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学 位 論 文 要 旨

SUMMARY OF DOCTORAL THESIS

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Title: A fundamental study on the “catch mechanism” of a sea cucumber body wall using the glycerinated model
（題目：グリセリンモデルを使用したナマコ体壁キャッチ機構の基礎的研究）

The principal component of the body wall of sea cucumber is a dermis consisting of collagen fibers, proteoglycans, a microfibrillar network, glycoproteins, nerve fibers and neurosecretory cells. Sea cucumber body wall changes its stiffness by ionic environments. The stiff state can be held for a long time, and the mechanism concerned is known as “catch mechanism” of connective tissue in Echinodermata phylum. The ions that have a strong influence on “catch mechanism” are Ca^{2+} and K^{+}, and it is experimentally confirmed that Ca^{2+} stiffens while K^{+} softens the body wall. However, it is difficult to know what ions are involved in the “catch mechanism” and how it works, because intact tissues include several types of living cell which may also have affected by ionic concentration. Then, I decided to examine the direct effects of ions on the mechanism using the glycerinated body wall treated with 50% glycerin to clarify how the ions effect changes of stiffness. It is thought that the glycerinated body wall is a suitable model to test the direct effect of ions to the “catch mechanism”. A special measuring device was designed to record the rate of elongation with pen recorder due to the effects of ions. In these measurements, changes of stiffness were expressed as elongation lines. Downward curvilinear lines on a record paper, reflected elongation of the sample, and showed the sample in a limp state, whereas near-horizontal trends reflected a stiff state. In this physiological tests, the glycerinated body walls showed high elongation rate during addition of 10mM EDTA. It is thought that EDTA is a chelating agent to remove the action of Ca^{2+} that remains in the sample. Next, the effect of Ca^{2+} was measured on the sample and the sample became stiff when 10mM $CaCl_2$ was applied instead of 10mM EDTA. However, 1mM $CaCl_2$ solution did not stiffen the sample. Next, the distribution of collagen fibers were counted among the tissues treated with, (a) 10mM EDTA (first step), (b) 1mM $CaCl_2$, (c) 10mM $CaCl_2$, and (d) 10mM EDTA (last step). The number of collagen fibers were 1.7 times more in $CaCl_2$ treated tissues than in 10mM EDTA treated one. Thus, (1) glycerinated body wall is a suitable model to study the direct effect of ions from out side to the “catch mechanism”, (2) these samples retain the main part of the mechanism and have no cellular elements which interfere the direct effects to the mechanism, and (3) 10mM $CaCl_2$ is the appropriate to stiffen the sample. The intact body wall became soft during the addition of EDTA as a chelating agent of Ca^{2+}, but EDTA is not included in the native tissue, and other ions may affect with Ca^{2+} antagonistically to soften the body wall. It is reported that K^{+} softens the body wall of sea cucumber, but K^{+} has a property to denature the protein and is low concentration in sea water. Sea cucumber lives in high density of sea water where Na^{+} concentration is 500mM or more. It is not certain how many concentration of Na^{+} is included in the body fluids, but Na^{+} has the possibility to release the “catch”. Then, the effect of Na^{+} on the glycerinated body wall was decided to examine. When 150mM→100mM→ 50mM→10mM→150mM NaCl solution was applied to the sample one after another, the elongation rate according to the concentration was shown. However, the effect on the elongation was hardly observed in 10mM NaCl solution, but 50mM NaCl solution was the lowest concentration to be well effective. Next, the effect with 50mM NaCl solution and 10mM $CaCl_2$ solution were examined. Consequently, the sample was first elongated with 50mM NaCl solution, and when 50mM NaCl solution was exchanged next for 10mM $CaCl_2$ solution, the sample became stiff. The sample elongated again when 50mM NaCl was applied instead of 10mM $CaCl_2$. Na^{+} and Ca^{2+} seemed to affect by contending each other. However, Mg^{2+} had

slight effect to harden the sample. It can be said that it is a new finding that the glycerinated model made the samples soft by Na^{+} and stiff by Ca^{2+} and what effect appears by combining these several kinds of ions, can be an interesting part of research in the future. The comparative observation on the surface of collagen fibers were done with the negative staining to confirm changes in banding patterns. As a result, the pattern on the surface of the collagen fibers between a soft and a hard sample seemed to be somewhat different. However, it is at present uncertain whether the difference of these banding patterns reflect presence/absence of Na^{+}, Ca^{2+} or Mg^{2+} directly.