Molecular Cloning of cDNA and Chromosomal Assignment of the Gene for Human Phenylethanolamine N-Methyltransferase, the Enzyme for Epinephrine Biosynthesis*

(Received for publication, December 14, 1987)

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Phenylethanolamine N-methyltransferase (PNMT; EC 2.1.1.28) catalyzes the synthesis of epinephrine from norepinephrine, the last step of catecholamine biosynthesis. To isolate a cDNA clone for human PNMT, we first isolated a cDNA clone for bovine adrenal medulla PNMT using mixed oligodeoxyribonucleotide probes whose synthesis was based on the partial amino acid sequence of tryptic peptides from the bovine enzyme. By screening a bovine adrenal medulla cDNA library, a cDNA clone with an insert of about 200 base pairs (bp) was isolated. This clone consisted of 84 bp of carboxyl-terminal coding region, which contained amino acid sequences corresponding to two tryptic peptides, and about 100 bp of 3′-untranslated region. Using this cDNA fragment as the probe, we screened a human pheochromocytoma cDNA library and isolated a cDNA clone with an insert of about 1.0 kilobase pairs, which contained the complete coding region of the enzyme. Northern blot analysis of human pheochromocytoma poly(A)+ RNA using this cDNA insert as the probe showed a single RNA species of about 1,000 nucleotides, suggesting that this clone is a full-length cDNA. Determination of the nucleotide sequence revealed that human PNMT consists of 282-amino acid residues with a predicted molecular weight of 30,853, including initial methionine. The amino acid sequence of the human PNMT was highly homologous (88%) to that of the bovine enzyme. Chromosomal assignment of the gene for human PNMT was carried out using mouse-human somatic cell hybrids. The PNMT gene was assigned to chromosome 17.

* This work was supported by Grant-in-Aid for Scientific Research on Priority Areas, Ministry of Education, Science, and Culture, Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: PNMT, phenylethanolamine N-methyltransferase; SDS, sodium dodeyl sulfate; HPLC, high performance liquid chromatography; bp, base pairs; kb, kilobase pairs.

to form epinephrine, using S-adenosyl-L-methionine as the methyl donor (1, 2). The enzyme is found predominantly in chromaffin cells of adrenal medulla, in which epinephrine is synthesized as a hormone. It is also distributed in adrenergic neurons of the medulla oblongata and hypothalamus in the brain (3-5), where epinephrine functions as a neurotransmitter. Although the details of epinephrine neuron function in the brain are not yet clearly understood, these neurons may be involved in important neurophysiological functions such as cardiovascular and neuroendocrine regulation of the central nervous system (6). Several lines of evidence for the involvement of brain epinephrine neurons in blood pressure regulation have been presented in studies using genetically hypertensive rats, in which both PNMT activity and epinephrine level were elevated in discrete regions of the medulla oblongata and hypothalamus (7-10). Development of the enzyme activity is regulated by glucocorticoids in embryonic adrenal gland (11, 12), and the enzyme activity in cultured chromaffin cells and in superior cervical ganglia is induced by nerve growth factor (13, 14). The enzyme has been purified to homogeneity from bovine and rabbit adrenal medulla (15-18). It is a monomeric protein with a molecular weight of 30,000-31,000 (16, 18), and the enzymatically active isoforms have been noted in bovine and rabbit adrenal medulla (15, 18, 19).

Molecular cloning of the enzyme's cDNA should facilitate further studies on hormonal and developmental regulation of PNMT gene expression and genetic analysis of the isoforms as well as the structural basis of the enzyme reaction. As a first step, we describe here the complete nucleotide sequence of human PNMT cDNA and the deduced amino acid sequence of the enzyme. We also describe for the first time the chromosomal assignment of the gene for this enzyme.

MATERIALS AND METHODS

RESULTS

Purification and Partial Amino Acid Sequences of Bovine Adrenal PNMT—PNMT was purified to homogeneity from the 100,000 × g supernatant of bovine adrenal medulla by means of DEAE-Sepharose chromatography followed by ammonium sulfate fractionation and gel filtration on a Sephadex
G-100 column. The purified preparation showed a single band on SDS-polyacrylamide gel electrophoresis and had a specific activity of 7,600 nmol/h/mg protein. This is comparable to values reported for purified enzymes (15, 18). About 100 g of adrenal medulla yielded about 30 mg of the pure enzyme. The pure enzyme preparation was reduced and carboxymethylated for trypsin digestion. The tryptic peptides were separated on a reverse-phase HPLC. Table I (see Miniprint) shows the amino acid sequences of four tryptic fragments. An attempt to sequence the amino-terminal region of the bovine enzyme was unsuccessful due to blocking of the amino terminus.

**Isolation and Identification of cDNA Clone Encoding Bovine Adrenal PNMT**—An amino acid sequence of peptide T4 seemed to be the most suitable for synthesizing oligodeoxyribonucleotide probes, because only 16 kinds of nucleotides could represent all possible nucleotide sequences corresponding to this amino acid sequence. The following mixed tetramer was chemically synthesized:

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Phe Phe Thr Trp Ala
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Bovine adrenal medulla cDNA library was first screened by colony hybridization with these synthetic probes. We isolated two hybridization-positive clones from about 5000 transformants. The inserts of these two clones had the same size (about 500 bp), suggesting that they are identical. Therefore, only one clone, named pbPNMT 14, was subjected to nucleotide sequence analysis. As shown in Fig. 1 (Miniprint), pbPNMT 14-insert cDNA contained nucleotide sequences corresponding to tryptic peptides T3 and T4 and poly(A)* tail, although the NH2-terminal amino acid residue of T3, Thr, was absent from the cDNA clone. In addition, the tryptophanyl residue in T4, which had been determined by amino acid sequencer, was found from the nucleotide sequence to actually be arginyl. For maximum homology, only one gap was inserted at the amino-terminal portion of the protein due to possible misreading of the ATG triplet encoding the initial methionine, and the nucleotide sequence corresponding to the COOH-terminal region is underlined. A stop codon, TAG, is shown by **. The deduced amino acid residues are numbered at right margin beginning with the initiating methionine.

![Fig. 3. Nucleotide sequence of cDNA insert of the clone, pbPNMT 901, encoding human phenylethanolamine N-methyltransferase and its deduced amino acid sequence.](image)

This clone contains 846 bp of a single long open reading frame, starting with an ATG at position 1–3 and ending with a TGA stop codon at position 847–849. The nucleotide sequence corresponding to the COOH-terminal region of the protein at position 766–849 including the stop codon was found to be highly homologous to that of the bovine PNMT cDNA, pbPNMT 14, indicating that this is a cDNA clone encoding human PNMT. The assignment of this initiation site is supported by the fact that the nucleotide sequence preceding the ATG codon, GCAGC, agrees well with the consensus sequence CCACC, in which adenine at position -3 is highly conserved, found in many eukaryotic mRNA (33, 34). This open reading frame codes for a protein with 282-amino acid residues and a predicted molecular weight of 30,853, including the initial methionine. The clone had only 1 bp 5′-untranslated and 84 bp 3′-untranslated sequences. A consensus polyadenylation signal (AATAAA) (95) is located 11 nucleotides upstream of the poly(A)* tail.

**Comparison of Amino Acid Sequence and Amino Acid Composition of Human and Bovine PNMT**—The nucleotide sequence of cDNA for bovine adrenal PNMT and the deduced amino acid sequence have recently been reported (36). However, we found this sequence to be incorrect in the COOH-terminal portion of the protein due to possible misreading of the nucleotide sequence (see “Discussion”). Fig. 4 (Miniprint) shows the amino acid sequence of human PNMT together with that of the bovine enzyme, of which the latter was corrected by assuming sequence homology. For maximum homology, only one gap was inserted at the carboxy-terminal.
region in human PNMT. The sequences differ only at 33 positions, and about 88% homology was observed between the two species. The amino acid sequence was completely conserved in positions 25–47, 52–83, and 171–229, while a certain divergence was observed at positions 4–24, 111–145, and 272–283. The areas of conservation would be important for the enzymatic function.

The amino acid compositions of human and bovine PNMT predicted from the nucleotide sequences were compared (Table II). The human and bovine enzymes were very similar, reflecting a high degree of sequence homology (88% homology, Fig. 4). The amino acid composition of the purified enzyme almost completely agreed with that deduced from the cDNA sequence of the bovine enzyme modified from the original sequence (see “Discussion”). Relatively high contents of arginine, glutamic acid, and leucine were observed.

RNA Blot Analysis—In order to examine the length of PNMT mRNA, poly(A)^+ RNA extracted from human pheochromocytoma was subjected to Northern blot analysis using the ^32P-labeled pHPNMT 901 insert as the probe. As shown in Fig. 5 (Mini-print), a single band corresponding to about 1.0 kb was observed, almost the same size as the cDNA insert of pHPNMT 901, suggesting that the cDNA insert described above is a full-length cDNA.

Chromosomal Assignment of Human PNMT Gene.—To carry out chromosomal assignment of PNMT gene, 15 DNAs from mouse-human somatic cell hybrids with selected human chromosomes were subjected to Southern blot analysis using the pHPNMT 901 cDNA insert as a probe. EcoRI-digested human genomic DNA gave a single band at 21 kb, while mouse DNA also gave a signal at 25 kb due to cross-hybridization. The presence of this human-specific band in these hybrid cells is listed in Table III. From the karyotype analysis data for the hybrid cells, the human PNMT gene was assigned to chromosome 17.

DISCUSSION

This paper describes the isolation and nucleotide sequence of a full-length cDNA clone encoding human PNMT. This cDNA clone was isolated by cross-hybridization with a partial bovine cDNA fragment in a clone, pbPNMT 14, which was also isolated in the present work. The identity of the latter clone was established by the presence of amino acid sequences of two tryptic peptides determined by direct analysis (see Fig. 1). The complete primary structure of human PNMT was deduced from the nucleotide sequence of the cDNA. The enzyme consisted of 282 amino acid residues with a molecular weight of 30,853, including initial methionine, which is in good agreement with the value (30,000–31,000) determined by SDS-polyacrylamide gel electrophoresis for the purified enzyme (16, 18).

Recently, Baetge et al. (36) reported the nucleotide sequence of cDNA encoding bovine adrenal PNMT. The nucleotide sequence reported by Baetge et al. (36) differs somewhat from our sequence corresponding to the COOH-terminal portion of the bovine enzyme. Comparison of the amino acid sequence of bovine PNMT deduced by Baetge et al. (36) with our amino acid sequence of tryptic peptides obtained from the pure preparation of the bovine enzyme revealed that peptides T1 and T2 could be assigned to amino acid positions 30–33 and 154–171, respectively (Fig. 4). However, peptides T3 and T4 could not be identified in Baetge’s sequence, although the carboxyl-terminal sequence of peptide T4, -Ala-Gln-Lys-Lys-OH, was assigned to their amino acid position 278–281. When our nucleotide sequence for the bovine cDNA clone, pbPNMT 14, is compared with their sequence in the region correspond-
Human Phenylethanolamine N-Methyltransferase cDNA

The present paper describes for the first time the chromosomal assignment of PNMT gene. The genes for the enzymes of the catecholamine pathway, tyrosine hydroxylase, dopamine β-hydroxylase, and PNMT, are located on different chromosomes: chromosome 11 (43), 9 (44), and 17, respectively. The cloned cDNA for human PNMT reported here provides a useful tool for studies on regulation of gene expression.

Acknowledgments—We thank Dr. M. C. Yoshida of Hokkaido University, Japan, for providing us mouse-human somatic cell hybrids.

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**Human Phenylethanolamine N-Methyltransferase cDNA**

Phenylethanolamine N-methyltransferase (PNMT) cDNA was isolated from human lung tumor tissue by screening a cDNA library. The sequence was confirmed by comparison with published data.

### Mouse-human somatic cell hybrid clones with selected human chromosomes

| Hybrid clone | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X | Y | Human chromosome |
|--------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1-4          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 1-5          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 1-6          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2-2          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2-3          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3-4          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 7-1          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 1B1-24       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3A6          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3B5          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 7A12         | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 7D2          | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| A/B7-5       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| H/B6-3       | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| H/F1a        | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ = present; − = not present.

**TABLE I**

| Human chromosome | Hybrid clone |
|------------------|-------------|
| 1-4              | +           |
| 1-5              | +           |
| 1-6              | +           |
| 2-2              | +           |
| 2-3              | +           |
| 3-4              | +           |
| 7-1              | +           |
| 1B1-24           | +           |
| 3A6              | +           |
| 3B5              | +           |
| 7A12             | +           |
| 7D2              | +           |
| A/B7-5           | +           |
| H/B6-3           | +           |
| H/F1a            | +           |

**TABLE III**

Mouse-human somatic cell hybrids with selected human chromosomes

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**TABLE II**

| Human chromosome | Hybrid clone |
|------------------|-------------|
| 1-4              | +           |
| 1-5              | +           |
| 1-6              | +           |
| 2-2              | +           |
| 2-3              | +           |
| 3-4              | +           |
| 7-1              | +           |
| 1B1-24           | +           |
| 3A6              | +           |
| 3B5              | +           |
| 7A12             | +           |
| 7D2              | +           |
| A/B7-5           | +           |
| H/B6-3           | +           |
| H/F1a            | +           |

+ = present; − = not present.
Human Phenylethanolamine N-Methyltransferase cDNA

Fig. 2. The restriction map of cDNA insert of the clone, pPHMNT 901, and the sequence strategy. The restriction map displays only relevant restriction endonuclease sites. Approximate arrows show the direction and extent of sequence determinations. The protease cocktail region is indicated by an open box.

Fig. 3. Amino acid sequence comparisons of human and bovine phenylethanolamine N-methyltransferase. Amino acid sequence of the human enzyme is from Fig. 1, and that of the bovine enzyme is from Sapolsky (5). (A) with correction of the original sequence as described in Discussion. The bovine enzyme sequence before correction is also aligned for reference in (B).(C) Portions of the corresponding sequences. Identical amino acid sequences are illustrated by boxes.