Analysis of the Proteinaceous Components of the Organic Matrix of Calcitic Sclerites from the Soft Coral Sinularia sp.

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Abstract

An organic matrix consisting of a protein-polysaccharide complex is generally accepted as an important medium for the calcification process. While the role this “calcified organic matrix” plays in the calcification process has long been appreciated, the complex mixture of proteins that is induced and assembled during the mineral phase of calcification remains uncharacterized in many organisms. Thus, we investigated organic matrices from the calcitic sclerites of a soft coral, Sinularia sp., and used a proteomic approach to identify the functional proteins. We purified eight organic matrix proteins and performed in-gel digestion using trypsin. The tryptic peptides were separated by nano-liquid chromatography (nano-LC) and analyzed by tandem mass spectrometry (MS/MS) using a matrix-assisted laser desorption/ionization (MALDI) – time-of-flight-time-of-flight (TOF-TOF) mass spectrometer. Periodic acid Schiff staining of an SDS-PAGE gel indicated that four proteins were glycosylated. We identified several proteins, including a form of actin, from which we identified a total of 183 potential peptides. Our findings suggest that many of these peptides may contribute to biocalcification in soft corals.

Introduction

Soft corals contain small spicules of CaCO3 called “sclerites”, which are biomineralized structures composed of an organic matrix and a mineral fraction [1,2,3]. They define key parameters for the structural design of a soft body [3]. The formation of biominerals by living organisms is fastidiously controlled by proteins and polysaccharides [4,5] that ensure the development of crystals with a particular composition and morphology, guarantee the manufacture of structures with strictly defined shapes, and dictate the internal and external physicochemical properties of the resulting materials [5,6,7]. Prominent among these are intracrystalline proteins, which are distributed throughout the individual biominaler crystals and play a vital role in dictating the texture and physical properties of calcified tissues of most marine organisms [6]. The organic matrix associated with the sclerites of soft corals primarily consists of proteins. Therefore, to better understand the calcification process of endoskeletal sclerites in marine organisms, functional biomolecules should be identified. We applied a proteomic approach, one of the best ways to identify functional molecules, to identify the proteins thought to be involved in the biocalcification of endoskeletal sclerites and to gain a more complete understanding of the calcification process.

We purified organic matrix proteins from the sclerites of a soft coral, Sinularia sp., and performed in-gel digestion using trypsin. The tryptic peptides were separated by a nano-LC and analyzed by MS/MS using a highly powerful MALDI-TOF-TOF mass spectrometer. Although marine organisms have been reported to contain carbonic anhydrase [9,10,11,12], calcium-binding and glycosylated proteins [1,10,13,14], this is the first report of the identification of actin in sclerites. Actin is a protein that has not previously been shown to play a role in the biocalcification process of other corals except in the planula larvae of the scleractinian Galaxea fascicularis [15], but it has been suggested that actins reach the area of biomineralization as a by-product of the secretion of other proteins involved in calcification and may not be directly involved in the biomineralization process [17]. Our results cannot decide so far whether soft coral sclerites might be formed through a biological process using the actin protein, similar to other biomolecules that were already reported in this group.

Actin is a protein that functions with myosin in muscle contractions [16]. It helps to construct and maintain the cytoskeletal structure within cells and forms microfilaments in mammalian cells. Actin also contributes to biological processes such as sensing the environment, internalizing membrane vesicles, moving over surfaces, and cell division. These cellular activities are complex; they depend on the interactions of actin monomers and...
filaments with numerous other proteins. As such, actin is a central player in cell shape and movement and participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, and the establishment and maintenance of cell junctions and cell shape [16]. An important, unresolved question is whether actin has a function in the incorporation of extracellular materials during the calcification process in marine organisms. Recently, actin was identified in the intracellular organic matrix of sea urchin tests and spines [17] and in the planular larvae of scleractinian corals [15] but its direct involvement in the biocalcification process has yet to be demonstrated. Here, we found that actin proteins in the sclerites of *Sinularia sp.*, which also contain the mineral calcite [14,18], are secreted together with an organic matrix and subsequently transported to the outside of the cell where extracellular calcification occurs. This process is similar to sclerite calcification in other octocorallians [19,20]. Mature sclerites are completely free of cellular materials and ultimately become extracellular structures. We are tempted to exclude actin contamination from the surrounding cells as an explanation for this finding due to the many peptide fragments identified as actin from a complex protein mixture that was purified from silver-stained protein bands using our newly established matrix protein purification technique [1,14]. However, verification of these proteins using a number of techniques (e.g., CBB staining, western blotting, silver staining, and analyses by nanoLC-MS/MS using a MALDI-TOF-TOF) was done several times on the same protein bands with the same results.

The sensitivity and high-throughput nature of modern mass spectrometry (MALDI-TOF-TOF) enables the implementation of a proteomic approach to explore the entire protein set of a complex mixture at once. Consequently, we used such an approach for an overview analysis of the matrix proteins contained in the soft coral *Sinularia sp.* The total soluble organic matrix (SOM) and the eight main purified proteins (dubbed SSCL-150, SSCL-100, SSCL-75, SSCL-63, SSCL-42, SSCL-33.5, SSCL-31, and SSCL-14 according to their predicted molecular weights) were investigated. Approximately 180 internal peptide sequences were determined by de novo sequencing. The sequence analysis strongly suggested that the protein migrating at approximately 150 kDa (i.e., SSCL-150) potentially played a role in the sclerite calcification process. Additionally, the peptide sequences corresponding to the SSCL-75 and the SSCL-14 bands matched proteins annotated as thioredoxin, hypothetical and fluorescent proteins.

**Results and Discussion**

**Calcified Endoskeletons (Sclerites)**

The sclerites of *Sinularia sp.*, when observed under a scanning electron microscope (SEM), appeared irregularly shaped; some were rod-like, and others were egg-shaped. The sclerites varied in size from approximately 100 to 250 μm. (Fig 1). These sclerite shapes are quite different from a previously identified specimen from the same family, Alcyoniidae [1,3,13,21]. Although frequently detected in other soft coral sclerites [1,13], spurs were not observed in the sclerites of *Sinularia sp.* (Fig 1B). Because the shape of these sclerites was completely different from other soft coral sclerites [1,3,13,14,20], we investigated sclerites representing each of the different shapes to determine whether they indeed induced calcite mineralogy as do sclerites in other soft corals. To confirm the mineral composition, we performed micro-Raman spectroscopy on the different shapes and FTIR analysis of the powdered samples of the sclerites. The results of both analyses clearly indicated calcitic mineralogy (Fig 1D, E; see figure legend for details).

Sclerites are usually found in the axis as well as the cortex of a colonial skeleton, although they may also be present in the tentacles, pharynx and upper part of the autozoids [22]. Notably, sclerites are found in the subclass Octocorallia, including members of the Gorgonacea and Alcyonacea; all sclerites in this subclass are composed of calcite [14,20]. We noted that the sclerite shape in *Sinularia sp.* was different from that of other Alcyonacea [3,23] and Gorgonacea [24]. Scale-shaped or rounded sclerites with a spherulitic structure were formed by gorgonians while spindles and rod-shaped spicules were common among gorgonians and pennatulids. These observations clearly suggest that sclerite shapes are species-specific and that within a species they generally differ according to the anatomical site at which they are formed. Very recently, a crystallographic structure [25] and a distribution pattern of calcite mineral [26] in soft coral sclerites have been reported in detail. However, although morphological studies of sclerites have been previously reported and form the basis of octocoral taxonomy, the complete biochemical and proteomic analyses of these important skeletal elements has been lacking to date. Here, we present the results of such analyses.

**Proteinaceous Organic Matrices**

After decalcification of calcitic sclerites, the SOM comprised 0.04% of the sclerite weight and emerged as a white fluffy material. SDS-PAGE (12%) analysis of the decalcified samples revealed eight bands of proteins with apparent molecular masses of 150, 100, 75, 63, 42, 33.5, 31 and 14 kDa. The electrophoresis pattern of SOM stained with CBB, alongside standard proteins of known molecular weights, is shown in Figure 2A. Spectrometric analysis indicated that partial sequences of many different proteins were present in the stained bands, suggesting that these bands corresponded to a mixture of proteins rather than a single protein.

Among these bands, five proteins with apparent molecular masses of 150, 75, 63, 42 and 14 kDa were the most abundant (Fig 2A, Lane Si). An additional band at 100 kDa was very weak. To confirm the purification, the proteins on the SDS–PAGE were transferred to a polyvinylidene difluoride (PVDF) membrane using a western blot technique (Fig 2B, WB). To identify any glycosylated proteins, a new SDS-PAGE was subsequently performed under the same experimental conditions as those described above. After staining the SDS-PAGE gel with PAS stain, four of the eight dominant sclerite proteins (SSCL-150, SSCL-63, SSCL-50, SSCL-31) were shown to be glycosylated (Fig 2C, Lane 2).

These results revealed that the proteinaceous organic matrices obtained from the sclerites of *Sinularia sp.* were more abundant than those of the closely related *Lobophytum crassum*, for example [13]. PAS staining indicated that four major bands of proteins contained glycoproteins (Fig 2C, Lane 2). Glycoproteins are known to function as inhibitors or stabilizers by binding the precursor amorphous CaCO₃ (ACC), which acts as a template for the transition of ACC to structured crystals on an insoluble or soluble organic matrix [27]. Of the eight proteins isolated, four showed strong glycosylation, revealing the potential function of these proteinaceous matrices. In addition, actin and thioredoxin sequences were obtained from glycosylated protein bands indicating that these two important proteins are glycosylated. Glycoproteinaceous matrices are commonly found in other calcifying systems [28]. Tompa et al. [29] described the insoluble matrix of snail egg shells as being a sulfated glycoprotein of 53 kDa. Based on studies of proteins in other animals, it appears that actin is also
glycosylated [30]. However, this is the first study to identify actin as one of the glycosylated proteins found in the organic matrix of a calcifying marine organism. As mentioned above, glycosylated proteins are important for the biomineralization process. Evidence from octocorallians and from our previous reports [1,13,14] suggest that at least one protein involved in biomineralization in calcifying marine organisms must be a glycoprotein. Therefore, the functional role(s) of glycosylated proteins in calcifying organisms, and in particular within the octocorallians, definitely requires further investigation. The presence of an organic matrix or sheath appears to be a requirement for all calcifying systems. Kingsley et al. [28] reported that mature sclerites contained 5.9% organic material by weight, most of which was protein; an observation supported by our results. Gel electrophoresis revealed the presence of many proteins along with their amino acid compositions. It has been reported that protein is always present in scleractinian coral skeletons [31] and that it comprises approximately 0.3% of the dry skeleton by weight [31,32].

Proteomic Analyses

To identify the proteins precisely, we visualized all purified proteins by silver staining (Fig. 3) followed by digestion with trypsin. The peptide sequences determined by the de novo interpretation of MS/MS spectra are shown in Table 1 for the main protein band SSCL-150. The peptide sequences shown in Table 1 were identified with 90–99.9% confidence. Those with the closest homology were identified from both the NCBI and the Anthozoa databases. An example of an MS/MS spectrum for actin is shown in Figure 4. A screenshot for actin obtained from ProteinPilot™ is shown in Figure S1 with the fragment peaks numbered and labeled; the spectra, with parent ions indicated by a red arrow, are shown in Figure S2. The MS/MS spectra for this protein revealed that the main protein identified in Sinularia sp. was actin. In total, we obtained approximately 183 actin peptide fragments from the SSCL-150 band (Table S1).

We also identified a mixture of thioredoxin (nine peptides) and several chain (A, E) proteins from the SSCL-75 band (Table 2). The MS/MS spectra of a thioredoxin peptide (m/z = 1821.89) is
shown in Figure 5. A screenshot for thioredoxin obtained from ProteinPilot® is shown in Figure S3 with the fragment peaks numbered and labeled; the spectra, with parent ions indicated by a red arrow, are shown in Figure S4. This study is the first to identify thioredoxin, which usually functions as an antioxidant, in soft coral sclerites. However, SSCL-75 was not identified as a glycoprotein by periodic acid Schiff analysis, suggesting that this protein has a limited function in the biocalcification process. Some other proteins, such as hypothetical and fluorescent, might have functional roles in biocalcification [33,34,35], although the putative function of the hypothetical proteins could not be defined.

In this study, we used a combination of biochemistry and proteomics to characterize the organic matrices of sclerites in the soft coral Sinularia sp. Our results revealed that the organic matrix of these sclerites was composed of eight principal, discrete, proteinaceous fractions, including four glycoproteins, several predicted proteins, and thioredoxin, which may play a role in the biocalcification of the sclerites. During our database mining, we noted that sequences indicative of actin were associated with a significant number of diverse species (Table 1 and S1) implying that this protein in soft corals is homologous with that of many other animals. These partial sequences match not only with known proteins from the NCBI database but also with unknown proteins because the transcriptome/genome of Sinularia sp. is not currently available; these partial sequences could be part of another protein.

To date, a few actin-binding proteins were identified in marine organisms [36,37,38], including a high molecular weight (250 kDa) actin-binding protein from a sea urchin egg cytoplasmic extract [36]. In another report, Mann et al. [17] noted that actin was present in the test and spine matrices of sea urchins. However, in this report, the authors hypothesized that this protein may have reached the mineralization space as by-products of the secretion of specific matrix proteins or of mineral precursors, or alternatively were may have been derived from damaged cells of the cell layer confining the mineralization space.

Many different proteins which modify actin polymerization are thought to regulate actin assembly and the formation of the spectacular variety of actin structures [37,39,40,41,42,43,44]. The effort to determine how assembly and interconversion of the diverse polymeric forms of actin are regulated has led to the discovery of many actin-binding proteins [36,45,46,47]. There appears to be no limitation to the ranges of molecular weight of actin-binding proteins, because many high molecular weight (90–270 kDa) actin-binding proteins have already been identified in diverse species [30,36,42,43,49,50], including marine organisms [36]. These findings are consistent with the present report.

There is a great abundance of Sinularia sp. in Okinawa, but organisms have not yet been identified at the species level because of a lack of primary information. It might be possible to determine the species by mitochondrial RNA/DNA studies, and we believe

Figure 2. Electrophoretic analysis of matrix proteins extracted from the calcitic sclerites. (A) SDS-PAGE fractionation with CBB staining after purification of the proteins. An eluate (derived from 10 g of sclerites) was run on a 12% polyacrylamide gel. M, protein marker; Si, purified proteins of Sinularia sp. The arrows indicate protein bands. (B) Western blotting (WB) of matrix proteins on a PVDF membrane; proteins correspond to those on the SDS–PAGE gel shown in A. The arrows indicate same purified proteins with the same numbers (1–8) as shown in A. The eight purified proteins were named according to their origin (Sinularia sp. sclerite; SSCL) and their apparent molecular mass as follows: SSCL-150, SSCL-100, SSCL-75, SSCL-63, SSCL-42, SSCL-33.5, SSCL-31, and SSCL-14. (C) SDS-PAGE gel with PAS staining to identify the glycoproteins in the soluble organic components of sclerites. An eluate (derived from 10 g of sclerites) was run on a 12% polyacrylamide gel. Lane 1, protein marker; Lane 2, four glycoproteins were identified (indicated by arrows) by periodic acid Schiff staining.

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Sequences were deduced from MS/MS spectra. The peptide sequences were generated with 90–99.9% confidence, and the best matches were identified from both the NCBI and the Anthozoa databases.

doi:10.1371/journal.pone.0058781.t001

Figure 3. Silver staining of purified matrix proteins. Lanes 1–8, eight bands of proteins with the apparent molecular masses of 150 (SSCL-150), 100 (SSCL-100), 75 (SSCL-75), 63 (SSCL-63), 42 (SSCL-42), 33 (SSCL-33), 31 (SSCL-31) and 14 (SSCL-14) kDa, respectively. M, protein marker. Each arrow indicates a protein band. Arrowheads indicate constant contaminants of control samples by SDS-PAGE. This contamination did not interfere with the analysis of the proteins. Blue arrows indicate the proteins that were subjected to in-gel trypsin digestion for identification by MALDI-TOF-TOF analysis.

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that the primary sequences obtained here will be a useful first step for identifying species of these abundant organisms. Combining proteomics with transcriptomics data could be helpful for such work. In addition, these results might be useful for further studies in which the genes that encode these proteins could be amplified using RACE experiments to isolate the full-length DNA sequences and, in turn, the complete amino acid sequences of the detected proteins. Additionally, the results of this study could be used for designing commercial antibodies against the actin protein to label and locate it within the organic matrix of the sclerites. Overall, the present study is preliminary; however, the peptide sequences obtained here may serve as a road map for further studies dealing with organic matrix and calcification.

Except for a few reports on mollusk shells [9,10,51,52,53] and sea urchins [17,54,55], few proteomics analyses have been performed on calcifying marine organisms. In particular, the

Table 2. A list of proteins identified by MS/MS spectra from silver-stained SSCL-75.

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The proteins were obtained with 80–90% confidence, and the best matches were identified from the NCBI database.

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protein composition of the organic matrix of scleractinian and octocoral biominerals is poorly characterized. Our present data now contribute to closing this gap thus enabling a broader comparative proteomic approach for studying organic matrices in marine organisms.

**Experimental Section**

**Specimen Collection**

Colonies of *Sinularia sp.* were collected by scuba diving to depths ranging from 10 to 20 meters at low tide near the Sunabe coast of Chatan, Okinawa, Japan. No specific permits were required for the described field studies. The field studies did not involve endangered or protected species.

The collected colonies were vigorously washed with tap water in the laboratory to remove excess salts and other unwanted substances. The colonies were then cut into small pieces using sharp scissors, washed with tap water again, and stored at −20°C until use.

**Isolation of Endoskeletons**

Calcified endoskeletons (sclerites) were isolated from each coral colony (*Sinularia sp.*) according to the series of mechanical and chemical treatments described by Rahman et al. [14]. Briefly, pieces of coral colony were ground five to six times in a mixer machine (BM-FE08, Zojirushi, Japan) and washed with tap water until the sclerites were obtained. The collected sclerites were cleaned by stirring in 1 M NaOH for 2 h followed by vigorous stirring in a 10% sodium hypochlorite (NaOCl) bleaching solution for 30 min to remove fleshy tissues and debris; following these washes, the sclerites were considered to be completely tissue-free. The treated samples were washed under tap water to remove the cleaning solutions. Finally, the samples were washed with MilliQ water five times to remove any additional contaminants. The sclerites were examined using a microscope (Nikon ECLIPSE E200, Japan) to determine whether they were completely free of tissues and other contaminants.

**Scanning Electron Microscopy (SEM)**

The completely cleaned mature sclerites were subjected to observation under an SEM. Mechanically and chemically treated sclerites were examined for the shape and effect of chemicals during the separation of sclerites from the soft tissues. All specimens were placed in a sample holder, dried for a few days, and coated with palladium gold using an ion coater (E-1010, Hitachi, Japan). The samples were examined under an SEM (Hitachi S-3000 N, Japan) operated at 15 kV.

**Extraction of Proteinaceous Organic Matrices from Sclerites**

To extract the proteinaceous organic matrix components from the sclerites, we used the protocol described in our previous works [1,14]. The mechanically and chemically cleaned (see method for isolation of endoskeletons above) sclerites were decalcified in 0.5 M ethylenediaminetetraacetic acid (EDTA)-4 Na (pH 7.8),...
Each lyophilized sample was weighed (2 mg) and mixed with water was changed five times during this period. The membrane (UC36-32-100, Viskase Companies, Inc., Japan). The samples were injected using a peristaltic pump (NeoLab Mini Pump) at a flow rate of 1 drop/s to separate the soluble macromolecules. The Sep-Pak® Plus C₁₈ cartridges were activated by methanol (4 mL/3 times) prior to the application of the samples. The samples were washed with distilled water (DW) (6 mL/2 times) after activation. After passing the samples through the cartridges, most of the EDTA were assumed to remain in the cartridges. To completely remove the EDTA, we passed DW (6 mL/5 times) through each cartridge followed by 10% acetonitrile (2 mL/3 times). Finally, the absorbed macromolecules were eluted in 50% acetonitrile (2 mL/3 times). The eluted macromolecules were frozen at −80°C and subsequently lyophilized.

(2) Each lyophilized sample was weighed (2 mg) and mixed with 0.4 mL of 99% chloroform (CHCl₃), 0.2 mL of 100% methanol (MeOH), and 0.4 mL of H₂O, and then centrifuged (1500 rpm) using an IKA® VORTEX (GENIUS 3) three times for six min each to remove the lipids and other unwanted substances. The sample was then concentrated by centrifugation in an Amicon Ultrafree-MC (MilliQ) column using a Minispin Plus centrifuge (Eppendorf) and dried using a VORTEX (GENIUS 3) three times. At this point, the sample was ready for gel electrophoresis.

(3) Each dried, purified matrix protein sample was dissolved in Laemmli sample buffer (Bio-Rad) at a ratio of 2:1 (sample buffer to protein sample) and denatured at 100°C for three min. The samples were then subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [56] for approximately 1 h. The Precision Plus SDS-PAGE standard (Bio-Rad) was used as the protein marker for the electrophoresis. After electrophoresis, the gel was stained with Coomassie Brilliant Blue (CBB) (1% CBB R-250 in 40% MeOH and 7.5% acetic acid) and destained (10% MeOH and 7.5% acetic acid) to visualize the protein bands. The purification of proteins was further checked by silver staining and western blotting.

Western Blotting on PVDF Membranes

The purified sample underwent electrophoresis with SDS-PAGE using the same technique as described above. After undergoing electrophoresis, the gel was kept in 100% MeOH for 15 s and shaken in buffer for 10 min. Subsequently, the Mini Trans-Blot® (Bio-Rad) was used to transfer the sample onto a polyvinylidene difluoride (PVDF) clear membrane at 350 mA and 60 V for 4 h. The membrane was stained with CBB (1% CBB, 50% MeOH and destained [50% MeOH, 5% acetic acid] for visualization of the bands; the membrane was then washed with distilled water and dried overnight.

Periodic Acid Schiff (PAS) Staining

PAS staining was used in the SDS-PAGE gel to identify glycopolypeptides. This staining was conducted using the Schiff (fuchsinsulfite) reagent according to the method developed by Segrest et al. [57]. A strong red/purple staining of a band indicated the presence of a glycopolypeptide.

Protein Digestion and MALDI-TOF-TOF Analysis

After electrophoresis and CBB (R-250) staining, we excised all protein bands from the gel and performed electrophoretic treatments for further purification of the proteins, following protocols described in our previous work [1]. After electrophoretic treatment, we performed SDS-PAGE again and subsequently visualized all purified proteins by silver staining (Bio-Rad product). The silver-stained purified proteins were excised from the gel and cut into smaller pieces prior to further processing. The gel pieces were washed with 50% 50 mM ammonium carbonate buffer (pH 8.0), 50% acetonitrile, and pure acetonitrile, in sequence. After the final wash, the gel pieces were dried under a vacuum. The cysteines were reduced with dithiothreitol (DTT) and alkylated with iodoacetamide. The proteins were digested in-gel with trypsin (Promega; Madison USA) overnight, and the gel pieces were extracted with 50% acetonitrile in 0.1% trifluoroacetic acid (TFA).

The nano-LC ( Dionex RSLC, Sunnyvale, USA) was set up with solvents A (95% H₂O, 5% acetonitrile, 0.1% TFA) and B (80% acetonitrile, 20% H₂O, 0.1% TFA). A spotting ( Dionex Probot, Sunnyvale, USA) was set up with a matrix consisting of 2 mg/ml α-Cyano-4-hydroxycinnamic acid (CHCA) in 70% acetonitrile with 0.1% TFA and a fraction size of 12 min/s. The peptide samples were separated using the following gradient: 0 to 5 min, 5% solvent B; 5 to 65 min, 5%–50% solvent B; and 65 to 66 min, 50%–95% solvent B.

The peptides separated by nano-LC were analyzed in MS reflector mode. The mass range for MS acquisition was 700 to 4000, and 2000 shots were summed for one spectrum. Precursors for MS/MS fragmentation were selected automatically, selecting a maximum of 20 precursors per fraction with a minimum signal to noise ratio of 50. MS/MS spectra were smoothed using the Savitzky-Golay filter method [58] before interrogating the databases. MS and subsequent MS/MS analyses were performed using the AB SCIEX TOF/TOF™ 5800 System (AB SCIEX, Foster City, USA).

Mass Spectrometry and Database Searching

All MS/MS spectra were analyzed using the following databases: (1) Mascot® using the NCBI database (NCBI 2010) with all entries, (2) Mascot® using the sub-database Anthozoa (extracted from NCBI), (3) ProteinPilot™ 3.0 Software (AB SCIEX, Foster City, USA) using the NCBI database with all entries, (4) ProteinPilot™ using the sub-database Anthozoa (extracted from NCBI), (5) de novo sequencing using the Denovo Explorer™ and a BLAST search against NCBI 2010, and (6) de novo sequencing using the Denovo Explorer™ and a BLAST search against the Anthozoa database.

For the MS/MS database search, the MS/MS data (i.e., fragment masses) were compared against theoretical fragment...
masses calculated from all protein sequences in the database. For the de novo sequencing analysis, the MS/MS spectra were used to create a sequence tag (i.e., the mass difference between fragment peaks in the MS/MS spectra could be explained by differences in amino acid composition). The amino acid sequences were then compared against the protein database.

The number of coral proteins in the database is limited. The ProteinPilot software using the Paragon algorithm offers the possibility of searching for amino acid substitutions, which increases the chance of finding proteins by homology. In addition, the Mascot search engine was used to find further evidence to confirm the identity of the peptides. In the current circumstances, where proteins are identified based on a low number of peptides due to poorly populated databases, identification based on two different search algorithms provides a higher confidence than if only one algorithm was considered.

Supporting Information

Figure S1 Screenshot of actin peptide obtained from ProteinPilotTM.

(PDF)

Figure S2 The parent ion of actin. (A, B) The parent ion on the spectra (red arrows).

(PDF)

References