IMPROVEMENT OF GEL STRENGTH MEASURING DEVICE AND ITS APPLICATION TO MYOSIN GEL

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INTRODUCTION

Myosin is the major protein of striated muscle myofibrils and it plays an important role in water holding and binding properties in processed meat. When myosin is heated at appropriate salt concentrations and pH levels, it forms a gel. Many types of gel strength measuring devices are available in the market, however, most of them are not that sensitive for weak gels and unsuitable for the small volume of samples. Yasui et al. (1979) developed a shear modulus tester for measuring gel strength of muscle protein. Their device requires only about 4 ml of the sample and is quite sensitive to measure weak gel strength. However, the operation of their device is not so easy. We have improved Yasui's gel strength measuring device to reduce sample volume and to achieve easy operation, and applied it to evaluate heat-induced myosin gel.

MATERIALS AND METHODS

Myosin was prepared and purified by the method of Offer et al. (1973) from rabbit back and white portion of leg muscles. Myosin was stored as ammonium sulfate precipitate at 4°C until use. It was dialyzed against 0.5 M KCl to remove ammonium sulfate and clarified by centrifugation just before use. Protein concentration was determined by UV absorption at 280 nm. Outline of the gel strength measuring device is shown in Fig. 1. A rectangular stainless steel blade (7.5 x 19 mm), glued on a needle, was fitted into a 10 g load cell by a Leur connector. A load cell was connected to a strain amplifier. A cuvette which contained 2 ml of protein solution was placed in a thermostatically controlled cell holder on a stage. The position of the stage was adjusted so that the blade immerse in the sample at a depth of 5 to 10 mm. After heating at 65°C for appropriate time, the stage was lowered by 1 or 2 mm. The force detected by a load cell was recorded and then the rigidity was calculated from the following equation:

rigidity = $(980.7PH)/(2Sd)$ dyne/cm^2

where P is the weight (g) required to pull the blade in the gel by a distance (d cm), H is the thickness of the gel on both sides of the blade, 2 is a constant factor, and S (cm^2) is the area of blade immersed in the gel. The improved device required only 2 ml (or less) of a protein solution and it gave successful results.

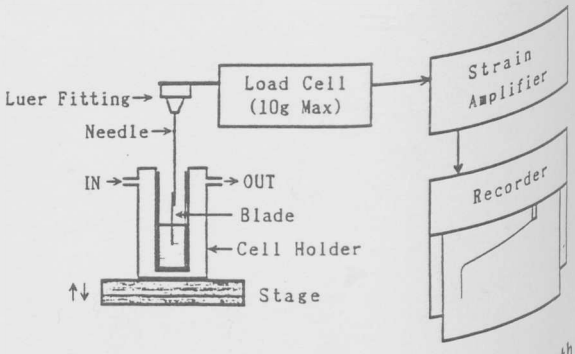

FIGURE 1. Outline of the gel strength measuring device.

RESULTS AND DISCUSSION

Gel strength measuring device

The temperature of the sample was monitored from 0 to 20 min during heating (Fig. 2). The temperature of circulatory water hold to be maintained about 69°C to obtain the final temperature of 65°C because of the heat-loss during circulation. The temperature of the sample raised

rapidly and reached 65°C after 5 min of heating. The myosin solution became turbid after a couple of minutes of heating, indicating the formation of gel.

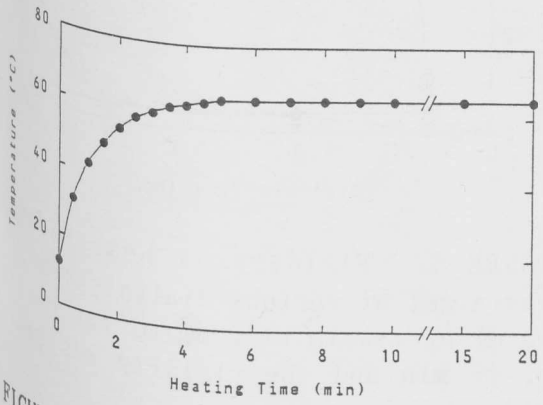

FIGURE 2. Changes in temperature of the sample during heating.
Two ml of 0.1 M KCl and 20 mM K phosphate in a glass cuvette (1 x 1 x 4 cm) was held in a thermostatically controlled cell holder. The temperature was measured every 30 sec.

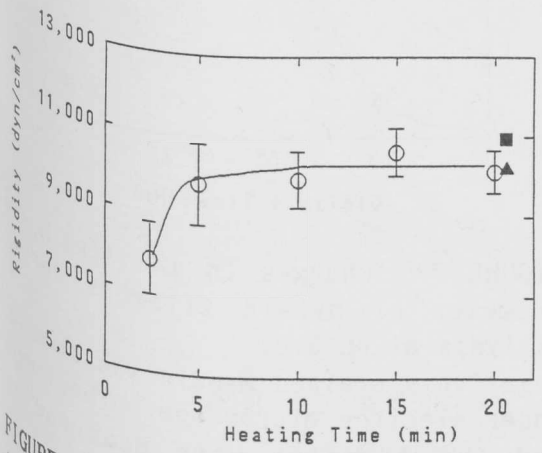

FIGURE 3. Effect of heating time on the rigidity of myosin.
Myosin in 0.1 M KCl and 20 mM K phosphate (pH 6.0) and 2.5 mg/ml was heated at 65°C. The stage was lowered by 2 mm with a speed of 10 mm/min. Bar indicates standard deviation. ■ and ▲ are the values obtained by Yasui's band type viscometer.

The rigidity of myosin in 0.1 M KCl and pH 6.0 was measured at appropriate intervals after heating (Fig. 3). Although the gelation occurred at 2.5 min of heating, it was found to be weak. Rigidities of the gels formed from 5 to 20 min of heating were almost the same, but the standard deviation in the 5 min sample was greater than those of others, suggesting the formation of gel was not completed.
Fig. 4 shows the rigidity of myosin in 0.5 M KCl and pH 6.0. Rigidity at this KCl concentration was considerably lower than that at 0.1 M. In this case, rigidity reached its maximum value just after 10 min. The results in Fig. 3 and 4 suggest that 15 min heating is sufficient to measure rigidity with this device.

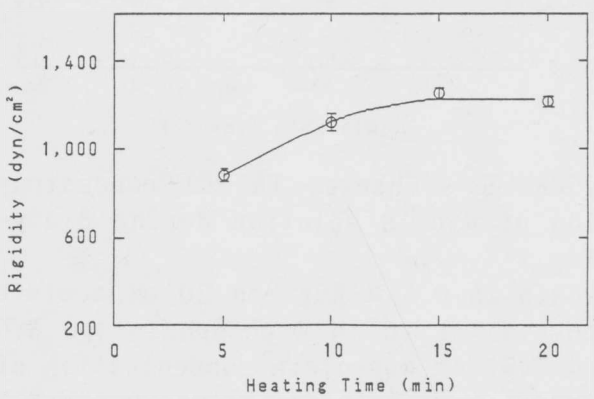

FIGURE 4. Effect of heating time on the rigidity of myosin.
Myosin in 0.5 M KCl and 20 mM K phosphate (pH 6.0) and 4.0 mg/ml was heated at 65°C. The stage was lowered by 1 mm with a speed of 10 mm/min. Bar indicates standard deviation.

Effect of the downward speed of the stage on the measurement of rigidity was examined. The stage speed from 5 to 40 mm/min had no effect on the rigidity.

Effect of salt concentration and pH on the rigidity of myosin gel

It is known that myosin aggregates to form filament at low ionic strength

salt solutions (Huxley, 1963). The length and thickness of filaments are varied by the final ionic strength and pH levels of the solution (Kaminer, 1969).

Myosin in 0.5 M KCl was dialyzed against 0.1 M KCl and Fig. 5 shows the changes in KCl concentration by dialysis. KCl concentration rapidly decreased from the beginning of dialysis to 1 hr and it reached approximately 0.1 M after 2 hr.

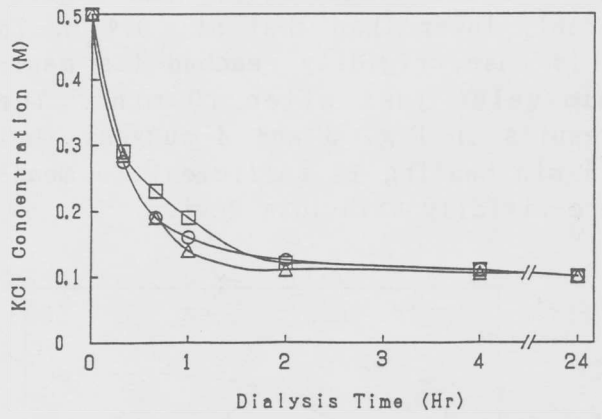

FIGURE 5. Changes in KCl concentration of myosin solution during dialysis.
Myosin in 0.5 M KCl and 20 mM acetate (pH 5.5) or 20 mM K phosphate (pH 6.0 and 6.5) at a protein concentration of 5 mg/ml was dialyzed against 0.1 M KCl and 20 mM buffer at 4°C. △ ;pH 5.5, ○;pH 6.0, and □ ;pH 6.5.

The samples at pH 5.5 and 6.5 showed low rigidities throughout the dialysis time, except that the sample at pH 6.5 at 24 hr. On the other hand, the rigidity of the sample at pH 6.0 rapidly increased from 0.66 to 1 hr of dialysis and it gradually increased until 4 hr (Fig. 6).

The formation of myosin filament by decreasing the salt concentration at pH 6.0 was observed under electron microscope and the length and the diameter of the filaments were measured. As shown in Fig. 7, the filaments were grown up after 0.3 hr and they reached maximum value after 1 hr of dialysis. The rapid growth in filament length was observed from 0.3 to 0.66 hr of dialysis. In contrast, the growth of diameter of a filament was almost linear up to 0.66 hr and the filament gained maximum diameter at 24 hr of dialysis. These results

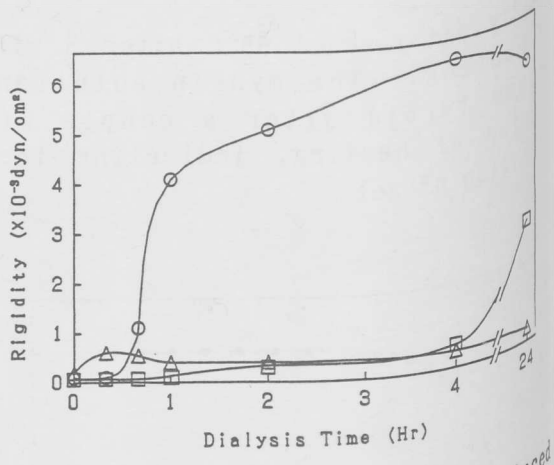

FIGURE 6. Rigidity of heat-induced myosin gel at various dialysis time. Two ml of myosin at 2 mg/ml was heated for 15 min and the rigidity was measured.

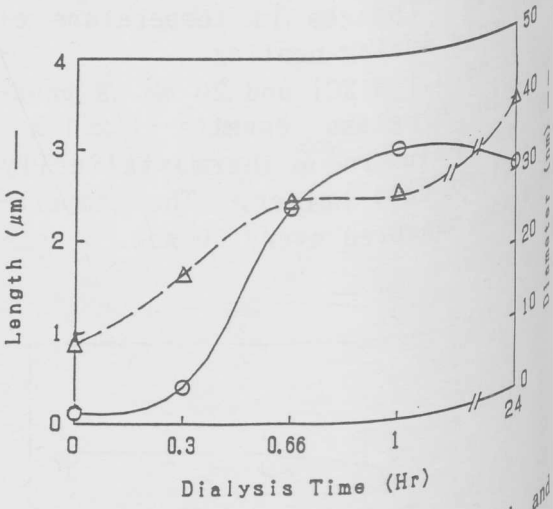

FIGURE 7. Changes in the length and diameter of myosin filament during dialysis at pH 6.0.
Negatively stained myosin was observed under electron microscope. The length and the diameter were measured on enlarged prints.

suggest that the growth in length and thickness of myosin filaments do not occur concomitantly.

As we have reported (Yamamoto et al. 1988), the speed of lowering the salt concentration affects the rigidity of heat-induced myosin gel. Rapid dilution formed shorter filaments which produced weak gels having aggregated structure. On the other hand, gradual decrease in salt concentration produced longer filaments and their heat-induced gels had a fine network structure and gave high rigidity.

The present study and our earlier studies suggest that the rigidity of heat-induced myosin gel strongly depend on the degree of aggregation of myosin molecule into filaments before heating.

CONCLUSION

Gel strength measuring device designed by Yasui et al. was improved to be more effective and convenient and also to reduce the sample volume. The device was sensitive enough even for weak gels less than 1,000 dyn/cm^2. Two ml of a sample solution was enough to measure the rigidity and this device would be useful for small and/or weak gels such as muscle protein gels.

The rigidities of heat-induced myosin gels increased by lowering the salt concentration. Myosin formed strong gels at pH 6.0, however, the gels formed at pH 5.5 and 6.5 were weak. It can be concluded that the gel strength of myosin depends on the state of myosin before heating.

ACKNOWLEDGMENT

We wish to thank Dr. W.C.D. Livera, Department of Food Science, Rakuno Gakuen University, for reading the manuscript.

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