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Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: Peptide isolation and cloning of a cDNA

(antibacterial peptides/antifungal peptides/bovine trachea/respiratory mucosa)

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ABSTRACT **Extracts of the bovine tracheal mucosa have an abundant peptide with potent antimicrobial activity. The 38-amino acid peptide, which we have named tracheal antimicrobial peptide (TAP), was isolated by a sequential use of size-exclusion, ion-exchange, and reverse-phase chromatographic fractionations using antimicrobial activity as a functional assay. The yield was ≈2 μg/g of wet mucosa. The complete peptide sequence was determined by a combination of peptide and cDNA analysis. The amino acid sequence of TAP is H-Asn-Pro-Val-Ser-Cys-Val-Arg-Asn-Lys-Gly-Ile-Cys-Val-Pro-Ile-Arg-Cys-Pro-Gly-Ser-Met-Lys-Gln-Ile-Gly-Thr-Cys-Val-Gly-Arg-Ala-Val-Lys-Cys-Cys-Arg-Lys-Lys-OH. Mass spectral analysis of the isolated peptide was consistent with this sequence and indicated the participation of six cysteine residues in the formation of intramolecular disulfide bonds. The size, basic charge, and presence of three intramolecular disulfide bonds is similar to, but clearly distinct from, the defensins, a well-characterized class of antimicrobial peptides from mammalian circulating phagocytic cells. The putative TAP precursor is predicted to be relatively small (64 amino acids), and the mature peptide resides at the extreme carboxyl terminus and is bracketed by a short putative propeptide region and an in-frame stop codon. The mRNA encoding this peptide is more abundant in the respiratory mucosa than in whole lung tissue. The purified peptide had antibacterial activity *in vitro* against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia,* and *Pseudomonas aeruginosa.* In addition, the peptide was active against *Candida albicans,* indicating a broad spectrum of activity. This peptide appears to be, based on structure and activity, a member of a group of cysteine-rich, cationic, antimicrobial peptides found in animals, insects, and plants. The isolation of TAP from the mammalian respiratory mucosa may provide insight into our understanding of host defense of this vital tissue.**

The respiratory epithelium of mammals is a complex tissue responsible for numerous physiological functions, one of which is forming a key barrier to potentially harmful environmental threats. Multiple defense mechanisms have been identified, which protect the respiratory tract from inhaled agents that are known to be responsible for airway disease, such as infectious agents, gases, and particulates (1). These multiple defenses are the result of a combination of anatomical design of the airway, together with the physiological role of local and circulating cells.

Recent isolation and characterization of antimicrobial peptides in a variety of species and tissues has unveiled an additional component of animal host defense (for recent reviews, see refs. 2–4). These various peptides, which can be classified into families based on common sequences, secondary structure, and/or sites of activity, are believed to participate in defense against potential microbiological pathogens. Cecropins were the first well-characterized family of structurally related antimicrobial peptides that were found in a wide distribution of insects (2, 5–7). They are coordinately expressed in the fat body of insect larvae following infection or injury. In vertebrates, the magainin family of antimicrobial peptides has been isolated from glands of the skin and gastrointestinal tract of *Xenopus laevis* and are thought to form the basis for a defense system of the amphibian mucosal surfaces against infection (refs. 8–10; K. S. Moore, C.L.B., M.B., K. Turner, H.E., and M.Z., unpublished data). Defensins are peptides found in phagocytic cells isolated from several mammalian species including man and may be characterized by eight invariant residues within the sequence (for a recent review, see ref. 3). They possess antimicrobial activity *in vitro* against bacteria, fungi, and enveloped viruses and may contribute to the "oxygen-independent" defense pathways of these cells (11). Expression of defensin in a nonmyeloid tissue source, the mouse small intestinal crypt cells, has also been reported (12). Cecropins, magainins, and defensins all share the properties of being cationic and membrane active, and evidence suggests that their antimicrobial activity is secondary to their ability to selectively disrupt membranes, possibly by channel formation (13–17).

As an extension of our studies on the antimicrobial peptides, we proposed that the ciliated respiratory mucosa of mammals might contain peptide-based antimicrobial activity, to complement other defense systems of the airway. In this paper we describe the isolation of an abundant peptide found in extracts of the bovine tracheal mucosa, which was isolated on the basis of potent antimicrobial activity. We have named this molecule tracheal antimicrobial peptide (TAP). In addition, we report the cloning of a cDNA encoding this peptide.**

MATERIALS AND METHODS

Tissue. A segment of adult bovine trachea just proximal to the carina and ≈40 cm in length was obtained fresh from a local meat processing plant. The tissue was immediately

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Abbreviation: TAP, tracheal antimicrobial peptide.

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. M63023).

placed on wet ice and processed within 2–3 hr. Preliminary experiments indicated that immediate processing of tissue gave no significant further improvement in yield. The epithelium and adherent connective tissue, dissected on ice from the underlying connective tissue and cartilage, was placed immediately in liquid nitrogen. The frozen tissue was then stored at −70°C for periods up to several months before further processing.

Protein Isolation. The frozen tracheal epithelium was pulverized with a mortar and pestle under liquid nitrogen. The frozen tissue powder was placed in boiling 10% (vol/vol) acetic acid, and boiling was continued for 10 min. The solution was allowed to cool to room temperature and centrifuged at 23,000 × *g* for 30 min at 10°C. All subsequent procedures were performed at room temperature. The resulting supernatant was divided into 30-ml aliquots, and each aliquot was applied to a C_{18} Sep-Pak cartridge (Millipore). The cartridges were washed with 0.1% trifluoroacetic acid (vol/vol) in H_2O (buffer C) and then eluted with 4 ml of a solution of acetonitrile in buffer C (60:40, vol/vol; buffer D). The cartridge eluates were dried, resuspended in 1–2 ml of 6 M guanidinium hydrochloride/20 mM Tris·HCl (pH 7.4), and applied to a Bio-Gel P-30 column (40 cm × 2.5 cm diameter; Bio-Rad) equilibrated with 50 mM ammonium formate (pH 4.1). The exclusion limit was 40 kDa. The column was developed with the same buffer, and each fraction (2 ml) was lyophilized, resuspended in water (0.1 ml), and assayed for antimicrobial activity as described below. The active fractions were pooled and applied to a sulfoethyl ion-exchange HPLC column (Poly LC, Columbia, MD). A 45-min linear elution gradient from buffer A to buffer B was employed at a flow rate of 1 ml/min. Buffer A was 25% (vol/vol) acetonitrile/5 mM potassium phosphate (pH 5.3), and buffer B was identical to buffer A except that it also contained 1 M NaCl. Preliminary experiments established that only a fraction eluting at 26 min contained significant antimicrobial activity. In subsequent isolations, this fraction was applied to a reverse-phase HPLC column and fractionated by using a linear gradient of buffer C to buffer D at 1 ml/min. The peak fraction eluting at 28.5 min was lyophilized and resuspended in H_2O at an approximate concentration of 0.5 mg/ml.

Protein Sequence Analysis. The isolated peptide was subjected to amino acid analysis by using an amino acid analyzer with automated hydrolysis (model 420/130; Applied Biosystems). The numerical calculations for specific amino acids were determined by using an Applied Biosystems model 920A data analysis module and an Applied Biosystems hydrolysis test peptide as a standard. Sequence analysis was determined by the Edman degradation method on a pulsed liquid-phase sequencer (Applied Biosystems model 477A/120). The carboxyl-terminal portion of the peptide was isolated by HPLC following cleavage with cyanogen bromide (18). Cysteine residues were identified by sulfhydryl reduction followed by reaction with 4-vinylpyridine (19), prior to sequence analysis. Protein sequence analysis and amino acid analysis were performed by the Protein/Nucleic Acid Core Facility, Department of Pediatrics, University of Pennsylvania School of Medicine.

Mass Spectroscopy. The molecular mass of the unreduced peptide was independently determined by fast-atom bombardment on a JEOL HX110 mass spectrometer at 1000 resolution (Structural Biochemistry Center, University of Maryland, Baltimore County) and on a Vacuum Generators Analytical ZAB 2-SE high-field mass spectrometer (MScan, West Chester, PA).

PCR Amplification. The PCR product was obtained by using the degenerate oligonucleotide 5′-GAGCTCDGTIC-CDATYTGYTTCAT-3′ (B = C, G, or T; D = A, G, or T; H = A, C, or T; Y = C or T) as an antisense primer, a 1:1 mixture of 5′-GAATTCAAYCCHGTBAGITGYGTT-3′ and 5′-GAATTCAAYCCHGTBTCYTGYGTT-3′ as sense primers, and a pool of bovine tracheal cDNA (prior to size fractionation, see below) as a template. Oligonucleotides were made by the Protein/Nucleic Acid Core Facility. The general protocol for PCR amplification (20) was modified as follows: the final concentration of template cDNA was 0.2 ng/ml and that of primers was 1 μM; initial denaturation was at 94°C; 30 cycles of amplification were done by cycling 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C. Bands were purified by electroelution after electrophoresis in polyacrylamide gels.

cDNA Cloning. The techniques used for cDNA library construction have been described (21, 22), and reagents were from Invitrogen (San Diego) unless noted otherwise. Total mRNA was isolated from bovine tracheal epithelium tissue (23), and poly(A)$^+$ RNA was then selected by using oligo(dT)-cellulose (5 Prime → 3 Prime, Inc.). The cDNA was size-fractionated by agarose gel electrophoresis, and a fraction (300–3000 base pairs) was recovered by electroelution. Approximately 5×10^6 independent phage were obtained, and 10^6 phage were plated at a density of 3×10^4 per 150-mm plate on a lawn of C600 hfl$^-$ *Escherichia coli*. Plaque lifts were made by using Colony/Plaque Screen filters (DuPont), and the filters were screened sequentially by using standard techniques (24) and three probes: 5′-AACCCTGTCTCCT-GTGTGCGCAACAAGGGCATCTGTGTGCCCAT-3′, 5′-AACAAGGGCATCTGTGTGCCCATCCGCTGCCCTG-GCTCCATGAAGCAGATTGG-3′, and the PCR product PCR-BT40.1. The standard conditions for hybridization and washing (24) were modified: 50°C, 6× standard saline citrate (SSC) for hybridization and washing with the oligonucleotide probes; and 37°C, 5× SSC/20% (vol/vol) formamide and 55°C, 6× SSC for hybridization and washing, respectively, for the double-stranded probe.

Northern Blot Analysis. RNA was fractionated by agarose gel electrophoresis in the presence of formaldehyde and blotted to nylon membranes (Nytran; Schleicher & Schuell) by the capillary technique (24). Radioactively labeled DNA probes were hybridized to the immobilized RNA in 20% (vol/vol) formamide/5× SSC/5× Denhardt's solution/0.1% (wt/vol) SDS at 42°C and washed in 0.1× SSC/0.1% SDS at 65°C.

Antimicrobial Assays. Antimicrobial activity was determined during purification by the plate assay described by Zasloff (8). A concentrated aliquot of each fraction (2–5 μl) was spotted onto a lawn of *E. coli* strain D31 (5) on a Petri dish containing bactotryptone at 10 g/liter, yeast extract at 5 g/liter, 0.75% (wt/vol) agarose (Sigma), 25 mM Tris (pH 7.4), and 50 mM NaF and incubated overnight at 37°C. Minimal inhibitory concentration of the peptide was determined by a modification of the method described by Soravia *et al.* (10). Briefly, 2.5×10^4 bacteria in 0.2 ml of 0.25× trypticase soy broth (Difco) were incubated with increasing concentrations of the peptide in static 96-well microtiter plates (Corning) overnight at 37°C.

RESULTS

Isolation of the Peptide. The bovine tracheal epithelium was extracted in acid and size fractionated by gel filtration on Bio-Gel P-30 (Fig. 1*A*). The elution profile of the tracheal extract shows two peaks of UV absorbance (monitored at 220 nm). Antibacterial activity was assayed by using *E. coli* strain D31 for fractions 13 (void volume) to 60 (included volume); fractions 32–36 showed significant activity, as evidenced by clear zones of killing on a lawn of *E. coli* (data not shown). These fractions contained predominantly small peptides (i.e., <5000 Da) when analyzed by SDS/PAGE and silver stained for protein (data not shown). The antimicrobial fractions were pooled and then fractionated by ion-exchange HPLC

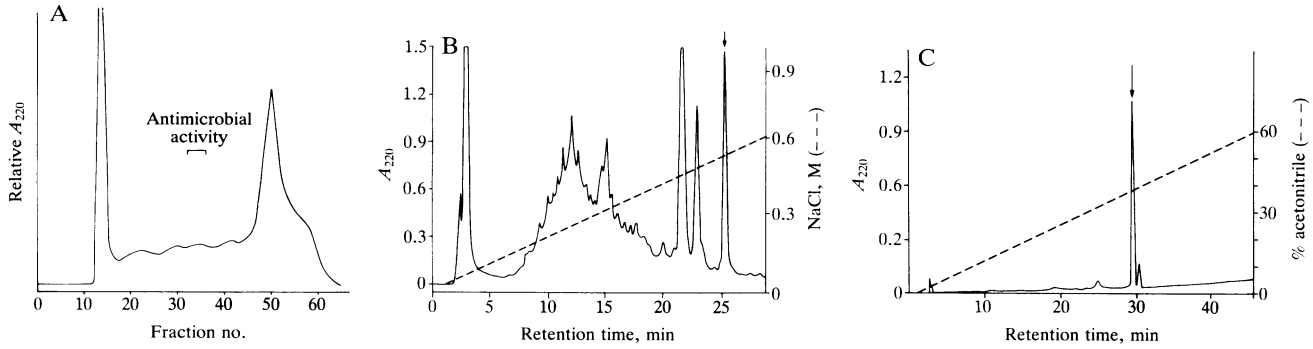

FIG. 1. Purification of the antimicrobial peptide from bovine trachea. (*A*) Gel-filtration chromatogram. Concentrated acid extracts of the bovine tracheal epithelium were applied to a Bio-Gel P-30 column. The column was developed with 50 mM ammonium formate (pH 4.1). UV absorbance was monitored at 220 nm, and concentrated aliquots were assayed for antibacterial activity. Fractions containing antimicrobial activity are marked. (*B*) Ion-exchange HPLC chromatogram of antimicrobial gel-filtration fractions from *A*. The active fractions from the gel-filtration column in *A* were pooled and subjected to ion-exchange HPLC. A linear gradient of increasing NaCl concentration (0–0.6 M) in 25% (vol/vol) acetonitrile/5 mM potassium phosphate (pH 5.3) was employed at a flow rate of 1 ml/min. The antimicrobial fraction is marked with an arrow. (*C*) Reverse-phase HPLC chromatogram of the antimicrobial fraction from ion-exchange HPLC. The ion-exchange fraction eluting at 26 min was applied to a reverse-phase column and fractionated by using a linear gradient of acetonitrile in aqueous 0.1% trifluoroacetic acid at 1 ml/min. The peak fraction eluting at 28.5 min was isolated.

(Fig. 1*B*). An isolated peak at 26 min (arrow) contained the only detectable antimicrobial activity. This peak was collected and further purified by reverse-phase HPLC (Fig. 1*C*). There was a single superimposed peak of UV-absorbing material (arrow) and antimicrobial activity (data not shown) eluting at 28.5 min [37.3% (vol/vol) acetonitrile]. This material was subjected to amino acid compositional analysis,†† and the overall yield was ≈2 μg/g of epithelium (wet weight). The purity of the peptide was >95% as assayed by analytical HPLC, amino-terminal sequence analysis, and capillary gel electrophoresis (data not shown). Mass spectral analysis indicated that the molecular mass of the isolated peptide is 4085 Da.

Peptide Sequence Analysis. The purified peptide was subjected to pulsed liquid-phase protein sequence analysis. Twenty-nine of the 38 amino-terminal residues were analyzed directly, and the more carboxyl-terminal residues were analyzed following cyanogen bromide cleavage (Fig. 2*A*).

cDNA Cloning and Sequence Analysis. Degenerate oligonucleotide primers were designed corresponding to amino acids 1–6 and 21–26, as shown in Fig. 2*B*. These primers were used for PCR by using bovine tracheal cDNA as template DNA and were expected to amplify the nucleotide sequence encoding amino acids 1–26. The principal DNA product was 90 base pairs in length, as expected based on peptide primary structure and the selected oligonucleotide primers. This indicated that cDNA encoding the peptide was present in the library and yielded a DNA template for probe synthesis.

The cDNA library from bovine tracheal epithelium (≈10^6 independent λgt10 phage) was screened by using three different probes in parallel: the PCR product and two "best-guess" synthetic oligonucleotide probes whose sequence was based on the peptide sequence (ref. 25; see *Materials and Methods*). Only clones that hybridized with 2 of the 3 probes (of which there were 15) were considered positive, and 7 of these clones were taken for further analysis. Several positive clones were plaque purified, and the size of the inserts were all ≈400 base pairs. One of the inserts was subcloned into Bluescript plasmid (Stratagene), and DNA sequence analysis was performed. The sequence for the cDNA clone pBT40-4.4 is shown in Fig. 2*C*.

Northern Blot Analysis. RNA obtained from whole lung and isolated tracheal mucosa was subjected to Northern blot analysis by using the cDNA insert pBT40-4.4 as a probe (Fig. 3). Under stringent conditions the cDNA probe recognized an abundant message of ≈400 nucleotides in bovine trachea mRNA, along with a less abundant species of the same size in bovine lung RNA. Both lanes had identical amounts of

††Amino acid composition was determined after hydrolysis of 200 pmol of purified peptide. Results are expressed as mol of amino acid per mol of protein: Asx, 2.17 (2); Glx, 0.83 (1); Ser, 1.31 (2); Gly, 3.51 (4); His, 0.00 (0); Arg, 3.09 (4); Thr, 0.94 (1); Ala, 0.97 (1); Pro, 2.93 (3); Tyr, 0.00 (0); Val, 4.59 (5); Met, 1.25 (1); Cys, 3.77 (6); Ile, 2.54 (3); Leu, 0.00 (0); Phe, 0.00 (0); Lys, 3.82 (5). The theoretical number of residues, in parentheses, is based on the peptide sequence presented in Fig. 2*A*.

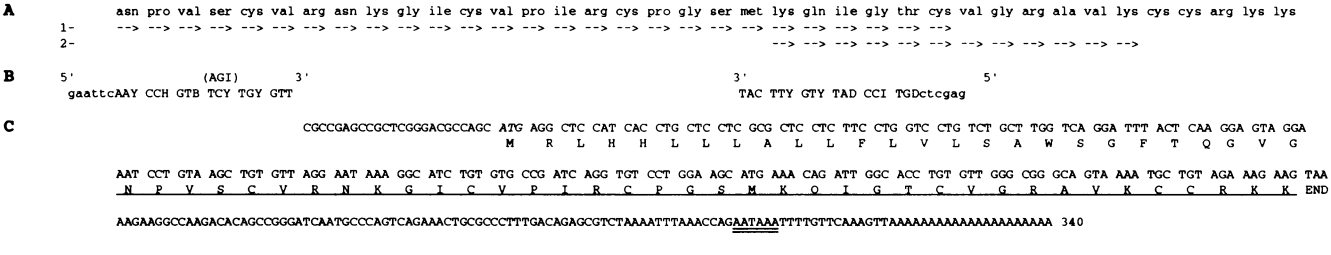

FIG. 2. Amino acid sequence of TAP and related nucleotide sequences. (*A*) Amino acid sequence of TAP based on a combination of peptide amino acid sequence analysis, mass spectral analysis, and cDNA sequence analysis. The arrows indicate the results from Edman degradation analysis: sequence 1 is from direct amino-terminal analysis, and sequence 2 is following cyanogen bromide cleavage and HPLC purification of the internal peptide. Cysteine residues were determined after reduction and treatment with 4-vinylpyridine. (*B*) Nucleotide sequence of degenerate oligonucleotides used for PCR amplification. A 1:1 mixture of two pools of degenerate oligonucleotides was used as an upstream primer. The two pools were identical in composition except for the changes noted in parentheses. Lowercase letters denote 5′ flanking sequences included in the oligonucleotide to aid in the subcloning. (*C*) Nucleotide sequence and deduced amino acid sequence of TAP putative precursor. The nucleotide sequence is from dideoxynucleotide sequence analysis of the isolated cDNA clone pBT40-4.4. Amino acid numbering starts with the presumed initiation codon. The mature peptide, beginning at residue 27, is underlined. The polyadenylylation signal is double underlined.

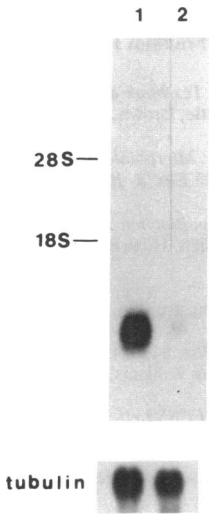

FIG. 3. Northern blot analysis of TAP message. RNA isolated from whole lung and isolated tracheal mucosa was subjected to Northern blot analysis using the cDNA probe pBT40-4.4 (*Upper*) and bovine α-tubulin (*Lower*) under stringent conditions. Both lanes had identical amounts of RNA as evidenced by ethidium bromide staining of the gel (data not shown). The positions of ribosomal bands are indicated. Lane 1, 10 μg of total bovine trachea RNA; lane 2, 10 μg of total bovine lung RNA.

RNA as evidenced by ethidium bromide staining of the gel (data not shown) and by hybridization to a bovine α-tubulin probe (G.D., M.Z., and C.L.B., unpublished results) (Fig. 3).

Antimicrobial Activity of the Isolated Peptide. The purified peptide was tested on several strains of bacteria to determine its antimicrobial activity *in vitro*. The results shown in Table 1 indicate that the peptide has antimicrobial activity against both Gram-positive and Gram-negative bacteria. In addition, the peptide has significant activity against the fungus *C. albicans*. The observed activity is of the same order of magnitude as synthetic magainin 2 carboxyamide (8) in the assay used here.

DISCUSSION

Acid extracts of the bovine tracheal mucosa are found to have an abundant peptide with potent antimicrobial activity (Fig. 1). The peptide was isolated by a combination of size-exclusion, ion-exchange, and reverse-phase chromatographic fractionations using antimicrobial activity against a strain of *E. coli* as a functional assay. We have named this peptide tracheal antimicrobial peptide (TAP).

The isolated peptide was characterized by amino acid sequence (Fig. 2*A*) and compositional analysis.†† Mass spectral analysis indicated that the molecular mass of the peptide is 4085 Da. Since the partial sequence determined by protein sequence analysis suggested a molecular mass of 3443, we predicted that the sequence was lacking five carboxyl-terminal residues. We decided to deduce the remainder of the peptide sequence by analysis of a cloned cDNA and found our prediction to be correct.

The cDNA corresponding to this peptide was cloned (Fig. 2*C*) and was found to contain an open reading frame encoding 64 amino acids. We conclude that the 38 carboxyl-terminal residues of this open reading frame correspond to the isolated peptide based on several observations. First, the 33 amino acids determined from the amino acid sequence data align perfectly with residues 26–59 of the deduced sequence (Fig. 2 *A* vs. *C*). Second, the amino acid composition agrees favorably with the deduced amino acid sequence.†† Finally, the observed average molecular mass of the isolated protein, 4085 Da, is in agreement with the deduced sequence and indicates that the six cysteine residues all participate in intramolecular disulfide bonds. The predicted pI of the unreduced peptide is 13.0, and there are no aromatic residues, both consistent with the observed protein data.

Table 1. Antimicrobial activity of TAP and synthetic magainin 2 carboxyamide (8)

Organism (ATCC no.)	Minimal inhibitory concentration, μg/ml: Magainin 2-NH_2	Minimal inhibitory concentration, μg/ml: TAP
Escherichia coli D31	3–6	12–25
Klebsiella pneumoniae (13883)	3–6	12–25
Staphylococcus aureus (25923)	25–50	25–50
Pseudomonas aeruginosa (27853)	6–12	25–50
Candida albicans (14053)	25–50	6–12

Minimal inhibitory concentrations were determined by incubating ≈2.5 × 10^4 bacteria in 0.25× trypticase soy broth with the appropriate peptide at 50, 25, 12, 6, or 3 μg/ml as described in *Materials and Methods*. Bacterial growth was assessed by optical density measurement at 600 nm. Control incubations in the absence of peptide and incubations in the absence of bacteria served to set baseline values.

Formal searches of the National Biomedical Research Foundation protein data base (release 21.0) (26) using modifications of FASTP (27) (MACVECTOR, IBI; and FASTDB, IntelliGenetics) and a nucleotide-based search of the GenBank data base (release 60.0) using the University of Wisconsin Genetics analysis software (28) found no sequences with significant similarity.

When assayed *in vitro* against several different strains of microbes, including some that are respiratory pathogens, TAP has broad-spectrum activity (Table 1). While TAP was most active against Gram-negative bacteria such as *K. pneumoniae*, significant antimicrobial activity was also seen when applied to *C. albicans*, indicating that its spectrum of activity includes both bacteria and fungi. The activity is comparable to that of synthetic magainin 2 carboxyamide, a potent analog of an antimicrobial peptide isolated from frog skin (8) (Table 1). The inhibitory concentrations observed with TAP were also similar to those observed with defensins (19, 29–31). However, it has been shown that assay conditions profoundly affect the microbicidal potency of the peptides (ref. 3; C.L. B., G.D., and M.Z., unpublished observations), and therefore direct comparisons of peptide potency between studies cannot be made.

The isolated cDNA is 340 bases in length, similar to the estimated size (400 nucleotides) of the strong signal observed on Northern blots, suggesting that the clone is nearly full length. The cDNA sequence (Fig. 2*C*) contains an open reading frame of 64 codons, extending from base 25 to base 216. The context of the first, presumably initiating, AUG(CCAGCAUGA) agrees reasonably well with the consensus sequence (CCACCAUGG) described by Kozak (32). An

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TAP                          N P V S C V R N K G I C V P I R C P G S M K Q I G T C V G R A V K C C R K K

DEFENSIN
CONSENSUS(3)                           - - C - C R - - - C - - - E R - - G - C - - - G - - - - - C C - - -
SAPECIN(37)          A T C D L L S G T G I N H S A C A A H C L L R G N R G G Y C N G K A V C V C R N
PHORMICINS(38)       A T C D L L S G T G I N H S A C A A H C L L R G N R G G Y C N G K G V C V C R N
ROYALISIN(39)      V T C D L L S F K G Q V N D S A C A A N C L S L G K A G G H C E K G V C I C R K T S F K D L W D K Y F
BACTENECIN(36)                                                       R L C R I V V I R V C R
TACHYPLESIN(40)                                              K W C F R V C Y R G I C Y R R C R
THIONIN BTH6(43)                 K S C C K D T L A R N C Y N T C R F A G G S R P V C A G A C R C K I I S G P K C P S D Y P K
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FIG. 4. Sequence comparison of basic, cysteine-rich antimicrobial peptides. The sequence data were obtained from the indicated references (numbers given in parentheses).

in-frame stop codon immediately follows the last residue of the mature peptide. Interestingly, the entire predicted precursor is devoid of acidic residues. There is an AAUAAA polyadenylylation signal (33) preceding the poly(A) tail by 14 bases.

The overall design and size of the predicted precursor of TAP is strikingly similar to the precursor of another vertebrate antimicrobial peptide, pGLa (64 residues) (34). There is a relatively hydrophobic sequence, 20 residues in length, found at the amino terminus of both the TAP and pGLa putative precursors, which could be a signal sequence. However, Schlenstedt and Zimmermann (35) reported that transport of pGLa into vesicles is via a signal recognition particle-independent mechanism, and it is the size of the precursor that may determine this mechanism of transport. This suggests that TAP might be transported into secretory vesicles by a similar mechanism.

Based on its amino acid composition and bioactivity, TAP seems to belong to a family of basic, cysteine-rich peptides with antimicrobial activity found throughout the animal and plant kingdoms (Fig. 4). These include mammalian defensins (3) and bactenecins (36), insect defensins [sapecins (37) and phormacins (38)], insect royalisin (39), crustacean tachyplesins (40), and plant thionins (41–43). Most similar to TAP are the defensins, basic peptides of 30–34 amino acids with three disulfide bonds. TAP shares with defensins the spacing of the second, third, and fourth cysteines, a glycine residue between the third and fourth cysteine, and an adjoining pair of cysteine residues near the carboxyl terminus of the peptides (Fig. 4). Yet, of the 14 characterized defensins from both myeloid and nonmyeloid tissues, all have 11 highly conserved amino acid residues, including 6 invariantly spaced cysteines. Aside from the limited similarity noted, TAP shares no other consensus residues (Fig. 4). Furthermore, the 5′ nucleotide sequence of all defensin cDNAs are strikingly conserved even across species (44), and no similarity in this region is found in the TAP cDNA. Sequence comparison with the other cysteine-containing antimicrobial peptides shows very limited similarity (Fig. 4). However, the identification of a group of basic, cysteine-rich peptides with functional similarities among widely diverse species suggests some degree of evolutionary conservation of antimicrobial defense.

Although the cell of origin of TAP has not yet been determined, the results of the Northern blot analysis suggest that much more of the message is present in the trachea than the lung. This suggests that the TAP message may be produced primarily in a tracheal cell. Although it is most common to find mammalian antimicrobial peptides in myeloid-derived cells, it is worth noting that defensin mRNA has been found in a nonmyeloid cell, the Paneth cell, in the small intestinal epithelium (12). While the actual function of TAP *in vivo* is not known, its microbicidal activity *in vitro* suggests that it may participate in a mucosal defense system. The isolation of this peptide from the mammalian respiratory mucosa provides an opportunity to further our understanding of host defense of this vital tissue.

We are indebted to Drs. Connie Mack and Catherine Fenselau (Structural Biochemistry Center, University of Maryland, Baltimore County) and Dr. Sharon Kelly (MScan) for performing mass spectral analysis and help with the interpretation. We also acknowledge our colleagues Dr. Eric Rappaport and Roger Wood for synthesis of oligonucleotides used in this study and Dr. Dorothy MacDonald for advice on antimicrobial assays. We thank Moyer Packing Company, particularly Brian Dolan for assistance in obtaining tracheal tissue. We also appreciate the critical comments on this manuscript from Drs. Saul Surrey and Dorothy Reiley. This work was supported in part by a Postdoctoral Fellowship from the National Cystic Fibrosis Foundation (to G.D.) and grants from The G. Harold and Leila Y. Mathers Charitable Foundation and The Ben Franklin Partnership.

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