

Contraction and Stiffness Changes in Collagenous Arm Ligaments of the Stalked Crinoid *Metacrinus rotundus* (Echinodermata)

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Abstract. Shortening and stiffness were measured simultaneously in the aboral ligament of arms of sea lilies. Arm pieces were used from which oral tissues (including muscles) were removed, leaving only collagenous ligaments connecting arm ossicles. Chemical stimulation by means of artificial seawater with an elevated concentration of potassium caused both a bending movement and stiffness changes (either softening or stiffening). The movement lasted for 1.5–10 min, and bent posture was maintained. The observation that contraction was not necessarily associated with softening provided evidence against the hypothesis that the shortening of the aboral ligaments was driven by the elastic components that had been charged by the oral muscles and released their strain energy at the softening of the aboral ligaments. The speed of ligamental shortening was slower by at least one order of magnitude than that of muscles. Acetylcholine (10^{-5} – 10^{-3} M) caused both contraction and softening. We conclude that the aboral ligament shows two mechanical activities based on different mechanisms: one is active contraction and the other is connective tissue catch in which passive mechanical properties show mutability. We suggest that there is neural coordination between the two mechanisms.

Introduction

Echinoderms are unique in possessing mechanically active collagenous connective tissues. The best-known example is catch connective tissue (mutable connective tissue), which changes its passive mechanical properties under ner-

vous control (Motokawa, 1984; Wilkie, 1996). Catch connective tissues are found in various anatomical locations in all the classes of echinoderms, and they have been regarded as one of the major features that characterize the phylum Echinodermata (Motokawa, 1988). The catch connective tissue stiffens or softens. Such changes in passive mechanical properties become apparent only when an external force is applied and the reaction of the tissue to this force is measured.

Another kind of mechanically active connective tissue was recently found in echinoderms (Birenheide and Motokawa, 1996, 1998; Birenheide *et al.*, 2000). The collagenous ligaments in the arms and cirri of crinoids contract in response to chemical stimuli such as cholinergic agents and seawater with an elevated concentration of potassium. Although the mechanism of contraction has yet to be elucidated, it is evident that muscles are not directly involved in the contraction because no muscle cells were found in these ligaments (Birenheide and Motokawa, 1994; Birenheide *et al.*, 2000).

There remains the possibility that muscles are indirectly involved in the contraction of connective tissues. The aboral ligaments of crinoid arms are disposed in an antagonistic position to the muscles in arm articulations (Fig. 1a). Crinoid arms consist of a row of ossicles connected by ligaments and muscles (Fig. 1b). The face of each ossicle bears a fulcrum that corresponds to the fulcrum of the adjacent ossicle. Muscles are found only on the oral (upper) side of the fulcrum, whereas collagenous ligaments are found both orally and aborally. When muscles on the oral side of the fulcrum contract, the arm bends in the oral direction. The arm also shows aboral bending. Because the aboral ligaments are the only mechanically strong element on the aboral side of the fulcrum, they are no doubt responsible for

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aboral bending. The conventional explanation has invoked elastic recoil (Birenheide and Motokawa, 1994). According to this view, elastic energy is stored in the aboral ligaments by passive stretch when oral bending is produced by the muscles. This strain energy is then released when the muscles relax, and the aboral ligaments shorten to cause aboral bending of the arm. This explanation regards the aboral ligament as being an antagonizing spring to the oral muscle.

The aboral ligaments are not, however, a simple spring. Arm preparations from which all the oral muscles have been removed keep a rather straight posture and show aboral bending upon chemical stimulation (Birenheide and Motokawa, 1996). Some locking mechanism for keeping the straight posture—and thus the charged state of the spring—would seem to be necessary, otherwise the aboral ligaments would spring back at the moment when the antagonizing muscles are removed. A possibility is that the ligament stores strain energy as an expanded spring by stiffening the tissue and releases it by softening, which results in shortening of the ligament. The “spring-with-a-lock” hypothesis for the contraction of crinoid arm ligaments seems to be a reasonable one because the arm ligaments contain fibrillin-like, and thus possibly elastic, microfibrils (Birenheide and Motokawa, 1994) and show mutability of stiffness (Birenheide and Motokawa, 1998). The hypothesis is also parsimonious because otherwise some unknown actively contractile machinery would have to be postulated.

The present study was designed to test the spring-with-a-lock hypothesis. We measured shortening and stiffness of the aboral ligaments simultaneously. The hypothesis predicts that softening should precede shortening. The results we obtained were contrary to the prediction and thus support our previous suggestion that the ligaments actively contract without the help of muscles (Birenheide and Motokawa, 1998; Birenheide *et al.*, 2000).

Materials and Methods

Specimens of the stalked crinoid *Metacrinus rotundus* Carpenter, 1882, were dredged from depths of 100–150 m in Suruga Bay off Numazu, Japan. Collected specimens were transported to our laboratory in Tokyo, where they were held in a tank containing recirculating seawater. The tank was kept dark and the water temperature was maintained at 14 °C. Nineteen individuals were used for experiments. They were used within 2 months after capture.

The arm of *Metacrinus rotundus* consists of a series of ossicles that are connected by ligaments and muscles. The skeletal joint of the ossicles is a transverse ridge that acts as a fulcrum. The muscles are found only on the oral side of the fulcrum, while ligaments are found on both sides (Fig. 1a). The aboral ligament was used in the present study. The aboral ligament consists of two parts, the main ligament and the much smaller ligament housed in a fossa. The results

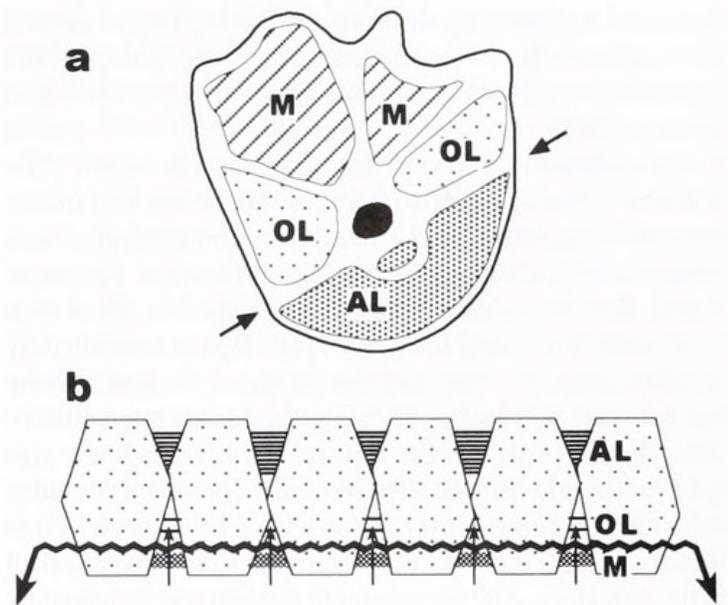


Figure 1. The articulation surface of an ossicle of *Metacrinus rotundus* (a), and the side view of an excised arm piece (b). Oral direction is to the top in a and to the bottom in b. (a) A transverse ridge (arrows) that acts as a fulcrum is arranged at an angle of about 60° to the oral-aboral plane. The ridges on the distal and the proximal side of each ossicle are arranged at angles of 60° and –60°, respectively. The central hole houses a brachial nerve. On the oral joint surface, two muscle bundles (M) and two bundles of oral ligaments (OL) insert into the ossicle. On the aboral joint surface, aboral ligaments (densely hatched area), which consists of a main aboral ligament (AL) and a small fossa ligament, insert into the ossicle. No muscles are found on the aboral side of the fulcrum. (b) An arm piece with six ossicles that are connected with each other by ligaments and muscles. The oral side, including muscle bundles, was removed from the arm piece with a razor blade, and the remaining oral tissues between the ossicles (arrows) were cut up to the fulcrum.

reported here refer to the combined properties of the two ligaments. An arm piece (length *ca.* 6 mm) containing five to seven ossicles was dissected from a sea lily. The oral side, containing coelomic canals, muscles, and most of the oral ligaments, was removed using a razor blade (Fig. 1b). The remaining oral tissue between ossicles was cut up to the fulcrum. The adjacent ossicles were thus connected by a mechanically strong aboral ligament and by a mechanically weak epidermis overlying the ligament, and also by a mechanically weak brachial nerve housed in a hole in the center of the ossicle.

The experimental setup is shown in Figure 2. The proximal end of the arm piece was firmly fixed to a holder by both cyanoacrylate glue and mechanical clamping. The double fixing ensured that there would be no slippage between the sample and the holder when the sample was subjected to a push. The arm piece was held horizontally with the aboral side upward. A small L-shaped stainless steel plate weighing 140 mg was glued to the free end of the arm, and seawater was introduced to a trough. The position of the plate, and thus that of the arm tip, was recorded by an eddy current sensor (E2CA-AN4E, Omron, Japan) located in the floor of the trough. In this setup, any active contraction of

the aboral ligaments results in an upward bending of the arm piece against the force of gravity; large softening of the ligaments would cause the arm piece to bend downward under gravity.

We constructed a device that allowed us to measure the stiffness of the ligament without restraining the free movement of the sample (Fig. 2). The device consisted of a force gauge (KSP-2-120-E-4, Kyowa, Japan) to which a probe of 1 mm diameter was attached. The gauge was fixed to a linear motion actuator (c-sx-30, THK, Japan) controlled by a microcomputer. The actuator produced vertical motion that allowed positioning of the probe in increments of 10 μm . The movements were controlled *via* a computer program written in BASIC. The program took data provided from the movement sensor and positioned the probe so that it was always 2 mm above the stainless steel plate attached to the arm piece. Any movement of the arm was followed by an immediate corresponding movement of the probe so that the distance between the probe tip and the specimen remained constant. At intervals determined by the experimenter, the probe was lowered until it touched the stainless steel plate. From this point the probe was lowered further for 0.2 mm, which caused downward displacement of the arm tip by the same amount. The force resulting from this downward displacement was recorded. The probe was then retracted to its position above the specimen. The speed of the probe was 4.2 mm/s, which was 100 times faster than the fastest arm-tip movement observed. The probe touched the arm for less than 91 ms. The bending stiffness was calculated as the peak force divided by the maximum excursion of the arm tip during the push, expressed as the

percentage of the control value. The device thus enabled us to record stiffness changes and arm movement simultaneously.

The sample was left in a trough for 10 min, and two successive downward pushes, separated by an interval of about 100 s, were applied to check that the stiffness and the baseline position of the arm tip were maintained. The stiffness at the second push was taken as the control value S_0 . The stiffness change ΔS , expressed as a percentage, was calculated as follows: $\Delta S = 100 \times (S_1 - S_0)/S_0$, where S_1 is the stiffness after stimulation. A stiffness decrease was thus shown as a negative value. Chemicals for stimulation were introduced within 1 min after the second push. The speed of elevation of the arm tip was designated as the bending speed. The peak bending speed was the maximum speed of the upward bending of the arm tip. The average bending speed in artificial seawater with an elevated potassium concentration was calculated as follows. The peak height of the bending of the arm tip from the baseline was taken as 100%. The average bending speed was defined as 80% of the maximum excursion divided by the time needed to bend from 10% to 90% of the peak height. The reaction time for contraction was the time that elapsed from the application of chemical to the beginning of bending.

Artificial seawater (ASW) in the trough was constantly circulated *via* a pump through a water bath to keep the temperature at 14 °C. The composition of ASW was as follows (in mmol/l): NaCl, 433.7; KCl, 10.0; CaCl₂, 10.1; MgCl₂, 52.5; NaHCO₃, 2.5. The pH of all the solutions was adjusted to 8.2. ASW whose potassium concentration was raised to 100 mM (KASW) was prepared by reducing the sodium concentration so as to keep osmolarity constant. Acetylcholine solution (ACh) was prepared by diluting acetylcholine chloride (Nacalai Tesque, Japan) to the desired concentration in ASW. To rinse out the trough, both KASW and ACh were exchanged with ASW using the circulation pump.

Results

Control experiments

We performed control experiments to ensure that our experimental setup did not influence the movement or stiffness of the arm. When an arm piece glued to the stainless steel plate was left in seawater, the plate was kept in the same position for at least 30 min in most cases. After the 10-min resting period, a little drift of the position was observed in some samples; such samples were not used for experiments. Repeated stiffness measurements without chemical stimulation were performed. A typical result is given in Figure 3a, in which the upper trace is for the position of the arm tip, and the lower trace is for the force. The vertical bars in the upper trace show the downward deflection of the arm pushed by a probe. The upward

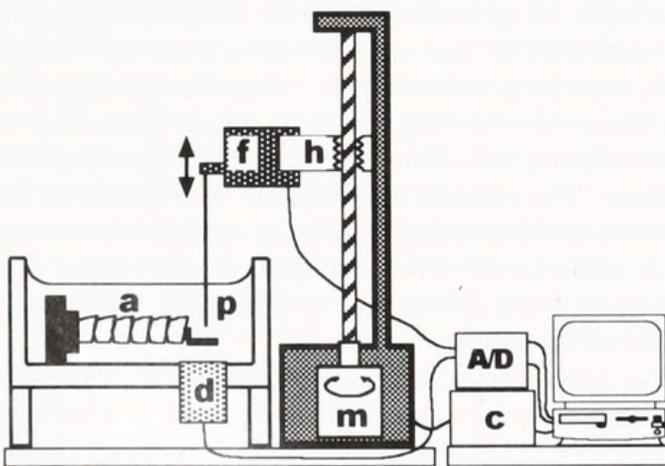


Figure 2. Schematic drawing of the experimental setup. An arm piece (a), whose size is drawn exaggerated, is held horizontally and aboral side up in a trough. A force gauge (f), to which a probe (p) is attached, is mounted on the moving head (h) of a linear motion actuator driven by a stepping motor (m). The vertical position of the moving head and thus that of the probe tip is precisely controlled. The position of the L-shaped metal plate glued to the distal end of the arm piece is monitored by a displacement sensor (d). Both force and displacement signals are fed to a computer through an analog-to-digital converter board (A/D). Feedback signals from the computer are given to the motor *via* a motor controller (c).

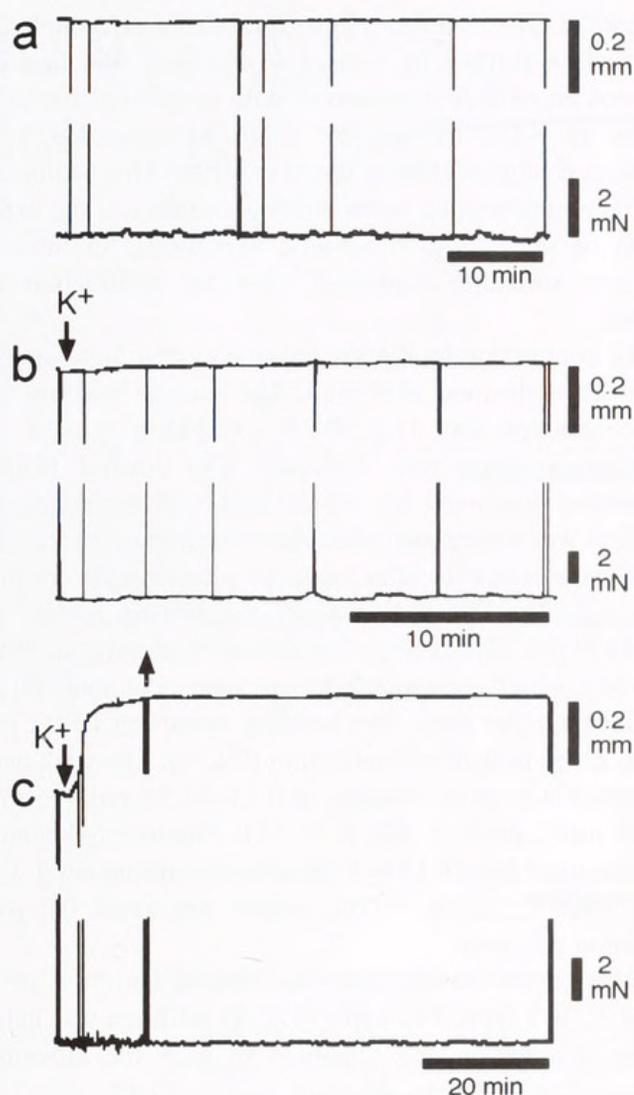


Figure 3. Control experiments. Upper traces show displacement, and lower traces show force. The vertical bars in the upper trace denote the passive downward movement of the arm when pushed, and the corresponding vertical bars in the lower trace denote the passive force exerted by the ligament in response to the push. (a) An example demonstrating that repeated pushes did not cause contraction or changes in stiffness. (b and c) Responses to chemical stimulation with artificial seawater with an elevated concentration of potassium (KASW) of a previously frozen arm piece (b) and a fresh arm piece (c). The arm pieces in b and c were cut from the same arm. The frozen sample did not respond, while the fresh one responded with contraction and stiffness decrease. In this and the following figures, a down-pointing arrow shows the introduction of a chemical, and an up-pointing arrow indicates a wash with artificial seawater.

deflections, corresponding to the downward bars, are the reaction forces to the pushes. The upper trace remained horizontal, which shows that the position of the arm tip remained the same after repeated pushes. The similar height of the upward vertical deflections shows that the stiffness remained almost the same after repeated pushes. When the first push was taken as the control, the stiffness change measured at the second push, applied 100 s after the first, was $0.63\% \pm 4.16\%$ (average \pm SD, $n = 18$). The range was -8% to $+8\%$. The average was not statistically different from 0%, which implied no changes in stiffness (one-

sample t -test, $P > 0.05$). After being pushed, the arm tip in most samples sprang back to almost the same position it held before the push. In some samples, however, small plastic deformations remained. Thus the averaged position after a push was a little lower than that before the push. It was $-10.2 \pm 24.9 \mu\text{m}$ (average \pm SD, $n = 18$) when the initial position was taken as 0 and downward shift was expressed as negative, although the average value was not statistically different from 0 (one-sample t -test, $P > 0.05$). The range was -70 to $+25 \mu\text{m}$. The quick downward bending and release of the arm piece necessary for stiffness measurement thus did not provoke any active contractions or any subsequent stiffness changes. Based on the results above, the response to chemical stimulation was classified as "no contraction" when the upward excursion of the arm tip was less than $+25 \mu\text{m}$ and as "no change in stiffness" when it was less than $\pm 8\%$.

For each arm that was dissected, two pieces were removed: one piece was used soon after dissection, and the other was frozen at -20°C overnight. The frozen sample was thawed and tested. The once-frozen, and thus no longer alive, samples did not respond to KASW. The stiffness and the position of the arm tip remained constant after repeated pushes (Fig. 3b). The fresh, unfrozen samples responded to KASW (Fig. 3c).

Responses to high-potassium seawater

Stimulation with KASW provoked two responses simultaneously. One was stiffness change, and the other was aboral bending due to the shortening of the ligament against the force of gravity. The combination of contraction and the direction of changes in stiffness was variable. The most frequent response was one in which both aboral bending and softening were observed (Fig. 3c and Fig. 4). In Figure 5, the relation between the maximal excursion of the contraction and the maximal stiffness change in a response was plotted for 20 samples stimulated by KASW. Most dots were found in the upper left quadrant, which corresponds to contraction with softening. Contraction was observed in 16 samples, 12 of which also showed softening. Although this seems to support the "spring-with-a-lock" hypothesis, there were marked exceptions. In three cases, contraction was associated not with softening but with stiffening (upper right quadrant in Fig. 5). In the case shown in Figure 6a and in the other two cases, the stiffness increased during contraction and remained so after the wash with seawater. The stiffness never fell below its value before stimulation, although some fluctuations were observed. Figure 6b shows an exceptional response. KASW caused contraction that started 1 min after stimulation. The stiffness measured at that time showed a small increase of 4%, which was classified as no change in stiffness according to our criterion. The contraction appeared to have almost reached a plateau

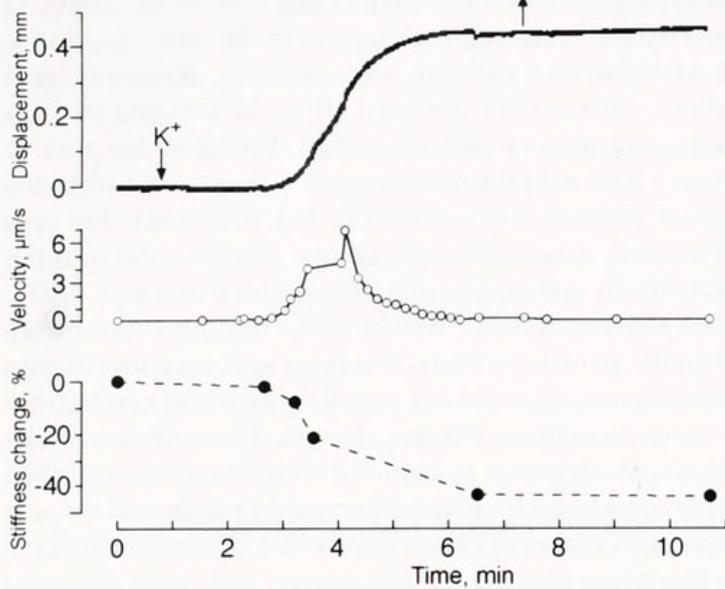


Figure 4. Typical response to artificial seawater with an elevated concentration of potassium (KASW). The upper trace is upward displacement of the arm tip, the middle trace is speed of upward bending of the arm tip, and the lower trace is softening. Both contraction and stiffness decrease were observed.

when KASW was washed out. The wash was followed by a further contraction accompanied by marked stiffening. This response is a clear demonstration that contraction was not associated with tissue softening. The contraction at wash was, however, not observed in other samples. Four arm pieces did not show contraction but did show stiffness changes. Figure 6c is such an example in which the stiffness almost doubled. Figure 6d is an example of tissue softening without contraction. In this example, the arm piece showed, instead of upward contraction, a little downward movement about 1 min after KASW stimulation. This arm piece did not fully spring back after a push by the probe but showed a persistent plastic deformation. Both the downward movement and the plastic deformation were probably produced by flow of the softened tissue due to gravity and to pushes with the probe. All the results above strongly suggested that the contraction and stiffness changes were separate responses, both independently activated by KASW. All the possible combinations—contraction with softening, contraction with stiffening, contraction with no stiffness changes, stiffening with no contraction, and softening with no contraction—were observed.

The most frequent response to KSW was contraction associated with softening, which was observed in 60% of the samples. Close inspection of the time courses of the contractile response and the softening response in these samples also supported the conclusion that these were independent of one another. In Figure 7, the maximum contraction height was taken as 100% and the maximum softening was taken as -100% in a response to KASW. In general, the extent of the softening increased as contraction

proceeded. The two were, however, not tightly coupled. In the sample marked by crosses in Figure 7, the first plot showed an evident contraction with a small stiffness increase of 6.7%, though this value was regarded as no stiffness change according to our criterion. This example of contraction preceding softening was contrary to the expectation of the spring-with-a-lock hypothesis. In most responses, softening continued after the contraction had ceased.

The contraction by KASW became evident in about 30 s after the application of stimuli. The average reaction time for contraction was 31.5 ± 7.8 s (\pm SD, $n = 13$). The contraction curve was S-shaped. The upward bending movement continued for 1.5–10 min, and the raised arm position was maintained after movement stopped. The arm remained raised even after the wash with seawater for more than 1 h (Fig. 3c). The distance the arm tip moved was 40–1140 μ m. The average was 384 ± 70 μ m (mean \pm SD, $n = 16$), which corresponded to an average of about 60 μ m of elevation per joint. The bending speed reached a peak value in the middle of contraction (Fig. 4). The peak bending speed was quite variable, at 0.35–43.52 μ m/s (6.47 ± 10.18 μ m/s, mean \pm SD, $n = 17$). The average bending speed ranged from 0.12 to 5.26 μ m/s; the mean was 1.35 ± 1.28 μ m/s (\pm SD, $n = 16$), which was about 0.2 μ m/s elevation per joint.

Softening of the ligament was observed in 70% of the samples. In a typical example (Fig. 4), stiffness was halved during contraction and remained so after the movement ceased. The stiffness decrease was $-35.8\% \pm 17.8\%$

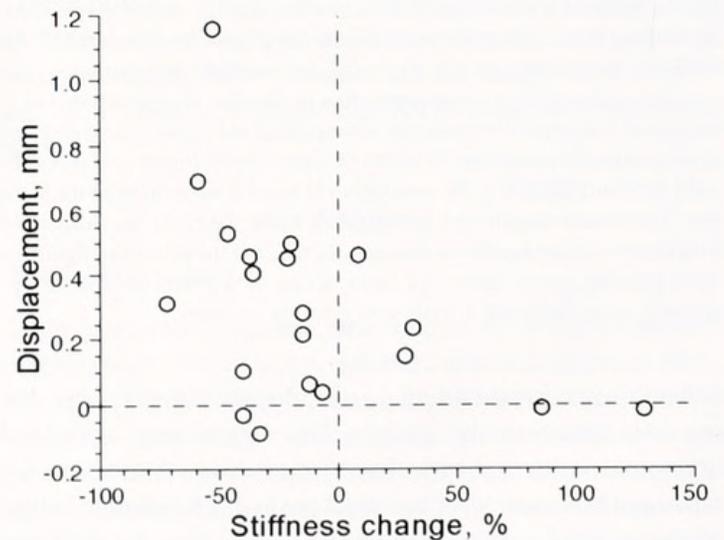


Figure 5. Relation between the maximum stiffness change and the maximum upward displacement of the arm tip in a response to artificial seawater with an elevated concentration of potassium (KASW). The former was plotted on the x-axis and the latter on the y-axis. Positive values of stiffness change denote stiffening, and negative values denote softening; positive values of displacement denote contraction, and negative values denote downward movement due to gravity.

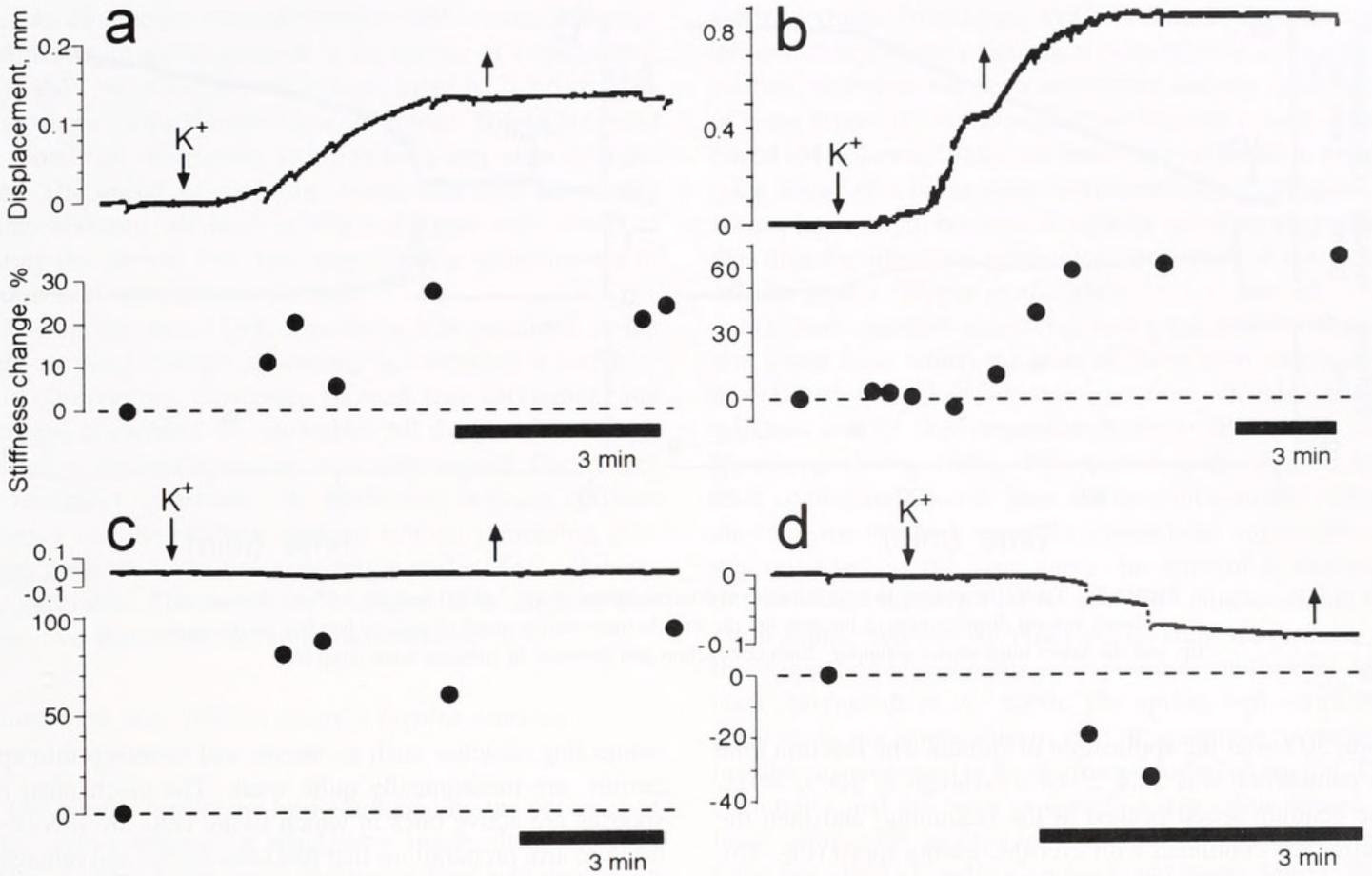


Figure 6. Responses to artificial seawater with an elevated concentration of potassium (KASW). The upper trace shows upward displacement of the arm tip, and the lower trace shows stiffness changes. Various combinations of contractile response and stiffness changes were observed. (a) Contraction associated with stiffening; (b) contraction without stiffness change in KASW, and contraction with stiffening when KASW was washed out; (c) no contraction, but marked stiffening; (d) no contraction, but softening.

(mean \pm SD, $n = 14$). The stiffness remained decreased long after the KASW was washed out (Fig. 3c).

Responses to acetylcholine

We treated arm pieces with 10^{-5} – 10^{-3} M solutions of acetylcholine in seawater. ACh 10^{-4} M and 10^{-3} M had similar effects, and 10^{-5} M provoked weaker responses. The typical response was contraction associated with softening (Fig. 8a, b). As in KASW, however, various combinations of contraction and stiffness changes were observed. Among 34 samples tested with 10^{-3} M ACh, 23 showed contraction with softening, 7 showed contraction without stiffness changes, and 4 showed softening without contraction. In 10^{-4} M ACh, 3 samples out of 8 showed contraction and softening, 2 showed contraction without stiffness changes, 2 showed no contraction but softening, and 1 neither contracted nor changed stiffness. Here again, contraction was not necessarily associated with tissue softening. It should be noted that the stiffness change was always softening; no stiffening was observed in ACh.

Contraction initiated by 10^{-3} M ACh became evident in

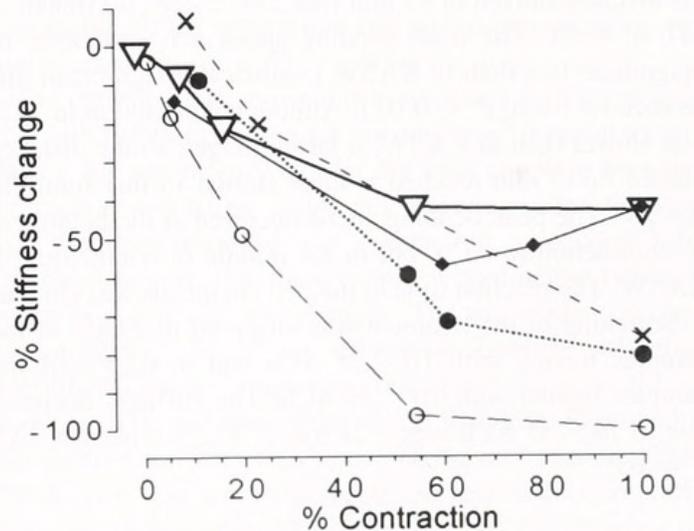


Figure 7. The relative degree of softening plotted against the relative degree of contraction in the samples that showed both softening and contraction to artificial seawater with an elevated concentration of potassium (KASW). Twelve samples showed both softening and contraction, but five examples are given here; the rest show curves similar to one of the curves shown in this figure. The final amount of contraction was taken as 100%, and the final amount of softening was taken as -100% . The time course of softening did not parallel that of contraction.

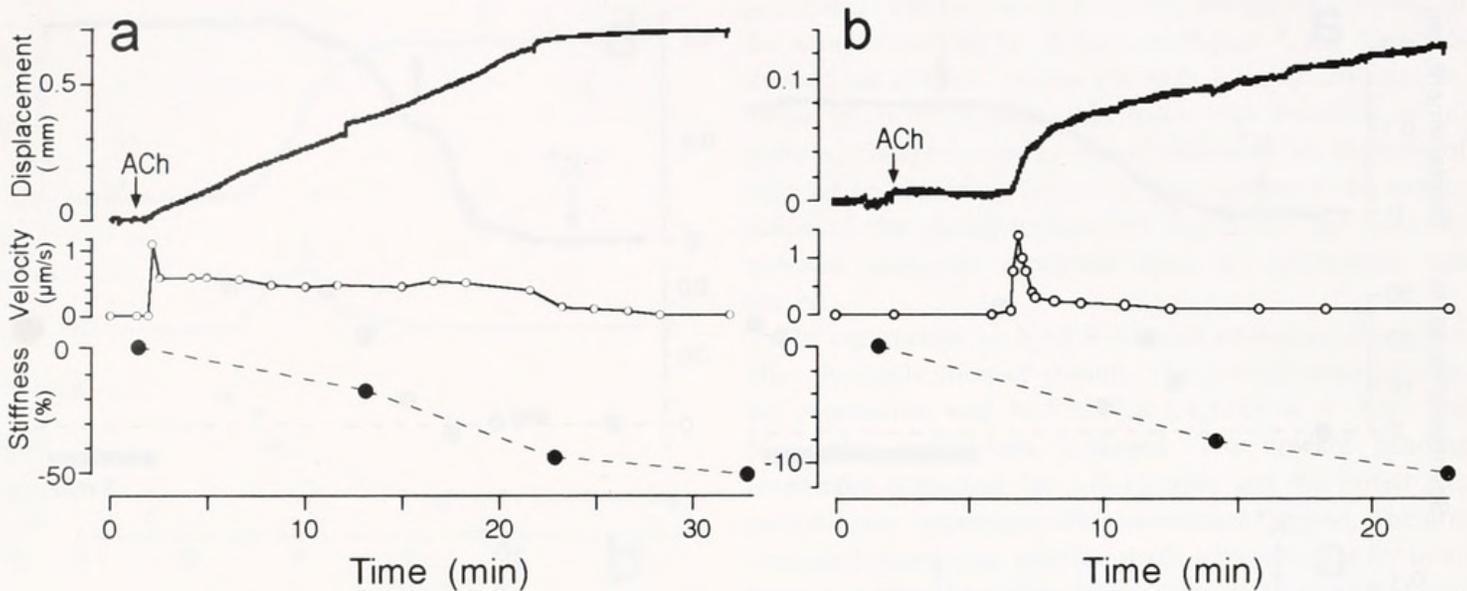


Figure 8. Typical responses to acetylcholine at concentrations of $10^{-3} M$ (a) and $10^{-4} M$ (b). The upper trace shows upward displacement of the arm tip, the middle trace shows speed of upward bending of the arm tip, and the lower trace shows softening. Both contraction and decrease in stiffness were observed.

about 30 s after the application of stimuli. The reaction time for contraction was 28.4 ± 3.4 s (average \pm SD, $n = 7$). The bending speed peaked at the beginning, and then the contraction continued with ever-decreasing speed (Fig. 8b). The upward movement continued for more than 15 min. The arm remained raised even after ACh was washed out. After the peak in bending speed, contraction with a steady bending speed was observed for 10–20 min in some cases (see Fig. 8a). The peak bending speed in $10^{-3} M$ ACh was $0.72 \mu\text{m/s}$ on average (SD = 0.42, $n = 7$, range 0.16–1.23), and the distance moved in 15 min was $259 \pm 228 \mu\text{m}$ (mean \pm SD, $n = 8$). The peak bending speed was one order of magnitude less than in KASW (statistically significant difference by *t*-test, $P < 0.01$). Although contraction in ACh was slower than in KASW, it lasted longer, so the distance moved in 15 min reached a value similar to that found in KASW. The peak bending speed occurred at the beginning of contraction in ACh, but in the middle of contraction in KASW. The reaction time to the two chemicals was similar.

Softening of the ligament was observed in 79.4% of the samples treated with $10^{-3} M$ ACh and in 62.5% of the samples treated with $10^{-4} M$ ACh. The stiffness decrease due to $10^{-3} M$ ACh was $-28.6\% \pm 11.7\%$ (mean \pm SD, $n = 6$).

Discussion

The simultaneous measurement of isotonic contraction and tissue stiffness revealed that the arm of a stalked crinoid from which arm muscles had been removed simultaneously shortened and changed in stiffness in response to chemical stimulation. Ligaments are undoubtedly responsible for these two mechanical activities, because other soft tissues

connecting ossicles, such as nerves and covering thin epidermis, are mechanically quite weak. The mechanical responses are active ones in which living cells are involved, because arm preparations that had been frozen and rethawed never responded. The effectiveness of ACh in evoking these responses suggests the involvement of neural elements. Seawater with an elevated potassium concentration (KASW) possibly exerted its effects through cellular depolarization of neural elements and of some effector cells that are involved in the mechanical responses.

Evidence against the “spring-with-a-lock” hypothesis

The most frequent response was shortening of the arm in association with tissue softening. This result may well be taken as evidence for the “spring-with-a-lock” hypothesis in which the source of contraction is attributed to an extended spring that, after being stretched by the antagonizing muscles, then releases the strain energy stored in the stiffened tissue so that tissue softening causes shortening of the ligaments. This hypothesis predicts that softening must precede shortening. We obtained, however, examples contradicting this prediction. Some arm pieces showed shortening without stiffness changes, and some showed shortening associated with tissue stiffening. The latter was quite contrary to expectations based upon the hypothesis. Even among the examples of shortening with softening, inspection of the time course of the response revealed that shortening sometimes became evident before stiffness decreased. These results clearly showed that contraction did not necessarily require a foregoing softening, thus providing definitive evidence against the spring-with-a-lock hypothesis.

The time course of the shortening speed also suggested

that the contraction was not simple elastic recoil. Although the maximum speed occurred in the middle of a contraction in KASW but at the start of contraction in ACh, no changes in stiffness corresponded to this difference. The ACh caused an initial fast contraction followed by a long slow contraction. The speed of the slow contraction was sometimes rather constant, although stiffness changes were observed during this period. This also suggested the independence of contraction and stiffness changes.

The spring-with-a-lock hypothesis was premised on the tight coupling between shortening and decrease in stiffness. Our observations, however, showed that shortening and changes in stiffness are separable. All the possible combinations of the two responses were encountered. The variety of responses, especially the shortening without stiffness changes and the stiffness changes without shortening, provides good evidence that contraction and stiffness changes are separable. The variety of responses also suggests that these two depend on different mechanisms.

Contraction and stiffness changes involve separate mechanisms

The present results are best explained by the presence of some active contractile machinery inside the ligaments. Stiffness control and active shortening may well depend on the same mechanism, as in most animals in which muscles are responsible for posture control, which involves both movement and stiffness changes. Active shortening implies force production, which would increase the resistance to stretch, causing an increase in stiffness during contraction. In the present study, however, most of the responses were contraction with a decrease in stiffness. Therefore, it is unlikely that contraction and stiffness changes share a common mechanism in these arm ligaments.

In a study of the stress-relaxation behavior of the cirral ligament of *Metacrinus rotundus*, we found that the collagenous ligament showed both stiffness changes and contraction (Birenheide *et al.*, 2000). The two responses were separable, although both were under cholinergic control. In the present study, we suggest that the same two responses are also under cholinergic control in arm ligaments.

Our present report provides the first measurement of stiffness changes in the arm ligaments of stalked crinoids. Such changes have already been reported in stalkless crinoids (Birenheide and Motokawa, 1998). The ability to change their passive mechanical properties seems to be a common character of the collagenous ligaments at the articulations of crinoids, since stiffness changes have also been reported in the ligaments of cirri (Wilkie, 1983; Birenheide *et al.*, 2000) and of stalks (Wilkie *et al.*, 1993, 1994). Stiffness changes serve to maintain body posture. They very likely share a common mechanism—connective tissue catch—which is found widely throughout the phylum

Echinodermata (Motokawa, 1984; Wilkie, 1996). Although the mutability of the mechanical properties of collagenous connective tissues has been established and the importance of these properties in the supportive function is well appreciated (Motokawa, 1988), the molecular mechanism underlying connective tissue catch is incompletely understood. It seems, however, to involve the cellular secretion of proteins that directly affect the mechanical properties of the extracellular matrix (Tipper *et al.*, 2003).

We have reported shortening and force development in arm joints from which the muscles have been removed in the stalked crinoid *Metacrinus rotundus* and also in the stalkless crinoid *Oxycomanthus japonica* (Birenheide and Motokawa, 1996, 1998). The present study showed that such contractions derive from active contraction of collagenous ligaments. Non-muscular contractions in crinoids are not restricted to the arm joints. In spite of a thorough ultrastructural investigation, we found no muscle cells in the cirral joints, and yet we observed bending movements of these joints of *M. rotundus* in response to cholinergic agonists (Birenheide *et al.*, 2000). The spring-with-a-lock hypothesis is not applicable to cirri if antagonizing muscle bundles are supposed to be the force-producing engine. The coelomic canal has been proposed as a possible source of force production in crinoid arms and cirri (Holland and Grimmer, 1981; Candia Carnevali and Saita, 1985). This idea is not applicable to the present arm preparation from which the coelomic canal has been removed. Because of their mechanical weakness, other soft tissues connecting ossicles, such as the epidermis and brachial nerves, are unlikely to generate contractile forces. Therefore, we conclude that, both in arms and in cirri, the ligaments are responsible for the contraction and probably possess a common mechanism for active force production.

The present study shows that contraction is often associated with softening. Although we employed atypical examples of this as evidence against the tight coupling between contraction and tissue softening, the observation that the most frequent response was contraction associated with softening suggests that there is some coordination between contraction and stiffness changes. The association of contraction with softening is reasonable because softening probably facilitates shortening; otherwise, kinks would be produced. The stiffness of the aboral ligaments is probably also coordinated with contraction of the oral muscles. This may be one reason that the observed responses were variable. The variety of the responses suggests the presence of sophisticated control. A cholinergic system seems to be involved in the coordination between the contraction and the stiffness changes of ligaments.

The largest bending speed observed was 43.5 $\mu\text{m/s}$. A rough calculation from this value suggests that the maximum shortening speed of the ligament itself is 0.05 l_0/s , where l_0 is the length of the aboral ligament when the arm

is straight. This is slower by one order of magnitude than reported for echinoderm muscles (Tsuchiya, 1985). The multi-joint structure of the arms, however, compensates for the slowness, because the speed of arm tip movement possibly increases in proportion to the number of joints.

The present study establishes that the collagenous ligaments of stalked crinoids show active contraction under nervous control. Among the animal kingdom, only crinoids have been documented to have such connective tissue contractions. The force-producing mechanism has yet to be elucidated.

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