Rubber-Like Elasticity and Volume Changes in the Isolated Spasmoneme of Giant *Zoothamnium* sp. under Ca^{2+}-Induced Contraction

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ABSTRACT Using glycerinated spasmoneme of giant *Zoothamnium* sp., the physical properties of spasmoneme before and after Ca^{2+}-induced contraction (pCa 4.5) were investigated. The volume change of spasmoneme contraction was measured under zero tension. The length and diameter decreased by about 50% of their initial value as a result of contraction, which means that contraction is nearly isotropic. Thus the volume of spasmoneme decreased drastically by 86% of its original value. The swollen ratio of extended and contracted spasmoneme were 0.07 and 0.37, respectively. Tension-extension relationships of extended and contracted spasmonemes were measured. By applying the theory of rubber elasticity, the number of segments of a chain in originally extended spasmoneme was only 3.3, i.e., the chain was almost a straight one. On the other hand, the number of segments of a chain in contracted spasmoneme was more than 100, i.e., the chain was essentially a random one. Furthermore, the total number of chains in single spasmoneme was the same in extended and contracted spasmoneme. This means that the interchain cross-links of chains were not influenced by addition or removal of Ca^{2+}. Moreover, the molecular weight of a chain is estimated to be at most about 50 kd. By considering all these results, it is concluded that the contractile mechanism of spasmoneme originates in the intramolecular folding and unfolding induced by Ca^{2+} binding and detaching.

INTRODUCTION

The glycerinated stalks of *Vorticellid* ciliates (*Vorticella*, *Carchesium*, and *Zoothamnium*) contract by addition of Ca^{2+} and extend by removal of Ca^{2+}. The contraction is produced by contraction of spasmoneme, a rod-shaped intracellular fibrous organelle. Reextension of the stalk is due to the elastic tension of the stalk's outer sheath. This contraction and extension can be repeated many times by changing the free Ca^{2+} concentration in the medium without hydrolysis of ATP or any other organic substrate (Hoffmann-Berling, 1958; Amos, 1975; Asai et al., 1978). The binding of Ca^{2+} to spasmoneme is thus thought to be the immediate source of energy for contraction. In the case of stalk contraction and extension of live *Vorticellid* ciliates, there must be a mechanism of Ca^{2+} release and restoration in the cytoplasm of stalks. Indeed, Favard and Carasso (1965; Carasso and Favard, 1966) observed by electron microscope membrane structures of endoplasmic reticulum located inside the contractile mass of spasmoneme fibrils. Thus, an indirect energy source for spasmoneme contraction and extension cycles will be the ATPase-driven Ca^{2+} restoration.

Allen (1973) reported in the electron microscopic study of *V. convallaria* that contracted spasmoneme is composed of bundles of 2- to 4-nm filaments. In extended spasmoneme, the structure of the bundles of filaments is unknown because electron microscopic study of extended spasmoneme has not been successful. In *Vorticella* and *Carchesium*, the extended spasmoneme is birefringent and the contracted spasmoneme is not (Schmidt, 1940; Amos, 1975). But in giant *Zoothamnium*, the birefringence of extended spasmoneme is very weak (0.2×10^{-3}), whereas that of *Carchesium* is 4×10^{-3} (Weis-Fogh and Amos, 1972; Amos, 1975). Both extended and contracted spasmoneme of *Vorticellid* ciliates have high elasticity (Weis-Fogh and Amos, 1972; Moriyama et al., 1996; Asai et al., 1998); thus, spasmoneme is thought to be a rubber-like substance that changes its length with changes in $[Ca^{2+}]$.

In this study, we made three important measurements of the physical properties of the glycerinated spasmoneme of a new species of giant *Zoothamnium*. The first is the measurement of volume change under zero tension as a result of spasmoneme contraction. The volume or length decrease of spasmoneme during contraction was previously reported to be 20–40% (Jones et al., 1970; Amos, 1972, 1975; Sugi, 1961). However, these measurements were made under conditions in which the spasmonemes were developing tension to bend the outer sheath spirally, so the measurements were not ideal for physical analysis of the contractile material. In contractile material, the measurements of isometric tension and the volume changes under zero tension are significant. Therefore, we consider our measurement to reveal more truly the properties of the material.

The second advance is measurement of the swollen ratio, defined here as the ratio between the unswollen volume and the swollen volume, of extended and contracted spasmoneme. This is the first measurement of the swollen ratio in the extended and contracted spasmoneme.

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The third type of important measurement was of the tension-extension relationships in extended and contracted spasmonemes. These were interpreted here using the theory of rubber elasticity. In 1972, Weis-Fogh and Amos attempted to analyze the force-extension curves of spasmoneme of *Zoothamnium geniculatum*, but they reported no statistics and their study was only preliminary. Moreover, the spasmonemes of *Z. geniculatum* they used were isolated from the stalk and enveloped in cytoplasm whose volume was about half that of the spasmoneme itself. So the isolated spasmonemes they acquired were not suitable for the kinds of measurements sought in this report. In contrast, the spasmonemes of giant *Zoothamnium* sp. that we discovered were isolated from the stalk without cytoplasm. For this reason, we were able to make accurate measurements of tension-extension curves of both extended and contracted spasmonemes.

By analyzing these tension-extension curves using the theory of rubber elasticity, we have estimated the number of segments in a chain between two cross-linkers and the molecular weight of a chain in a spasmoneme. In this analysis, data about the volume change and the swollen ratio of spasmoneme and the partial specific volume of a protein were used. Although analyses like these have been done for keratin (Arai et al., 1991), for the outer sheath of *Carchesium* sp. (Hawkes, 1976), and so on (Weis-Fogh, 1961), this is the first application, to our knowledge, of the theory of rubber elasticity to a biological motile organelle. The result of this analysis suggests the contractile mechanism of spasmoneme.

MATERIALS AND METHODS

Organisms and glycerol treatment

Giant *Zoothamnium* sp. were found and collected in a small river near Isanuma Pond in Kawagoe, Japan, but long culture ($>$ 3 weeks) was unsuccessful. This species has several features differing from those of *Z. geniculatum*, such as the density of zooids in mature colonies, its shape, and the protein constitution of isolated spasmoneme. Therefore, we consider this *Zoothamnium* to be a new species or a family of *Z. arbuscula* Ehrenberg (Müller, 1980), which is closely related to and sometimes confused with *Z. geniculatum*. These observations and morphology of the giant *Zoothamnium* species (probably called *Z. arbuscula* Complex) will be published elsewhere.

The collected colonies of giant *Zoothamnium* were immersed in a medium of 0.1% saponin, 4 mM EDTA-2Na, 0.1 M KCl, and 20 mM MOPS, pH 6.8, at 0°C for 45 min, followed by rinsing with a washing solution containing 0.1 M KCl, 4 mM EDTA-2Na, and 20 mM MOPS, pH 6.8. The colonies were then placed in a new medium containing 35% glycerol, 0.1 M KCl, 4 mM EDTA-2Na, 0.1 M KCl, and 20 mM MOPS, pH 6.8, at 0°C for 60 min. Glycerinated colonies were preserved in a medium containing 50% glycerol, 4 mM EDTA-2Na, and 20 mM MOPS, pH 6.8, at -20°C for at least 1 month (Asai et al., 1978).

Isolation of spasmoneme

A giant spasmoneme was isolated from a stalk of the glycerinated colony using fine forceps under a dissection microscope. The spasmoneme was stretched to about twice its original length during this isolation. After the isolation, it was immediately restored to its original length by its own elasticity.

Method of measuring the tension-extension curves

The isolated spasmoneme was glued to two glass needles held in micromanipulators (Narishige, Tokyo) by silicone sealant (SE9186-L-Clear, Toray Dow Corning silicone, Tokyo), as shown in Fig. 1, in a Lucite chamber under a dissecting microscope. One of the two glass needles was very rigid and was used for stretching the spasmoneme; the other was flexible and was used for tension measurement. The Hooke's elastic constant of the flexible needle was 3–6 μg/μm.

Elongation of the isolated spasmoneme was performed by moving the rigid glass needle at a selected speed. The spasmoneme was elongated until it became twice its original length, and then it was relaxed by returning the rigid glass needle to the initial position at the same speed. This elongation and relaxation took usually about 12–18 s. The microscopic image of elongation and relaxation was monitored with a camera (C2400, Hamamatsu Photonics, Hamamatsu, Japan). The displacements of the two glass needles were measured in real time by means of a double-channel position detector (Width analyzer C3161, Hamamatsu Photonics). The time resolution was 1/30 s. From the position data, it was possible to measure the length of isolated spasmoneme (the distance between the two glass needles) and the tension (= [displacement of the flexible needle from an equilibrium position] $\times$ [Hooke's elastic constant of the needle]). The exact and detailed tension-extension curves of isolated spasmoneme were thus obtained. All experiments were performed at room temperature.

Measurement of volume change and swollen ratio

The volume change of a glycerinated spasmoneme in the stalk during contraction was measured. A glycerinated colony of a giant *Zoothamnium* sp. was pinned down with two glass needles in a Lucite chamber as in Fig. 2 *A*. Fig.2 *B* shows the contracted spasmoneme in the stalk. Contraction was induced by changing the solution from an extending solution (4 mM EGTA, 0.1 M KCl, and 20 mM MOPS, pH 6.8) to a contracting solution (4 mM EGTA, 0.1 M KCl, and 20 mM MOPS, pH 6.8); Ca^{2+} was added to adjust the free Ca^{2+} concentration to pCa 4.5. A value of 10^6 M^{-1} was employed as the apparent stability constant of Ca^{2+}-EGTA. Because of the fixing of the distal end and upper part of stalk by the two glass needles, the stalk could not bend. So the contractile tension of the spasmoneme broke the tendon connecting the spasmoneme with the distal end of the stalk, and

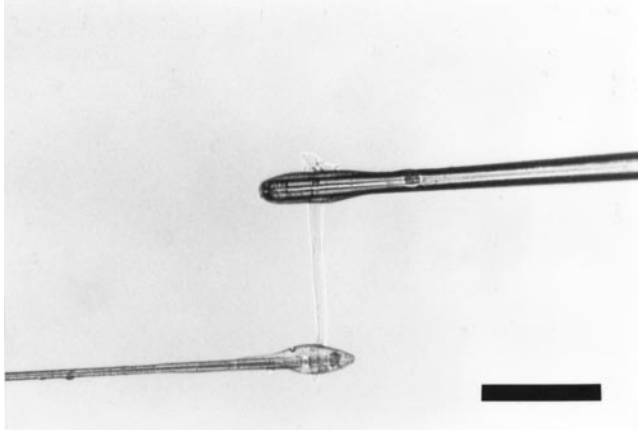

FIGURE 1 The isolated spasmoneme was glued to two glass needles. The upper glass needle is rigid and the lower one is flexible for the tension measurements. Bar, 500 μm.

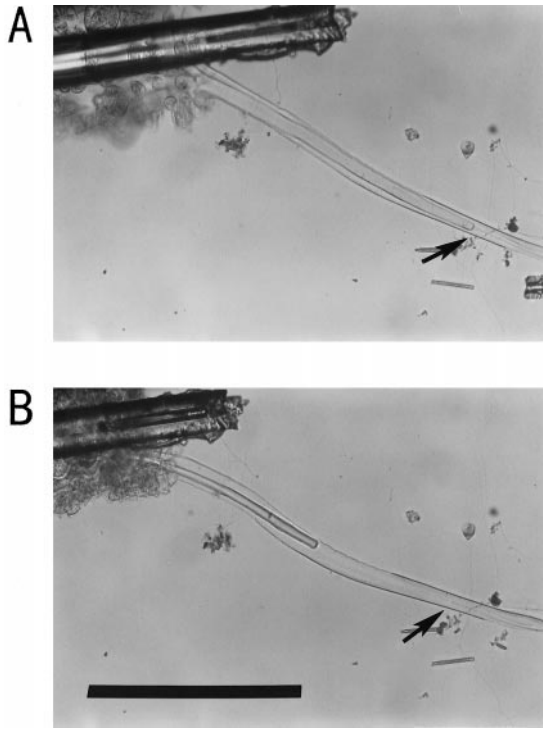

FIGURE 2 Contraction of giant *Zoothamnium* sp. spasmoneme within the stalk. The glycerinated colony of a giant *Zoothamnium* sp. was fixed by two glass needles. (The glass needle fixing the distal end of the stalk is not seen in this figure.) (*A*) The extended spasmoneme in an extending solution (absence of Ca^{2+}). (*B*) The contracted spasmoneme in a contracting solution (presence of Ca^{2+}, pCa 4.5). Because of fixation by the two glass needles, the stalk could not be bent by the Ca^{2+}-induced contraction of spasmoneme. The contractile tension of the spasmoneme therefore broke the tendon connecting the spasmoneme with the distal end of the stalk (*arrowhead*), and the spasmoneme contracted in the straight stalk under zero tension. Bar, 500 μm.

the spasmoneme contracted in the stalk under zero tension. The sizes of these extended and contracted spasmonemes were measured under both an ordinary light microscope and a phase contrast microscope. In this observation, the refraction of light passing through the outer sheath of the stalk was neglected, because the outer sheath did not deform during the contraction of spasmoneme, and also because Sugi (1961) reported that the optical deformation was considered negligible in the case of *Carchesium* stalks.

The volume change of isolated spasmoneme was measured in the same way as were the tension-extension curves. The sizes of extended and contracted spasmonemes were measured under zero tension.

In measuring the swollen ratio of isolated spasmoneme, the solution in the chamber was removed to observe the unswollen spasmoneme. To avoid the distortion of spasmoneme by surface tension during removal of the solution, the spasmoneme was attached to two rigid glass needles, as mentioned above. The rigid glass needles also prevented length change of spasmoneme. So only the diameter of spasmoneme was decreased by the evaporation of water in it. The unswollen spasmoneme easily returned to the initial swollen state by re-addition of solution.

RESULTS

Volume change

Table 1 shows the volume change of spasmoneme both isolated and not isolated. We measured the volume change in both situations in order to know the damage to the spasmoneme by isolation from the stalk.

Within the stalk, the length and diameter of the spasmoneme during Ca^{2+}-induced contraction were reduced by 47% and 48% respectively, so the volume reduction of spasmoneme was 86% (SD = 2.5; N = 13) under zero tension. In the case of isolated spasmoneme, the length and diameter reductions were 40% and 44% respectively, so the volume reduction of spasmoneme was 81% (SD = 4.4; N = 15) under zero tension.

When the contracting solution was changed to extending solution in the chamber, the contracted spasmoneme elongated actively. The length and diameter were returned to 91% (SD = 5; N = 7) and 96% (SD = 5; N = 7) of their initial extended values without and with outer sheath, respectively.

Swollen ratio

The swollen ratios of extended and contracted spasmonemes were measured in the same way as the volume change. To avoid extensive distortion of spasmoneme by surface tension during removal of solution, the spasmoneme was attached to two rigid glass needles so that the length of spasmoneme was fixed and only its diameter narrowed.

Table 2 shows the swollen ratio of spasmoneme. The swollen ratio of the extended spasmoneme was 0.072 and that of the contracted spasmoneme was 0.37. This means that the Ca^{2+}-induced volume decrease was the same as the spasmoneme-containing water decrease, as the volume

TABLE 1 Change in spasmoneme length and diameter

	EGTA		pCa 4.5		
	length (μm)	diameter (μm)	length (μm)	diameter (μm)	Volume change
average	900	31	480 (−47%)	16 (−48%)	−86%
SD (N = 13)	(90)	(7.2)	(62)	(4.5)	(2.5)

	Isolated spasmoneme		
	length change	diameter change	Volume change
average	−40%	−44%	−81%
SD (N = 19)	(12)	(5.6)	(4.4)

TABLE 2 Measurement of spasmoneme volume in swollen and unswollen states

	EGTA diameter (μm)		pCa 4.5 diameter (μm)	
	swollen	unswollen	swollen	unswollen
average	33.2	8.9	17.1	10.4
SD ($N = 8$)	(5.4)	(1.8)	(2.6)	(1.6)
1/swelling ratio	0.072		0.37	
SD ($N = 8$)	(0.0097)		(0.064)	

change from extended to contracted spasmoneme was 0.19 ($0.37 \times 0.19 = 0.07$, nearly 0.072). Moreover, this agreement of the values shows the accuracy of this measurement.

Analysis of tension-extension curves

Fig. 3 shows approximately measured tension-extension curves at various pCa. The tension-extension curves of spasmoneme change their shapes in relation to the free Ca^{2+} concentrations, so the completely extended spasmoneme in an extending solution and the completely contracted spasmoneme in a contracting solution were measured to establish their tension-extension curves more exactly and in greater detail. The curves were analyzed using the theory of rubber elasticity and the change of internal structure of the spasmoneme was estimated.

The elongation of the isolated spasmoneme was performed by moving the rigid glass needle at a selected speed. The spasmoneme was elongated until its length became twice its original length; then it was relaxed by moving the rigid glass needle to its initial position at the same speed. Fig. 4 shows the tension-extension curves measured by this method. The curves of elongation did not correspond to those of relaxation, i.e., there was a hysteresis. The hysteresis was caused by internal friction between the molecules of spasmoneme or by irreversible mutual interaction between molecules in neighboring polymer chains. To test the influence of viscoelasticity, the time of elongation was

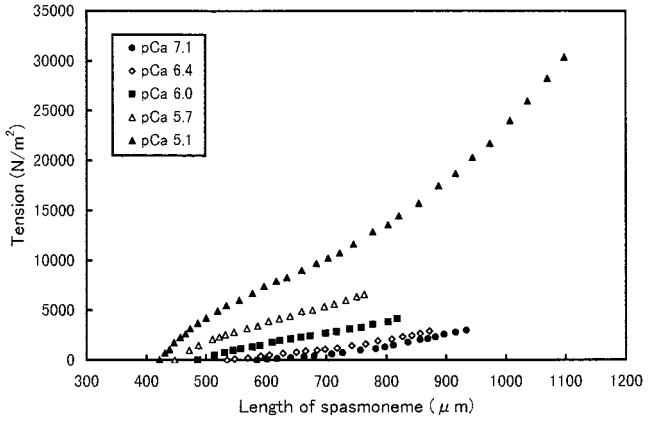

FIGURE 3 Approximately measured tension-extension curves at various pCa. The tension-extension curves of spasmoneme change their shapes in relation to the free Ca^{2+} concentrations.

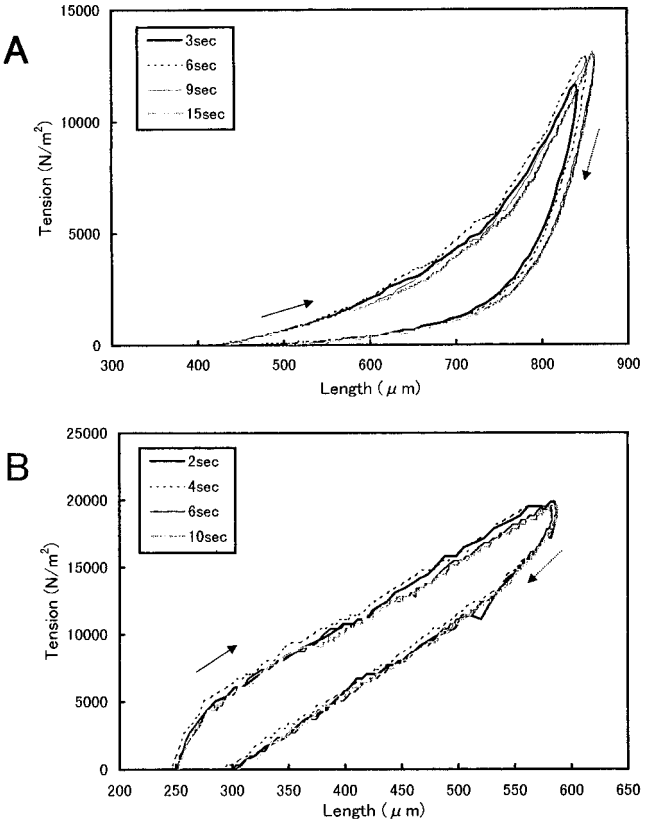

FIGURE 4 Time dependence of tension-extension curves measured by a more exact method (see text). (*A*) Tension-extension curves of extended spasmoneme in an extending solution (absence of Ca^{2+}). Arrow shows the curve of spasmoneme elongation; arrow with broken line shows that of spasmoneme relaxation. The time of elongation varied from 3 to 15 s. (*B*) Tension-extension curves of contracted spasmoneme in a contracting solution (presence of Ca^{2+}, pCa 4.5). Arrows are same as those of *A*. The time of elongation varied from 2 to 10 s.

varied from 3 to 15 s in extended spasmoneme and from 2 to 10 s in contracted spasmoneme. In this range of times, the tension-extension curves showed no significant difference (Fig. 4), so the influence of viscoelasticity was ignored.

Both the extended (no free Ca^{2+}) and contracted ($10^{-4.5}$ M Ca^{2+}) spasmoneme were described by expressions from the generalized theory of rubber-like elasticity (see Treloar (1975) and Fig. 6). In this theory, the tension F at fractional extension λ is

$$F = G\nu^{1/3}\frac{\sqrt{n}}{3}\left[L^{-1}\left(\frac{\lambda}{\sqrt{n}}\right) - \lambda^{-3/2}L^{-1}\left(\frac{1}{\sqrt{\lambda n}}\right)\right] \qquad (1)$$

where ν is the swollen ratio ($\nu = 0.072$ and 0.37, respectively, for extended and contracted spasmoneme), n is the number of freely-jointed segments in a chain, and $L^{-1}(x)$ is the inverse Langevin function of x. A "segment" is a nonflexible (rigid, rod-like) polypeptide in a spasmoneme. The elastic constant is $G = NRT$, where N is the number of chains (each of n segments) or, alternatively, the number of interchain cross-links, per unit volume. Thus, if the average molecular weight of a segment is m and that of a chain is M,

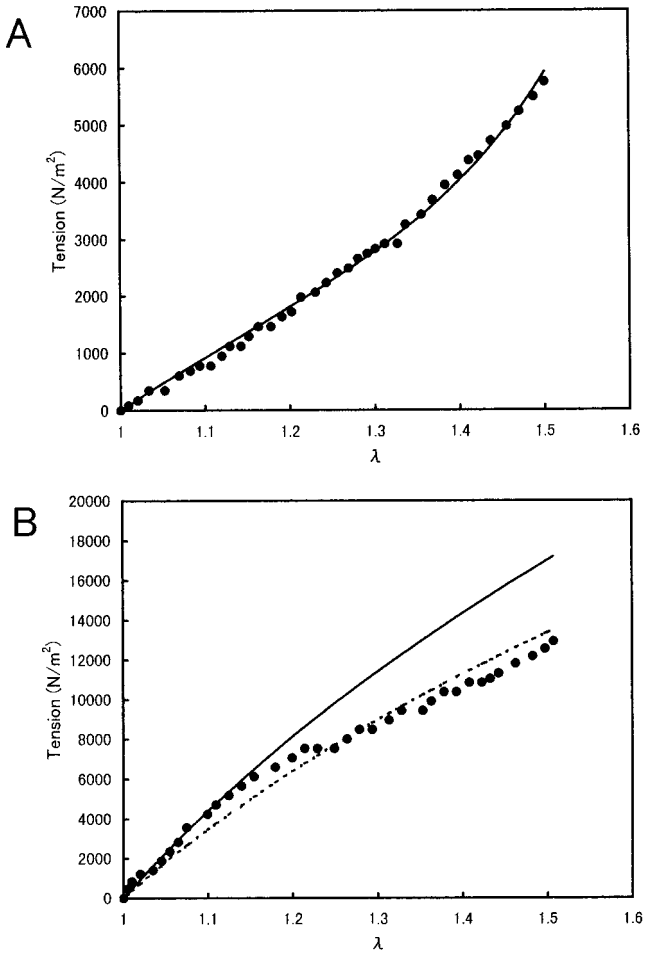

FIGURE 5 Typical tension-extension curves. (*A*) Plot indicates the tension-extension curve of extended spasmoneme (one of the typical curves among 13 samples). The line has been fitted to Eq. 1 for this sample. (*B*) Plot indicates the tension-extension curve of contracted spasmoneme (one of the typical curves among 13 samples). The solid line has been fitted to Eq. 3 using data from $\lambda = 1.0$–1.1 for this sample. The broken line is the fit using data from $\lambda = 1.0$–1.5. In the latter fit, the fitted molecular weight of a chain between cross-linking points was about 70 kd, whereas that using $\lambda = 1.0$–1.1 was 50 kd. In the analysis of tension-extension curves, the experimental data at small elongations are better fitted by the theory of rubber elasticity than those at large elongations. And the difference is not so serious in the sense of intramolecular folding.

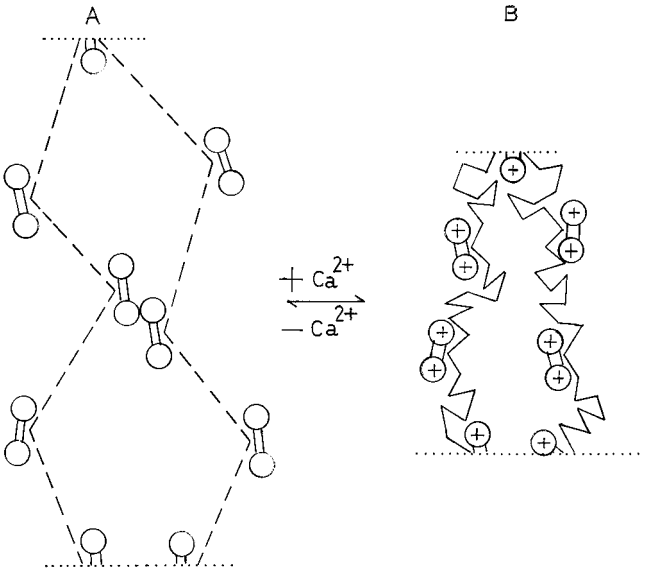

FIGURE 6 A rubber-like elastic model of two chains, one (*A*) in the absence of Ca^{2+} for $n = 4$ and one (*B*) in the presence of Ca^{2+} for a very large n. The dumbbell-shaped model represents Ca^{2+}-regulated and less flexible protein, spasmin (18–23 kd). The squares represent cross-linkers to interconnecting chains. The broken line (about 12.5 kd) and solid line (very low molecular weight) are thermally flexible units in *A* and *B*, respectively.

then $M = n \times m$. Eq. 1 can be expanded in a Taylor series to

$$F = G\nu^{1/3}\left[\left(\lambda - \frac{1}{\lambda^2}\right) + \frac{3}{5n}\left(\lambda^3 - \frac{1}{\lambda^3}\right) + \frac{99}{175n^2}\left(\lambda^5 - \frac{1}{\lambda^4}\right) + \frac{513}{875n^3}\left(\lambda^7 - \frac{1}{\lambda^5}\right) + \cdots\right] \quad (2)$$

Using independently measured values of ν and λ, we found values of N and n which, upon substitution into Eq. 2, generated values of F best resembling (on a nonlinear least-squares criterion) the experimentally measured values of F. The range of λ fitted was restricted to $1.0 < \lambda < 1.5$. (Although λ can exceed 2.0, crystallization at large λ is an insuperable difficulty.)

Eq. 1 or Eq. 2 is applied to the contracted as well as to the extended state. In severe contraction, however, n has to become very large (so as to generate very few segments) and the series converges too soon to estimate n accurately. For example, when $\lambda = 1.1$, the ratio of the second to the first term is 0.01 when $n = 100$, whereas for $\lambda = 1.1$ the ratio is 0.1 when $n = 10$. Thus, in the contracted state it is hard to estimate n. The tension as a function of extension is then given by the limiting case of Eq. 2, viz.,

$$F = G\nu^{1/3}\left(\lambda - \frac{1}{\lambda^2}\right) \quad (3)$$

For contracted spasmoneme, ν is found to be 0.37 (approaching the specific volume of dry proteins, 0.76).

In this and in previous work by the authors and others, it is shown that the active element in this primitive form of contractility exhibits long-range "rubber-like" elasticity, shortens upon binding a charged ligand (e.g., Ca^{2+} ions), and relaxes when the ligand is removed by Ca^{2+} pumping system (e.g., Ca^{2+}-activated ATPase in endoplasmic reticulum). In the present work the ligand must be removed by Ca^{2+} chelator, EGTA, because we used glycerinated spasmoneme.

It is historically interesting that before the discontinuous and impulsive nature of muscle contraction was known, physical biochemists had developed an analysis appropriate for a continuous system, such as ours, in which entropic-

electrostatic chains shortened on binding an oppositely-charged ligand (Botts et al., 1951; Morales and Botts, 1953).

The experimental data were fitted to Eq. 2 by a nonlinear least-squares fitting and the values of G and n were determined. In this analysis, only the data from $\lambda = 1.0$ to $\lambda = 1.5$ was used, because a spasmoneme elongated over twice its original length is drastically changed by the crystallization of internal molecules. Fig. 5 shows the typical tension-extension curves of extended and contracted spasmoneme. The results for 13 samples are shown in Table 3.

The analysis showed that the contracted spasmoneme was composed of random chains of over 100 segments ($n > 100$) and that the extended spasmoneme was composed of nonrandom chains with an average of only 3.3 segments ($n = 3.3$, (SD = 0.45, $N = 13$)). Moreover, the chain number per contracted spasmoneme is 9.5 mol/m^3 (SD = 1.6, $N = 13$), i.e., 9.5 mM/l, which is also the cross-link number per unit volume; the chain number of extended spasmoneme is 2.0 mol/m^3 (SD = 0.72, $N = 13$). Whereas the volume of spasmoneme becomes 19% (= 0.19) after contraction, the total number of cross-links ($9.5 \times 0.19 = 1.8$, nearly 2.0) does not change during spasmoneme contraction. Because the partial specific volume of proteins is usually 0.70–0.74, the density of unswollen spasmoneme is $1/0.7 \times 10^6$ g/m^3. Using the swollen ratio of extended spasmoneme (= 0.07), the density of swollen spasmoneme except water is 10^5 g/m^3. As the chain number per extended spasmoneme is 2.0 mol/m^3, the molecular weight of a chain between cross-linking points is 50 kd.

These results suggest that the contractile mechanism of spasmoneme originates in the intramolecular folding and unfolding induced by Ca^{2+} binding and detaching.

DISCUSSION

Volume change

There are some reports about the shape change and the volume change of the contractile element, spasmoneme, of *Vorticellid* ciliates. Jones et al. (1970), after taking high speed films of *Vorticella* contraction, reported that spasmoneme shortened by less than 10%. But Amos (1975) doubted the report because of the small size of the *Vorticella* stalk in relation to the resolution of the film. Amos (1972, 1975) observed that the length of *Carchesium* spasmoneme was reduced to 36% of its initial length, and that in *Z. geniculatum* the corresponding value is 45%. Sugi (1961) observed the contraction of *Carchesium* stalk carefully and reported that the spasmoneme shortened to 22–33% of its initial length and its volume decreased by 24–38% of its initial value during contraction. Jones and Amos did not mention the diameter change of spasmoneme. Only Sugi measured the diameter of spasmoneme, and he reported that the cross-section of spasmoneme became larger as a result of contraction. All of these measurements were carried out on spasmoneme within the spirally contracted stalk, where it was very difficult to measure dimensions accurately. Moreover, the contracted spasmonemes developed an unknown tension required to bend the stalk spirally. Therefore, these measurements were inadequate for examining the properties of the contractile material. In this paper, we report our measurements of the volume change of isolated spasmoneme of giant *Zoothamnium* sp. under zero tension. We also measured the volume change, as a result of contraction, of the spasmoneme within the straight extended stalk under zero tension. This giant *Zoothamnium* was found to be a much better specimen than *Z. geniculatum* for the accurate measurement of volume change. In our measurements of isolated spasmoneme of giant *Zoothamnium*, both the length and the diameter decreased by about 50% of their initial value as a result of contraction. This means that the volume decrease of spasmoneme is by 86% of its initial value. This value is far larger than that for *Carchesium* (24–38%) measured by Sugi (1961). The difference may be due to the difference between species and to the tension development of the *Carchesium* spasmoneme in the spirally contracted stalk. But it is more significant for the investigation of the physical properties of spasmoneme to measure its contraction under zero tension, as we did for this study.

The length and diameter decreases are the same, so the contraction of *Zoothamnium* spasmoneme is isotropic. This fact may seem inconsistent with the birefringence of extended spasmoneme. But it was reported that the birefringence of extended spasmoneme of giant *Zoothamnium* is very weak (0.2×10^{-3}), whereas that of *Carchesium* is 4×10^{-3} (Amos, 1975). So, in the giant *Zoothamnium*, the fibrous structure in the extended spasmoneme is not so much clustered in one direction as it is in *Carchesium*. As a result, the contraction of spasmoneme of giant *Zoothamnium* is isotropic. Moreover, this isotropic contraction is consistent with the theory of rubber elasticity used for the analysis of the tension-extension curves of spasmoneme.

TABLE 3 Parameters in the model of rubber-like elasticity

	extended spasmoneme 13 samples	contracted spasmoneme 13 samples
n	3.3	>100
SD	(0.45)	
N (mol/m^3)	2.0	9.5
SD	(0.72)	(1.6)
G (N/m^2)	4900	23,000

Little damage to spasmoneme by isolation from the outer sheath

The volume of the spasmoneme isolated from the outer sheath became 19% of its initial value during contraction (reduction of 81%), whereas that of the spasmoneme within the outer sheath became 14% of its initial value during contraction (reduction of 86%). So isolation decreased the contractile ability by 6% (81/86 = 94%) of its initial value. In *V. convallaria* the tension of the isolated spasmoneme

was decreased by over 90% by isolation (Moriyama et al., 1996). The diameter of giant *Zoothamnium* spasmoneme (30 μm) is much larger than that of *Vorticella* (1 μm) and *Carchesium* (5 μm), so the damage during isolation is thought to be very small. For this reason, the use of isolated giant *Zoothamnium* spasmoneme is superior for investigating physical properties. And it is already confirmed that the glycerol treatment of spasmoneme does not influence its ability to contract (tension development) (Moriyama et al., 1998).

Swollen ratio

Weis-Fogh and Amos (1972) reported that the swollen ratio of glycerinated spasmoneme of *Z. geniculatum* was 0.15, but the method of measurement was not reported. In our study we measured the diameter reductions of both extended and contracted spasmonemes, fixed by two rigid glass needles. The diameter reduction was induced by the evaporation of the immersing water. The swollen ratio in our measurement was 0.07 for extended spasmoneme and 0.37 for contracted spasmoneme. The volume of contracted spasmoneme became 19% (= 0.19) of the extended value during the Ca^{2+}-induced contraction of isolated spasmoneme. Thus, the dry mass of extended and contracted spasmonemes is the same ($0.19 \times 0.37 = 0.07$). This agreement testified to the accuracy of the measurements.

The constancy of the dry volume means that there was excretion of water or saline from the interior of spasmoneme during contraction. This can be interpreted in two ways. One is excretion by volume reduction of spasmoneme; that is to say, Ca^{2+} induces the contraction of spasmoneme and its volume decreases, so the inner water or saline is ejected. The other interpretation is that Ca^{2+} makes the inner of spasmoneme hydrophobic, causing excretion of the inner water or saline, which induces the contraction of spasmoneme. Actually, the Ca^{2+}-binding protein spasmin, which is one of the components of spasmoneme, becomes hydrophobic when it binds to Ca^{2+} (Asai et al., 1998). However, we cannot determine the mechanism of contraction only from the fact of water or saline excretion. As a matter of fact, both phenomena, namely saline excretion and hydrophobicity change, may simultaneously facilitate the Ca^{2+}-induced spasmoneme contraction.

The contractile mechanism of spasmoneme

Muscle contraction occurs as a result of interaction of actin and myosin, and ciliary movement occurs as a result of interaction of dynein and tubulin. Those well-known motile systems are driven by chemical energy liberated from ATP hydrolysis. But the mechanism of spasmoneme contractility, including the striated flagellar roots of flagellated or ciliated eukaryotic cells (also known as rhizoplasts or fibrous system II roots) (Salisbury, 1983; Melkonian, 1980), operates independently of ATP or other organic fuels. The immediate energy for contraction of spasmoneme and rhizoplast is liberated from the Ca^{2+} binding to the spasmin or centrin, which are 20-kd Ca^{2+}-binding proteins. So we have expected that the contractile mechanism of spasmoneme is simpler than that of muscle or cilia and have investigated it to clarify its mechanism.

Isolated spasmoneme exhibits high elasticity in both extended and contracted states, and the tension-extension curves of spasmoneme change their shape in relation to the free Ca^{2+} concentration. Of course, the change of curve shape reflects an internal structural change of spasmoneme induced by Ca^{2+}. We applied the theory of rubber elasticity for the analysis of the tension-extension curves in order to estimate the change of internal structure of spasmoneme. According to the thermodynamic equation of state for elastic deformation, the tension F at length l is

$$F = \left(\frac{\partial U}{\partial l}\right)_T - T\left(\frac{\partial S}{\partial l}\right)_T,$$

where U is the internal energy, S is the entropy, and T is the absolute temperature. In the theory of rubber elasticity, the tension F is caused by the change of entropy only. Strictly speaking, we should consider not only the entropy elasticity

$$-T\left(\frac{\partial S}{\partial l}\right)_T,$$

but also the energy elasticity

$$\left(\frac{\partial U}{\partial l}\right)_T.$$

To examine the participation of the internal energy in elasticity

$$\left(\frac{\partial U}{\partial l}\right)_T,$$

we must investigate whether or not the force at constant extension is proportional to the absolute temperature. Unfortunately, we still have not succeeded in measuring such a small force change. We should note that keratin, elastin, and resilin are all rubber-like proteins; the basis of their elasticity was shown to be entropy elasticity (Meyer and Haselbach, 1949; Hoeve and Flory, 1958; Weis-Fogh, 1961).

The number of segments of a chain in an originally extended spasmoneme in the absence of Ca^{2+} was only 3.3, which means that each chain was almost straight. On the other hand, the number of segments of a chain in contracted spasmoneme in the presence of Ca^{2+} was > 100, which means that each chain was essentially random. Furthermore, the total number of chains in a single spasmoneme was the same in extended and contracted spasmoneme. This means that the cross-links between chains were not influenced by the addition or removal of Ca^{2+}. The molecular weight of a chain between cross-linking points is estimated to be at

most about 50 kd. This molecular weight is almost the maximum value estimated by us, because in spasmoneme there are also endoplasmic reticulum and mitochondria, which may not contribute to spasmoneme elasticity. If the percentage of 2- to 4-nm filaments in spasmoneme is 50%, the molecular weight of a chain becomes 25 kd. Naturally enough, in the cases of natural rubber and synthetic rubber-like substance, the molecular weight of a chain between cross-linking points, which is estimated applying the theory of rubber elasticity, coincides with that estimated by chemical reaction.

It is concluded from all these results that the contractile mechanism of spasmoneme originates in the intramolecular folding and unfolding induced by Ca^{2+} binding and detaching. Spasmoneme is composed mainly of 20-kd Ca^{2+}-binding proteins, spasmins (Yamada and Asai, 1982). Thus it is suggested that the intramolecular folding and unfolding will occur in these spasmins, or that such folding and unfolding will occur in any other proteins but be regulated by spasmins. In any case, it is a proven fact that the intramolecular folding and unfolding by Ca^{2+} binding and detaching is the essential factor of spasmoneme contraction.

Having in mind three central features of a contractile system, we believe that in this paper we have identified and analyzed two of them. We have found that the elasticity of extended spasmoneme is effectively described by a system of molecular chains with unitary segments of 50 kd, whereas on ligation of Ca^{2+}, the segments fold in such a way that their segments became thermally flexible. If contractile systems like these, consisting of two kinds of rubber, are to operate reversibly, there must be a mechanism whereby the ligated ions are removed via chelation by EGTA in the glycerinated model or are pumped out into another compartment of the living organism.

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