mobility of the radicals, which promotes cross-linking, insolubilization, and loss of enzymatic activity.

Low water activity not only prevents the recombination of protein radicals, but also prevents cross-linking involving soluble intermediates, such as malonaldehyde. A lesser degree of visually observable browning occurred at low water activities (LeRoux, 1969), and the fluorescence of samples recovered from incubated model systems also was least at the low water activities (Table IV).

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Structural Changes in Actomyosin Induced by Ca^{2+} Ions

Ryo Nakamura

To study the structural changes of actomyosin induced by Ca^{2+} ions, viscometric and light scattering experiments were made on solutions of F-actin, natural actomyosin, and synthetic actomyosin. The viscosity of both F-actin and actomyosin decreased with the addition of Ca^{2+} ions. The amounts of Ca^{2+} ions needed to decrease the viscosity of actomyosin, however, were much smaller than that of F-actin. Although the particle weight of actomyosin did not change at all, its root-mean-square radius $(r^2)^{1/2}$ increased with the addition of small amounts of Ca^{2+} ions such as 100 μM. Both viscosity and the $(r^2)^{1/2}$ value of actomyosin were not affected by the addition of 1 mM Mg^{2+} ions. Discussions are included about these Ca-induced changes of actomyosin and the possible role of Ca^{2+} ions in the meat tenderization phenomenon during post-mortem aging.

Recently Ca^{2+} ions have been shown to have an important role in the meat tenderization phenomenon during postmortem aging (Davey and Gilbert, 1969; Busch et al., 1972a,b; Nakamura, 1972). Although some workers prefer to consider that Ca^{2+} ions activate a muscle protease and cause the meat tenderization phenomenon (Busch et al., 1972b; Penny, 1974; Penny et al., 1974), there is a possibility that Ca^{2+} ions bind to some muscle proteins, change their properties, and increase the tenderness of meat. In a previous work (Nakamura, 1974), to study the effect of Ca^{2+} ions on the properties of muscle proteins, Ca-induced change of viscosity was studied about F-actin which is one of the main components of myofibril. The result obtained from the work was that the viscosity of F-actin decreased largely with the addition of small amounts of Ca^{2+} ions and this phenomenon was explained by the decrease in the hydration of F-actin induced by Ca binding. Structural changes induced by Ca^{2+} ions were also reported about F-actin (Yanagita et al., 1974) and thick filaments (Morimoto and Harrington, 1974).

The purpose of this paper is to study the structural changes of actomyosin induced by Ca^{2+} ions. The differences in Ca-induced changes between actomyosin and F-actin are also studied.

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MATERIALS AND METHODS

Preparation of F-actin, Myosin, and Actomyosin. All muscle proteins used in this experiment were prepared from chicken breast muscle immediately after death. F-actin was prepared as described previously (Nakamura, 1974); G-actin was extracted from the acetone powder and further purified by the method of Spudich and Watt (1971). Myosin was prepared according to the method of Perry (1955). Synthetic actomyosin was made by mixing 1 part of F-actin to 4 parts of myosin by weight in 0.6 M

KCl. Natural actomyosin was prepared according to the method of Endo (1964). The pH of F-actin was adjusted to 5.5 with 20 mM acetate buffer and that of both synthetic and natural actomyosin was adjusted to 7.0 with 20 mM Tris-HCl buffer.

Viscosity Measurements. Viscosity measurements were carried out in an Ostwald viscosimeter with an outflow time for water of about 120 s in a water bath at 25 ± 0.05 °C. When the effect of Ca^{2+} ions was studied, the viscosity was measured 20 min after addition of Ca^{2+} ions. The result was expressed as the specific viscosity (η_{sp}) or the reduced viscosity (η_{red}) according to the following relationship: $\eta_{sp} = \eta_{solution}/\eta_{solvent} - 1$ or $\eta_{red} = \eta_{sp}/C$ (C is concentration).

Light Scattering Measurements. Light scattering measurements were made with a photoelectric light scattering photometer (Shimazu Co. Ltd., Type PG-21) at a wavelength of 4360 Å, and the angular distribution of the scattered light was measured between 35 and 110°.

In the light scattering experiment, the removal of dust from the sample solution and the suppression of the protein–protein interaction are the most important procedures. These procedures are often very difficult and rather time consuming, so in this experiment, some improvements were made about these procedures.

For the removal of dust, the experiments were made according to the method of Schmidt et al. (1974) with slight modifications; briefly, to remove gross impurities, protein solutions were first centrifuged for 20 min at 20000g. The cells were filled with solvent through a Millipore filter, after which the unfiltered concentrated protein solution was added. As the amounts of unfiltered solutions were 0.2–0.3 ml, which is a negligible volume in comparison with the cell content (approximately 32 ml), interference as a result of dust particles was expected to be small. Justification of this assumption was ascertained by the reproducible result obtained. For the suppression of the protein–protein interaction, the experiments were made only at a low protein concentration (0.04–0.08 mg/ml), following the suggestion of Kamata and Nakahara (1973). Results obtained with this method were almost the same as those obtained with the Zimm plot method.

A value of $dn/dc = 0.200$ was used for both synthetic and natural actomyosin according to Gergely (1956) and $dn/dc = 0.227$ was used for F-actin according to Steiner et al. (1951). In order to determine the shape of the proteins, $P^{-1}(\theta)$ was plotted against $V = 3t_g\gamma_0 \sin^2 \theta/2$, where $t_g\gamma_0$ is the initial slope and $P^{-1}(\theta) = R_0/R_\theta$. The experimental curves were compared with the theoretical ones for monodisperse random coils (Peterlin, 1953) and stiff rods (Benoit, 1953).

Measurements of the Protein Concentration. Protein concentration was measured with the micro-Kjeldahl method, the factor 6.2 being used for converting nitrogen values to protein. Measurements were made after dilution of stock solutions. This is an essential procedure for a highly viscous solution such as the concentrated actomyosin solution to reduce the error of dilution.

RESULTS AND DISCUSSION

The specific viscosity of both synthetic and natural actomyosin decreased with the addition of Ca^{2+} ions and a larger change was noted in synthetic actomyosin (Figure 1). As various kinds of myofibrillar proteins in addition to actin and myosin are present in natural actomyosin, a Ca-induced change of actomyosin might be suppressed by these proteins. The viscosity decrease of natural actomyosin, however, was larger than the experimental error. The reduced viscosity of synthetic actomyosin also decreased with the addition of small amounts of Ca^{2+} ions (Figure 2). On the other hand, Mg^{2+} ions did not change the viscosity of actomyosin at a concentration of 1 mM, and the effect of Ca^{2+} ions was not affected by the addition of 1 mM Mg^{2+} ions. Although the viscosity was also measured at pH 5.5, almost the same results as those at pH 7 were obtained.

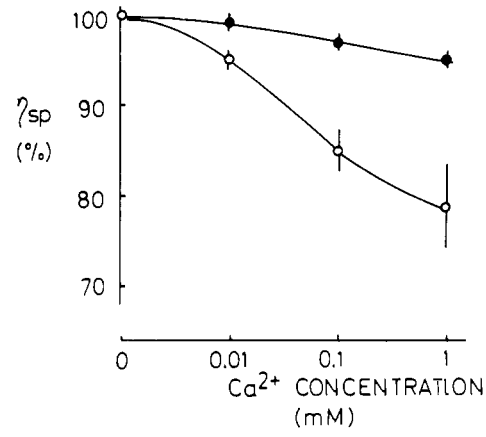

Figure 1. Viscosity change of actomyosin induced by Ca^{2+} ions: (•) natural actomyosin; (○) synthetic actomyosin. The vertical bars show the range of viscosities of four different preparations. The concentration of actomyosin was 0.5 mg/ml.

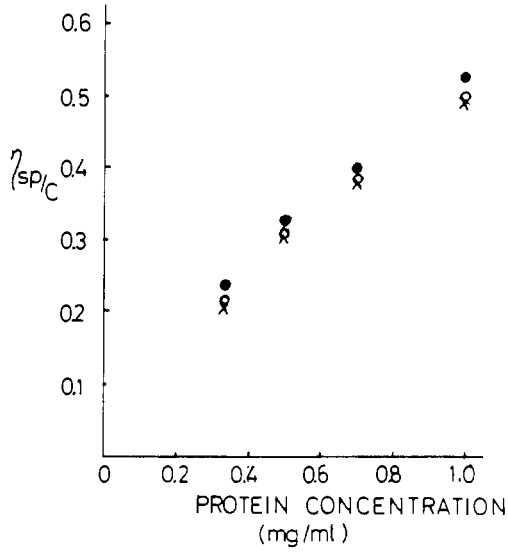

Figure 2. Reduced viscosity of actomyosin in the presence of Ca^{2+} ions: (•) without Ca^{2+} ions; (○) with 100 μM Ca^{2+} ions; (x) with 1 mM Ca^{2+} ions.

The viscosity of F-actin was also shown to decrease with the addition of Ca^{2+} ions (Nakamura, 1974), but this change in viscosity depended on the concentration of the protein (Figure 3); the larger amounts of Ca^{2+} ions were needed to decrease the viscosity at higher protein concentrations. The concentration of Ca^{2+} ions needed to decrease the viscosity of both actomyosins, however, did not vary with the concentration of protein within the range between 0.3 and 1 mg/ml. Furthermore, the amounts of Ca^{2+} ions needed to decrease the viscosity of both actomyosins were very small compared with F-actin. Since the reduced viscosity of F-actin is higher than that of actomyosin at the same protein concentration (compare Figure 3 of the previous work (Nakamura, 1974) with Figure 3 of this work), the protein–protein interaction of F-actin might be stronger than that of actomyosin. The differences in the Ca-induced viscosity change between F-actin and

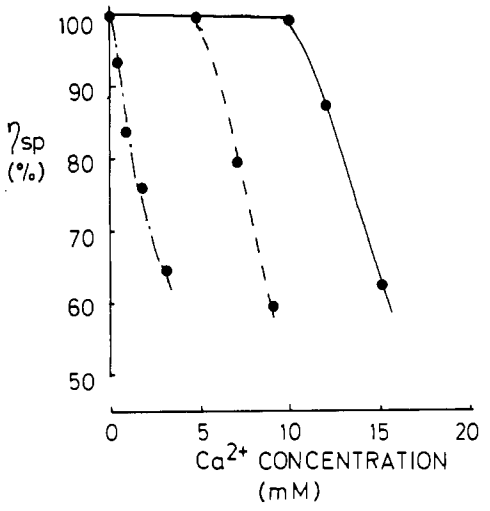

Figure 3. Viscosity change of F-actin induced by Ca^{2+} ions. The concentrations of F-actin were as follows: (●–●) 0.5 mg/ml; (●- - -●) 0.3 mg/ml; (●· · ·●) 0.2 mg/ml.

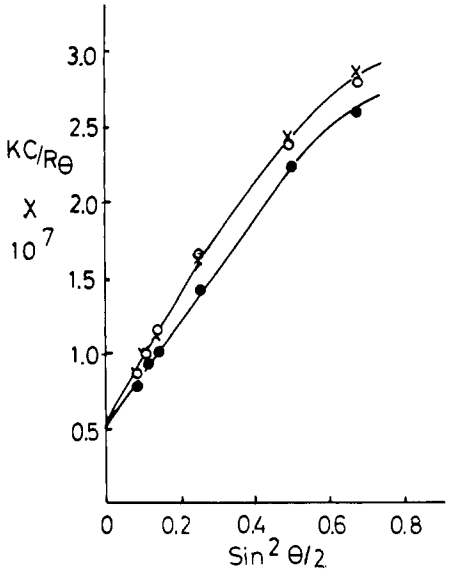

Figure 4. KC/R_θ vs. $\sin^2\theta/2$ plot for synthetic actomyosin in the presence of Ca^{2+} ions: (●) without Ca^{2+} ions; (○) with 100 μM Ca^{2+} ions; (x) with 1 mM Ca^{2+} ions. The concentration of protein was 0.06 mg/ml.

actomyosin might be explained by the difference in the intermolecular forces of these proteins; in the presence of Ca^{2+} ions, F-actin molecules form aggregates by their strong intermolecular forces, but actomyosin molecules change their properties without the formation of aggregates. To study the Ca-induced changes of the actomyosin molecule, the effect of the intermolecular forces must be removed. Intrinsic viscosity is usually measured for such a purpose. However, enough extrapolation could not be made about the actomyosin solutions used in this experiment, so a precise value could not be obtained. Further analysis was made using light scattering experiments, in which the protein concentration was so small that the protein–protein interaction was largely removed.

The difference in the Ca-induced changes between F-actin and actomyosin was also recognized from the results of the light scattering experiments. The slope of the KC/R_θ vs. $\sin^2\theta/2$ plot for both actomyosins increased with the addition of Ca^{2+} ions, although the intercept of the vertical did not change at all (Figures 4 and 5). Such actomyosin behavior differed largely from that of F-actin;

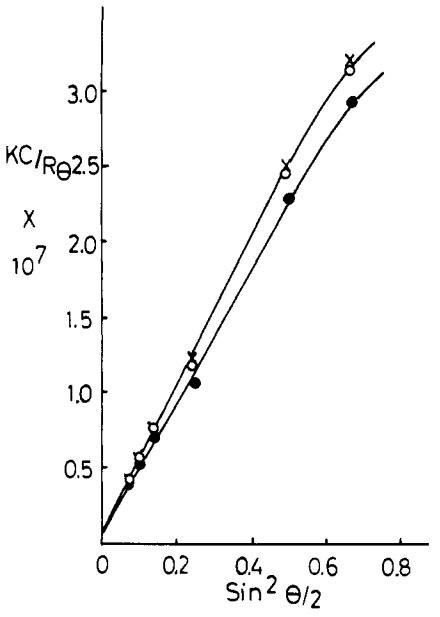

Figure 5. KC/R_θ vs. $\sin^2\theta/2$ plot for natural actomyosin in the presence of Ca^{2+} ions: (●) without Ca^{2+} ions; (○) with 100 μM Ca^{2+} ions; (x) with 1 mM Ca^{2+} ions. Concentration of protein was 0.04 mg/ml.

Table I. Ca-Induced Change in the Size and Shape of Actomyosin and F-actin

Expt no.	Particle wt × 10^{-7}			$(r^2)^{1/2}$ × 10^{-3} (Å)		
	Synthetic Actomyosin					
	[Ca]			[Ca]		
	0	100 μM	1 mM	0	100 μM	1 mM
1	1.7	1.7	1.7	1.3	1.4	1.4
2	2.2	2.2	2.2	1.4	1.5	1.5
3	2.5	2.5	2.5	1.6	1.7	1.7
4	1.2	1.2	1.2	1.6	1.7	1.7
	Natural Actomyosin					
	[Ca]			[Ca]		
	0	100 μM	1 mM	0	100 μM	1 mM
1	33	33	33	3.9	4.1	4.1
2	50	50		7.4	7.6	
3	50	50		6.7	6.9	
	F-actin					
	[Ca]			[Ca]		
	0	1 mM	10 mM	0	1 mM	10 mM
1	1.4	2.0	5.0	3.0	3.0	4.5
2	4.0	4.5	12.5	3.8	3.8	4.9
3	1.7	1.7	2.0	1.5	1.5	1.5

the intercept of the vertical decreased and the slope of the KC/R_θ vs. $\sin^2\theta/2$ plot did not change with the addition of either 10 mM Ca^{2+} ions (Nakamura, 1974) or 1 mM Ca^{2+} ions (Nakamura, 1975). These differences were definitely shown by the calculation of both the $(r^2)^{1/2}$ value and the particle weight of these proteins (Table I); the $(r^2)^{1/2}$ values of both actomyosins increased a little with the addition of Ca^{2+} ions as small as 100 μM although their particle weights did not change at all in the presence of 1 mM Ca^{2+} ions. The $(r^2)^{1/2}$ value of F-actin did not change with the addition of 1 mM Ca^{2+} ions and its particle weight increased a little.

The data on particle weight show that with the addition of Ca^{2+} ions the actomyosin molecule does not aggregate although the F-actin molecule aggregates easily. The data on $(r^2)^{1/2}$ values show that a Ca-induced change of actomyosin may be either the change in the shape, the increase of polydispersity, or both. To study this change of the $(r^2)^{1/2}$ value of actomyosin, the angular dependence of light

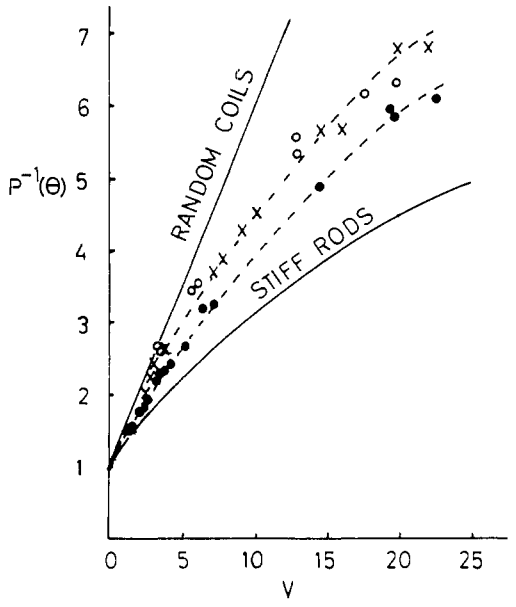

Figure 6. Comparison of observed angular dependence of light scattering of synthetic actomyosin with theoretical curves for random coils and stiff rods: (•) without Ca^{2+} ions; (○) with 100 μM Ca^{2+} ions; (x) with 1 mM Ca^{2+} ions.

scattering was compared with the theoretical curves for monodisperse random coils and stiff rods (Figure 6). Figure 6 shows that the actomyosin curve is between two theoretical ones and this result is almost the same as that of Gergely (1956). Addition of Ca^{2+} ions displaces the curve near that of random coils. If the increase of polydispersity were the main event caused by Ca^{2+} ions, the curve should be near that of stiff rods rather than that of random coils. However, as the change toward random coils induces the decrease of the $(r^2)^{1/2}$ value in a monodisperse system, one cannot explain the result of Figure 6 from the viewpoint of simple shape change in a monodisperse system.

Morimoto and Harrington (1974) found that Ca^{2+} ions increased the sedimentation coefficient of the thick filaments isolated from rabbit muscles. Although they could not explain this phenomenon sufficiently, one suggestion was made in their report; Ca^{2+} ions bind to the DTNB light chain, make the thick filaments more flexible, and alter its sedimentation coefficient. The Ca-induced change of actomyosin found in this work is relatively small and seems to be too complicated to predict the real changes induced by Ca^{2+} ions. However, this change is induced by the same low concentration of Ca^{2+} ions as that of Morimoto and Harrington (1974), so one might speculate that the actomyosin molecule in this experiment becomes flexible through the binding of Ca^{2+} ions.

As shown in Table II, Mg^{2+} ions did not affect the $(r^2)^{1/2}$ value of actomyosin at the concentration of 1 mM. The effect of 5 mM Mg^{2+} ions on $(r^2)^{1/2}$ value was still lower

Table II. Ca-Induced Change in the $(r^2)^{1/2}$ Value of Synthetic Actomyosin in the Presence of Excess Mg^{2+} Ions

Addition of Ca^{2+} or Mg^{2+} Ions	$(r^2)^{1/2}$ with Ca^{2+} or Mg^{2+}/$(r^2)^{1/2}$ without Ca^{2+} or Mg^{2+}
None	1.0
1 mM Mg^{2+}	1.0
3 mM Mg^{2+}	1.02
5 mM Mg^{2+}	1.07
100 μM Ca^{2+}	1.08
1 mM Mg^{2+} + 100 μM Ca^{2+}	1.08
5 mM Mg^{2+} + 100 μM Ca^{2+}	1.08

than that of 100 μM Ca^{2+} ions. Addition of 100 μM Ca^{2+} ions in the presence of 1 mM Mg^{2+} or 5 mM Mg^{2+} ions increased the $(r^2)^{1/2}$ value of actomyosin. More work should be done to explain the mechanism of the meat tenderization phenomenon based on the results obtained in this work. However, since the amounts of Ca^{2+} ions needed to cause these changes are as small as those found in muscles during the conditioning period (Nakamura, 1973) and since the large amounts of Mg^{2+} ions which are usually present in muscles do not relate to these changes of actomyosin, there is a possibility that the actomyosin molecule in muscles may change its structure with increased Ca^{2+} ions.

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