変性コイアクトミオシンの表面張力,曳糸性とゲル化

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Surface Tension, Spinnability, and Gelation of Denatured Carp Actomyosin Preparation

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Actomyosin preparation was treated by denaturating agents. When $NH_2C(=NH)NH_2HSCN$ was added to actomyosin preparation in water or 0.6 M KCl, surface tension and spinnability showed the maximum around 3 M, decreased at 6 M and decreased farther at 8 M. When $CO(NH_2)_2$ was added to actomyosin preparation, surface tension and spinnability showed the maxima at 2 M and around 8 M. It was suggested from the great changes of surface tension and spinnability that structure change (unfolding) of actomyosin was induced with the treatments by these denaturating agents.

When actomyosin preparation in water or 0.6 M KCl was treated by $NH_2C(=NH)NH_2HSCN$ or $CO(NH_2)_2$ and dialysed against water to remove the denaturating agent, 6 M-treatment produced firm gel, 4 M-treatment produced medium gel, and 8 M-treatment produced soft gel. It was concluded that the local unfolding of actomyosin molecule by 6 M-treatment was more favoured for the gelation of actomyosin than the complete unfolding by 8 M-treatment. When actomyosin preparation was treated by $NH_2C(=NH)NH_2HSCN$ and dialysed against 0.6 M KCl in place of water, the gel made by 6 M-treatment was softer than the gels made by 4 M- and 8 M-treatments.

The secondary structures of myosin[1,2] and paramyosin[3,4] at low concentrations (0.1–3 mg/ml) in solution were destroyed with the treatment by the denaturating agent such as $CO(NH_2)_2$ or $NH_2C(=NH)NH_2HCl$ and recovered with the dialysis for the removal of the denaturating agent. In a diluted solution of myosin, the myosin molecules stay well apart. When the myosin concentration is increased to a certain extent, some molecules interact with each other and the others are still isolated. When the myosin concentration is further increased, all molecules come into contact with each other. When the myosin solution at such a high concentration is treated by the denaturating agent and dialysed to remove this agent, a conformational change will be induced to form a gel network structure. Niwa *et al.*[5] applied this idea to the gelation of heat-denatured myosin, and made fish myosin gels with the treatment by $CO(NH_2)_2$ and the dialysis. Nakayama *et al.*[6] also applied this idea to the gelation of minced sardine meat, using $NH_2C(=NH)NH_2HSCN$ as a denaturating agent. Though the effect of denaturating treatment on myosin suspension and minced fish meat has been studied so far, the effect of the concentration of denaturating agent was not investigated yet in relation to the texture (strength) of fish protein gel induced by denaturating treatment. If the change of the secondary structure of actomyosin molecule is induced by denaturating treatment, physicochemical properties of actomyosin preparation (*e.g.* surface tension and spinnability) will reflect the structure change of actomyosin. These physicochemical changes will relate to the texture of actomyosin gel formed by the denaturating treatment and the dialysis. Therefore, the effect of the concentration of denaturating agent was investigated in relation to the change of surface tension and spinnability of actomyosin preparation and the texture of actomyosin gel formed by the denaturating treatment and the dialysis.

Materials and Methods

Preparation of Actomyosin Suspension and Solution

Carp actomyosin was prepared by Takashi's procedure.[7] The actomyosin precipitation was prepared by the dilution with 10 volumes of water and the centrifugation. This actomyosin precipitation was dialysed against water or against 0.6 M KCl, 20 mM Tris-maleate buffer (pH 6.8). After the dialysis, more than 35 mg/ml actomyosin suspension in water or actomyosin solution in

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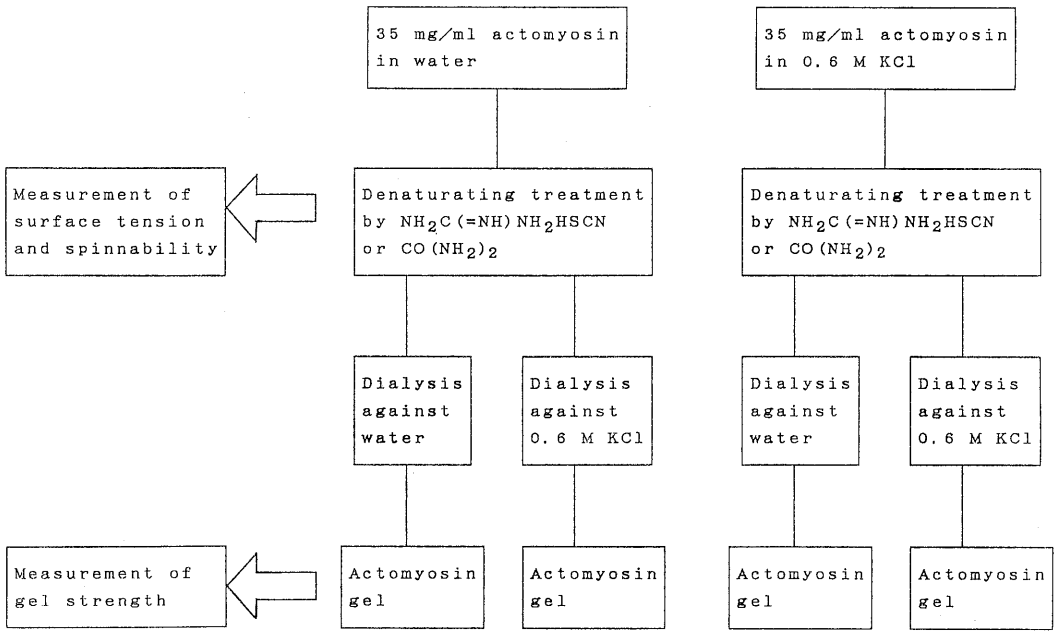

Fig. 1. Schematic diagram of denaturating treatment, dialysis, and gelation of actomyosin preparations.

0.6 M KCl was obtained as shown in Fig. 1. The protein concentration of the suspension in water was calculated from its moisture content determined by drying to constant weight at 120°C. The protein concentration of the solution in 0.6 M KCl was determined by biuret method with the calibration by means of the micro-Kjeldahl method. In each case, actomyosin concentration was adjusted to exactly 35 mg/ml.

Denaturating Treatment

Actomyosin preparations (in water and in 0.6 M KCl) were treated by denaturating agent $NH_2C(=NH)NH_2HSCN$ or $CO(NH_2)_2$ for 3 h. During this treatment the gentle stir was applied by a glass rod to prevent the foam formation. The concentration of denaturating agent was between 1 M and 9 M (molality). Then these actomyosin preparations were offered to the measurement of surface tension and spinnability (Fig. 1).

After the denaturating treatment for 3 h, actomyosin preparation was dialysed against 100 volumes of water or 0.6 M KCl to remove denaturating agent and urge irreversible renaturation of actomyosin. The dialysing solution was changed once a day and the dialysis was continued for four days. If actomyosin gel was formed after the dialysis, it was offered to the measurement of gel strength (Fig. 1 and the section after the next).

Measurement of Surface Tension and Spinnability

A rheometer (Rheoner RE3305, Yamaden Co., Ltd.) was remodeled as shown in Fig. 2 to measure surface tension and spinnability of actomyosin preparations. The load sensor was remodeled to be sensitive to 1 mg by the use of additional amplifier. The force values detected by this load sensor were automatically stored in a personal computer (NEC 9801 VX21) through A/D converter (EMAC 8–1, EMAC Co., Ltd.), and analysed by our own computer program.

Surface tension was the maximum force induced by the touch of platinum plate to the surface of actomyosin preparation when the sample table was raised at 0.5 mm/s. In one case the rise of the sample table was stopped when platinum plate just touched the surface of actomyosin preparation (stop operation) while in another case it was not stopped at that moment but was continued successively (nonstop operation). The stop operation is a standard method for the measurement of surface tension. The delayed response was detectable in this operation. The application of nonstop operation could measure the instantaneous response of surface tension instead of the delayed response. Therefore the difference of the surface tension at stop and nonstop operations showed the extent of the delayed response due to the viscosity. The speed of sample table 0.5 mm/s was most suitable to detect the difference

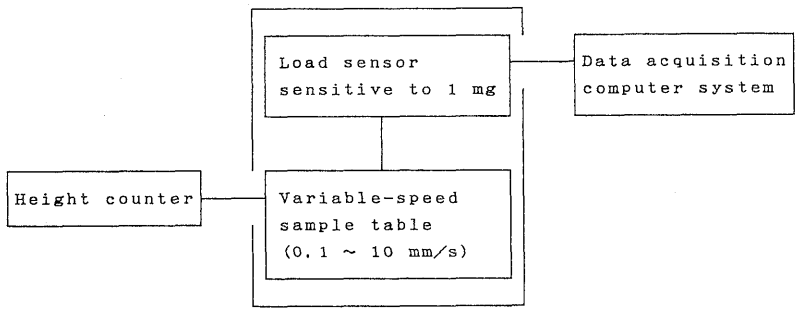

Fig. 2. Measuring apparatus of surface tension and spinnability remodeled by T. Nakayama and Y. Watanabe.

at stop and nonstop operations.

Nakagawa[8,9] and Hironaka *et al.*[10] measured the spinnability of extracts of *Hibiscus manihot* and Chinese yam, which was expressed as the maximum length of a liquid thread spun by a rod raised out of the liquid. The spinnability of the former extract was 10 mm. In our spinnability measurement, a rod measuring 3 mm diameter was used and the immersed length of a rod was adjusted as we monitored the position of sample table by Nakayama-Watanabe type Height Counter.[11] After a rod was immersed exactly 5 mm deep into actomyosin preparation, it was pulled up by lowering the sample table at 10 mm/s. As long as the induced force was detected by the load sensor (Fig. 2), the duration of the lowering of sample table was measaured by computer, and the duration was converted to the distance of the lowering of sample table. The spinnability of actomyosin preparation was shown by this distance less the immersed length of a rod. As the speed of sample table was increased, the spinnability values increased. Therefore the highest speed of our apparatus 10 mm/s was selected in the present experiment.

The measurements of surface tension and spinnability were carried out at 15°C according to the preliminary experiment.[6]

Measurement of Gel Strength

When firm actomyosin gel was formed with the denaturating treatment and the dialysis, gel strength was judged by the creep measurement. When soft actomyosin gel was formed, gel strength was judged with the touch of a finger and visual observation (See the footnote*[3] of Table 1). For the creep measurement, a columnar sample of actomyosin gel was prepared by means of cutter blades set in parallel. Its height and diameter were 1.30 cm and 1.58 cm, respectively. Creep behaviors were measured by Rheoner at 15°C, applying 1 g force for 300 s. The results were automatically stored in a personal computer (NEC 9801 VX21) and were displayed as creep compliance curves.

Results and Discussion

Surface Tension and Spinnability of Actomyosin in Water

Surface tension and spinnability of actomyosin suspension in water were shown in Fig. 3 to examine the effect of denaturating treatment. Standard deviations of surface tension values were less than 0.1×10^{-2} N/m and those of spinnability values were less than 0.2 mm. When $NH_2C(=NH)NH_2HSCN$ was added to actomyosin suspension, the maximum value of surface tension was observed at 3, 4, and 5 M range (the bottom of Fig. 3). On the effect of this denaturating agent, the maximum value of spinnability was observed at 2 and 3 M range (the top of Fig. 3). The values of surface tension and spinnability were lower at 6 M and farther lower at 8 M. It was found from the large difference at stop and nonstop operations that the treatment by 1 M and 2 M agent induced the high viscosity in actomyosin suspension.

When $CO(NH_2)_2$ was added to actomyosin suspension, the first maximum of surface tension was observed at 2 M and the second maximum at 7, 8, and 9 M range, in the case of stop operation (the bottom of Fig. 3). It should be noted from the comparison of stop and nonstop operations that the high viscosity was induced in the vicinity of 2 M and at 6 M. On the effect of this denaturating agent, the first maximum of spinnability was observed at 2 M and the second maximum at 7 and 8 M range (the top of Fig. 3). Spinnability was minimum at 6 M.

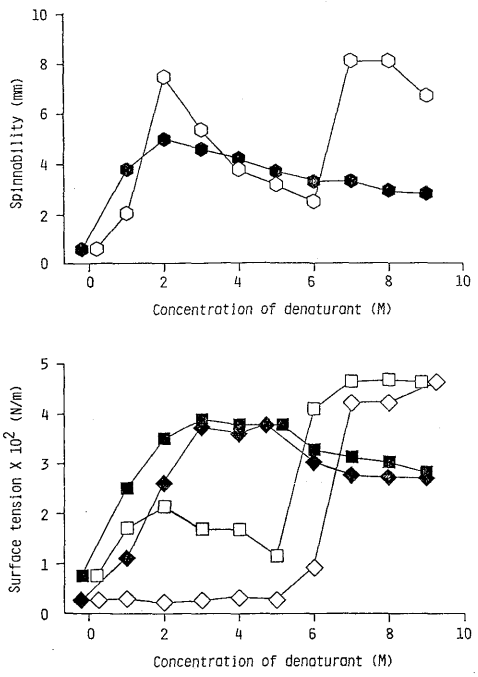

Fig. 3. Effect of denaturating treatment on surface tension and spinnability of actomyosin suspension in water.
$NH_2C(=NH)NH_2HSCN$
■, Surface tension at stop operation.
◆, Surface tension at nonstop operation.
⬢, Spinnability.
$CO(NH_2)_2$
□, Surface tension at stop operation.
◇, Surface tension at nonstop operation.
○, Spinnability.

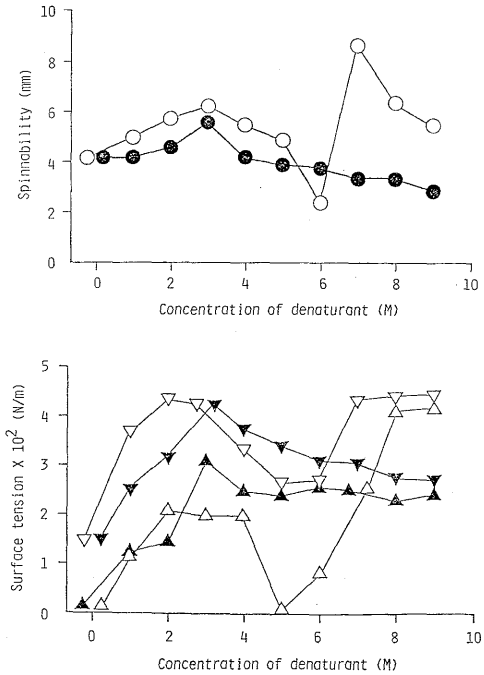

Fig. 4. Effect of denaturating treatment on surface tension and spinnability of actomyosin solution in 0.6 M KCl.
$NH_2C(=NH)NH_2HSCN$
▼, Surface tension at stop operation.
▲, Surface tension at nonstop operation.
●, Spinnability.
$CO(NH_2)_2$
▽, Surface tension at stop operation.
△, Surface tension at nonstop operation.
○, Spinnability.

Surface Tension and Spinnability of Actomyosin in 0.6 M KCl

Surface tension and spinnability of actomyosin solution in 0.6 M KCl were shown in Fig. 4 to examine the effect of denaturating treatment. The general tendency of actomyosin solution was almost the same as that of actomyosin suspension, except that the value of surface tension of the sample with the treatment by 1, 2, 3, and 4 M $CO(NH_2)_2$ was much higher in the case of actomyosin solution (Fig. 4), and except that very high value of spinnability of the sample with the treatment by 2 M $CO(NH_2)_2$ was detected in the case of actomyosin suspension (Fig. 3).

The following result was obtained on the actomyosin solution. When $NH_2C(=NH)NH_2HSCN$ was added to actomyosin solution, the maximum value of surface tension was observed at 3 M (the bottom of Fig. 4). On the effect of this denaturating agent, the maximum value of spinnability was observed also at 3 M (the top of Fig. 4). The surface tension at stop operation and the spinnability were lower at 6 M and even lower at 8 M. It should be noted from the comparison of stop and nonstop operations that actomyosin solution showed high viscosity irrespective of the concentration of $NH_2C(=NH)NH_2HSCN$ used as a denaturating agent.

When $CO(NH_2)_2$ was added to actomyosin solution, the first maximum of surface tension was observed at 2 and 3 M range and the second maximum at 7, 8, and 9 M range, in the case of stop operation (the bottom of Fig. 4). On the effect of this denaturating agent, the first maximum of spinnability was observed at 3 M and the second maximum at 7 M (the top of Fig. 4). Spinnability was minimum at 6 M. It should be noted from the comparison of stop and nonstop operations that actomyosin solution showed high viscosity irrespective of the concentration of $CO(NH_2)_2$ used

as a denaturating agent.

Gels Prepared from Actomyosin in Water

In this section, the starting material for the preparation of actomyosin gel was an actomyosin suspension in water (the top left of Fig. 1).

When the actomyosin suspension was treated by 4, 6, and 8 M $NH_2C(=NH)NH_2HSCN$ and dialysed against water to remove this denaturating agent, an actomyosin gel was formed. Actomyosin gel made with the treatment by 6 M agent was the firmest because its creep compliance (*i.e.* deformation) was the smallest (the left of Fig. 5). It was shown from the comparison of compliances that 6 M-treatment produced a firm gel, 4 M-treatment produced medium gel, and 8 M-treatment produced a soft gel.

The surface tension was approximately 7.2×10^{-2} N/m for water or 0.6 M KCl with any concentration of denaturating agent. As the concentration of $NH_2C(=NH)NH_2HSCN$ was increased in actomyosin suspension, the surface tension increased up to 3.8×10^{-2} N/m at 4 M agent, and then gradually decreased in Fig. 3. Kato and Nakai[12] reported that the presence of protein in water lowered surface tension to 5.4×10^{-2}—6.4×10^{-2} N/m showing a liquid state, but an adequate explanation was not give in molecular basis. It was considered from our result that the actomyosin suspension changed from semi-solid state to liquid-like state as the concentration of agent was increased up to 4 M, and with further increase of agent the suspension changed toward semi-solid state again. The suspensions which existed in liquid-like state around 4 M agent showed relatively high spinnability. It was suggested that the effective structure change of actomyosin which induced the gel formation occurred around the 4 M agent.

Actomyosin gel was not firm yet with the treatment of 4 M $NH_2C(=NH)NH_2HSCN$ (the left of Fig. 5) where the surface tension and the spinnability were relatively high (Fig. 3), and the effective structure change of actomyosin began to occur. Actomyosin gel became the firmest with the treatment by 6 M $NH_2C(=NH)NH_2HSCN$ where the surface tension and the spinnability were medium, and the effective structural change proceeded to some extent. Actomyosin gel was not firm with the treatment by 8 M $NH_2C(=NH)$-NH_2HSCN where the surface tension and the spinnability were relatively low, and the effective structural change proceeded further.

When actomyosin suspension was treated by 4, 6, and 8 M $CO(NH_2)_2$ and dialysed against water to remove this denaturating agent, actomyosin gel was formed but was softer than the gel made with the treatment by $NH_2C(=NH)NH_2HSCN$ (the comparison between the right and the left of Fig. 5). Among the actomyosin gels made with the treatment by $CO(NH_2)_2$ the gel made with the treatment by 6 M agent was the firmest because its creep compliance was the smallest (the right of Fig. 5). It was shown from the comparison of

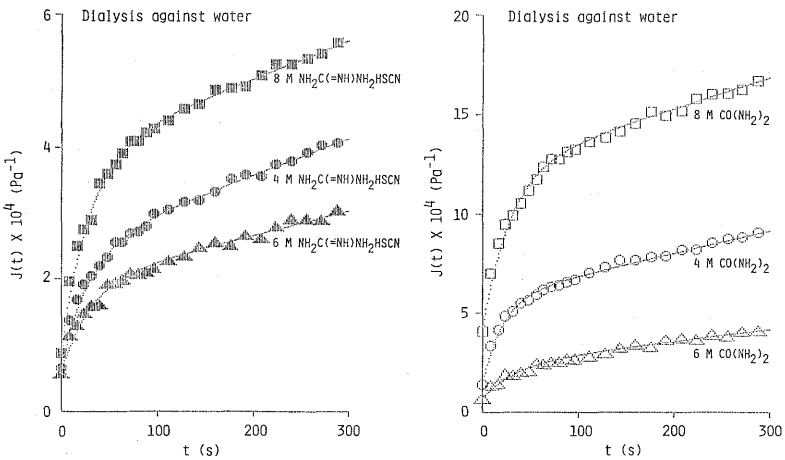

Fig. 5. Creep behavior of gels prepared from actomyosin in water. For the gel preparation, actomyosin was treated by denaturating agent and dialysed against water.
t, Time.
J(*t*), Creep compliance.

the compliances that 6 M-treatment produced firm gel, 4 M-treatment produced medium gel, and 8 M-treatment produced soft gel.

As the concentration of $CO(NH_2)_2$ was increased in actomyosin suspension, the surface tension at stop operation increased up to 2.1×10^{-2} N/m at 2 M agent, and then decreased to 1.1×10^{-2} N/m at 5 M agent in Fig. 3. This change was attended with the increase and decrease of the viscosity as detected by the difference at stop and nonstop operations, and also with the increase and decrease of the spinnability. It was considered from these results that the actomyosin suspension changed from semi-solid state to liquid-like state, and then to semi-solid state again as the concentration of agent was increased up to 5 M. It was suggested that the first step of structure change was completed at 5 M agent. With 6 M agent, the delayed response of surface tension (stop operation) showed the very large value, but the instantaneous response (nonstop operation) showed still the small value. The difference of these values was due to the high viscosity. The spinnability was the minimum. These results were followed by the very large surface tensions at both stop and nonstop operations and the very large spinnability with 7 M or 8 M agent. Therefore it was suggested that the second step of structure change began to occur at 6 M agent and was completed with 7 M or 8 M agent. Nakagawa[9] reported that the spinnability was observed when dynamic viscosity was between 0.1 Pa s and 1 Pa s. In our experiment, the spinnability was not proportional to the viscosity (the difference at stop and nonstop operations) but was related to viscosity change.

Actomyosin gel was not firm yet with the treatment by 4 M $CO(NH_2)_2$ (the right of Fig. 5) where the first step of structure change was almost completed (Fig. 3), and became the firmest with the treatment by 6 M $CO(NH_2)_2$ where the second step of structure change began to occur. Actomyosin gel was not firm with the treatment by 8 M $CO(NH_2)_2$ where the second step of structure change was completed. It was concluded that the local unfolding of actomyosin molecule (the local melting of the helical structure) was more favoured for the gelation of actomyosin than the complete unfolding (the complete melting of helical structure). This conclusion is applicable to our previous results obtained in the gelations induced by KSCN[13] and $NH_2C(=NH)NH_2HSCN$.[6]

When actomyosin suspension was treated by 4, 6, and 8 M $NH_2C(=NH)NH_2HSCN$ and dialysed against 0.6 M KCl in place of water, actomyosin gel was also formed but was softer than the gel made by the dialysis against water (the comparison between Fig. 6 and the left of Fig. 5). The contribution of instantaneous compliance to total compliance was larger in the former gel with the treatment by 6 and 8 M $NH_2C(=NH)NH_2HSCN$ than in the latter. It was shown from the comparison of compliances in Fig. 6 that among the actomyosin gels made by the dialysis against 0.6 M KCl, the gel made with the treatment by 6 M $NH_2C(=NH)NH_2HSCN$ was softer than the gels made with the treatments by 4 and 8 M agent. This result was contrary to that obtained by the dialysis against water.

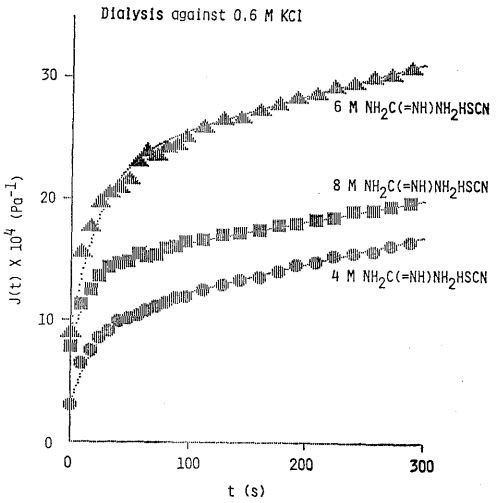

Fig. 6. Creep behavior of gels prepared from actomyosin in water. For the gel preparation, actomyosin was treated by denaturating agent and dialysed against 0.6 M KCl.
t, Time.
$J(t)$, Creep compliance.

When actomyosin suspension was treated by 4, 6, and 8 M $CO(NH_2)_2$ and dialysed against 0.6 M KCl in place of water, actomyosin gel was also formed but was so soft that its creep measurement was impossible. The textures of these gels were judged with the touch of a finger and a visual observation, but the difference in gel strength was not found.

Gels Prepared from Actomyosin in 0.6 *M KCl*

In the previous section, the starting material for the preparation of actomyosin gel was actomyosin suspension in water. In this section, the starting material was actomyosin solution in 0.6 M KCl (the top right of Fig. 1). When actomyosin solution was

Table 1. Strength of gels prepared from actomyosin in 0.6 M KCl*1

Starting material	Denaturating agent	Dialysing solution	Gel strength judged by touch*2 and appearance*3
Actomyosin in 0.6 M KCl	$NH_2C(=NH)NH_2HSCN$		
	4 M	Water	++
	6 M	Water	+++
	8 M	Water	+
	$CO(NH_2)_2$		
	4 M	Water	+
	6 M	Water	++
	8 M	Water	+
	$NH_2C(=NH)NH_2HSCN$		
	4 M	0.6 M KCl	+
	6 M	0.6 M KCl	−
	8 M	0.6 M KCl	+
	$CO(NH_2)_2$		
	4 M	0.6 M KCl	−
	6 M	0.6 M KCl	−
	8 M	0.6 M KCl	−

*1 For the gel preparation, actomyosin was treated by denaturating agent and dialysed against water or 0.6 M KCl.
*2 +++, firm; ++, medium; +, soft.
*3 −: It was extremely soft and failed to keep its columnar shape when it was taken out from dialysis tube.

treated by 4, 6, and 8 M $NH_2C(=NH)NH_2HSCN$ or $CO(NH_2)_2$ and dialysed against water or against 0.6 M KCl, actomyosin gel was also formed but was so soft that its creep measurement was impossible. Therefore in this section, the strength of actomyosin gel was judged with the touch of a finger and a visual observation.

When actomyosin solution was treated by $NH_2C(=NH)NH_2HSCN$ and dialysed against water, 6 M-treatment produced a firm gel, 4 M-treatment produced a medium gel, and 8 M-treatment produced a soft gel as shown in Table 1. When actomyosin solution was treated by $CO(NH_2)_2$ and dialysed against water, the resulting gel with the treatment by 6 M agent was firmer than the gels with the treatments by 4 and 8 M agent. When actomyosin solution was treated by $NH_2C(=NH)NH_2HSCN$ and dialysed against 0.6 M KCl in place of water, the resulting gel with the treatment by 6 M agent was much softer than the gels with the treatments by 4 and 8 M agent on the contrary. When actomyosin solution was treated by $CO(NH_2)_2$ and dialysed against 0.6 M KCl in place of water, the resulting gels were extremely soft and it was impossible to examine the dependence of gel strength on the agent concentration.

In the dependence on the agent concentration, the results obtained by using actomyosin solution as the starting material (Table 1), were very similar to those obtained by using actomyosin suspension (Figs. 5 and 6), except that the absolute value of gel strength was much smaller in the former results than in the latter. Therefore, we conclude that the dependence of gel strength on the agent concentration was not influenced by the starting material (actomyosin suspension or actomyosin solution) but was influenced by the dialysing solution (water or 0.6 M KCl).

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