

# LATTICE SWELLING WITH THE SELECTIVE DIGESTION OF ELASTIC COMPONENTS IN SINGLE-SKINNED FIBERS OF FROG MUSCLE

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**ABSTRACT** Changes in the 1,0 lattice spacing during trypsin (0.25  $\mu\text{g/ml}$ ) treatment in mechanically skinned single fibers of frog muscle was examined by an x-ray diffraction method at various sarcomere lengths. The resting tension of a relaxed fiber was decreased by trypsin treatment but the stiffness of a rigor fiber was not, suggesting that elastic components were selectively digested. With progression of the digestion, the lattice spacing increased remarkably at longer sarcomere lengths and finally became independent of the sarcomere length. The increase in the lattice spacing was proportional to the decrease in the resting tension. These results support our previous suggestion (Higuchi, H., and Y. Umazume, 1986, *Biophys. J.*, 50:385–389) that the lattice spacing decreases at long lengths due to compressive force exerted by a lateral elastic component that connects thick filaments to an axial elastic component. Consequently, it is unlikely that the decrease in the lattice spacing is determined by a decrease in the repulsive force between thick and thin filaments with stretching a fiber.

## INTRODUCTION

The lattice spacing in skinned fibers decreases with stretching (Matsubara and Elliott, 1972) and continues to decrease even in the fiber stretched beyond overlap between thick and thin filaments (Higuchi and Umazume, 1986). From the proportional relation between 1,0 lattice spacing,  $d_{1,0}$ , and resting tension over a wide range of sarcomere lengths, Higuchi and Umazume (1986) suggested that the decrease in  $d_{1,0}$  with stretching could be attributed to a compressing force exerted by a lateral elastic component connecting thick filaments, which is itself proportional to a resting tension.

Maruyama et al. (1977) observed that the resting tension in a frog skinned fiber decreased after treating a fiber with a low concentration of trypsin and suggested that trypsin digested elastic components. Therefore, it is possible to digest selectively the elastic components using an appropriate concentration of trypsin. According to our previous suggestion,  $d_{1,0}$  should therefore increase by reducing resting tension after selective digestion of the elastic components. To examine this possibility, we determined the relation between the resting tension and  $d_{1,0}$  in single-skinned fibers treated with trypsin.

## MATERIALS AND METHODS

### Fiber Preparation

A single fiber of the semitendinosus muscle of frog (*Rana catesbeiana*) was mechanically skinned (Natori, 1954) in a relaxing solution (90 mM KCl, 5.2 mM  $\text{MgCl}_2$ , 4.3 mM  $\text{Na}_2\text{ATP}$ , 4 mM EGTA, 10 mM PIPES, pH 7.0). To destroy the sarcoplasmic reticulum, segments of skinned

fibers ( $\sim 5\text{-mm}$  long and  $120\text{--}170\text{-}\mu\text{m}$  diam) were immersed for  $\sim 15$  min in relaxing solution containing 0.5% Brij-58 at a sarcomere length,  $L$ , of  $\sim 2.5\text{ }\mu\text{m}$ . All experiments were performed at  $20^\circ\text{C}$ .

### Measurement of the Resting Tension

A segment of skinned fiber was placed in the experimental trough and was mounted horizontally via stainless steel shafts between a tension transducer (UL2-240; Shinkoh Inc., Tokyo, Japan) and a micromanipulator (MM-33; Narishige Scientific Inst. Lab., Tokyo, Japan) (Higuchi and Umazume, 1985). After mounting the fiber,  $L$  was adjusted to a given value ( $2.1\text{ }\mu\text{m} \leq L \leq 5.0\text{ }\mu\text{m}$ ) determined with an optical diffraction method. The digestion of the elastic components was initiated by replacing the relaxing solution with a trypsin solution, i.e., the relaxing solution containing 0.25  $\mu\text{g/ml}$  trypsin (Sigma type III, 9200 BAEE U/mg trypsin).

### Measurement of Rigor Stiffness

Axial stiffness of the rigor skinned fiber was measured by a sinusoidal method. A sinusoidal vibration (0.2% of the fiber length) at 30 Hz was applied to one end of the fiber by a servo motor (model G100PD; General Scanning, Watertown, MA) and the force was detected by a strain gauge (model AE801; Aksjeselskapet, Horten, Norway) connected to the other end. The resonant frequency of the force measurement system was 700 Hz. The fiber was incubated for 10 min in a rigor solution (110 mM KCl, 1.2 mM  $\text{MgCl}_2$ , 4.0 mM EGTA, and 10 mM PIPES, pH 7.0) at  $L = 2.1\text{ }\mu\text{m}$ . Then the fiber was immersed in a rigor solution containing 0.25  $\mu\text{g/ml}$  trypsin. The phase angle between length and force sinusoids was  $\sim 5^\circ$  in both trypsin treated and untreated fibers. This result showed that the rigor fibers behave as an approximately elastic structure. Thus, the rigor stiffness was defined as the peak-to-peak amplitude of the force divided by the amplitude of the applied sinusoidal stretch. The elastic modulus of the untreated fibers in rigor was  $\sim 1.2 \times 10^7\text{ Nm}^{-2}$ .

### X-ray Diffraction

The single-skinned fiber was mounted in a chamber and  $L$  was adjusted to an appropriate value by the method described elsewhere (Higuchi and

Umazume, 1986). The trypsin treatment was initiated by replacing the relaxing solution with the trypsin solution at ~10 and 20 min after adjusting the sarcomere length to  $L \leq 3.0 \mu\text{m}$  and  $L \geq 3.5 \mu\text{m}$ , respectively. (As will be referred to, separate tension experiments had revealed that these times of 10 and 20 min were enough for the complete relaxation of the tension induced by the stretch.) 5 or 10 min after initiation of the treatment, the digestion was terminated by exchanging the trypsin solution with an inhibitor solution, i.e., the relaxing solution containing  $100 \mu\text{g/ml}$  trypsin inhibitor (Sigma type S-1). In the inhibitor solution, the gradual decrease in the resting tension ceased within 30 s, suggesting that the inhibition was complete. 5 min after termination of digestion, the chamber was connected to a peristaltic pump (SJ-1221; ATTO, Tokyo, Japan) to circulate the relaxing solution. Then the equatorial x-ray diffraction pattern was recorded for 40–60 min exposure on x-ray film (DEF-5; Eastman Kodak Co., Rochester, NY) by a double-mirror Franks camera (Franks, 1955). The x-ray source was a rotating-anode generator (model RU-200; Rigaku Electric Co., Tokyo, Japan) with a fine focus on a copper target ( $0.154 \text{ nm}$  in wavelength). The specimen-to-film distance was  $227 \text{ mm}$ . To obtain the  $d_{1,0}$  values, the positions of the 1,0 reflections on the films were measured with a comparator (V-12; Nikon Inc. Tokyo, Japan). Details are found elsewhere (Umazume et al., 1986).

## RESULTS

### Decrease in the Resting Tension by Trypsin Treatment

The resting tension was measured in the trypsin solution at  $2.1 \mu\text{m} \leq L \leq 5.0 \mu\text{m}$ . The stress relaxation of the resting tension after stretching the fibers to  $L \leq 3.0$  and  $\geq 3.5 \mu\text{m}$  almost finished within ~5 and 20 min, respectively. The trypsin treatments were initiated 10 and 30–50 min after adjusting  $L$  at  $L \leq 3.0 \mu\text{m}$  and  $\geq 3.5 \mu\text{m}$ , respectively. Although stirring the solution slightly accelerated the decrease in the resting tension, the measurement was made without stirring to avoid mechanical artifacts. Fig. 1 represents the decrease in the resting tension with progression of the trypsin treatment. The decay rates, which were normalized with respect to the initial resting tension, decreased as  $L$  was increased. For example, times for the resting tension to decrease to 10% of the initial values were ~7, 10, and 13 min at  $L = 3.0, 3.5$ , and  $5.0 \mu\text{m}$ , respectively. The skinned fibers in the trypsin solution tore within 10–20 min at  $2.1 \mu\text{m} \leq L \leq 4.0 \mu\text{m}$  and 20–30 min at  $L = 5.0 \mu\text{m}$ . During the trypsin treatment, the sarcomere length did not change appreciably.

### Effect of Trypsin on Rigor Stiffness

We examined whether the decrease in the resting tension could be attributed solely to the selective digestion of elastic components. If structural proteins other than the elastic proteins are digested by trypsin, the rigor stiffness should decrease. The rigor stiffness at  $L = 2.1 \mu\text{m}$ , however, did not change appreciably in the rigor solution containing trypsin; the ratios of rigor stiffness after the trypsin treatment for 5, 10, and 15 min to that before were  $0.99 \pm 0.01$ ,  $0.97 \pm 0.03$ , and  $0.96 \pm 0.02$  (mean  $\pm$  SD, three fibers measured), respectively. The fiber broke down when it was reincubated in the relaxing solution after

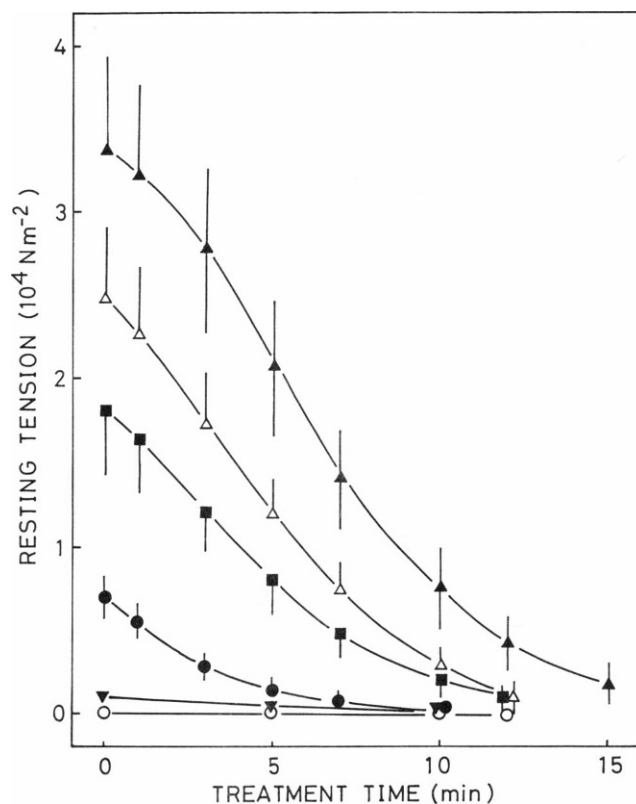


FIGURE 1 The time course of a change in the resting tension with trypsin treatment. The trypsin treatment was started at time zero in a relaxing solution containing  $0.25 \mu\text{g/ml}$  trypsin.  $L = 2.1$  (○),  $2.5$  (▼),  $3.0$  (●),  $3.5$  (■),  $4.0$  (△), and  $5.0 \mu\text{m}$  (▲). The tension was measured in three fibers at each  $L$ . Symbols and bars; means and SD.

15–20-min trypsin treatment. These results suggest that the elastic components were digested selectively at the trypsin concentration used in the present study.

### Change in $d_{1,0}$

We measured  $d_{1,0}$  in fibers treated for 5 or 10 min with trypsin at  $2.1 \mu\text{m} \leq L \leq 5.0 \mu\text{m}$  (Fig. 2). After treatment for 5 min the  $d_{1,0}$  hardly decreased at  $L \leq 3.0 \mu\text{m}$ , but markedly at  $L \geq 3.0 \mu\text{m}$  with stretching the fibers although this decrease was less than in untreated fibers with trypsin.  $d_{1,0}$  did not vary with  $L$  after the 10-min treatment. After both treatments, an increase in the width of the intensity profile and a decrease in the integrated intensity of the 1,0 reflection was observed at all  $L$ 's studied.

### Relation between $d_{1,0}$ and Resting Tension

The relation between  $d_{1,0}$  and the resting tension of skinned fibers treated with trypsin was obtained from the data in Figs. 1 and 2, and is shown in Fig. 3. The solid line in Fig. 3 represents the regression line that had been obtained in skinned fibers without the trypsin treatment (Higuchi and Umazume, 1986);  $d_{1,0} = -1.87T + 41.9$ , where  $T$  is the resting tension in  $10^4 \text{ Nm}^{-2}$  and  $d_{1,0}$  is in nanometers. The  $d_{1,0}$  values after the trypsin treatment were also propor-

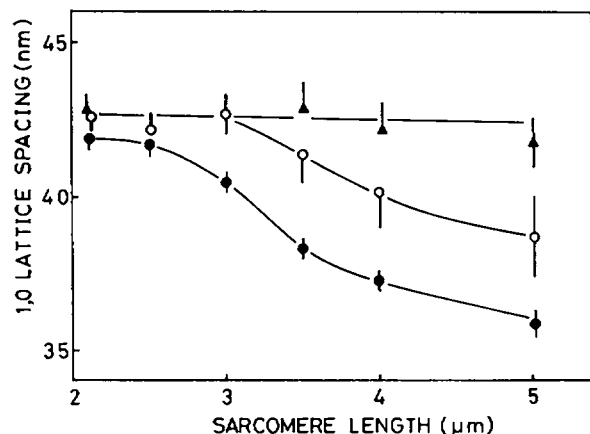


FIGURE 2 Changes in  $d_{1,0}$  with the trypsin treatment. At each symbol,  $d_{1,0}$ 's were measured in three fibers for 5-min (O) and 10-min (▲) treatments with trypsin. Filled circles are for skinned fibers without the trypsin treatment given in Higuchi and Umazume (1986). Symbols and bars; means and SD.

tional to the resting tension, but all data were  $\sim 1$  nm above the regression line.

## DISCUSSION

### Resting Tension

Natori (1954) showed that the mechanically skinned fiber developed a resting tension when stretched and to account for this suggested that elastic components were present in the sarcoplasm. The elastic components in the skinned fiber have been attributed to elastic proteins (Maruyama et al., 1977; Wang et al., 1979). In the present study, a 7–13 min treatment with  $0.25 \mu\text{g/ml}$  trypsin reduced the resting tension to 10% of that without the treatment. We

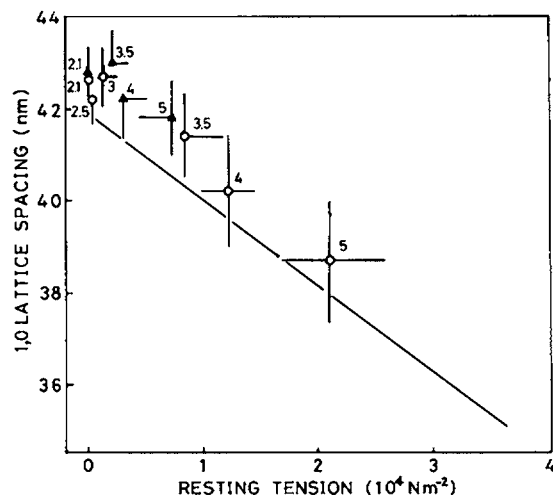


FIGURE 3 Relation between the resting tension and  $d_{1,0}$  after trypsin treatment. Data were taken from Figs. 1 and 2. O, 5-min and ▲, 10-min trypsin treatment. Numbers indicate the sarcomere length. The solid line shows the regression line on skinned fibers without trypsin treatment, which was obtained in Higuchi and Umazume (1986).

checked the possibility that structural proteins other than the elastic components were hydrolyzed. Since the rigor stiffness was hardly affected by the treatment, it is unlikely that appreciable digestion occurred in thick and thin filaments and Z-line. Furthermore SDS PAGE and electron microscopic studies in skinned fibers showed very little, if any, digestion of proteins other than  $\alpha$ -connectin (also called titin 1) by the same treatment as ours (Yoshioka et al., 1986). These results strongly suggest that an elastic component,  $\alpha$ -connectin, is selectively digested by the mild trypsin treatment, which results in the decrease in the resting tension.

The decay rate of the resting tension depended on the sarcomere length during the treatment (Fig. 1): The time course of the decrease in the resting tension was slower at longer sarcomere lengths. One of the possible reasons for the length dependence may be that the structure of elastic components changes with stretching and this change affects the digesting process. An SDS PAGE study that showed the slower digestion of  $\alpha$ -connectin at longer sarcomere lengths (Yoshioka et al., 1986) is consistent with this interpretation.

### Lattice Spacing

The lattice spacing,  $d_{1,0}$ , should be determined by a balance between repulsive and compressing forces on the filaments. As had been suggested by Elliott (1968), one of these would be an electrostatic repulsive force. Matsubara and Elliott (1972) showed that  $d_{1,0}$  decreased as skinned fibers of frog muscle were stretched, and Elliott (1973) attributed this to a reduction in the electrostatic repulsive force between thick and thin filaments; such a force, if it existed, should have been decreased with the decrease in the overlap length of the thin and thick filaments. We confirmed the length-dependence of  $d_{1,0}$  and extended the study (Higuchi and Umazume, 1986); showing that the gradual decrease in  $d_{1,0}$  continued beyond filament overlap (Fig. 2) and was proportional to the resting tension developed by the fibers. From these and other results, we proposed that a lateral elastic component connects thick filaments to an axial elastic component, so that the lateral elastic component exerts a compressing force that depends on the resting tension of the axial elastic component. Thus, the decrease in  $d_{1,0}$  with stretching could result from an increase in the compressing force.

In the present study,  $d_{1,0}$  was not influenced by stretching, when elastic components in the fibers had been selectively digested by the trypsin (Fig. 2). This result is consistent with our previous hypothesis but is difficult to explain from Elliott's view that the length-dependence of  $d_{1,0}$  is ascribable to changes in an electrostatic repulsive force between thick and thin filaments. Therefore, even if such repulsive force exists, it appears to be a minor factor in the decrease in  $d_{1,0}$  on stretching skinned fibers. It should be noted that there may very well be electrostatic

forces between thick and thin filaments. The present conclusion is only that such forces, which would depend directly on the amount of overlap of the thick and thin filaments, are unlikely to be important for the lattice dynamics in skinned fibers.

The increase in  $d_{1,0}$  with the reduction of the resting tension by the trypsin treatment supports our hypothesis. But the relation between the resting tension and  $d_{1,0}$  after the trypsin treatment was found to deviate systematically from that before treatment: all points obtained after the treatment fell above the regression line obtained before the treatment (Fig. 3). This indicates that the force determining the lattice spacing also consists of a component that is independent of the resting tension as well as a component proportional to it. The component independent of resting tension was reduced, at least partially, by our digestion. The structural element underlying this component might be elastic filaments surrounding the myofibrils (Wang and Ramirez-Mitchel, 1983), or a lateral elastic component that exerts the compressing force on thick filaments even at slack sarcomere length of 2.1  $\mu\text{m}$ .

An increase in the width of the intensity profile and a decrease in the integrated intensity of 1, 0 reflection were also observed after the trypsin treatment. These changes can be ascribed to disorder of the lattice structure of thick filaments due to the digestion of the elastic components.

From the results obtained in the previous and present studies, we suggest that a lateral elastic component bound to the axial one contributes to the resting tension and thereby influences the dimensions of the lattice.

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Note Added in Proof: After the present paper had been submitted, a paper on a similar subject (Horowitz et al., 1986) came to our attention. It

showed that passive and active tension generated by chemically skinned fibers were reduced with the radiation inactivation, and suggested that the tension reduction was produced by the destruction of elastic proteins such as titin and neblin. Thus the present method appears to be the chemical counterpart of their study in some respects.

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