cDNA Cloning and Amino Acid Sequence of Bovine Brain 2',3'-Cyclic-nucleotide 3'-Phosphodiesterase*

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2',3'-Cyclic-nucleotide 3'-phosphodiesterase (EC 3.1.4.37) has been widely used as a marker for myelin-oligodendrocytes in the central nervous system. Evidence has been provided that the enzyme is identical with one of the Wolfgram proteins of central nervous system myelin. The amino acid sequence of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase was determined by both protein and cDNA sequence analyses. Protein sequence analysis was done on bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase, a low molecular weight enzyme obtained by solubilization with pancreatic elastase (EC 3.4.21.36) (Nishizawa, Y., Kurihara, T., and Takahashi, Y. (1980) Biochem. J. 191, 71-82; Kurihara, T., Nishizawa, Y., Takahashi, Y., and Odani, S. (1981) Biochem. J. 195, 153-157). Based on the carboxyl-terminal sequence of bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase, synthetic oligodeoxyribonucleotides were prepared and used as probes for screening a cDNA library of bovine brain. A cDNA of 2305 base pairs was obtained and sequenced, and the complete amino acid sequence of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase was deduced. Bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase deduced contains 400 amino acids including initiation methionine and has a molecular weight of 44,850. Bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase corresponds to the 236 amino acids of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase. RNA blot analysis revealed a single-species mRNA of about 2600 bases.

The protein composition of central nervous system myelin is relatively simple, consisting largely of myelin basic protein, proteolipid protein, and the Wolfgram proteins (Lees and Brostoff, 1984). In addition, at least one enzyme, 2',3'-cyclic-

The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; bp, base pairs; CNP, 2',3'-cyclic-nucleotide 3'-phosphodiesterase; SSC, 0.15 M NaCl and 0.015 M sodium citrate (pH 7.0); Mops, 3-(N-morpholino)propanesulfonic acid; TPCK, 1-1-tosylamido-2-phenylethyl chloromethyl ketone.

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Dedicated to the late Dr. Stanford Moore.
which had been mostly determined by protein sequence analysis.

**EXPERIMENTAL PROCEDURES AND RESULTS AND DISCUSSION**

The protein sequence analysis of bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase is described in the Miniprint Section. Primary cleavages of the protein were carried out with cyanogen bromide and trypsin. In the case of the trypsin digestion, the lysines were blocked with citraconyl groups. Two methionine as well as 3 arginine residues are clustered in the carboxyl-terminal 40 residues of bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase (Fig. 1). The sequence of the carboxyl-terminal region was therefore revealed by peptides of the above cleavages. Two sequences in the carboxyl-terminal region of bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase, Ala-Lys-Lys-Met-Glu-Val and Gly-Tyr-Tyr-Gly-Lys-Gly (residues 364-369 and 375-380, respectively), were used for synthetic oligonucleotide probes.

The cDNA library of 20,000 transformants prepared from the poly(A)^+ RNA of bovine brain was screened with synthetic oligonucleotides I by hybridization. Two positive clones were found; one of these also hybridized with synthetic oligonucleotides IIb. The plasmid DNA was prepared from the clone that hybridized with both oligonucleotide probes. The plasmid contained a cDNA insert of about 1200 bp. The region that hybridized with synthetic oligonucleotide probes was located by DNA blot analysis, and the nucleotide sequence of this region was determined. The terminal 90 bp of the insert corresponded to the carboxyl-terminal 30 amino acids of bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase. The termination codon, TGA, was found 48 bp downstream from the nucleotides coding for the carboxyl-terminal isoleucine of the elastase enzyme. The plasmid analyzed above was designated pCNP1.

The cDNA library of 20,000 plus 10,000 (total 30,000) transformants was then screened with a 730-bp PstI fragment of cDNA isolated from pCNP1. Additional seven 2',3'-cyclic-nucleotide 3'-phosphodiesterase clones were obtained by this procedure; and their plasmids, pCNP2-8, were prepared. Restriction map analysis indicated that the size of the cDNA covered by the inserts of these plasmids is about 2300 bp. The two overlapping cDNA inserts from pCNP1 and pCNP4 seem to represent the entire cDNA obtained. The nucleotide sequence of the cDNA was determined by the strategy presented in Fig. 2.

Fig. 3 shows the complete nucleotide sequence of the combined cDNA inserts from pCNP1 and pCNP4. The consensus polyadenylation signal, AATAAA, as well as part of poly(A) is present at the 3' end. The only open reading frame extends from the 5' end to TGA at nucleotides 1201-1203. Bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase corresponds to the 236 codons from GCA at nucleotides 1201-1203 to ATA at nucleotides 1153-1155. The greater part of the elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase had been sequenced by protein sequence analysis (Fig. 1), and the rest was deduced from the cDNA sequence. The amino acid compositions of 20 peptides from the elastase enzyme (Tables I-III) correspond closely with those calculated from the sequence. The molecular weight of the elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase calculated from the sequence was 26,120.

There are two ATG codons at nucleotides 1-3 and nucleotides 178-180 upstream in the reading frame of the elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase. Both ATG codons are surrounded by sequences homologous to those sequences which often flank functional initiation sites (Kozak, 1981). The ATG at nucleotides 1-3 was assigned as the initiation site since this is the first ATG triplet and a possible binding site for 18 S rRNA is present upstream of the ATG. The corresponding RNA sequence 5'-CUUCCGCA-3' at nucleotides -10 to -3 is complementary to the sequence 3'-GAAGGGCU-5' near the 3' end of mammalian 18 S rRNA (Chan et al., 1984). The free energy of the hybridization as
**Table IV**

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<th>Amino acid composition of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase</th>
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A molecular weight of 45,000 was assumed, and the data of Drummond et al. (1978) were recalculated. The numbers in parentheses are from the sequence deduced from the data of Sprinkle et al. (1979). The methionine is included.

**Fig. 4.** Poly(A)^+ RNA blot analysis. Lane 1, 2.5 µg of poly(A)^+ RNA from bovine cerebellum; lane 2, 5 µg of poly(A)^+ RNA from rat whole brain. The upper and lower arrows indicate the positions of 28 S and 18 S rRNAs, respectively. The 730-bp PstI fragment of cDNA from pCNP1 was used as the probe. The same result was obtained with the 1270-bp PstI fragment of cDNA from pCNP4, which contains most of the coding region.

**Fig. 3.** cDNA sequence and deduced amino acid sequence of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase. The nucleotides are numbered in the 5' to 3' direction, beginning with the first nucleotide of the initiation methionine codon ATG; the nucleotides on the 3' side of nucleotide 1 are indicated by negative numbers. The possible 18 S RNA-binding site and the consensus polyadenylation signal are underlined. The initiation methionine may be removed in vivo after translation; the methionine seems to be efficiently removed when the second residue is serine. The translation product deduced from the cDNA sequence contains 400 amino acids (Fig. 3). The molecular weight calculated from the sequence, 44,580 (44,720 excluding initiation methionine), is close to that determined by SDS-PAGE for the major component of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase (45,000 by Drummond and Dean, 1980; 48,000 by Sprinkle et al., 1980). The amino acid composition calculated from the sequence agrees well with that calculated in Table IV.

The possibility that the initiation site is upstream of the 5' end of the cDNA obtained has not completely been excluded. However, available data do not support this possibility. 1. The molecular weight of bovine elastase 2',3'-cyclic-nucleotidase is 3258. 2. The initiation methionine is removed in vivo after translation; the methionine seems to be efficiently removed when the second residue is serine. The translation product deduced from the cDNA sequence contains 400 amino acids (Fig. 3). The molecular weight calculated from the sequence, 44,580 (44,720 excluding initiation methionine), is close to that determined by SDS-PAGE for the major component of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase (45,000 by Drummond and Dean, 1980; 48,000 by Sprinkle et al., 1980). The amino acid composition calculated from the sequence agrees well with that calculated in Table IV.

The possibility that the initiation site is upstream of the 5' end of the cDNA obtained has not completely been excluded. However, available data do not support this possibility. 1. The molecular weight of bovine elastase 2',3'-cyclic-nucleotide 3'-phosphodiesterase is 2425. 2. The initiation methionine is removed in vivo after translation; the methionine seems to be efficiently removed when the second residue is serine. The translation product deduced from the cDNA sequence contains 400 amino acids (Fig. 3). The molecular weight calculated from the sequence, 44,580 (44,720 excluding initiation methionine), is close to that determined by SDS-PAGE for the major component of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase (45,000 by Drummond and Dean, 1980; 48,000 by Sprinkle et al., 1980). The amino acid composition calculated from the sequence agrees well with that calculated in Table IV.

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tide 3'-phosphodiesterase calculated from the sequence (26,120) is smaller than that determined by SDS-PAGE (29,000-30,000). The molecular weight of the intact enzyme may also be smaller than that determined by SDS-PAGE (45,000-48,000); at least it is unlikely that the molecular weight of bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase is larger than that determined by SDS-PAGE. 2) There are 48 phenylalanine, 3 lysine, and 3 arginine codons between nucleotide -59, the 5' end of the cDNA obtained, and nucleotide -1 in the reading frame of 2',3'-cyclic-nucleotide 3'-phosphodiesterase (Fig. 3). If the initiation site is just upstream of nucleotide -59, the amino acid composition of the translated protein calculated does not match well with the amino acid composition analyzed (Table IV).

Poly(A)" RNA blot analysis revealed a single-species mRNA coding for bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase (Fig. 4). The size of the mRNA is about 2600 bases. The cDNA contains 2305 bp including part of poly(A) at the 3' end (Fig. 3). The full-length cDNA would contain an additional 100-200 bp in the 5' nontranslated region.

The amino acid composition presented in Table IV indicates that 2',3'-cyclic-nucleotide 3'-phosphodiesterase is a whole is less hydrophobic than expected from the difficulty in solubilization. Bovine 2',3'-cyclic-nucleotide 3'-phosphodiesterase is solubilized by the cleavages at the carboxyl sides of residues 149 and 385 with elastase. Both of the amino- and carboxyl-terminal regions removed, however, do not have long hydrophobic segments that can be used as anchors in lipid bilayers (Fig. 5). The possibility is suggested that the enzyme is bound to other hydrophobic proteins, most likely to proteins through local hydrophobic interactions. Highly hydrophobic sequences of 4 amino acid residues, that might be involved in this binding, are present at residues 32-35, 97-100, and 124-127 in the amino-terminal region. These sequences are in predicted β-sheet structures (Fig. 6), which may favor the interactions in the cytoplasmic space of myelin membrane (Laursen et al., 1984). The fact that 2',3'-cyclic-nucleotide 3'-phosphodiesterase is solubilized by guanidinium chloride also supports the above possibility. The positive charge localized in the carboxyl-terminal 40 residues may stabilize the 2',3'-cyclic-nucleotide 3'-phosphodiesterase molecule on the surface of lipid bilayers.

cDNA cloning offers a way to analyze the regulation of 2',3'-cyclic-nucleotide 3'-phosphodiesterase gene expression during myelin formation. The regional distribution and developmental change of the enzyme mRNA in the central nervous system are now under study. cDNA will also facilitate the isolation and characterization of the structural gene encoding 2',3'-cyclic-nucleotide 3'-phosphodiesterase. It should be noted, however, that the cDNA has species specificity similar to that found for the antibody against 2',3'-cyclic-nucleotide 3'-phosphodiesterase (Nishizawa et al., 1985).

Acknowledgment—We thank Professor Irving Zabin for his support and advice in protein sequence analysis.

REFERENCES

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Continued on next page.
**Supplementary Material**

To determine the ability of bovine cerebrocortical fraction IIA (Lot 1701) to stimulate the growth of mouse neuroblastoma cells (M11), we cultured the cells in the presence of varying concentrations of this fraction. The results are shown in the graph below. As expected, the highest cell counts were observed at the highest concentrations of the fraction, indicating a dose-dependent effect on cell proliferation. The data suggest that this fraction could be a potential therapeutic agent for neurodegenerative diseases or for promoting neurogenesis in the brain.
2’,3’-Cyclic-nucleotide 3’-Phosphodiesterase

Overall, 65% of the elastase CNP residue were sequenced. All the peptides analyzed except CT2, CT6 and CT9, which were not sequenced, can be placed in the sequence presented in Fig. 1. All the peptides analyzed, except CT2, satisfy the specifications of the three types of cleavage used.

Fig. 5. Hydrophilicity profile of bovine CNP.

The procedure of Hopp and Woods (1981) was used. Averaging segment length was octapeptide. The numbers in abscissa indicate the numbers of amino acid residues (Fig. 3). The arrows indicate the cleavage sites by elastase.

Fig. 6. Predicted secondary structure of bovine CNP.

The procedure of Chou and Fasman (1978) was used. The secondary structure is shown under the numbers of amino acid residues (Fig. 3). 

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