Chapter

Active Deformation in the Tunic of *Halocynthia roretzi*: How the Tissue Composed of Cellulose Responds to Stimuli and Deforms

Yoko Kato

Abstract

Halocynthia roretzi, belonging to class Ascidiacea, has highly pure and crystalline cellulose Iβ, and sulfated chitin in its tunic. Cells, including hemocytes in the open circulatory system, are scattered in the tunic. The tunic, which maintains its thickness by continuous proliferation and removal, can be classified into active tissues. Recently, it has been reported that various stimuli, such as mechanical stimuli and changes in the mechanical environment, could cause active deformations of the tunic without changes in the characteristics of the tissue structure, which would be associated with influx and efflux of water. In this chapter, the system associated with active deformation, tissue structure and flux of water in the tunic is shown, with reference to the previous reports.

Keywords: cellulose, sulfated chitin, active deformation, water, stimuli, adaptation, tissue structure

1. Introduction

Halocynthia roretzi, which is a solitary ascidian and of the class Ascidiacea (the subphylum Tunicata and the phylum Chordata) in marine habitats, is entirely covered with the tissue called tunic. An example of *Halocynthia roretzi* is shown in **Figure 1A**. The tunic, where blood vessels and various cells including hemocytes have been observed [1–3], shows the system to keep its thickness by continuous removal and secretion [1] and defense system by the secreted substances of the hemocytes [4–11]. While it has been reported that the species in Tunicata has cellulose in its tunic [12], whose elastic modulus is 143 GPa [13], cellulose Iβ in the tunic of *Halocynthia roretzi* shows pure and highly crystalline form [14]. Also, sulfated chitin, which is biocompatible as well as biodegradable [15], has been observed in the tunic [16, 17]. In addition to the aforementioned components, α-smooth muscle actin and elastic fiber, which are expected to directly influence the mechanical properties of the tunic, and nervous systems, have been observed [18]. In the meantime, the active deformation in the tunic of *Halocynthia roretzi*, caused by acetylcholine (neurotransmitter) [18], mechanical stimuli [18, 19], electric stimuli [20] and enzyme (α-chymotrypsin) [20], has been reported. The active deformation responding to the mechanical environment has been associated with change in mass

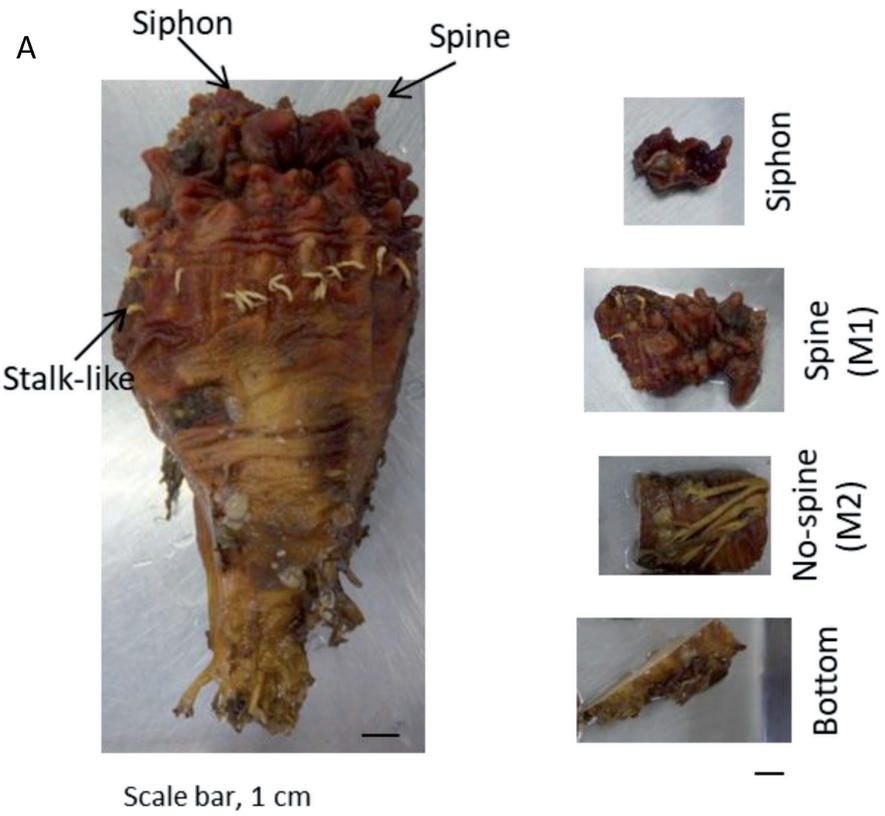

Figure 1.
Sample of Halocynthia roretzi. *A, entire image; B, the tunic sample in each category (siphon, M1 (tunic with spines), M2 (tunic without a spine) and bottom (thickest part)).*

of the tunic [21]. Because the change in mass of the tunic agreed with that in water content of the tunic, influx and efflux of water would be involved with the tunic deformation [21]. When the tunic sample was put into the seawater, the absorbance at 220 nm and 250–350 nm [22–27], which is influenced by the concentrations of nitrate and dissolved organic matter, was changed so that the substances released from the tunic would be added to the seawater [21, 28].

As **Figure 1** shows, the tunic tissue can be categorized by characteristics in shape: siphon, tubular parts where seawater is passing through; M1, tunic with spines; M2, tunic without a spine; and bottom, thickest part. While the mechanical stimuli caused a decrease in mass in every category, the tunic in the seawater at 5°C indicated an increase in the mass of the tunic, which became smaller as the position was closer to bottom [21]. While the outer layer and collapse of blood vessels could cause the difference in change of mass [21], the cells extracted from the tunic by centrifugation, kept in the seawater at 5°C for 10 days, showed motility [28] so that these cells would also influence change in mass. While the absorbance at 220 nm and 250–350 nm in the seawater used for keeping the tunic at 5°C was decreased after the removal of the tunic samples [28], the influence of the tunic category has been barely examined. Also, whether or not the cells in the tunic are obtained from all the tunic categories by centrifugation at the same degree has not been clear. If the effect of centrifugation on separating the cells from the tunic tissue is dependent on the tunic category, the characteristics of the tunic structure would be diverse and influence mass transfer.

In this chapter, why the tunic category, composed of siphon, M1, M2 and bottom, could influence the active deformation was examined. The absorbance of the seawater, which kept the tunic sample in each category separately, was evaluated by spectroscopic analysis in order to examine the change in the components of the

seawater. The seawater after removing the tunic sample was also evaluated in the same way. In the meantime, the hemocytes in each category of the tunic, which would secrete halocyamines (antimicrobial substance) [5] and hemagglutinin [10], were obtained by centrifugation to examine the influence of the tissue category on separating the cells from the tunic.

2. The following materials and methods have been followed to find out the findings

2.1 Change in mass and components from the tunic

The samples of *Halocynthia roretzi* were obtained from Yamanaka Inc. and Marutaki Suisan (Miyagi, Japan) (n = 3). The tunic was removed from other organs and cut into samples in each category (siphon, M1, M2 and bottom) by tweezers and trimming blades (feather trimming blade; Feather Safety Razor, Co. Ltd., Osaka, Japan) as **Figure 1B** shows. The sample in each category was put into the artificial seawater (Reef Crystals, Aquarium Systems, Sarrebourg, France) separately, and kept at 5°C for 10 days (Day 10) or 15 days (Day 15). The mass of the tunic, which was wrapped by paper (Kimwipe; Nippon Paper Crecia, Tokyo, Japan) for 10 s to remove water on the surface, was measured with the balance (UW420S; Shimadzu Corporation, Kyoto, Japan), in order to check whether or not the change in mass of the tunic sample agreed with that in the previous report [21]. After removing the tunic sample, two types of the seawater samples, filtrated (1001-150 (Whatman); GE Healthcare Japan, Tokyo, Japan) and not filtrated, were prepared. The two types of seawater samples were kept at 5°C for 10 days, 17 days or 30 days. The absorbance of the seawater at 190–1100 nm was measured by the spectrometer (UV-1280; Shimadzu Corporation, Kyoto, Japan) before and after removing the tunic sample. The absorbance at 220 nm and mean absorbance at 250–350 nm, which are influenced by the concentrations of nitrate and dissolved organic matter [21–27], and the peak absorbance around 970 nm, which was clearly observed, were used to evaluate the characteristics of the seawater. For the evaluation in the shape of the absorbance curve at 220–350 nm, the standard deviation of the absorbance at 250–350 nm, divided by the mean absorbance at the same range, and the mean absorbance at 250–350 nm, divided by the absorbance at 220 nm, which is named shape index, were used. Shape index was also used for estimating the change in the component ratio of the seawater.

2.2 Hemocytes

While there are several types of hemocytes in *Halocynthia roretzi* [11], the hemocyte secreting halocyamines and hemagglutinin could be obtained by the centrifugation (1000 G, 7 min) of hemolymph [5, 10]. Considering that effect of centrifugal force on separating the hemocyte from the tunic could be a parameter to evaluate the characteristics of the tunic structure, the tunic samples in each category were centrifuged in the previous report [5, 10] (n = 5). During the centrifugation, the tunic sample was put into the artificial seawater (Suprema21; Tomy, Tokyo, Japan). After removing the supernatant and tunic sample, the cells were obtained. Because the cells seemed damaged during counting the number by hemocytemeter, the number of the obtained cells was estimated by observation under the microscope (CX41-31PHP; Olympus, Tokyo, Japan).

3. Outcomes of the present study

3.1 Change in the mass of the tunic and components from the tunic

An example of a change in mass of the tunic sample is shown in **Figure 2**. The tunic bottom underwent smaller changes than those in other categories. The tendency, which was observed in all the samples, agreed with that in the previous report [21].

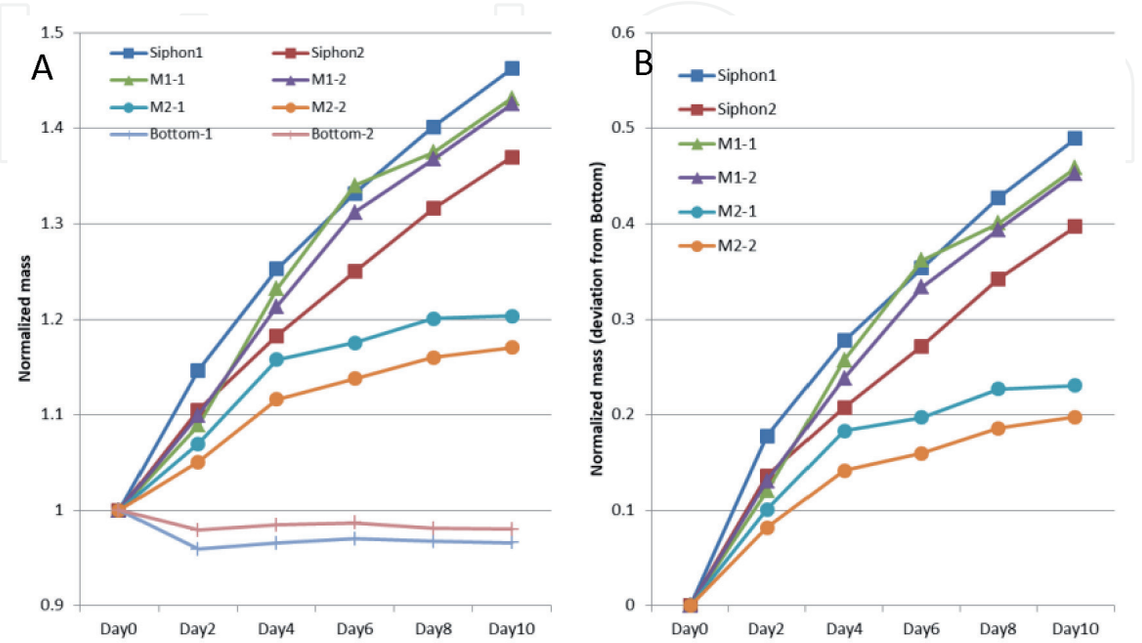

Figure 2.
Change in the mass of the tunic sample kept in the seawater at 5°C up to 10 days (day 10). A, normalized by the mass before the immersion; B, deviation from the normalized mass in bottom. All the samples indicated the same tendency.

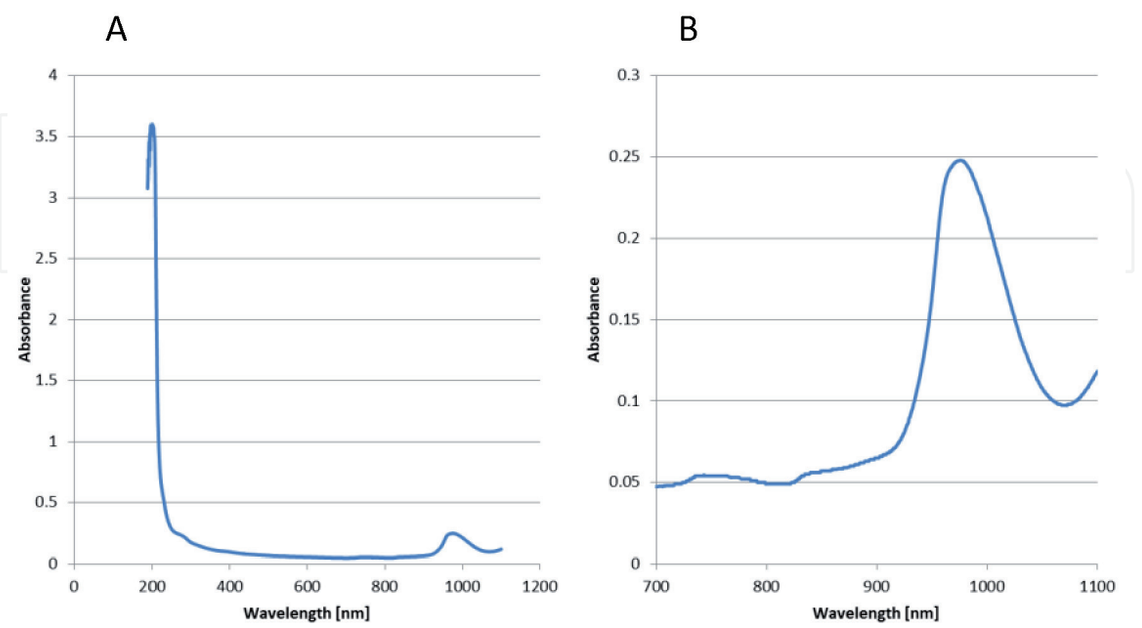

Figure 3.
Absorbance for the seawater containing the tunic sample (siphon) for 10 days at 5°C (190–1100 nm). This absorbance at 190–1100 nm was one of the results. A, entire range; B, around 1000 nm.

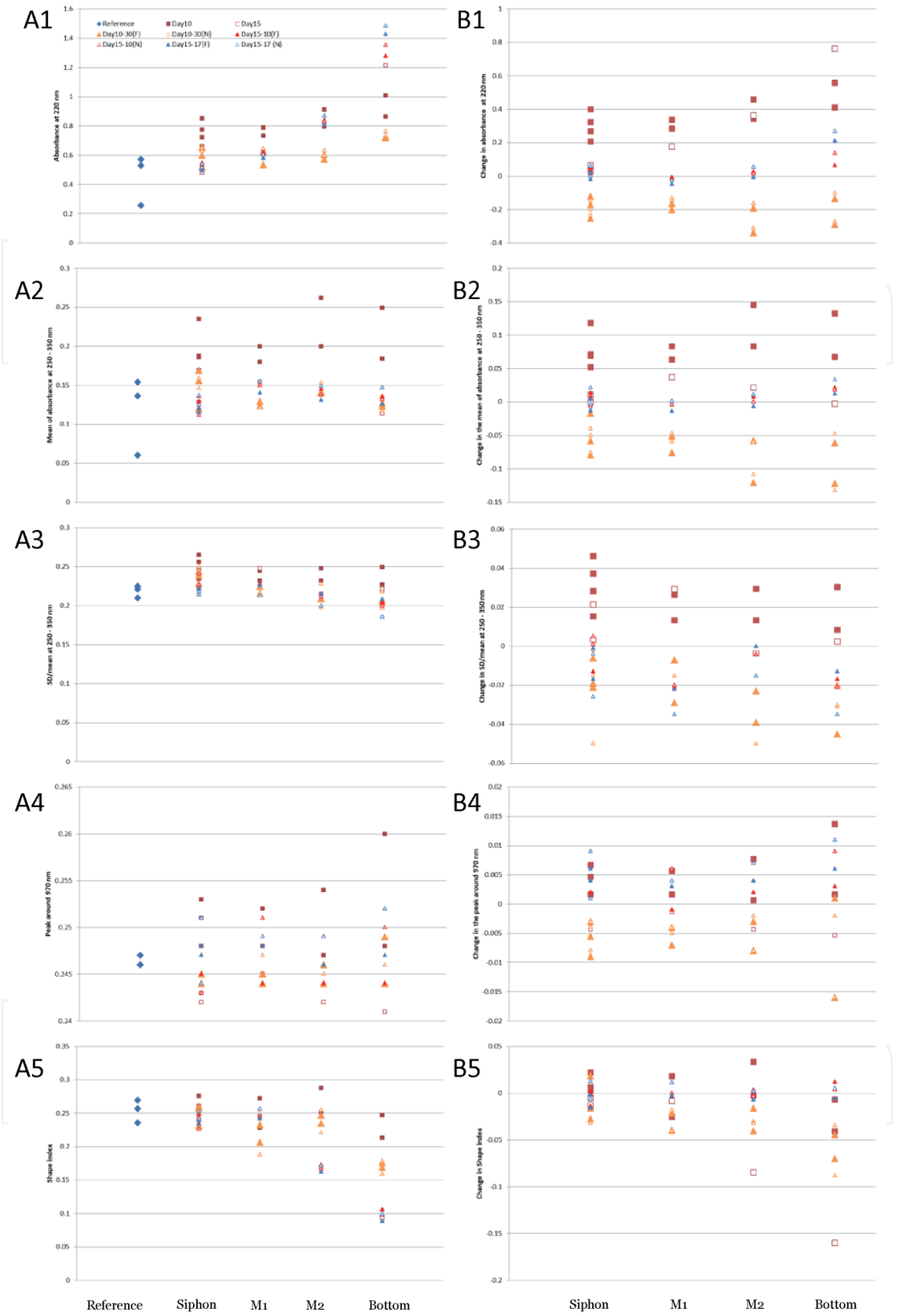

Figure 4.
Absorbance at the characteristic wavelength and related parameter. The absorbance at each wavelength and related parameter (A1–A5, left), and their change between the adjacent processes (B1–B5, right): before adding, keeping and removing the tunic samples in the seawater. The seawater samples labelled as follows: reference, without usage; day i (i = 10, 15), *keeping the tunic sample at 5°C for* i *days; day* i–j *(F or N)* (i = 10, 15, j = 10, 17, 30), *keeping the tunic sample at 5°C for* i *days and kept at 5°C after removing the tunic samples for* j *days with filtration (F), or without filtration (N).*

An example of the absorbance at 190–1100 nm is shown in **Figure 3**. The shape of the absorbance curve is almost the same in all the samples. The absorbance at the characteristic wavelength and related parameter, shape index, and their changes, caused by the adjacent process, in each seawater sample are shown in **Figure 4**. Considering the influences of the tunic sample categories (siphon, M1, M2 and bottom) on the absorbance, the absorbance and related parameter are indicated in each sample category. The mean value and change between the adjacent processes and their ranges through all the processes are indicated in **Figures 5** and **6**, respectively. As F**igures 4–6** show, the absorbance values at the characteristic wavelength and related parameters were changed by the tunic category as well as the presence and removal of the tunic samples. While the change in shape index between the adjacent processes was zero or less, other absorbance values and parameters increased before the removal of the tunic samples, and decreased after the removal, in all the tunic categories, as **Figure 5B** shows. Because the presence and absence of the tunic samples in the seawater directly influenced these parameters, and change in the component ratio of the seawater was kept through the processes, the substances released from the tunic sample would be partially degradable with progress in the change of the component ratio in the seawater. But the influences of the tunic category and process in other results were so complicated that they could be hardly explained in such a simple way. These results indicated that each category might have different systems to control its active deformation.

3.2 Cells

Figure 7 shows the cells from M1 by centrifugation (1000 G, 7 min). The cells were also obtained from the tunic samples of siphon and M2, but barely from bottom. Considering blood vessels in bottom and open circulation in the entire body, few cells in bottom would be hardly expected. Hence, there might be the

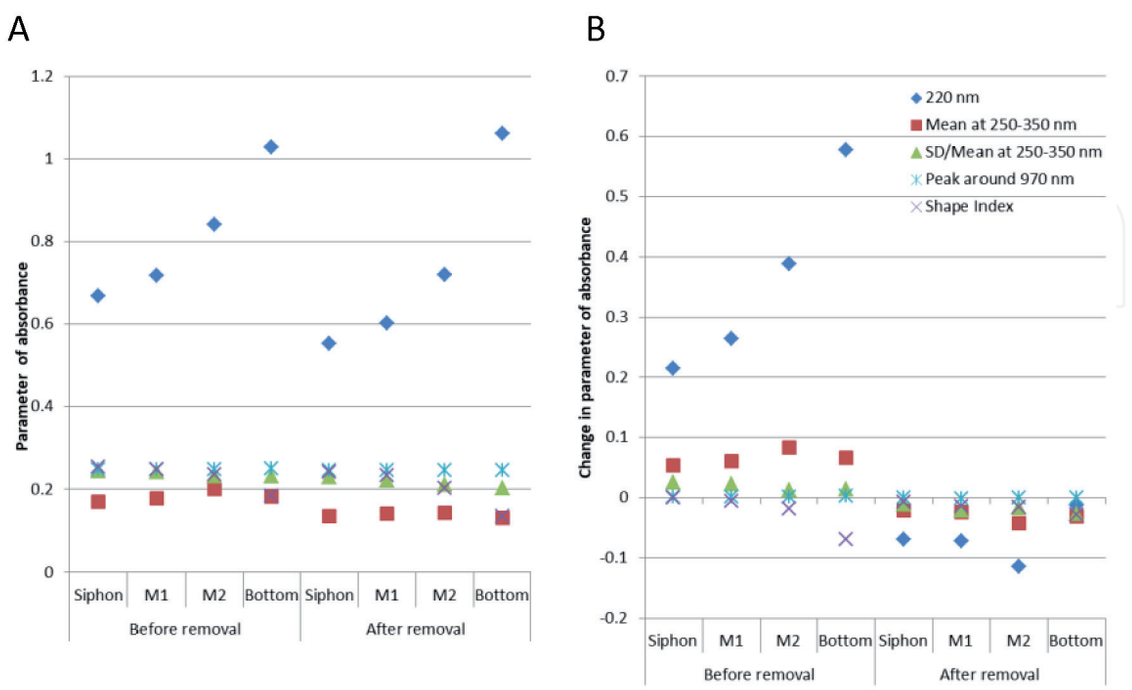

Figure 5.
Mean absorbance and related parameter. The parameter of absorbance (A) and its change between the adjacent processes (B), before and after the removal of the tunic samples in the seawater, are shown.

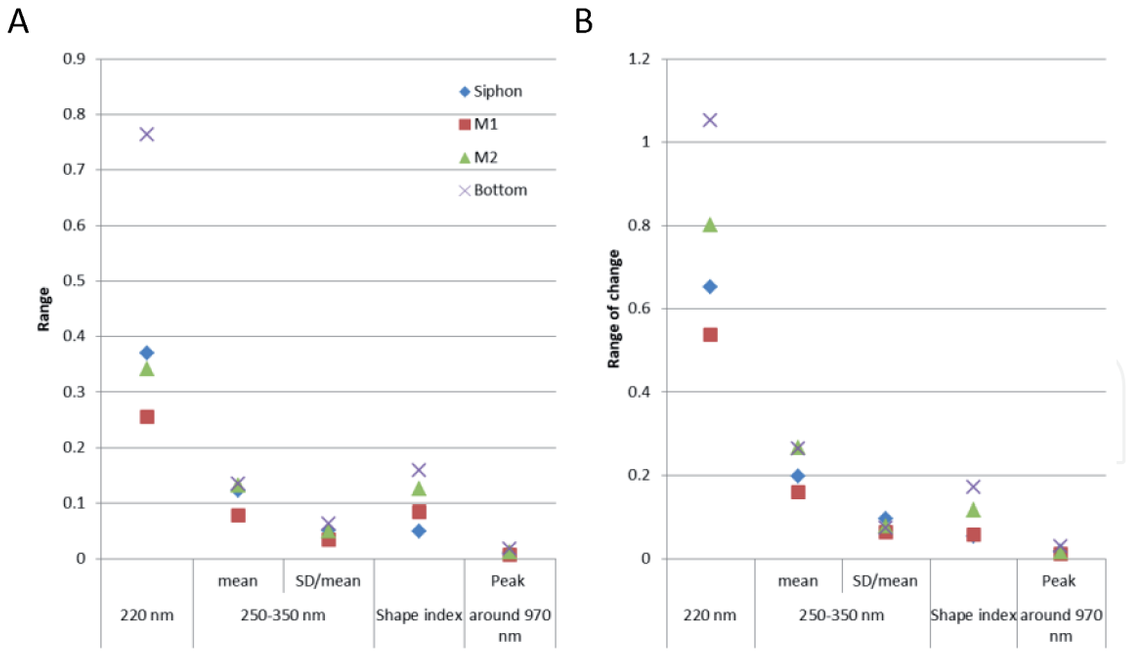

Figure 6.
Range of the parameter and change through all the processes. The range of the parameter (A) and change through all the processes from reference (B) are shown.

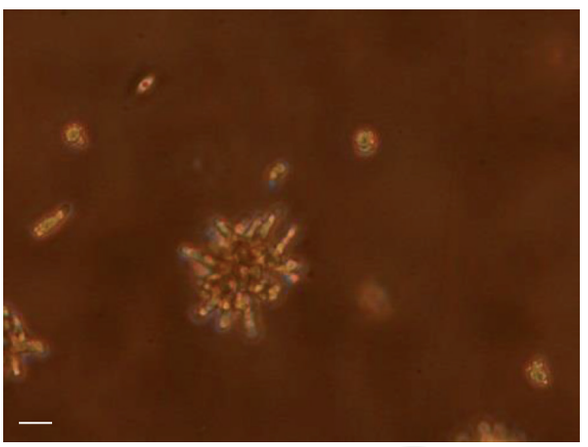

Figure 7.
Cells from M1. These cells were obtained by centrifugation (1000 G, 7 min). Scale bar, 20 μm.

characteristics of the tissue structure in bottom, which would cause cells to be hardly separated by an external force, but not in other categories of the tunic, siphon, M1 and M2.

4. Discussion of the findings as compared to earlier studies

In this chapter, the difference in the tunic categories, which are siphon, M1, M2 and bottom, was investigated to examine the system for active deformation in the tunic. Considering that influx and efflux from the tunic, which are associated with the active deformation of the tunic, would bring some components to the seawater, change in the components of the seawater was evaluated by the absorbance at the characteristic wavelength and related parameters. In all the tunic categories, these parameters, except shape index, which continuously decreased, were increased by

keeping the tunic in the seawater and decreased by removing them. These results indicated that the substances, released from the tunic, would disappear without continuous supply and keep the change in the component ratio of the seawater. The released substances would be degradable partially as well as reactive, associated with the change of the component ratio of the seawater. In the meantime, the influence of each tunic category on these parameters was complicated. Hence, the active deformation would be controlled by two types of substances, which would be in every category of the tunic sample, and specific in each category. The details of the substances will be investigated in the future.

In the meantime, the cells were obtained from siphon, M1 and M2 by centrifugation, but not from bottom. Considering the open circulation system and blood vessels in bottom, bottom would have cells, which would be hardly separated from the surrounding by centrifugation because of the characteristics in the tissue structure of bottom, different from those in other tunic categories. The result that change in mass of the tunic was smallest at bottom would agree with this unique feature of bottom. Why the cells in bottom are hardly obtained by centrifugation and how the cells in bottom can be obtained will be investigated in the future.

5. Conclusion

In this chapter, the active deformation of the tunic in *Halocynthia roretzi*, a solitary ascidian, was investigated by the substances released from the tunic, and cells obtained from the tunic by centrifugation. The absorbance at the characteristic wavelength and related parameter, except shape index, in the seawater were enhanced by keeping the tunic samples and decreased by removing them while shape index was continuously decreased. Hence, the substances released from all the tunic categories would be partially degradable, and reactive enough to stable change in the component ratio of the seawater. The difference in the influences of the tunic category on these parameters, which was complicated, would contribute to a difference in the active deformation in each tunic category. The cells in bottom were hardly obtained by centrifugation although those in other categories were successfully obtained. Hence, bottom would have the specific characteristics in the tissue structure that would keep the cells in the tunic firmly. Also, these characteristics in bottom would prevent change in mass of the tunic at bottom.

Author details

Yoko Kato
Faculty of Engineering, Tohoku Gakuin University, Tagajo, Japan

*Address all correspondence to: ykato@mail.tohoku-gakuin.ac.jp

References

[1] Berill NJ. The Tunicata with an Account of British Species. Pisces Conservation Ltd: Lymington; 1950. pp. 365

[2] Goodbody I. The physiology of ascidians. In: Russel FS, Yonge M, editors. Advances in Marine Biology. Vol. 12. Amsterdam: Elsevier; 1974. pp. 1-149. DOI: 10.1016/S0065-2881(08)60457-5

[3] Das SM. On the structure and function of the ascidian test. Journal of Morphology. 1936;**59**(3):589-600. DOI: 10.1002/jmor.1050590308

[4] Yokosawa H, Sawada H, Abe Y, Numakunai T, Ishii S. Galactose-specific lectin in the hemolymph of solitary ascidian, *Halocynthia roretzi*: Isolation and characterization. Biochemical and Biophysical Research Communications. 1982;**107**(2):451-457. DOI: 10.1016/0006-291X(82)91512-1

[5] Azumi K, Yokosawa H, Ishii S. Halocyamines: Novel antimicrobial tetrapeptide-like substances isolated from the hemocytes of the solitary ascidian *Halocynthia roretzi*. Biochemistry. 1990;**29**(1):159-165. DOI: 10.1021/bi00453a021

[6] Yokosawa H, Harada K, Igarashi K, Abe Y, Takahashi K, Ishii S. Galactose-specific lectin in the hemolymph of solitary ascidian, *Halocynthia roretzi*. Molecular, binding and functional properties. Biochimica & Biophysica Acta. 1986;**870**(2):242-247. DOI: 10.1016/0167-4838(86)90228-1

[7] Harada-Azumi K, Yokosawa H, Ishii S. N-acetyl-galactosamine-specific lectin, a novel lectin in the hemolymph of the ascidian *Halocynthia roretzi*: Isolation, characterization and comparison with galactose-specific lectin. Comparative Biochemistry and Physiology. B. 1987;**88B**(1):375-381. DOI: 10.1016/0305-0491(87)90130-1

[8] Azumi K, Yoshimizu S, Suzuki S, Ezura Y, Yokosawa H. Inhibitory effect of halocyamine, an antimicrobial substance from ascidian hemocytes, on the growth of fish viruses and marine bacteria. Experientia. 1990;**46**(10):1066-1068. DOI: 10.1007/BF01940675

[9] Azumi K, Yokosawa H, Ishii S. Lipopolysaccharide induces release of a metallo-protease from hemocytes of the ascidian, *Halocynthia roretzi*. Developmental and Comparative Immunology. 1991;**15**(1-2):1-7. DOI: 10.1016/0145-305X(91)90041-V

[10] Azumi K, Ozeki S, Yokosawa H, Ishii S. A novel lipopolysaccharide-binding hemagglutinin isolated from hemocytes of the solitary ascidian, *Halocynthia roretzi*: It can agglutinate bacteria. Developmental and Comparative Immunology. 1991;**15**(1-2):9-16. DOI: 10.1002/jez.1402650312

[11] Azumi K, Satoh N, Yokosawa H. Functional and structural characterization of hemocytes of the solitary ascidian, *Halocynthia roretzi*. Journal of Experimental Zoology. 1993;**265**:309-316. DOI: 10.1002/jez.1402650312

[12] Zhao Y, Li J. Excellent chemical and material cellulose from tunicates: Diversity in cellulose production yield and chemical and morphological structures from different tunicate species. Cellulose. 2014;**21**(5):3427-3441. DOI: 10.1007/s10570-014-0348-6

[13] Štrucova A, Davies GR, Eichhorn SJ. Elastic modulus and stress-transfer properties of tunicate cellulose whiskers. Biomacromolecules.

2005;**6**(2):1055-1061. DOI: 10.1021/bm049291k

[14] Nishiyama Y, Langan P, Chanzy H. Crystal structure and hydrogen-bonding system in cellulose Iβ from synchrotron X-ray and neutron fiber diffraction. Journal of the American Chemical Society. 2002;**124**(31):9074-9082. DOI: 10.1021/ja0257319

[15] Jayakumar R, New N, Tokura S, Tamura H. Sulfated chitin and chitosan as novel biomaterials. International Journal of Biological Macromolecules. 2007;**40**(3):175-181. DOI: 10.1016/j.ijbiomac.2006.06.021

[16] Anno K, Otsuka K, Seno N. A chitin sulfate-like polysaccharide from the test of the tunicate *Halocynthia roretzi*. Biochimica et Biophysica Acta. 1974;**362**(1):215-219. DOI: 10.1016/0304-4165(74)90043-9

[17] Wagner GP. Evolution and multi-functionality of the chitin system. EXS. 1994;**69**:559-577. DOI: 10.1007/978-3-0348-7527-1_33

[18] Kato Y. Active movement of the tunic in *Halocynthia roretzi*. Journal of Biomechanical Science and Engineering. 2010;**5**(2):163-174. DOI: 10.1299/jbse.5.163

[19] Kato Y. Mechanical senses and the tunic structure in *Halocynthia roretzi*. In: Proceedings of ISOPE-2011: 19-23 June 2011; Maui, California. Volume II. 2011. pp. 250-253

[20] Kato Y. The role of protein as a deformation controller in cellulose tissue. In: Proceedings of ASME 2012 International Mechanical Engineering Congress and Exposition; 9-15 Nov 2012; Houston. New York: ASME. 2012. pp. 607-613

[21] Kato Y. Deformation control and mass transfer in the tunic of *Halocynthia roretzi*. Open Chemistry Journal. 2018;**5**:1-17. DOI: 10.2174/1874842201805010001

[22] Armstrong FAJ, Boalch GT. Volatile organic matter in algal culture media and sea water. Nature. 1960;**185**:761-762. DOI: 10.1038/185761b0

[23] Armstrong FAJ, Boalch GT. The ultra-violet absorption of sea water. Journal of the Marine Biological Association of the United Kingdom. 1961;**41**:591-597. DOI: 10.1017/S0025315400016179

[24] Ogura N, Hanya T. Nature of ultra-violet absorption of sea water. Nature. 1966;**212**:758. DOI: 10.1038/212758a0

[25] Ogura N, Hanya T. Ultraviolet absorption of the sea water, in relation to organic and inorganic matters. International Journal of Oceanology and Limnology. 1967;**1**(2):91-102

[26] Foster P, Morris AW. The use of ultra-violet absorption measurements for the estimation of organic pollution in inshore sea water. Water Research. 1971;**5**:19-27. DOI: 10.1016/0043-1354(71)90059-5

[27] Collos Y, Mornet F, Sciandra A, Waser N, Larson A, Harrison PJ. An optical method for the rapid measurement of micromolar concentrations of nitrate in marine phytoplankton cultures. Journal of Applied Phycology. 1999;**11**:179-184. DOI: 10.1023/A:1008046023487

[28] Kato Y. Maintenance of the cell motility in the tunic of *Halocynthia roretzi*. In: Proceedings of 2018 Sustainable Industrial Processing Summit & Exhibition; Volume 6: New and Advanced Materials and Technologies; 4-7 November 2018; Rio de Janeiro. Quebec: FLOGEN. 2018. pp. 207-214