Dermcidin: a novel human antibiotic peptide secreted by sweat glands

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Published online: 5 November 2001, DOI: 10.1038/ni732

Antimicrobial peptides are an important component of the innate response in many species. Here we describe the isolation of the gene *Dermcidin*, which encodes an antimicrobial peptide that has a broad spectrum of activity and no homology to other known antimicrobial peptides. This protein was specifically and constitutively expressed in the sweat glands, secreted into the sweat and transported to the epidermal surface. In sweat, a proteolytically processed 47–amino acid peptide was generated that showed antimicrobial activity in response to a variety of pathogenic microorganisms. The activity of the peptide was maintained over a broad pH range and in high salt concentrations that resembled the conditions in human sweat. This indicated that sweat plays a role in the regulation of human skin flora through the presence of an antimicrobial peptide. This peptide may help limit infection by potential pathogens in the first few hours following bacterial colonization.

The epithelia of multicellular organisms represent a major barrier to the environment and provide the first line of defense against invading microorganisms. In the epithelia, antimicrobial peptides participate in the defense system of many organisms, including plants, insects, amphibians and mammals. They control microbial growth in the first hours after epithelial injury and during wound healing and are especially prevalent during some inflammatory skin disorders.

In mammalian skin, two classes of antimicrobial peptides have been identified: cathelicidins[1] and β-defensins[2]. They are expressed in human keratincytes after induction by inflammatory stimuli and function primarily in the response to injury. The cathelicidins are components of wound fluid[1] and are expressed by human skin keratinocytes at sites of inflammation in disorders such as psoriasis, nickel contact dermatitis and systemic lupus erythematosus[3]. Defensins are small (3–5 kD) cationic peptides that can be grouped into the α-and β-defensins. The α-defensins human neutrophil peptides 1–4 are expressed in human leukocytes and human defensins 5 and 6 are expressed in Paneth cells in the small intestine[4,5]. Human β-defensins 1–3 are found in various epithelial cells[4]. In mammalian skin, human β-defensins 2 and 3 are expressed after induction in keratinocytes in response to infection and inflammation[2,6]. Human β-defensins 1 and 2 show microbicidal activity predominantly against Gram-negative bacteria such as *Escherichia coli* and yeasts, whereas human β-defensin 3 is also effective against Gram-positive bacteria such as *Staphylococcus aureus*, a major cause of skin infections, particularly in atopic dermatitis.

We describe here the isolation of a new antimicrobial protein that has no homology to known antimicrobial peptides. This protein was specifically and constitutively expressed in sweat glands, secreted into the sweat and transported in sweat to the epidermal surface. This indicated that human sweat contains at least one antimicrobial protein, which may play a role in the regulation of human skin flora.

Results

Isolation and mapping of *DCD*

Screening of a subtracted cDNA library of primary melanoma and benign melanocytic nevus tissues with cDNA arrays identified a clone—which we later designated *Dermcidin* (*DCD*)—that had no homology to any published gene sequence[7]. Sequencing of overlapping polymerase chain reaction (PCR) products identified a full-length DCD cDNA of 458 bp with an open-reading frame of 330 bp that encoded 110 amino acid (aa) residues (see **Web Fig. 1** on the supplementary information page of *Nature Immunology* online). The gene consists of five exons and four introns and is expressed as a single transcript[7]. Two short overlapping peptides generated from the 5′ end of the gene are described as a human cachexia-associated protein[8,9] and a survival-promoting peptide for neuronal cells[10,11]. We determined the chromosomal localization of *DCD* by analyzing a collection of human-rodent somatic hybrid cells that contained defined human chromosomes or their fragments on a rodent background. First, a monochromosomal hybrid cell panel was investigated with human DCD-specific primers in PCR experiments. For detailed subchromosomal mapping, the same technique was used with radiation hybrid mapping panel. With these approaches, we were able to assign *DCD* to chromosome 12q13 between the markers D12S1896 and D12S1632 (lod score 14.11).

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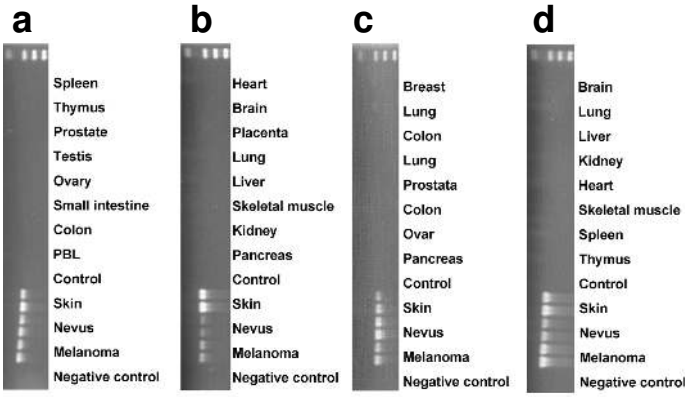

Figure 1. *DCD* expression was restricted to cells in the skin. RT-PCR analysis of various tissues with human MTC panels and *DCD*-specific primers. As shown, each sample was analyzed twice with 40 PCR cycles. For each sample, GAPDH could be successfully amplified (data not shown). (a) Tissue panel I (b) tissue panel II (c) tumor panel and (d) fetal panel.

Expression pattern of *DCD*

To determine the overall expression profile of *DCD*, a dot blot—in which RNA from 50 different tissues at different developmental stages were spotted onto a nylon membrane—was done with the use of labeled DCD cDNA as a hybridizing probe. No detectable signal was found in any of the 50 samples, which indicated that DCD had a restrictive expression pattern (data not shown). To determine whether DCD was expressed only at low amounts in human tissues or cell lines, we analyzed *DCD* expression with reverse transcribed–PCR (RT-PCR). DCD was highly expressed in human skin, melanocytic nevus tissue and cutaneous melanoma tissue (**Fig. 1**). However, expression was not detected in any of the 16 human tissues analyzed (spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood lymphocytes, heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) and was not expressed in several fetal and tumor tissues. In addition, in a panel that contained various parts of the human digestive system and several different tumor cell lines, no amplification product was detected by RT-PCR after 40 cycles (data not shown). These data indicated that *DCD* expression was restricted to cells in the skin.

To define the cell types that expressed DCD we used *in situ* hybridization, immunohistochemistry, immunofluorescence and immunoelectronmicroscopy. *In situ* hybridization showed that the gene was expressed in eccrine sweat glands within the dermis of human skin (**Fig. 2a**). With the use of a sense probe for *DCD* as a negative control no signals were detected (**Fig. 2b**). We used an antiserum raised in rabbits to carry out immunohistology on skin sections (**Fig. 2c**). Again, we saw strong staining on the eccrine sweat glands, but no expression on other skin cell types. Confocal laser scanning microscopy showed strong immunofluorescence staining in the secretory coils of eccrine sweat glands (**Fig. 2d**). In the secretory granules of apocrine sweat glands only weak and markedly reduced staining was seen (data not shown). Finally, we used immunoelectronmicroscopy to ultrastructurally localize DCD inside the eccrine sweat glands of normal axillary skin; we found that DCD was expressed in the dark mucous cells of the secretory coil (**Fig. 2e**). DCD localization was examined further and was found to occur within the golgi complex and in the secretory granules (**Fig. 2f**), which suggested DCD is a secreted protein.

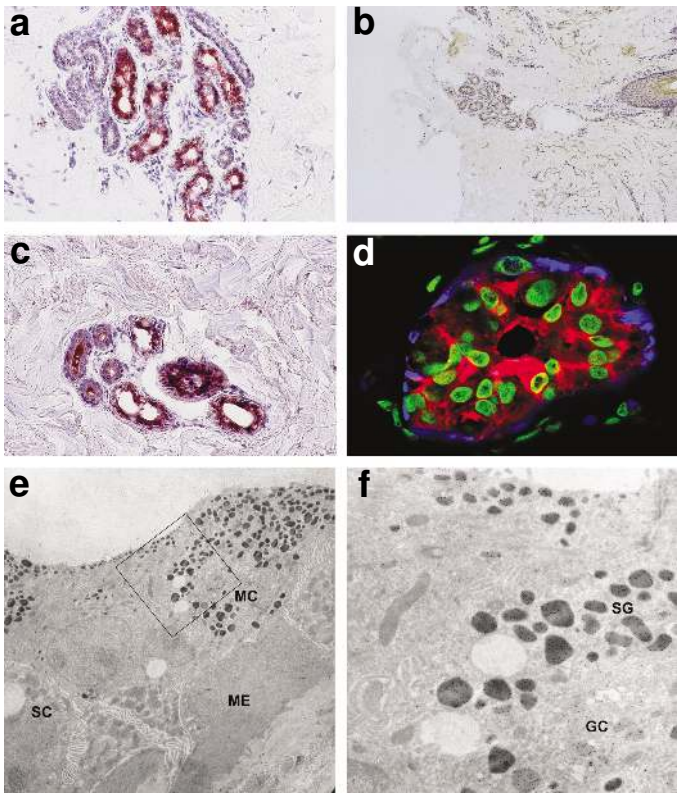

Figure 2. DCD was expressed in human eccrine sweat glands. (a,b) *In situ* hybridization with a digoxigenin-labeled sense probe that was transcribed *in vitro* (negative control) or an antisense probe for *DCD* showed that DCD was localized to the eccrine sweat glands. (c) The immunohistochemistry of DCD was assessed with rabbit anti-DCD serum and a secondary rabbit antibody. As a specificity control, the sections were incubated with a preimmune rabbit serum + the secondary antibody or the secondary antibody alone. (d) Immunofluorescence of a transected secretory coil of an eccrine sweat gland showed DCD^+ (red) serous cells surrounded by $actin^+$ myoepithelial cells (blue). Nuclei stained with YO PRO (green). Original magnification: ×800. (e,f) Immunoelectronmicroscopy was used to examine an eccrine sweat gland. (e) Secretory tubule of an eccrine sweat gland showed dark mucous cells (MC) with secretory granules, serous cells (SC) and a myoepithelial cell (ME). Original magnification: ×3000. (f) Higher magnification of e. Grouped gold particles, which revealed DCD localization, were found in the secretory granules (SG) and in the golgi complex (GC) of mucous sweat gland cells. Original magnification: ×12,000.

DCD is proteolytically processed

To determine whether DCD or DCD-derived peptides were secreted into human sweat, we isolated several protein fractions derived from human sweat after high performance liquid chromatography (HPLC) fractionation (**Fig. 3a**). To determine the peptide sequence in sweat fractions, we used Edman microsequencing and nanoelectrospray mass spectrometry. The mass spectrum of sweat fraction 4 showed an abundant signal that corresponded to a neutral mass of 4,702.57 Daltons. Edman analysis was used to identify aa 1–46 of this peptide, and tandem mass spectrometry was used to determine that the COOH-terminal sequence was V(I/L)DSV-COOH. Taken together these data showed that this peptide represented a processed form of the DCD protein that encompassed 47 aa of its COOH-terminal part (**Fig. 3b,c**). We termed this peptide DCD-1; it had a calculated neutral mass of 4,702.54 Daltons.

We analyzed sweat samples from four individuals and, from the data we obtained, estimated that the DCD-1 peptide was present in sweat at a concentration of 1–10 μg/ml. It remains to be determined whether processing of full-length DCD, which has a calculated molecular weight of

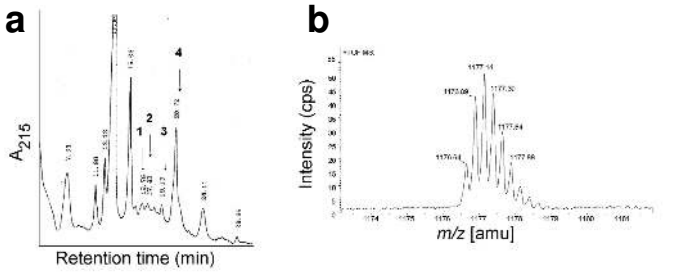

Figure 3. DCD was secreted into human sweat. (a) RP-HPLC analysis of human sweat. Antimicrobial assays were done with peak fractions 1–4. (b) Nanoelectrospray mass spectrometry of fraction 4 showed a quadruply charged signal at m/z=1176.64, which corresponded to a neutral mass of 4702.57 Daltons (calculated neutral mass 4702.54 Daltons). (c) Amino acid sequence of full-length DCD. The signal peptide is in italics; the peptide eluted from fraction 4 of human sweat is underlined. This peptide, designated DCD-1, encompassed aa 63–109 of full-length DCD.

9.3 kD without the signal peptide takes place in the sweat gland cells or after it has been secreted into sweat.

Antimicrobial activity of DCD-1 peptide

Antimicrobial peptides, such as the defensins, are produced as inactive precursor proteins that are proteolytically processed to give rise to active 25–45 aa peptides from the COOH-terminal region of the protein[12]. Although the amino acid sequence of DCD had no homology with other known antimicrobial peptides, the size of DCD and its processed peptides resembled the structural characteristics of the defensin family of antimicrobial proteins. Therefore, with purified peptides generated from the DCD amino acid sequence and the HPLC-purified sweat fractions 1–4, we analyzed the pathogens *E. coli*, *Enterococcus faecalis*, *S. aureus* and *Candida albicans* with antimicrobial assays.

The activity of two synthetic DCD-derived peptides (Y-P30 and DCD-1L) and a control peptide (DPI) were tested. The 30-aa peptide Y-P30 is derived from the NH_2-terminal end of DCD (aa 20–49)[10]; DCD-1L comprised 48 aa from the COOH-terminal end of the DCD protein; and DPI was a 30-aa control peptide without homology to DCD (**Fig. 4a**). All peptides were tested at concentrations of 0.1–100 μg/ml. Y-P30 and DPI had no toxic effect on the microorganisms (mean cell death was 5.6% with Y-P30 and 3.7% with DPI). However, DCD-1L, which was derived from the 3′ end of DCD and encompassed the DCD-1 peptide sequence plus the last leucine in the DCD sequence (**Fig. 4a**), showed dose- and time-dependent antimicrobial activity against the bacteria (**Fig. 4b**). It was highly effective against *E. coli, E. faecalis* and *S. aureus* and, at 10 μg/ml, killed 100% of the organisms after only 4 h of incubation. DCD-1L was also highly fungicidal, as shown by its toxic effect on *C. albicans* (**Fig. 4b**). The minimal inhibitory concentration (MIC) of the DCD-1L peptide, defined as the lowest concentration of peptide that prevented visible microbial growth after 4 h of incubation, was ~1 μg/ml for *E. coli, E. faecalis* and *S. aureus* and ~10 μg/ml for *C. albicans*. Increasing the incubation time to 18 h led to a greater microbicidal effect (data not shown). A shorter version of the DCD-1L peptide that encompassed 31 aa of the DCD COOH-terminal end had no toxic effect on the microorganisms (data not shown).

Sweat is acidic, with a pH of 4–6.8, and mainly consists of water, sodium (20–60 mM), chloride (20–80 mM), potassium (10 mM) and magnesium (1 mM). The antimicrobial assays were done in a sodium phosphate buffer at pH 7.4, as is used for defensins[13]. To strengthen support for the antimicrobial function of DCD and to show that the peptide was also active in sweat, we did the antimicrobial assays in several buffers that had a pH and ionic composition similar to that of human sweat. We tested phosphate buffers in which the pH varied between 5.5–7.4 or the sodium chloride varied between 25–150 mM; we also used a buffer with a pH of 5.5 or 6.5 that had a similar composition to human sweat (referred to as sweat buffer).

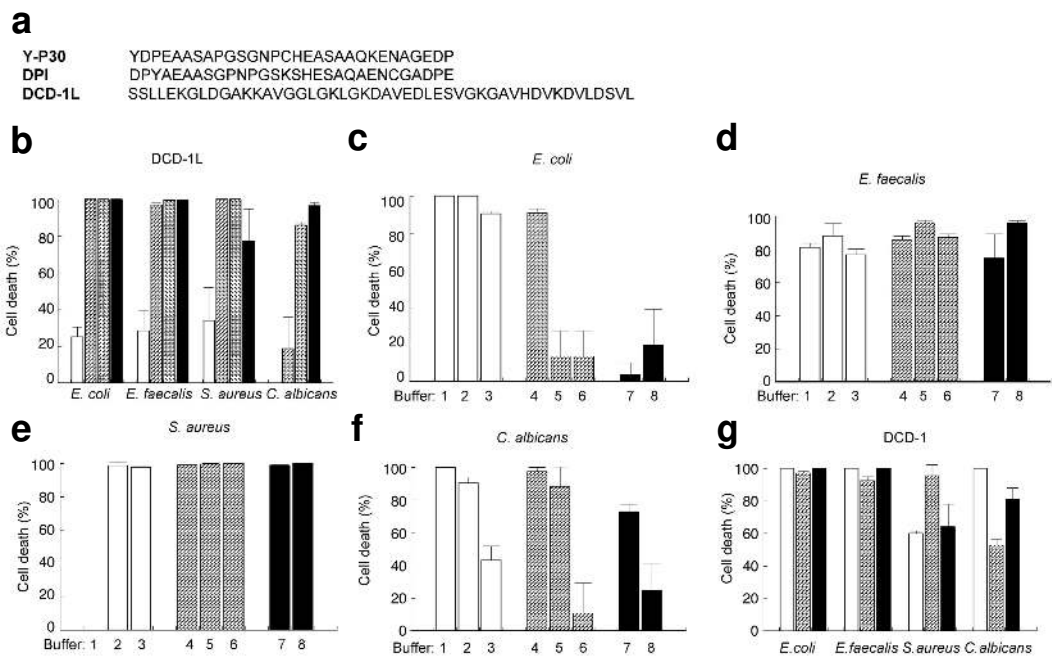

Figure 4. DCD had antimicrobial activity and was salt- and pH-insensitive. (a) Amino acid sequences of Y-P30, DCD-1L and DPI (the control peptide). (b) To assess DCD-1L activity, DCD01L was incubated with *E. coli*, *E. faecalis*, *S. aureus* and *C. albicans* for 4 h in 10 mM phosphate buffer (pH 7.4) at concentrations of 1 μg/ml (open bars), 10 μg/ml (checkered bars), 50 μg/ml (dotted bars) and 100 μg/ml (filled bars). The number of bacterial or yeast colonies were counted and percentage cell death calculated. (c–f) To assess DCD antimicrobial activity in different buffers over a broad pH range and salt concentrations, DCD-1L (10 μg/ml) was incubated for 4 h with various buffers + (c) *E. coli* (d) *E. faecalis* (e) *S. aureus* and (f) *C. albicans*. Buffer 1, sodium phosphate buffer pH 5.5; 2, sodium phosphate buffer pH 6.5; 3, sodium phosphate buffer pH 7.4; 4, sodium phosphate buffer pH 7.4 + 25 mM NaCl; 5, sodium phosphate buffer pH 7.4 + 100 mM NaCl; 6, sodium phosphate buffer pH 7.4 + 150 mM NaCl; 7, sweat buffer pH 5.5; 8, sweat buffer pH 6.5. (g) To assess the antimicrobial activity of an HPLC-derived sweat fraction (fraction 4 in Fig. 3, protein concentration, ~10 μg/ml), samples were incubated for 4 h with different microorganisms. The incubation buffers were 10 mM phosphate buffer pH 7.4 (white bars), sweat buffer pH 5.5 (checkered bars) and sweat buffer pH 6.5 (filled bars).

We found that the antimicrobial activity profile of the DCD-1L peptide was similar in pH 5.5 phosphate buffer or with sodium chloride concentrations of 25–150 nM and in sweat buffer compared to pH 7.4 phosphate buffer (**Fig. 4c–f**). Whereas the activity of DCD-1L in response to *E. coli* dropped when tested in sweat buffer or when the sodium chloride concentration increased to 100 mM (**Fig. 4c**), the antimicrobial activity of DCD-1L against *E. faecalis* and *S. aureus* did not change in the different incubation buffers. DCD-1L had no antibiotic activity against *S. aureus* in phosphate buffer at pH 5.5, although in sweat buffer of the same pH its activity was retained (**Fig. 4d–f**). In pH 5.5 or 6.5 phosphate buffer, in pH 5.5 sweat buffer or with sodium chloride concentrations at 25–100 mM, DCD-1L showed more activity against *C. albicans* than it did in pH 7.4 phosphate buffer (**Fig. 4f**).

Next, we tested the antimicrobial activity of four protein fractions derived from human sweat after HPLC fractionation (**Fig. 3b**). Whereas fractions 1–3 had no toxic effect on the microorganisms, fraction 4 killed all microorganisms in a dose-dependent manner (**Fig. 4g**). With mass spectrometry and peptide sequencing, we identified the DCD-1 peptide in fraction 4 (**Fig. 3c,d**). When sweat buffers were used as incubation buffers, the activity against the different microorganisms hardly changed. The antimicrobial activity of this peptide fraction against *E. coli* did not drop when sweat buffer, compared to phosphate buffer, was used for incubation. This contrasted with the results obtained with DCD-1L, which may have been due to differences in the peptide sequences because the last leucines in DCD-1 and DCD-1L differed. Thus, these data indicated that human sweat contained a proteolytically processed DCD peptide that had antibiotic activity against several microorganisms.

Discussion

We have described here the isolation of a gene encoding an antimicrobial peptide with a broad spectrum of activity and without any homology to known antimicrobial peptides. This protein was specifically and constitutively expressed in sweat glands, secreted into the sweat and transported to the epidermal surface. In sweat a proteolytically processed DCD peptide was present that has antimicrobial activity against a variety of pathogenic microorganisms. This activity was maintained over a broad pH range and in high salt concentrations, which resembled the conditions in human sweat.

Human sweat glands are divided into eccrine, apocrine and apoecrine; apoecrine glands share the properties of the eccrine and apocrine glands. The eccrine sweat gland is composed of epithelial and myoepithelial cells that are organized into the secretory coil, dermal duct and epidermal duct or acrosyringium. The secretory coil of the eccrine sweat gland is involved in the production of precursor sweat, which is isotonic or slightly hypertonic and is essentially an ultrafiltrate of plasma. The sweat then passes through the coiled duct, straight duct and acrosyringium to the epidermal surface. During passage through the intradermal duct the sweat is modified by selective resorption of sodium, which mainly occurs within the coiled duct[14].

The secretory unit consists of three cell types: clear (serous) and dark (mucous) cells, which are involved in secretion, and myoepithelial cells, which function as smooth muscle cells[14]. The clear cells secrete precursor sweat *via* the active transport of sodium into the lumen of the secretory coil, whereas the dark cells produce mucins, such as glycoproteins, that can be found along the eccrine gland lumina. The tubuli of eccrine glands are involved in reabsorption of sodium and chloride, which results in hypotonicity of the secreted sweat. Sweat mainly consists of water, sodium, chloride, potassium, urea and lactate. A variety of other substances may be found in sweat, including pharmacologically active substances, inhibitors, antigens, antibodies and drugs[14]. The majority of peptides in sweat are of small molecular weight. The concentration of high molecular weight proteins (>10 kD) is positively correlated with the sweat rate; this is due to a different degree of proteolytic degradation during the outflow of sweat, secretion of proteins from different intracellular storage sites or differing protein synthesis[14].

Immunoglobulin A[15], interleukin 1 (IL-1) and IL-8[16,17], IL-6 and tumor necrosis factor-α[18], transforming growth factor β receptor[19], epidermal growth factor[14] and a prolactin-inducible protein[19] have all been identified in human sweat. We determined that ~1–10 μg/ml of the peptide DCD-1 was present in sweat, a concentration that proved toxic to most microorganisms we tested. The antiserum we used for immunohistological analysis was directed against a peptide located in an antigenic region at the center of the DCD protein (aa 42–65). Thus, most likely, we had identified either a nonprocessed, full-length protein or a DCD-derived peptide that encompassed the antigenic region. The antibiotically active DCD-1 peptide lacked the antigenic determinant used for immunization and could not be detected by the antiserum. Therefore, it remains to be determined whether the full-length DCD protein had already been processed in the eccrine sweat gland cells or in the sweat. Because human sweat contains various proteases[21,22] it is likely that the DCD protein was processed there. The concentration of proteolytically processed peptide probably differs between individuals and is dependent on the concentration of proteolytic enzymes in the sweat as well as the rate at which an individual sweats.

We have shown here that the peptides DCD-1, which encompasses the COOH-terminal part of DCD (aa 62–109), and DCD-1L, which has an additional leucine at the COOH end, were toxic to various microorganisms in medium that had a similar pH and ionic composition to human sweat. In contrast to the activity profile of DCD-1, members of the defensin family are only active in the presence of low salt concentrations; they are inactive at high salt concentrations[23,24]. As modeling studies suggest, like many antimicrobial peptides—including insect cecropins, frog magainins and some mammalian cathelicidins—DCD is likely to adopt an amphipathic α-helix[25]. However, unlike most antimicrobial peptides—which are enriched in arginine or lysine residues and are therefore cationic[26]—antimicrobial DCD-1 has a net negative charge of –5. Therefore, the mode of action of this peptide in response to microorganisms is probably different to that of the cationic defensins, which can bind to anionic components of the target membrane and kill the microorganisms by pore formation and permeabilization of the cell membrane[24]. It remains to be determined whether DCD is toxic to microorganisms that are resistant to established antibiotic therapies.

In conclusion, DCD-1, a 47-aa peptide, was proteolytically processed from DCD and secreted by mucous cells of the sweat gland coil into the sweat. Under pH and salt conditions that were characteristic of human sweat, it was antimicrobially active against *E. coli, E. faecalis, S. aureus* and *C. albicans*. DCD showed no homology to known antimicrobial peptides and was characterized by a restrictive expression pattern that was limited to sweat glands. This peptide probably plays a key role in the innate immune responses of the skin.

Methods

Gene mapping. Human-rodent somatic cell hybrid (Coriell Cell Repositories, Camden, NJ) and radiation hybrid mapping panels (Stanford Human Genome Center, Stanford, CA) were used to determine the chromosomal localization of DCD.

RNA expression analysis of DCD. To detect DCD expression, RT-PCR was done with RNA from various tissues and cell lines. Isolation of RNA from benign melanocytic nevi, primary cutaneous melanoma and skin tissues was done with a modified procedure as described[27]. In addition, cDNA from the multiple tissue cDNA (MTC) panels human I and II, human fetal, human digestive system and human tumor (Clontech, Heidelberg, Germany) were analyzed by PCR. The PCR amplification mixture contained 5 μl of the template, 1×Taq PCR reaction buffer, 0.5 μl of Taq-polymerase (Amersham Pharmacia, Freiburg, Germany), 0.4 mM dNTP

and 0.4 μM of each primer. The primers we used were 5′–AGCATGAGGTTCATGACTCTC–3′ and 5′–CACGCTTTCTAGATCTTCGAC–3′. The PCR product was ~290 bp. As a control, GAPDH was amplified with the primers: 5′–AATGCCTCCTGCACCACC–3′ and 5′–ATGCCAGTGAGCTTCCCG–3′. The PCR conditions were 30 cycles for GAPDH or 30–40 cycles for DCD at 96 °C for 1 min, 54 °C for 1 min and 72 °C for 80 s. Each PCR was done at least twice and included two negative controls and at least one positive control. A 100-bp marker was used as a reference marker in agarose gel electrophoresis (MBI Fermentas, St. Leon-Rot, Germany).

***In situ* hybridization, immunohistochemistry, immunofluorescence and immunogold electron microscopy.** *In situ* hybridization was done—on 5-μm paraffin tissue sections that were fixed in formalin—with single-stranded antisense or control sense digoxigenin-labeled probes. The probes were synthesized by *in vitro* transcription of DCD cDNA in pBluescript SK with the DIG RNA labeling kit (Roche, Mannheim, Germany) and T3 and T7 RNA polymerase. Several melanocytic nevi, biopsies of normal skin and two primary melanoma were examined.

For the identification of DCD protein with immunohistology or immunofluorescence, 2- or 5-μm vertical paraffin sections of normal skin were dehydrated, blocked with normal horse serum and incubated for 1 h with a 1:3000 dilution of polyclonal rabbit antiserum to DCD. The antiserum was obtained after repeated immunization with the peptide KENAGEDPGLARQAPKPRKQRSSL, which was coupled to keyhole limpet hemocyanin. The sections were incubated with biotinylated anti–rabbit IgG (Vector, Burlingame, CA), followed by incubation with the Vectastain ABC-AP system (Vector), developed with neufuchsin and counterstained with hematoxylin. For immunofluorescence analysis, the sections were stained with polyclonal antiserum to DCD and incubated for 1 h with a 1:500 dilution of a Cy5-donkey anti–rabbit serum (Dianova, Hamburg, Germany). The myoepithelial cells of the secretory coil of eccrine sweat glands were then labeled with monoclonal anti-actin (Enzo Diagnostics, Loxo, Dossenheim, Germany), stained with Cy3-donkey anti–mouse serum (Dianova) and all nuclei were stained with YOPRO (Molecular Probes, Leiden, Netherlands). The sections were analyzed with a confocal laser scanning microscope (Leica TCS SP, Leica Microsystems, Bensheim, Germany) and magnified 250 times.

For ultrastructural localization of DCD, normal axilla skin was treated as described[28]. The sections were counterstained with uranyl acetate and lead citrate and examined with a ZEISS EM 109 electron microscope (Zeiss, Jena, Germany).

Peptide synthesis, HPLC analysis, mass spectrometry and protein sequencing. Peptides were synthesized by the solid phase method with the Fmoc/But-strategy on a MilliGen 9050 continuous flow synthesizer (Millipore, Bedford, MA). The cleaved, products were purified by gradient reversed phase (RP-HPLC) to a purity of ≥95 %. Peptide identity was confirmed by electrospray mass spectrometry on a Finnigan MAT 700 instrument (Finnigan Corp., San Jose, CA).

Isolation of proteins from human sweat was done as follows. Human sweat (25 ml) was lyophilized overnight to yield a colorless, hygroscopic solid product. The residue was dissolved in 500 μl of 10 % acetic acid in water and sonicated for 1 min. Nonsolubilized material was removed by centrifugation at 10,000*g* for 5 min. The supernatant (100 μl) was subjected RP-HPLC on a Nucleosil C18 column (125×4 mm) with 5-μm particles and a 120-Å pore size) with a flow rate of 1 ml/min. Solvent A was 0.055% aqueous trifluoroacetic acid; solvent B was 80% acetonitrile in 0.05% aqueous trifluoroacetic acid. A linear gradient of 0% B to 60 % B over 40 min was used. The resulting elution peaks were collected and concentrated with a speed vac to a volume of ~15 μl. Samples of 1 μl of each fraction were used for MALDI-TOF analysis (G2025A, Hewlett-Packard, Waldbronn, Germany), 2,5,-dihydroxyacetophenone was used as a matrix. The peak that eluted at 20.72 min was highly homogeneous and contained the DCD-1 peptide (which had a molecular mass of ~4.702 kD). This fraction was analyzed by automated Edman degradation with an Applied Biosystems 494 protein sequencer. (Applied Biosystems-MDS Sciex, Concord, Canada).

Nanoelectrospray mass spectrometry was done on an QSTAR Pulsar i QqoaTof mass spectrometer (Applied Biosystems-MDS Sciex) with medium Protana NanoES spray capillaries. For tandem mass spectrometry experiments, the resolution of the first quadrupole was set to transmit the isotopic envelope of the ion of interest. Nitrogen was used as the collision gas.

For Edman microsequencing, aliquots of the HPLC fractions were applied onto TFA-treated glass filter discs that were coated with 0.75 mg of polybrene, dried and sequenced in a protein sequencer Procise 494A (Applied Biosystems, Weiterstadt, Germany) following the manufacturer's protocols.

Antimicrobial assay. The antimicrobial activity of DCD-1L, Y-P30, DPI and four sweat fractions was analyzed with a colony-forming unit assay as described[13]; *E. coli*, *S. aureus*, *E. faecalis* and *C. albicans* were assessed. *E. coli* were grown in LB medium, *E. faecalis* and *S. aureus* in Columbia medium (Difco, BD Heidelberg, Germany) and *C. albicans* in caseinhydrolysate medium (Merck, Darmstadt, Germany). The bacterial and yeast concentrations were determined photometrically. Bacterial strains were cultured to an A_{600} of 0.4–0.7 and the yeast strain to an A_{450} of 0.4–0.6. Before analysis, we determined the concentration of the organisms in culture by plating different bacterial and yeast dilutions. At A_{600}, 1.0 corresponded to 8.2×10^8/ml for *E. coli*, 1.9×10^{10}/ml for *S. aureus* and 9.0×10^8/ml for *E. faecalis*. At A_{450}, 1 corresponded to 1.4×10^8/ml for *C. albicans*. Cells were washed twice with 10 mM sodium phosphate buffer (pH 7.4) and diluted to a concentration of 2×10^6 cells/ml (for *E. coli*, *E. faecalis* and *C. albicans*) or 2×10^7 cells/ml (for *S. aureus*) in phosphate buffer. Cells were incubated at 37 °C for 4 h with various concentrations of peptides or sweat fractions (protein concentration, ~10 μg/ml) in 200 μl of sodium phosphate buffer. The cells were diluted 1:50–500, and 50 μl and 100 μl of the solutions were plated in duplicate on agar plates. At least four plates from each experiment were evaluated and the mean number of colonies determined. The bactericidal activity of the tested reagents were expressed as: [1– (cell survival after peptide incubation)/(cell survival after control peptide incubation)] ×100, which represented the percentage of cells that were killed.

To examine the activity profile of DCD-1L and the antibiotic sweat fraction that contained DCD-1, we incubated the microorganisms for 4 h with 10 μg/ml of DCD-1L or sweat fraction under the following conditions. Phosphate buffer (10 mM) + either 25, 100 or 150 mM sodium chloride; phosphate buffer (10 mM) at pH 5.5, 6.5 or 7.4; sweat buffer (40 mM NaCl, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1mM Na-dihydrogenphosphate) at pH 5.5 or 6.5. Then antimicrobial activity was assessed as outlined above.

Genbank accession number. The Genbank accession number for the DCD cDNA sequence we identified is AF144011. Another group has also deposited a DCD sequence, the accession number for this data is AY044239.

Acknowledgements

We thank T. Iftner and colleagues for DNA sequencing; M. Schwarz and colleagues for the use of radioisotopes and Phosphorimager; B. Fehrenbacher and H. Bischof for electron microscopy; A. Nordheim for mass spectrometry; E. Maczey for technical assistance; and P. Gött, T. Paul, M. Stark and F. Lang for discussions. Supported by the Fortüne-program of the Eberhard-Karls-University Tübingen (432-0-1), the Bundesministerium für Bildung und Forschung (IZKF, Fö01KS9602) and the Deutsche Forschungsgemeinschaft (SFB510).

Received 8 June 2001; accepted 11 October 2001.

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