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Isolation and characterization of a novel protein toxin from fire coral

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Abstract

Fire corals (*Millepora* spp.) cause severe pain and inflammatory effects in humans upon contact, and the organs responsible for these effects are called nematocysts. Here, we isolated an active cytotoxin of ca. 18 kDa (MCTx-1) from nematocysts of *Millepora dichotoma* var. *tenera*. MCTx-1 was potently cytotoxic (EC_{50} value 79 ng/mL) towards L1210 mouse leukemia cells, hemagglutinated a 0.8% suspension of sheep erythrocytes (0.2 μg protein/mL) and was lethal in crayfish (LD_{50}, 106 μg/kg). We deduced the primary structure of MCTx-1 from the corresponding cDNA sequence and found that MCTx-1 is a novel dermatopontin that is an extracellular matrix protein in mammals. This is the first characterization of a proteinaceous toxin from fire coral.

Keywords: Fire coral; *Millepora*; Cytotoxin; Nematocyst; Toxin; Dermatopontin

Millepora spp. (family Milleporidae, class Hydrozoa, phylum Cnidaria) corals are abundant in shallow tropical and subtropical waters. *Millepora* spp. are called fire corals or stinging corals, because contact with them causes severe pain and a series of skin eruptions and blisters [1,2]. One report has indicated that the sting of *Millepora* can cause acute renal failure, nephritic syndrome, and pulmonary edema [3]. The causative agents of these conditions are proteinaceous toxins stored in nematocysts [4], which are unique extrusive organs in cnidarians that are involved in defense and the capture of prey. Nematocysts inject venom through needles in response to chemical or mechanical stimuli. Wittle et al. showed that purified toxins from *Millepora alcicornis* and *Millepora tenera* (first identified as *Millepora dichotoma* and later corrected) originated from nematocysts and that they elicited hemolytic and dermonecrotic responses accompanied by a rash that lasted several days [5–7]. Lethal factors with hemolytic activities have been recognized in *Millepora platyphylla* and *M. dichotoma* and a hemolytic toxin (31.5–33 kDa) has been isolated from *M. platyphylla* [8]. Moreover, milleporin-1 (32.5 kDa) with PLA_2 and hemolytic activities has been isolated from nematocysts of *M. platyphylla* [9]. However, proteinaceous toxins from *Millepora* spp. have not yet been characterized. We isolated and characterized a novel *Millepora* cytotoxin (*Millepora* cytotoxin-1; MCTx-1) from the nematocysts of *M. dichotoma* var. *tenera*. This is the first characterization of a proteinaceous toxin from a fire coral.

Materials and methods

Collection of fire corals. Millepora dichotoma var. *tenera* was collected at a depth of 3–5 m from the coast of Aka Island, Okinawa, Japan with the permission of the Okinawa prefectural government. Samples were immediately frozen after collection at −30 °C. A portion of the sample was treated with RNA*later*® (Ambion, Austin, TX) for total RNA preparation according to the manufacturer's instructions. The samples were identified by Dr. Yuri Latypov of Russian Academy of Science (The Institute of Marine Biology, Far East Division) and then deposited at the Laboratory of Aquatic Ecochemistry of the Tokyo University of Marine Science and Technology.

Isolation and extraction of nematocysts. Nematocysts were released from the fire coral exoskeleton by ultrasonic disruption (500 W, 38 kHz, ultrasonic cleaner MUS-40D, Eyela, Tokyo, Japan) in 1 M sodium citrate solution for 3 min. The skeleton and tissue were removed by filtration through a nylon stocking net to yield a filtrate containing zooxanthellae

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and nematocysts. The filtrate was gently centrifuged (20*g*, 2 min) to sediment undischarged nematocysts, which were repeatedly washed with 1 M sodium citrate. Crude venom extracted from the isolated nematocysts by vortex mixing in distilled water, was centrifuged (12,000*g*, 15 min) and the supernatant was filtered through a 0.45 μm membrane (Glass Microfibre filters; GF/F, Whatman, Maidstone, UK). The filtrate was referred to as the venom fraction.

Cytotoxicity test. Mouse leukemia L1210 cells (obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging, and Cancer, Tohoku University) were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 100 mg/L streptomycin, 100 U/mL penicillin, and 0.3 mg/mL glutamic acid at 37 °C in a humidified 5% CO_2 atmosphere. Cultured cells (100 μL) were seeded onto 96-well culture plates, and 10 μL of the venom fraction was added to each well. After 18 h at 37 °C in CO_2 incubator, the wells incubated for 6 h with 50 μL XTT working solution 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt, XTT sodium salt (Sigma, St. Louis, MO, USA) in RPMI-1640 medium (1 mg/mL) and 5-methylphenazinium methyl sulfate; PMS (Sigma) (38 μg/mL). Colorimetric changes in the XTT-reduction assay were then measured in a microtiter plate reader at dual wavelengths of 492 nm and 690 nm.

Molecular exclusion chromatography of crude venom. Crude extract was chromatographed and fractionated on a molecular exclusion column containing Superdex-200 (10 × 300 mm, GE Healthcare, Buckinghamshire, UK). Fractions were eluted with 0.3 M NaCl 10 mM phosphate buffer (pH 7.5) at a flow rate of 0.5 mL/min. The eluate was monitored at 210 nm using a UV detector. The activity of each fraction was examined using the cytotoxicity test. Samples dissolved in 0.01 M Tris–HCl buffer (pH 6.8), containing 2% SDS and 10% glycerin or 2% SDS, 10% glycerin and 2.5% dithiothreitol, were heated in a boiling water bath for 3 min and then resolved by sodium dodecyl–sulfate poly acrylamide gel electrophoresis (SDS–PAGE) on 12.5% polyacrylamide gels with Tris–glycine buffer to determine purity. Protein bands were visualized using a silver stain kit (Kanto-Kagaku, Tokyo, Japan). The molecular mass was estimated by comparison with the standards provided in a low molecular weight marker kit (GE Healthcare, Buckinghamshire, UK).

Isolation of MCTx-1. Crude fire coral venom from 10 g of isolated nematocysts was treated by ammonium sulfate saturation (separation steps: 0–35, 35–70, and 70–100%). The 35–70% precipitate was dissolved in phosphate buffer and then desalted and concentrated by ultrafiltration (Amicon YM-30, GE Healthcare). The desalted fraction was purified by elution with 10 mM phosphate-buffer (pH 7.5) containing 0.15 M NaCl through a column containing Superdex-200 (10 × 300 mm, GE Healthcare). The cytotoxic fraction was concentrated by ultrafiltration and isolated by molecular exclusion chromatography through a column of Superdex-75 (10 × 300 mm, GE Healthcare). The eluate was monitored at 210 nm using a UV detector. The protein concentration of the cytotoxic fraction was measured using BCA protein assay reagent (Pierce, Rockford, IL, USA) following the manufacturer's instructions and the purity was confirmed by SDS–PAGE.

Hemagglutination test. Purified fractions were incubated in 96-well round-bottom microplates with a 200 μL (0.8%) suspension of sheep red blood cells (Nihon Seibutsu Zairyo Center, Tokyo, Japan) in phosphate buffered saline (PBS) (+) for 6 h at room temperature. The minimum concentration of MCTx-1 required to cause detectable hemagglutination was determined.

Crayfish toxicity test. Purified cytotoxic fraction (10 μL) were injected intraperitoneally into crayfishes in quintuplicate. Lethality was examined 4 h later.

Phospholipase A_2 activity assay. Phospholipase A_2 (PLA_2) activity was examined using a $sPLA_2$ enzyme assay kit (Cayman Chemicals, Ann Arbor, MI, USA). Fractionated samples (10 μL) were incubated with 10 μL of 5,5′-dithio-bis-(2-nitrobenzonic acid) (DNTB, Cayman Chemicals), 5 μL of assay buffer (25 mM Tris–HCl containing 10 mM $CaCl_2$, 100 mM KCl, 0.3 mM Triton X-100 and 1 mg/mL albumin from bovine serum) and 200 μL of substrate buffer (10.66 mM/L diheptanoyl thiophosphatidylcholine, Cayman Chemicals) in microplate wells at room temperature. Positive standard curves were determined using 100 μg/mL of bee venom PLA_2 (Cayman Chemicals). The absorbance at 414 nm was measured every minute using a microplate reader.

Deglycosylation of MCTx-1. MCTx-1 was deglycosylated to examine whether it contains amino acid-linked sugar residues of N-linked or O-linked oligosaccharides. The extent of deglycosylation was assessed by mobility on SDS–PAGE using the Enzymatic Protein Deglycosylation Kit (Sigma) following the manufacturer's instructions. N-linked sugars were removed by digestion with the enzyme PNGase F, and O-linked sugars were removed using the enzymes, PNGase F and α-2 (3,6,8,9)-neuraminidase, respectively. The positive control glycoprotein was bovine fetuin that contains sialylated N-linked and O-linked oligosaccharides. MCTx-1 without enzyme digestion was the negative control. Samples and controls were incubated for 3 h at 37 °C and then mobility shifts were analyzed by SDS–PAGE.

Protein sequencing of MCTx-1. The thiol group of cysteines in MCTx-1 was pyridylethylated as follows: MCTx-1 (20 μg) was dissolved in 300 μL of 7 M guanidine hydrochloride, 50 mM dithiothreitol, 10 mM EDTA, and 0.5 M Tris–HCl buffer (pH 8.5). Samples were allowed to reach room temperature over 2 h, and then 2 μL of 4-vinylpyridine was added and mixture was reacted for 30 min at room temperature. The reaction was stopped by adding 5 μL of 99% formic acid and then the mixture was applied to reversed-phase HPLC (Resource RPC 1 ml; GE Healthcare) using a 2–80% acetonitrile gradient in water containing 0.05% trifluoroacetic acid at a flow rate of 1 mL/min to isolate pyridylethylated MCTx-1. The N-terminal amino acids of the pyridylethylated MCTx-1 were sequenced by automated Edman degradation using the protein sequencer PSQ-1 (Shimadzu, Kyoto, Japan).

Cloning of MCTx-1 cDNA. Total RNA was isolated from 10 g of frozen tissue samples using the TRIzol reagent (Invitrogen). For degenerative 3′-rapid amplification of cDNA ends (RACE), first-strand cDNA was synthesized from 5 μg of total RNA using the 3′RACE System for Rapid Amplification of cDNA Ends kit (Invitrogen) and the oligo dT-adapter primer. Degenerate primers were designed based on the internal amino acid sequences determined by protein sequencing. The first 3′-RACE reaction proceeded using a degenerate primer (5′-WSI CAR CAY AAY AAY TAY TAY G-3′ corresponding to SQHNNYYE, 5′-CAR CAY AAY AAY TAY TAY GAR G-3′ corresponding to QHNNYYED) and the abridged universal amplification primer (AUAP). Products were amplified using Ex Taq Polymerase (Takara Bio, Shiga, Japan) by incubation at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 50 s, and extension at 72 °C for 5 min. Secondary PCR products were subcloned into the pT7Blue T-vector (Novagen, San Diego, CA, USA) and sequenced using the Thermo Sequenase Cy5 Dye Terminator kit (GE Healthcare) and a Long-Read Tower DNA sequencer (GE Healthcare). The remaining 5′-terminal sequences were analyzed by 5′-RACE as follows: first-strand cDNA was synthesized from 5 μg of total RNA using the 5′RACE System for Rapid Amplification of cDNA Ends kit (Invitrogen) and a gene-specific primer (5′-GAA ACA CAG AAC ATA AGA AG-3′). The first 5′-RACE reaction was completed using a gene-specific primer (5′-AAT CCA TCG GGT GGG TCC ATC-3′) and the abridged anchor primer (AAP), followed by re-amplification of the PCR products using a gene-specific primer (5′-GTA TTA GCA GTA GAG CAT CTT G-3′) and AUAP. The amplification conditions comprised incubation at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 50 s and extension at 72 °C for 5 min. The secondary PCR products were subcloned into the pT7Blue T-vector and sequenced. We performed a homology search of the protein sequence database BLAST (http://blast.ddbj.nig.ac.jp/top-j.html).

Results

Isolation of nematocysts

Intact nematocysts (~3 g) were isolated from 500 g of fire coral by sonication followed by slow centrifugation.

Adding distilled water to the isolated nematocysts facilitated their complete discharge.

Isolation of MCTx-1

Several cytotoxins were separated by molecular exclusion chromatography of the crude venom on Superdex-200 (Fig. 1). We targeted a cytotoxin (MCTx-1) with a molecular weight of around 18,000. MCTx-1 was precipitated from crude venom in the 35–70% ammonium sulfate fraction and purified by consecutive molecular exclusion chromatography to yield active MCTx-1.

Bioactivities of MCTx-1

Isolated MCTx-1 was potently cytotoxic towards L1210 mouse leukemia cells (IC_{50} 79 ng/mL), lethal to crayfish (LD_{50} value 106 μg/kg), and hemagglutinated 0.8% sheep erythrocytes (0.2 μg protein/mL). MCTx-1 was not hemolytic to 0.8% sheep erythrocytes (<0.2 μg protein/mL) and did not contain phospholipase A_2 activity (<0.02 μmol/min/mL). A different fraction of the crude venom fraction separated by molecular exclusion chromatography of the crude venom contained potent phospholipase A_2 activity (Fig. 1).

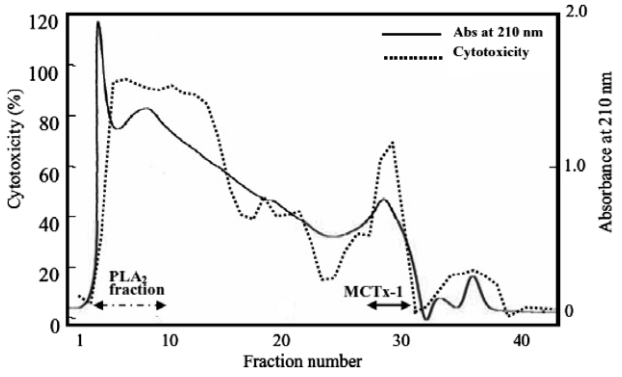

Fig. 1. Size exclusion chromatography of crude venom. Crude venom was applied to size exclusion chromatography (superdex-200). Separated fractions were assayed by cytotoxicity test (L1210 cell line). Dotted line shows cytotoxicity of each fraction. Phospholipase A_2 fraction is shown as an arrow of dash-dotted line. MCTx-1 fraction is shown as an arrow of solid line.

Deglycosylation of MCTx-1

We analyzed mobility shifts of MCTx-1 digested with PNGase and with both PNGase and α-2 (3,6,8,9)-neuraminidase using SDS–PAGE. These enzymes did not affect the mobility of these fractions compared with the negative control (intact MCTx-1) on SDS–PAGE. The results indicated that MCTx-1 is not a glycoprotein.

Protein sequencing of MCTx-1

The N-terminus sequence of MCTx-1 was revealed to be SKVNQYDQPFX. Tryptic digests of MCTx-1 were separated by reversed-phase HPLC and the sequences of five peptide fragments (A–E) were determined as SIGSIHDNX, YQLMXSYLNNX, GSXAWTSYX, FLVGMK, and SQHNNYYEDR, respectively.

Cloning and sequencing of MCTx-1

Molecular cloning clarified the nucleotide sequence of the full-length cDNA (920 bp) encoding MCTx-1 (Fig. 2). A poly(A) tail was located in the 3′-untranslated region, whereas stop codons were not apparent in the 5′-untranslated region upstream of the putative initiating methionine. The complete amino acid sequence of MCTx-1 was

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atcgaagcgaaaggtaattcgactgcagatcatccaggtgcaacaaaaatggttactttatacttacacgttcctatcttgttactcgtggtgataacagcacgagcagcgccgaaacct 120
                                                  M  V  T  L  Y  L  H  V  P  I  L  L  L  V  V  I  T  A  R  A  A  P  K  P  24

gatactcacaatccatttgacgaattatcttcggttgctgaaaaacaggatctgcattatggtgatcgttcaaggaaagatcccttcattgctcaaaatgatgttgggaacaatttcagg 240
 D  T  H  N  P  F  D  E  L  S  S  V  A  E  K  Q  D  L  H  Y  G  D  R  S  R  K  D  P  F  I  A  Q  N  D  V  G  N  N  F  R  64

gatggaactcaagaaaatcttactaaagttcggtcgaaagtaaatcaatatgatcaaccgttcacttttaaatgtcctctaggagaaacaatcaagagtattgggagtatacatgataac 360
 D  G  T  Q  E  N  L  T  K  V  R  S  K  V  N  Q  Y  D  Q  P  F  T  F  K  C  P  L  G  E  T  I  K  S  I  G  S  I  H  D  N 104
                                                                                                                  A

cattacgaagatcggcaatgggatatcgattgtaaaccagctggttatactatgggtatctcaacgtggtctccttatgcaaatgactatgacggcagcatgaactttgagtgcaatgaa 480
 H  Y  E  D  R  Q  W  D  I  D  C  K  P  A  G  Y  T  M  G  I  S  T  W  S  P  Y  A  N  D  Y  D  G  S  M  N  F  E  C  N  E 144

ggtagtgttgtcactggaatgagttctattcacgataattattacgaggatagacgctaccaattgatgtgttcttatctaaacaattggaagcgaggatcttgtgcctggacatcgtat 600
 G  S  V  V  T  G  M  S  S  I  H  D  N  Y  Y  E  D  R  R  Y  Q  L  M  C  S  Y  L  N  N  W  K  R  G  S  C  A  W  T  S  Y 184
                                                                                 B                          C

acaacttatgatgcaagttttgtcgaactaacaccaactgggaagttcctggttggaatgaaaagtcagcataataattactacgaagatcgaaaattcaagatgctctactgctaatac 720
 T  T  Y  D  A  S  F  V  E  L  T  P  T  G  K  F  L  V  G  M  K  S  Q  H  N  N  Y  Y  E  D  R  K  F  K  M  L  Y  C  *    222
                                              D                 E

cagttactacggatggacccacccgatggattatggatagtgattttcgcgatcttcttatgttctgtgtttcgaacaccttaacaatatattgtcttacatgcatgtcttatgataatt 840
gtcttataatgatgtaatacctaaatttttataaaaactaaggaaaatttccttgagaacaaaaaaaaaaaaaaaaaa                                          920
```

Fig. 2. Nucleotide and amino acid sequences of MCTx-1. The complete gene sequence of MCTx-1 and its translation product are illustrated. The asterisk indicates in-flame stop codon (TAA). The sequences of peptide fragments are underlined. The box indicates the N-terminal sequence of mature MCTx-1. Dash-dotted line indicates signal peptide. Three repeats of the same motif (S-X-H-X-N-X-Y-E-D-R) are shown with bold letter. Accession number of MCTx-1 is AB299385.

deduced from the full-length cDNA. The open reading frame comprised 666 bp, which encoded a protein of 222 amino acid residues. The deduced sequence between Ser^{76} and Phe^{85} was identical to the N-terminal 10 residues determined by the protein sequencer. Thus, the mature protein started from the Ser of the 76th residue. Sequences of fragments A–E determined by peptide mapping were located within the deduced amino acid sequence (Fig. 2). A similar amino acid motif (SXHXNXYEDR) was repeated three times (Fig. 2). The calculated isoelectric point (5.41) indicated that MCTx-1 is acidic. Analysis using SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP) showed that N-terminal segment up to residue 20 is a signal peptide (Fig. 2).

Discussion

Fire corals (*Millepora* spp.) are considered venomous marine animals since they damage the human body upon accidental contact. Nematocysts are organs that contain venom and a needle inside, and these are responsible for the *Millepora* sting. The preparation of pure venom from isolated nematocysts that are free of contaminants (especially zooxanthellae) is critical for unambiguously investigating which toxins are actually involved in *Millepora* stings. Here, we established a method to isolate nematocysts from *Millepora* coral using sonication and slow centrifugation in 1 M sodium citrate. This method allowed the simple isolation of crude venom, since adding distilled water to nematocysts in sodium citrate readily caused them to discharge. An osmotic change might be associated with this process.

We identified at least two cytotoxins and a hemolytic toxin in crude nematocysts extracts (data not shown), and isolated an 18 kDa cytotoxin (MCTx-1) with potent cytotoxicity toward L1210 mouse leukemia cells (IC_{50}, 79 ng/mL), lethality to crayfish (LD_{50}, 106 μg/kg), and hemagglutination activity towards 0.8% red sheep blood cells (0.2 μg/mL). MCTx-1 did not have either PLA_2 or hemolytic activity. Thus, MCTx-1 is different from millepoorin-1 (~32.5 kDa) and the hemolytic toxin formerly isolated from *M. platyphylla* [8,9].

According to the BLAST database, MCTx-1 belongs to the dermatopontin family. Dermatopontin was initially isolated as an extracellular matrix protein from bovine dermal extracts [10]. Thereafter, dermatopontins were isolated from humans, pigs, rats, and mice as extracellular matrix [11–13] and from invertebrates including a horseshoe crab, sponges, and snails [14–16]. However, MCTx-1 is the first toxic dermatopontin to be discovered.

The characteristic motifs of dermatopontins from mammals and from invertebrates differ (Figs. 3 and 4). All invertebrate dermatopontins have three repeats of the same motif (S-X-H-X-N-X-Y-E-D-R) as MCTx-1, whereas those of mammals have a different repeated motif (D-R-E/Q-W-X-F/Y) [17].

MCTx-1 had the highest homology (36%) with hemagglutinin/amebocyte aggregation factor from the horseshoe crab *Limulus polyphemus*. Of the known dermatopontins, only MCTx-1 and *Limulus* aggregation factor have hemagglutination activity. Bovine dermatopontin does not have hemagglutinating activity [17], whereas *Limulus* aggregation factor has PLA_2 activity [18] and MCTx-1 does not. We found here that *M. dichotoma* var. *tenera* has a protein(s) with PLA_2 activity that differed from MCTx-1 (Fig. 1).

Some proteins originating from snake and spider venoms have hemagglutinating activity [19–21]. Galactoside-

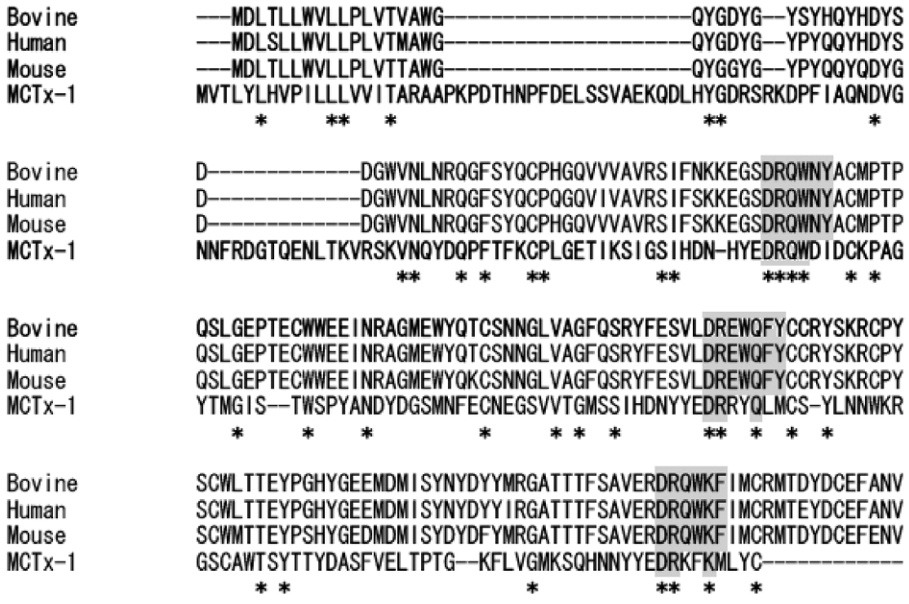

Fig. 3. Comparison of mammal dermatopontin homologs and MCTx-1. Accession numbers are as follows: Bovine, P19427; human, Q07507; mouse, Q9QZZ6; MCTx-1, AB299385. Repeating motifs (D-R-E/Q-W-X-F/Y) are shaded. Conserved residues are marked by asterisks.

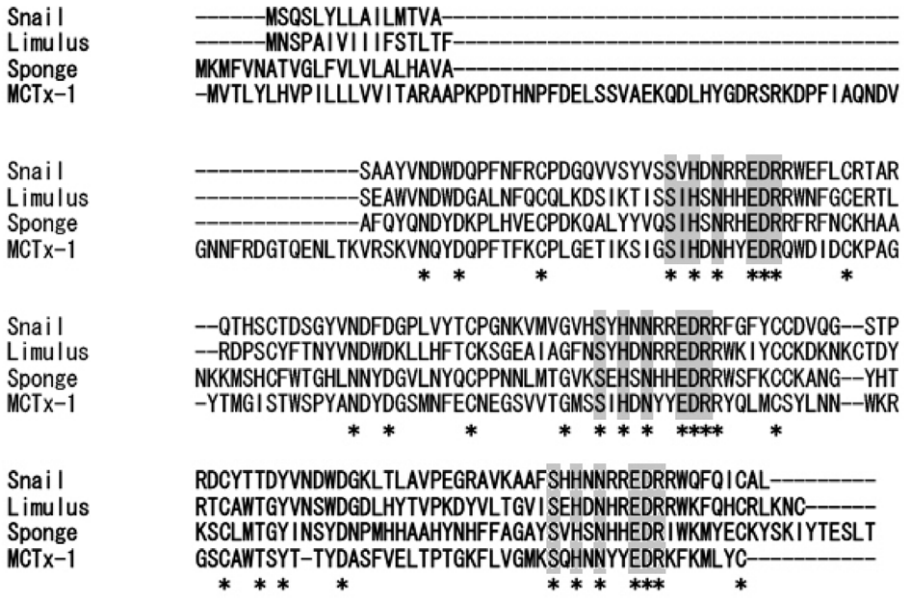

Fig. 4. Comparison of invertebrate dermatopontin homologs and MCTx-1. Accession numbers are as follows: Snail, Q3YJU8; limulus (horseshoe crab), Q01528; Sponge, Q966Y2; MCTx-1, AB299385. Repeating motifs (S-X-H-X-N-X-Y-E-D-R) are shaded. Conserved residues are marked by asterisks.

specific lectin (BJcuL) isolated from venom of the snake *Bothrops jararacussu* [22] has hemagglutinating activity and remarkable cytotoxicity [23]. Injection of BJcuL into mouse paws causes dose-dependent edema and vascular permeability [24]. Thus, BJcuL participates in the edema formation induced by snake bites. MCTx-1 also has hemagglutinating activity and potent cytotoxicity like BJcuL. Therefore, MCTx-1 might participate in the edema formation associated with *Millepora* stings.

We isolated and characterized MCTx-1 from *Millepora dichotoma* var. *tenera* that was potently cytotoxic towards L1210 mouse leukemia cells. This is the first example of the characterization of a proteinaceous toxin from fire coral.

Acknowledgments

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References

[1] A. Sagi, L. Rosenberg, P. Ben-Meir, D.J. Hauben, 'The fire coral' (*Millepora dichotoma*) as a cause of burns: a case report, Burns 13 (1987) 325–326.

[2] J.G. Camarasa, A.E. Nogues, E. Serra-Baldrich, Red Sea coral contact dermatitis, Contact Dermatitis 29 (1993) 285–286.

[3] G.V. Prasad, L. Vincent, R. Hamilton, K. Lim, Minimal change disease in association with fire coral (*Millepora* species) exposure, Am. J. Kidney Dis. 47 (2006) e15–e16.

[4] F.F.Y. Radwan, Comparative toxinological and immunological studies on the nematocyst venoms of the Red Sea fire corals *Millepora dichotoma* and *M. platyphylla*, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 131 (2002) 323–334.

[5] L.W. Wittle, R.E. Middlebrook, C.E. Lane, Isolation and partial purification of a toxin from *Millepora alcicornis*, Toxicon 9 (1971) 327–331.

[6] R.E. Middlebrook, L.W. Wittle, E.D. Scura, C.E. Lane, Isolation and purification of a toxin from *Millepora dichotoma*, Toxicon 9 (1971) 333–336.

[7] L.W. Wittle, E.D. TScura, R.E. Middlebrook, Stinging coral (*Millepora tenera*) toxin: a comparison of crude extracts with isolated nematocyst extracts, Toxicon 12 (1974) 481–486.

[8] K. Shiomi, M. Hosaka, N. Yanaike, H. Yamanaka, T. Kikuchi, Partial characterization of venoms from two species of fire corals *Millepora platyphylla* and *Millepora dichotoma*, Nippon Suisan Gakkaishi 55 (1989) 357–362.

[9] F.F.Y. Radwan, H.M. Aboul-Dahab, Milleporin-1, a new phospholipase A2 active protein from the fire coral *Millepora platyphylla* nematocysts, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 139 (2004) 267–272.

[10] P.J. Neame, H.U. Choi, L.C. Rosenberg, The isolation and primary structure of a 22-kDa extracellular matrix protein from bovine skin, J. Biol. Chem. 264 (1989) 5474–5479.

[11] A. Superti-Furga, M. Rocchi, B.W. Schafer, R. Gitzelmann, Complementary DNA sequence and chromosomal mapping of a human proteoglycan-binding cell-adhesion protein (dermatopontin), Genomics 17 (1993) 463–467.

[12] A.D. Cronshaw, J.R. MacBeath, D.R. Shackleton, J.F. Collins, L.A. Fothergill-Gilmore, D.J. Hulmes, TRAMP (tyrosine rich acidic matrix protein), a protein that co-purifies with lysyl oxidase from porcine skin. Identification of TRAMP as the dermatan sulphate proteoglycan-associated 22 K extracellular matrix protein, Matrix 13 (1993) 255–266.

[13] C.Y. Tzen, Y.W. Huang, Cloning of murine early quiescence-1 gene: the murine counterpart of dermatopontin gene can induce and be induced by cell quiescence, Exp. Cell Res. 294 (2004) 30–38.

[14] N. Fujii, C.A. Minetti, H.L. Nakhasi, S.W. Chen, E. Barbehenn, P.H. Nunes, N.Y. Nguyen, Isolation, cDNA cloning, and characterization of an 18-kDa hemagglutinin and amebocyte aggregation factor from *Limulus polyphemus*, J. Biol. Chem. 267 (1992) 22452–22459.

[15] J. Schütze, A. Skorokhod, I.M. Müller, W.E. Müller, Molecular evolution of the metazoan extracellular matrix: cloning and expression of structural proteins from the demosponges *Suberites domuncula* and *Geodia cydonium*, J. Mol. Evol. 53 (2001) 402–415.

[16] J.C. Marxen, M. Nimtz, W. Becker, K. Mann, The major soluble 19.6 kDa protein of the organic shell matrix of the freshwater snail *Biomphalaria glabrata* is an N-glycosylated dermatopontin, Biochim. Biophys. Acta 1650 (2003) 92–98.

[17] O. Okamoto, S. Fujiwara, Dermatopontin, a novel player in the biology of the extracellular matrix, Connect. Tissue Res. 47 (2006) 177–189.

[18] J.C. MacPherson, R.S. Jacobs, An 18.5 kDa protein from the amebocyte of *Limulus polyphemus*, homologous to the previously described amebocyte aggregation factor, expresses alternative phospholipase A_2 activity, Comp. Biochem. Physiol. B, Biochem. Mol. Biol. 127 (2000) 31–44.

[19] B. Lomonte, G. Rojas, J.M. Gutierrez, G. Ramirez, Isolation of a galactose-binding lectin from the venom of the snake *Bothrops godmani* (Godmann's pit viper), Toxicon 28 (1990) 75–81.

[20] S.P. Liang, X. Pan, A lectin-like peptide isolated from the venom of the Chinese bird spider *Selenocosmia huwena*, Toxicon 33 (1995) 875–882.

[21] F. Aragon-Ortiz, J.R. Brenes-Brenes, F. Gubensek, Characterization of a lectin-like protein isolated from *Lachesis muta* snake venom, Rev. Biol. Trop. 37 (1989) 79–83.

[22] D.D. Carvalho, S. Marangoni, B. Oliveira, J.C. Novello, Isolation and characterization of a new lectin from the venom of the snake *Bothrops jararacussu*, Biochem. Mol. Biol. Int. 44 (1998) 933–938.

[23] D.D. de Carvalho, S. Schmitmeier, J.C. Novello, F.S. Markland, Effect of BJcuL (a lectin from the venom of the snake *Bothrops jararacussu*) on adhesion and growth of tumor and endothelial cells, Toxicon 39 (2001) 1471–1476.

[24] P.C. Panunto, M.A. da Silva, A. Linardi, M.P. Buzin, S.E.S.F.C. Melo, S.M. Mello, J. Prado-Franceschi, S. Hyslop, Biological activities of a lectin from *Bothrops jararacussu* snake venom, Toxicon 47 (2006) 21–31.