ACIDIC PRECURSOR REVEALED IN HUMAN EOSINOPHIL GRANULE MAJOR BASIC PROTEIN cDNA

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Eosinophil granule major basic protein (MBP) comprises the crystallloid core of the eosinophil granule (1) and is a 13.8-kD single polypeptide rich in arginine with a calculated isoelectric point (pI) of 10.9. MBP is a potent toxin for helminths and various cell types in vitro, and it has been localized on damaged helminths and tissues in hypersensitivity diseases including bronchial asthma (2). A molecule indistinguishable from MBP has been localized to placental X cells and placental-site giant cells (3) and increases in maternal plasma just before labor (4). Human MBP contains 17 arginines, only one carboxylic acid-containing residue, and a high proportion of amino acids with hydrophobic side chains (5). It probably exists in solution as an extended molecule with a major fraction of its surface occupied by cationic charge. The cationic charge may attract MBP to the negatively charged cell surface of a target, whereupon the apolar residues insert into and perturb the lipid milieu. Previous studies suggest that the cationic nature of MBP is an important determinant of its cytotoxicity (6). Here we report on the nucleotide sequence of a cDNA representing human MBP mRNA from the promyelocytic cell line HL-60, which produces MBP (7). Analysis of the cDNA indicates that MBP is translated as a preproprotein with an acidic pro-portion.

Materials and Methods

Library. A λ GT10 HL-60 cDNA library containing 1.5 × 10^6 independent clones with an average insert size of 1 kb was provided by Dr. David Bentley (I. C. R. F. Laboratories, London, England).

Probes. Two mixed oligonucleotide probes (Table I), which contained all possible coding combinations for two nonoverlapping areas of MBP, were generated from the MBP amino acid sequence (5) by Probe program (DNAstar, Inc., Madison, WI). Probes were produced by a DNA synthesizer (series 380; Applied Biosystems, Inc., Foster City, CA) and purified over Sephadex G-50 columns.

Isolation and Nucleotide Sequence Determination of MBP cDNA. The λ GT10 HL-60 cDNA library was screened essentially as previously described (8). Phage were sequentially adsorbed onto 2–8 × 8-cm nylon membrane filters per each library plate. Both sets of filters were prehybridized in 5X SSPE (0.9 M NaCl, 50 mM NaH2PO4, 5.0 mM EDTA, pH 7.4), 0.2% SDS, and 0.005% denatured salmon sperm DNA for 2 h at 50°C with eight filters per 50 ml prehybridization fluid per bag. Filters were hybridized with 0.67 ng of labeled probe MBP1 per ml of fresh prehybridization fluid overnight at 50°C. Oligonucleotides were labeled (9)

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with γ \textsuperscript{32}P ATP 5,000 Ci/mmole (New England Nuclear, Boston, MA). Filters were washed at room temperature for 45 min in 1-liter 5X SSPE per 40 filters followed by a 1-min wash in fresh buffer at 50°C, slightly air dried, and exposed to Kodak XAR5 film with intensifying screens for 72 h at ~70°C. Filters were then hybridized with probe MBP2 under the same conditions as MBP1 after all positive signals had been removed from the filters by a 10-min 5X SSPE 70°C wash. Recombinant clones positive to both probes were selected from the library and plaque purified. Recombinant phage DNA was purified (10) and restricted with Eco RI, which resulted in a single insert fragment of ~900 bp, which was ligated into M13mp10 and sequenced by the method of Sanger et al. using \textsuperscript{[35S]}ATP (11).

### Results and Discussions

As is typical of many cDNAs, the nucleotide sequence of the isolated clone (Fig. 1) contained sequences similar to the consensus sequences associated with eukaryotic initiation (12) and polyadenylation signals (13). However, it did not contain the TTATTTAT consensus sequence in its 3'-untranslated region, which is prevalent among mRNAs encoding proteins related to the inflammatory response and is involved in the selective degradation of transiently expressed messengers (14). Primer extension reactions (15) using purified HL-60 mRNA (data not shown) indicated that the MBP cDNA is the same length as full-length message, and thus contains the entire coding sequence for the protein.

The longest open reading frame of the nucleotide sequence codes for a protein of 222 amino acids that includes a terminal sequence of 117 amino acids, which is identical to the amino acid sequence of MBP (5). However, the MBP sequence is preceded by a 15-amino acid putative signal peptide (16) typical of secreted proteins, and by a middle sequence of 90 amino acids; neither of these are present in the mature protein. These data suggest that MBP is initially translated as a pre-proprotein, which is cotranslationally modified to proMBP and posttranslationally modified to MBP.

MBP has 17 strongly basic amino acids, only one strongly acidic amino acid, and a pI of 10.9 (Table II). The pro-portion of proMBP has 26 strongly acidic amino acids, seven strongly basic amino acids, and a pI of 3.9. The combination of the acidic and basic peptides in proMBP has a slightly acidic pI of 6.2. Translation of MBP as a proprotein may mask the toxic effects of mature MBP and protect the
eosinophil from damage while the protein is processed through the endoplasmic reticulum. Heparin, an acidic mucopolysaccharide, is reactive with MBP (17) and inhibits its toxicity (2, 6), suggesting that MBP could be neutralized by an acidic precursor.
Several possibilities exist for the processing of proMBP. First, the acid portion could be cleaved during transport from the endoplasmic reticulum to the granule and toxic MBP stored in the granule. Second, proMBP could be initially stored in the granule and subsequently processed to toxic MBP, which then crystallizes to form the granule core. Third, proMBP could crystallize during granule maturation and form the granule core; immediately before secretion proMBP would be further processed to toxic MBP. Among these possibilities we favor the second, namely the initial storage of proMBP in the granule followed by processing to toxic MBP and crystallization of toxic MBP to form the granule core. Our preference for this mechanism is based on several experimental observations. First, basophilic staining granules (18) are present in eosinophilic myelocytes from bone marrow, but mature eosinophils do not show basophilia, suggesting that the cationicity of the granule increases as it develops. Second, prior studies of eosinophil granules (19) showed that lysis at pH2 reduces granule turbidity by 92–95% and liberates MBP; no evidence of a proMBP was found. Third, SDS-PAGE gels of acid-solubilized guinea pig eosinophil granule crystalloid cores showed only a single discrete band which migrated with molecular weight of toxic MBP (1). Because virtually all of the core substance was solubilized in this procedure, this observation suggests that toxic MBP forms the granule crystalloid. Overall, these data are in keeping with the hypothesis that proMBP is initially sequestered in the granule and processed to toxic MBP, which is stored in the crystalloid.

To determine if an acidic precursor is unique to MBP or is found in other toxic basic proteins, we initially used the Genetic Sequence Data Bank (20) and the Protein Identification Resource (21). A search of these data bases indicated that MBP showed little sequence homology with known genes and proteins. Next, we collected the cDNA sequences of toxic basic proteins from a literature search. None of the reported cDNAs from basic toxins codes for an acidic precursor; however, the cDNA of barley toxin a-hordothionin (22) (BTAH) codes for a nearly neutral precursor. Interestingly, the pI profile of BTAH is quite similar to MBP (Table II). The proportion of proBTAH has 11 strongly acidic amino acids, three strongly basic amino acids, and a pI of 3.6. BTAH has 10 strongly basic amino acids, no acidic amino acids and a pI of 9.6, while proBTAH has a pI of 7.6. Curiously, in BTAH, the signal peptide is immediately followed by the mature protein and then by the acidic

| Protein    | Number of amino acids | Number of strongly basic amino acids (K.R.) | Number of strongly acidic amino acids (D.E.) | pI  |
|------------|-----------------------|---------------------------------------------|---------------------------------------------|-----|
| Human MBP  | 117                   | 17                                          | 1                                           | 10.9|
| Pro-portion| 90                    | 7                                           | 26                                          | 3.9 |
| Pro        | 207                   | 24                                          | 27                                          | 6.2 |
| BTAH (22)  | 45                    | 10                                          | 0                                           | 9.6 |
| Pro-portion| 64                    | 3                                           | 11                                          | 3.6 |
| Pro        | 109                   | 13                                          | 11                                          | 7.6 |

Isoelectric points (pI's) calculated by Trans program (DNAstar, Inc.). BTAH, barley toxin a-hordothionin, MBP, major basic protein.
pro-portion (22). BTAH, a thionin, is lysine and cysteine rich and composes 4% of endosperm protein where it may be extrinsically associated with the endoplasmic reticulum (23). Although orally innocuous, thionins are toxic to laboratory animals by intraperitoneal injection, cultured mammalian cells, yeasts, and phytopathogenic bacteria (22). Thionins modify cell membrane permeability which induces a rapid leakage of ions and low molecular weight compounds that may be responsible for the inhibition of protein and nucleic acid synthesis by thionins (24). A function of the pro-portion of BTAH has not been suggested (22). One possibility is that the acidic pro-portion of BTAH may neutralize the basic mature protein as we have already suggested for the precursor of MBP, and that this mechanism may be found in other cells that produce cationic toxins that damage cell membranes.

Summary

Eosinophil granule major basic protein (MBP), a potent toxin for helminths and various cell types, is a 13.8-kD single polypeptide rich in arginine with a calculated isoelectric point (pI) of 10.9. A cDNA for human MBP was isolated from a λ GT10 HL-60 cDNA library. The nucleotide sequence of the MBP cDNA indicates that MBP is translated as a 25.2-kD preproprotein. The 9.9-kD pro-portion of proMBP is rich in glutamic and aspartic acids and has a calculated pI of 3.9, while proMBP itself has a calculated pI of 6.2. We suggest that MBP is translated as a nontoxic precursor that protects the eosinophil from damage while the protein is processed through the endoplasmic reticulum to its sequestered site in the granule core as toxic MBP, and we present results from the literature suggesting that other cationic toxins, which damage cell membranes, may also be processed from nontoxic precursors containing distinct anionic and cationic regions.

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