CALCIUM AND MAGNESIUM BINDING IN THE BODY WALL OF THE SEA CUCUMBER CUCUMARIA FRONDOSA

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The collagenous tissues of echinoderms have neurally regulated tensile properties. These mutable connective tissues are composed of relatively short (<1mm) tapered collagen fibrils in a nonfibrillar matrix. As is true of any discontinuous fiber reinforced material, the interfibrillar matrix has to transfer stress between the reinforcing collagen fibrils. The stress-transfer properties of the matrix thus control the tensile properties of the tissue, and it follows that neural regulation of tissue compliance is mediated by changes in matrix properties.

The predominant hypothesis for the mechanism by which echinoderms can reversibly change the compliance of their connective tissues is based on divalent ion mediation of collagen fibril interactions. This hypothesis derives exclusivlely from experiments in which artificial perturbation of tissue divalent ion content results in alterations in the tissue's mechanical properties. For example, chelation of divalent ions from the body wall of sea cucumbers greatly increases its compliance (Motokawa, Biol Rev. 59:255, 1984). We here report results of our initial studies that, for the first time, determine the normal ionic composition and divalent ion binding capabilities of the body wall in the sea cucumber Cucumaria frondosa.

C. frondosa in an ideal holothurian for these studies because the body wall lacks ossicles and because there is a conspicuous lack of podia in the ventral interambulacra. These two interambulacral areas were excised and stripped of the inner circular muscle layer. The body wall was then cut into roughly 2 x 4 x 6 mm pieces and the pigmented epidermal layer was removed with a razor blade. For measurement of in situ mineral content, blotted specimens were dried without further manipulation at 65°C. Five specimens from each of 5 animals were analyzed. To determine the proportion of bound mineral, specimens were first thoroughly washed with deionized water and then dried at 65°C. Binding experiments were performed on tissue specimens which had been washed with deionized water, treated with 4 mM ethylenediaminetetraacetic acid in 50 mM Tris-HCl, pH 8.0 to remove bound divalent ions, and again washed with deionized water. Specimens were incubated in artificial sea water (ASW: 10 mM Tris-HCl, 500 mM NaCl, 50 mM $MgCl_2$, 10 mM KCl) containing 0 to 50 mM $CaCl_2$. Following a 24 hr incubation at 12°C, the specimens were thoroughly washed with deionized water and dried. Minerals were eluted from the dried tissue specimens with 1N HCl for 24 hr and quantified by atomic absorption spectroscopy (Ca & Mg) or flame photometry (Na & K). For all the measured values given in Table 1, the coefficient of variation was less than 10%.

The total ion content of the body wall is given in columns 2 and 3 of Table 1. The bound ion content of the water washed tissue is given in column 4 of Table 1. The dry weights following extensive water washing are not comparable to fresh tissue dry weights, since some organic material is eluted in water. Nevertheless, these data indicate that the tissue preferentially

TABLE 1

	Sea Water mmol/L	Fresh Tissue mmol/Kg wet wt.	Fresh Tissue mmol/Kg dry wt.	Washed Tissue mmol/Kg dry wt.	Relative Avidity
Ca	8	8	40	38	4.75
Mg	48	57	285	134	2.79
K	10	24	120	9.5	0.95
Na	500	398	1,975	84	0.17

binds divalent cations, and binds more Ca then Mg. To illustrate this preferential binding, the fifth column in Table 1 gives the ratio of bound ion (mmol/kg dry weight) to the concentration of that ion in sea water (mmol/L). It is seen that the binding avidity is in the order Ca > Mg > K > Na.

The efficiency of mineral elution with increasing pH was determined by incubating specimens sequentially in water, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} and 1 N HCl and measuring the amount of calcium and magnesium that eluted at each concentration. Deionized water removed 63% of tissue magnesium and 38% of tissue calcium. Little additional magnesium or calcium was eluted at pH 6, 5, 4 or 3. The bulk of the remaining mineral was eluted at pH 2. Elution of the last 7 - 10% of the magnesium and calcium required 0.1 N HCl. These results establish that the mineral remaining after water washing is firmly bound to the tissue.

Treatment of water-washed specimens with EDTA removed 98% of bound calcium and magnesium. Calcium binding to these specimens in artificial sea water was concentration dependent between 0.5 and 50 mM. This binding showed complex concentration dependence that could not be attributed solely to single site binding. Nevertheless, it was interesting to note that the amount of calcium bound to tissue incubated in 10 mM $CaCl_2$ (37.4 mmol/kg dry wt.) was nearly identical to that measured in washed fresh tissue (38 mmol/kg).

These results show that calcium binding in the body wall of C. frondosa is both tight and specific. Our binding studies are consistent with the aforementioned mechanical experiments and with our unpublished observations that chelation of calcium is sufficient to produce free collagen fibrils from body wall. The pH at which the bound Ca and Mg were eluted from the tissues corresponds closely to the pK of sulfate groups on glycosaminoglycans (GAGs). This correspondence may be especially meaningful, because sulfated GAGs constitute a major fraction of the interfibrillar matrix of the body wall. Moreover, the principal GAG of the body wall of C. frondosa is closely and periodically associated with the surfaces of the collagen fibrils (Trotter and Koob, Bull. MDIBL 29:28, 1990).

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