

Differential Expression of Soluble and Membrane-bound Proteins in Soft Corals (Cnidaria: Octocorallia)

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Abstract. Current understanding of the ecological role of corals is largely based on extrapolations of physiological processes studied on coral colony fragments as model experimental units. Due to their modular nature, these organisms are assumed to be structurally and functionally homogeneous. Here we address this assumption in an investigation of the distribution of proteins in soft coral tissue regions. This is the first study comparing variations of protein content and protein profiles within colonial cnidarians. *Sarcophyton* sp and *Capnella gaboensis* specimens were sectioned into colony regions with different physiological potential, based on their proximity to endosymbiotic algae and/or direct contact with the external medium. The tissues were homogenized in either 'NP40 buffer' a solution efficient at extracting membrane-bound proteins, or 'Tris buffer' which is efficient at extracting soluble, cytosolic proteins. SDS-PAGE analysis of proteins showed significant intracolony differences of protein content ($p < 0.05$) and differences of protein profile ($p < 0.05$). This investigation indicated that the specific proteins have a different pattern of distribution ($p < 0.05$) when the tissues are processed using NP40 or Tris buffers. Different spatial expression of proteins suggests regional functional specialization within the structurally repetitive units of these modular organisms.

Keywords. Octocorals, protein, electrophoresis, modular organisms.

Introduction

Whole protein content is routinely measured as an index of physiologically active biomass of cnidarians in numerous investigations such as skeletal and cellular growth (Goreau 1959; Velimirov and King 1979; Barnes 1985; Allemand and Grillo 1992; Tentori et al. 2004); coral-zooxanthellae interactions (Michalek-Wagner and Willis 2001); and expression of specific proteins (Snyder and Rossi 2004; Phelan et al. 2006). Edmunds and Gates (2002) considered soluble protein an acceptable normalizing trait that reflects the spatio-temporal variability of biomass. Tentori and Allemand (2006) questioned the implicit assumptions that protein is equally distributed through coral tissues and interacts uniformly with all the tissues of the experimental coral specimens. Indeed, seasonal and stress-related variability in the protein content of colonial cnidarians has been shown in recent studies (Barneah et al. 2005; Ravindran and Ragukumar 2006). Further research on the chemical nature of colonial cnidarian tissues is needed to appreciate their functional complexity.

To understand the way the tissues of coral colonies interact with the environment, we need to consider their physical organization. Hard and soft corals adopt a range of growth forms from fine-arborescent to thick-massive structures. Hard and soft coral

specimens of comparable dimensions are common occurrence in healthy Indo Pacific reefs, but their internal compositions are remarkably different. All cells in the stony coral body are located close to the external medium and to their symbiotic algae; the tissues spread generally less than 1 mm thick over the skeleton (Quan-Young and Espinoza-Avalos 2006). The widely used water pik method developed by Johannes and Wiebe (1970) amongst coral researchers in the analysis of coral tissues highlights the assumed even distribution of functional components. By contrast, in the soft coral body the polyps form a mass several centimetres thick (Chevalier et al. 1984); the distribution of zooxanthellae is limited by penetration of light into the external tissues (Chin 1996). Clearly, the soft coral morphology increases the resolution needed to study the functional variability of tissues in the modular nature of colonial cnidarians.

The aim of this study is to investigate the spatial distribution of proteins in soft corals. Two zooxanthellate soft corals were studied: *Sarcophyton* sp and *Capnella gaboensis*. Colony regions with different potential of interaction with their endosymbiotic algae and the external medium were analyzed, we refer to these as *functional regions*. We hypothesized that the identified functional regions

would be equipped with different proteinic component. Specific protein bands obtained from tissue homogenates processed under different methods were compared.

Materials and Methods

The experimental organisms

Sarcophyton sp. was collected at 1-3 m depth, 5km off Broome, Western Australia and maintained for two weeks in aquarium conditions: 35 ppt salinity, light intensity of 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 26°C temp, fed once a week, 30% water exchanged weekly. *Capnella gaboensis* was collected at Fairlight, on the Sydney coast, at 6 m depth, this coral was processed on the day of collection.

Preparation of samples

Tissue samples from various functional regions of one *Sarcophyton* sp. colony and one *C. gaboensis* colony were sectioned as shown in Fig. 1A-B. The tissue homogenates were treated with two buffer solutions: NP40 buffer (40 mM Tris, pH 7.4, 1% Nonidet NP-40, 0.25% cholic acid, 150 mM NaCl, 1mM PMSF, 1 mg ml⁻¹ aprotinin, 1 mM NaF) that solubilises membrane-bound proteins; and Tris buffer (40 mM Tris, pH 7.4, 10 mM EDTA, 1 mM PMSF, 1 mg ml⁻¹ aprotinin), that solubilises cytosolic water-soluble proteins. Coral samples ranging from 0.2 to 0.9 g wet weight were homogenized on ice, in 0.5 ml of one of the buffer solutions using a Teflon grinder and kept for 30 min in ice and spun at 10,000 rpm, 4°C for 1 min. The coral extracts were stored at -20°C until time of analysis.

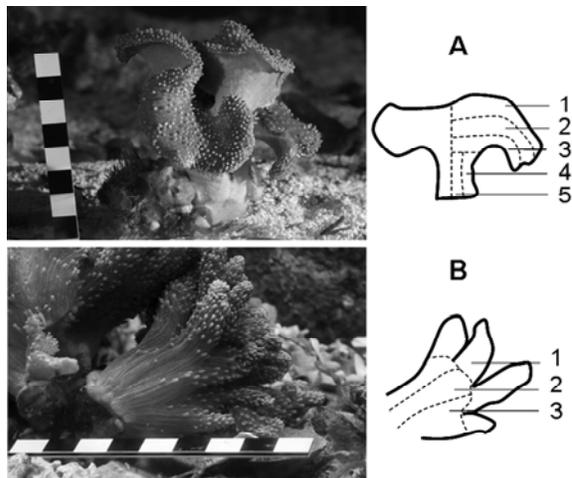


Figure 1. Soft coral colonies and functional regions investigated. (A) *Sarcophyton* sp.: capitulum upper (1); capitulum middle (2); capitulum lower (3); stem surface (4); stem subsurface (5). (B) *C. gaboensis*: lobe (1); stem surface (2), stem subsurface (3). Ruler marks = 1 cm.

Analysis of proteins

Total protein content was measured by colorimetry with bicinchoninic acid (BCA) as standard, and read at 526 nm using a microplate reader. Proteins were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Molecular marker and 3 replicate samples were loaded onto 12% Tris Glycine gels using 4 % stacking gel and run at 200V for approximately 1 h. The analysis of gel images was performed using the public domain NIH Image program (<http://rsb.info.nih.gov/nih-image/>). Using as a reference a molecular ladder, selected bands were analyzed and compared across all the resulting gels.

Experimental set up

Total protein content was analyzed in duplicate samples. The electrophoretic analysis was performed in triplicate samples. Two protein bands (approximate MW of 54 and 35 kDa) were compared amongst all treatments in each coral species. The effects of buffer and functional region on these two main specific protein bands were tested by one-way ANOVA.

Results

Intracolony distribution of proteins

The analysis of tissues showed decreasing protein concentrations from distal to proximal region of the colony and from surface to subsurface of the stem (Fig. 2A-B). The difference of protein content between regions was highly significant in both coral species (*Sarcophyton* sp ANOVA $p < 0.001$; *C. gaboensis* ANOVA $p < 0.001$). Tissue extracts prepared with NP40 and with Tris buffers showed similar general trends of variation of whole protein content. The two buffers produced a statistically significant difference of protein yield in *C. gaboensis* (ANOVA $p < 0.01$) but not in *Sarcophyton* sp (ANOVA $p > 0.05$).

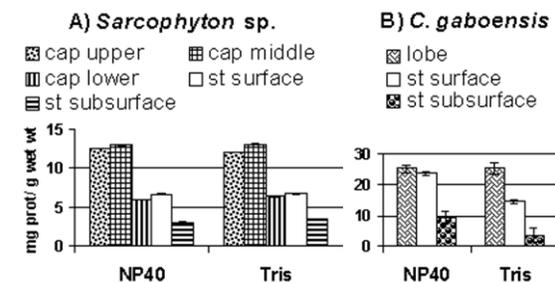


Figure 2. Total protein content in regions of the coral colony processed with NP40 and Tris buffers (n=2). (A) *Sarcophyton* sp. (B) *C. gaboensis*. Abbreviations: cap = capitulum; st = stem (mean \pm 1 sd).

Extraction of specific protein bands

SDS-PAGE on coral tissue extracts prepared with NP40 and Tris buffers produced at least 12 protein bands in the molecular weight range 216 to 7 kDa as revealed by Coomassie Blue (Fig. 3A-B). The protein bands with approximate molecular weights of 54 and 35 kDa and three light bands closely set in the range 17 to 7 kDa showed the greatest contrast between the protein profiles obtained by the two methods of extraction. The 54 and 35 kDa bands were analysed by densitometry

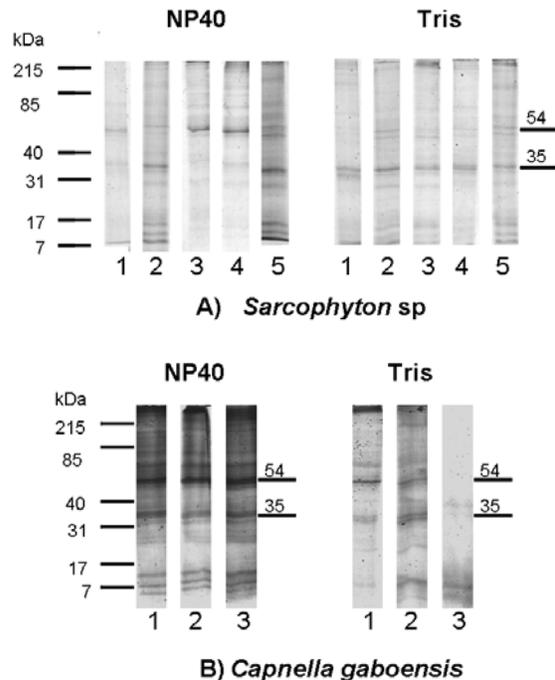


Figure 3. SDS-PAGE protein profiles of soft coral extracts. (A) *Sarcophyton sp.* Regions indicated by tracks: 1 = capitulum upper; 2 = capitulum middle; 3 = capitulum lower; 4 = stem surface; 5 = stem subsurface. (B) *C. gaboensis*. Regions indicated by tracks: 1 = lobe; 2 = stem surface; 3 = stem subsurface.

The density of specific protein bands 54 and 35 kDa was highly variable throughout both coral species. The method of protein extraction also produced significant variation in the content of these protein bands (Figs. 4-5). There were 3 functional regions in which one of the specific protein bands was not detected by one of the methods (54 kDa *Sarcophyton sp.* capitulum upper region in Tris; 35 kDa *Sarcophyton sp.* capitulum lower region in NP40; 54 kDa *C. gaboensis* stem subsurface region in Tris). The interaction between regions and extracts was significant in all cases except for protein band 35 kDa in *C. gaboensis* (see Tables 1 and 2).

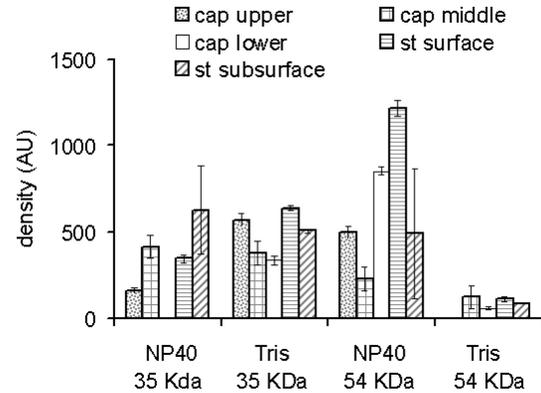


Figure 4. *Sarcophyton sp.* Comparison of two protein bands obtained by SDS-PAGE in coral samples prepared with NP40 and with Tris buffers (n=3). (A) Protein band 54 kDa; (B) Protein band 35 kDa. Density of bands is reported in arbitrary units (mean \pm 1 se).

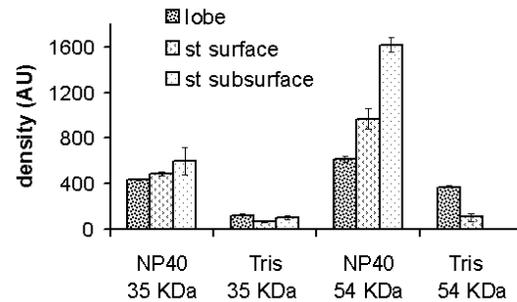


Figure 5. *C. gaboensis*. Comparison of two protein bands obtained by SDS-PAGE in coral samples prepared with NP40 and Tris buffers (n=3). (A) Protein band 54 kDa; (B) Protein band 35 kDa. Density of bands is reported in arbitrary units (mean \pm 1 se).

Discussion

This investigation contains the first comparative data of whole protein content as well as specific protein bands in tissues from various regions of soft coral colonies. The application and comparison of two different buffer solutions in the extraction of cnidarian proteins is also tested for first time.

In any organism, protein half-lives range from minutes to months depending on their function and location; their continuous renewal is the basis for biological adaptation (Hawkins 1991) yet few studies have considered the spatial variation of proteins in colonial cnidarians. The variation of protein content between polyp and stem regions in the soft corals *Sarcophyton ehrenbergi* measured as elemental carbon-to-nitrogen ratios (Tentori et al. 1997) and *Litophyton arboreum* measured as sclerite-to-protein weight ratios (Tentori et al. 2004) indicate that distal tissues are richer in protein than are proximal tissues. Protein content (measured as protein-to-biomass weight ratios) of soft corals reported in the present study confirm those results. Furthermore, our data

show significant differences in protein content throughout the colony. These data are indicative of different functional potential amongst the tissues involved.

Variability of Specific Proteins

Research on protein diversity of colonial cnidarians highlights the dynamic metabolism of these organisms. Electrophoretic protein profiles have been generated for various cnidarian species identifying stress proteins associated with heat shock (Black et al. 1995; Sharp et al. 1995); space competition (Rossi and Snyder 2001); dietary changes (Rossi et al. 2006); zooxanthella acquisition (Barneah et al. 2005); and microbial infection (Ravindran and Raghukumar 2006). In these studies two important aspects of the temporal variability of specific proteins in colonial cnidarians are appreciated: 1) the variation of specific proteins does not match the trend of variation of whole protein content (Black et al. 1995; Ravindran and Raghukumar 2006; Rossi and Snyder 2001; Rossi et al. 2006); 2) a change of specific proteins can be induced within hours in the laboratory or within several weeks under natural environmental conditions (Black et al. 1995; Sharp et al. 1995; Rossi and Snyder 2001; Barneah et al. 2005).

Table 1. *Sarcophyton* sp. one-way ANOVA. Specific protein bands in five functional regions (capitulum upper, capitulum middle, capitulum lower, stem surface, stem subsurface) in two different homogenate extracts (NP40 and Tris).

Source of variation	df	54 kDa		35 kDa	
		F	P	F	P
Extract	1	59.2	<0.001	10.9	<0.01
Regions	4	5.3	<0.01	6.2	<0.05
Interaction	4	5.1	<0.01	3.9	<0.05
Within total	20				
	29				

Table 2. *C. gaboensis* one-way ANOVA. Specific protein bands in three functional regions (lobe, stem surface, stem subsurface) in two different homogenate extracts (NP40 and Tris).

Source of variation	df	54 kDa		35 kDa	
		F	P	F	P
Extract	1	515.4	<0.01	102.0	<0.01
Regions	2	24.2	<0.01	1.4	>0.05
Interaction	2	99.2	<0.01	1.9	>0.05
Within total	12				
	17				

The spatial variability of proteins in cnidarians is less well documented. Rossi and Snyder (2001) reported the variation of stress proteins amongst tentacles of single polyps in anemones and corallimorpharians, they pointed out the differences between whole-organism and selected-tissue responses. Given the apparent morphological simplicity of colonial cnidarians, their protein analysis is commonly performed on whole experimental specimens or unspecified fragments of those specimens. We

suggest that detailed knowledge of the spatial variability of proteins within the cnidarian colony tissues could increase the resolution of the experimental response sought in physiological investigations.

In the present study, the protein bands, of approximate molecular weights of 54 and 35 kDa, stand out as a common feature in both *Sarcophyton* sp and *C. gaboensis* suggesting their involvement in a cellular activity or structural requirement common to both species. Yet the variable abundance of these protein bands indicates contrasting needs amongst the various functional regions analysed. Our results are evidence of tissue specialization in organisms that have been largely regarded as morphologically and functionally uniform.

Soluble and membrane-bound protein distribution

Specific protein analysis is commonly performed on the soluble fraction of tissue homogenates processed either in Tris-HCl (Black et al. 1995; Sharp et al. 1995; Bythell et al. 1995; Barneah et al. 2006) or in phosphate buffers (Harithsa et al. 2005; Ravindran and Raghukumar 2006; Takeda et al. 2000; Rossi and Snyder 2001; Rossi et al. 2006). Hashimoto et al. (2005) analysed both soluble and insoluble proteins obtained from Tris extracts of anthocodia (distal region of the polyp) of the soft coral *Clavularia viridis*; they demonstrated a different protein profile for each of the two fractions. Our results agree with that general finding.

The use of NP40 allows detection of membrane-bound proteins. To our knowledge, this is the first application of NP-40 to obtain proteins from cnidarian tissues. Our study revealed a significant difference of the protein profile of *Sarcophyton* sp and *C. gaboensis* depending on the extraction buffer used and colony region analysed (Tables 1 and 2).

The existence of different tissue regions in soft coral colonies is acknowledged in early taxonomic descriptions highlighting the site-specific shape of their sclerites (Verseveldt 1977). Furthermore, there is evidence of functional gradients in the soft coral body associated with feeding (Schlichter 1982; Schlichter et al. 1983), secondary metabolite production (Maida et al. 1993; Van Alstyne et al. 1994), gastrovascular circulation (Gateño et al. 1998), cell growth and tissue repair (Tentori et al. 2004), characteristics that are no doubt linked to the protein profile of the tissues involved. We suggest soft corals as model organisms that offer a natural experimental setting to enhance spatial resolution in cnidarian physiology.

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