCM 2 as a probe, three transcripts of 3.4 Kb, 2.0 Kb and 1.3 Kb were detected in rat brain (Fig.4). As the size of our cDNA in λPCM 1 is 1.6 Kb, it may correspond

to the middle sized mRNA. The properties of the longer (3.4 Kb) and the shorter (1.3 Kb) transcripts are not clear at present. It is also unknown whether these transcripts encode different species or only one species of protein carboxyl methyltransferase.

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