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Morphological Changes of Carp Actomyosin during Thermal Treatments

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Abstract

Morphological changes in carp actomyosin during thermal treatments were observed under an electron microscope. Rheological profiles were measured simultaneously. The structure of actomyosin arrowheaded filaments and straight filamentous F-actin were transformed with an increase in temperature over the range of 30-45°C. At the same time, aggregated masses appeared and seemed to be formed by aggregations of myosin released from actomyosin filaments. Similar morphological changes were observed when heating time extended from 3 to 12 h at 30°C. In the range of 50-85°C, the filamentous structures of actomyosin and F-actin were lost and the aggregated masses changed to coagulated masses with smooth outlines.

Introduction

It is known that myosin constituted myofibrillar proteins mainly contribute to the elasticity of surimi-based products¹⁻³). Frozen surimi is composed of myofibrillar proteins (namely actin and myosin)⁴), and actomyosin filaments were found in salt added frozen surimi (Ka-en surimi)⁵). When myofibrillar proteins were mixed with sodium chloride, the main components were dissolved and formed actomyosin sol, and the sol then changed to actomyosin gel with a fine network structure during *suwari* (setting incubation) and heat processing. No morphological changes of actomyosin filaments have yet been reported during thermal treatments. In the present paper, electron microscopic observation was carried out mainly to investigate the transformation of carp actomyosin during thermal treatments.

Materials and Methods

Preparation of sample

Actomyosin was prepared from carp dorsal muscle using the method of Takashi et al.⁶). An actomyosin solution (5-6 mg/ml for electron microscopic observation and 43.7 mg/ml for rheological profiles) was heated for 5 min at 25, 30, 35, 40, 45, 50,

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and 85°C, and cooled in an ice-water bath. Another solution (38.5 mg/ml) was heated for 1–48 h at 30°C, and cooled in an ice-water bath. Protein concentration was determined by the biuret method⁷⁾.

Electron microscopic observation

Diluted sample solutions (0.1–0.5 mg/ml) were prepared after thermal treatments and the samples were negatively stained with 2% uranyl acetate solution and observed under a Hitachi HU-12 type electron microscope with an accelerating voltage of 75 kV⁸⁾.

Rheological profiles

Hardness and adhesiveness of actomyosin samples during thermal treatments were measured by a Rheolometer (Iio Denki Co., RMT-1300). The diameter of the cylindrical plunger was 30 mm. The sample was packed in a small petri dish (inside size : 45 mm diameter and 12 mm height).

Results and Discussion

Fine actomyosin filaments with arrowhead structures were observed and were similar to those of freshly prepared actomyosin after treatment with a solution of actomyosin for 5 min at 25°C and 30°C (Fig. 1A and B). After 5 min at 35°C, intact actomyosin filaments with arrowhead structures remained similar to those at 30°C and below, but aggregated masses were significant (Fig. 1C). After thermal treatments for 5 min at 40°C, the arrowhead structure of actomyosin filaments were transformed, and aggregated masses became evident. Since naked F-actin filaments were observed, it is presumed that myosin was released from F-actin and formed the aggregated masses (Fig. 1D). At 45°C, no arrowhead filaments of actomyosin were apparent and the naked straight F-actin filaments were not found in any significant amount (Fig. 1E). Under the conditions of 50°C and 85°C, hardly any F-actin filaments were noticeable (Fig. 1F and G). The aggregated masses changed to coagulated masses with smooth outlines. It has not yet been ascertained whether the aggregated masses form the coagulated masses with an accompanying coagulation involving the F-actin filaments, or whether the F-actin filaments change to shortened filaments⁹⁾.

No changes in hardness were found after heating for 5 min at 25–35°C (Fig. 2), and the overall appearance was transparent and almost the same as before heating. The hardness of samples increased at 40°C, and the transparency was lost and changed to white, and coagulation was also apparent. At 45°C, the hardness increased beyond that at 40°C, and reached a maximum at 50–85°C. Released water was found on the surface of white coagulated samples in the range of 45–85°C. Adhesiveness of the sample decreased with an increase in the heating temperature, and was zero in the range of 45–85°C (Fig. 2).

An analysis of the electron microscopic observation in conjunction with gel hardness yields the following possible explanation. The arrowhead structure of actomyosin was transformed, and aggregated masses were found when gel hardness increased (at 40°C). No arrowhead structure was observed, and aggregated masses became abundant as the heating temperature rose to 45°C. When hardness was at

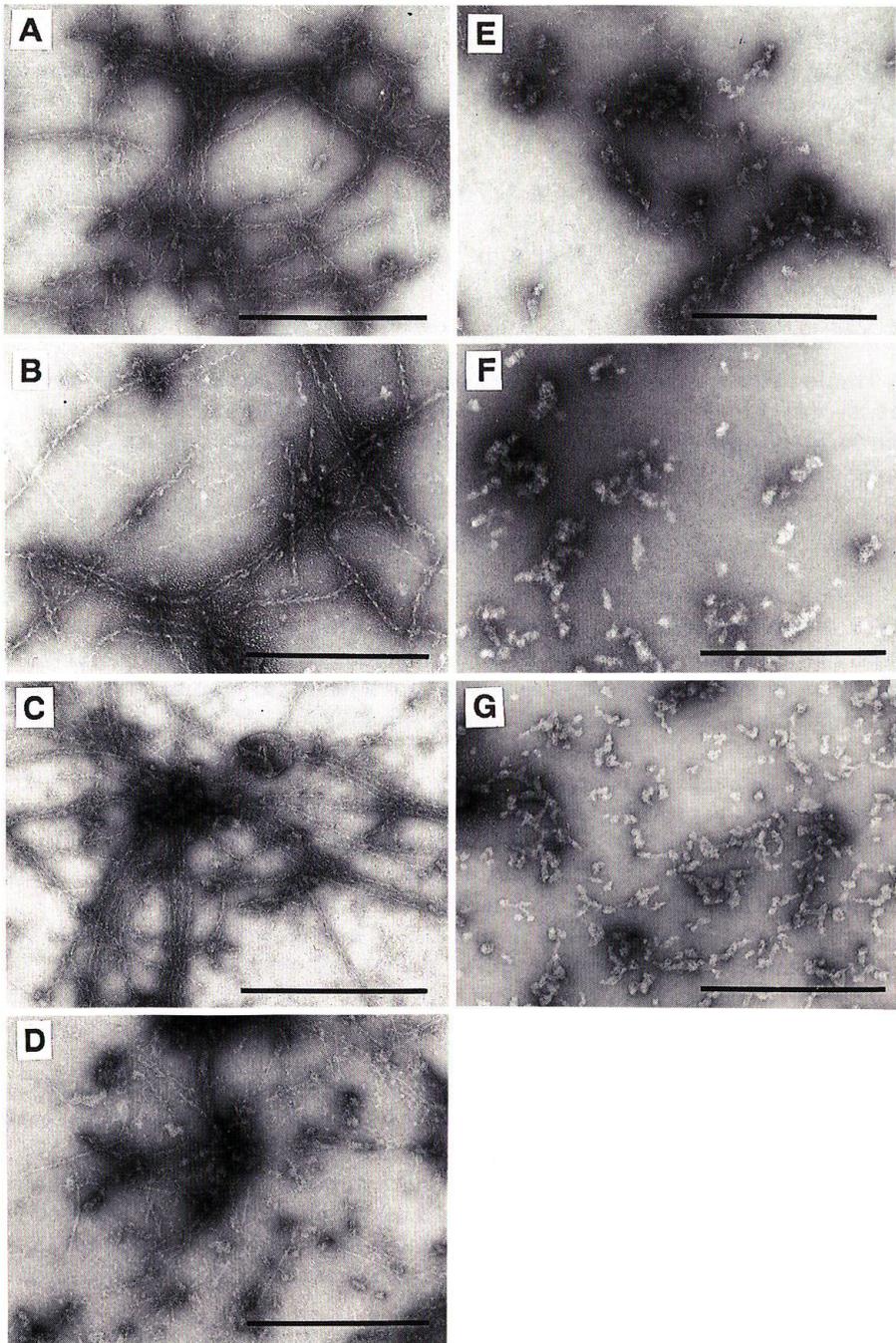


Fig. 1. Electron micrographs of carp actomyosin solutions heated for 5 min at different temperatures.

Heating temperatures are A, 25°C ; B, 30°C ; C, 35°C ; D, 40°C ; E, 45°C ; F, 50°C ; and G, 85°C. In all electron micrographs, the bars represent 500 nm.

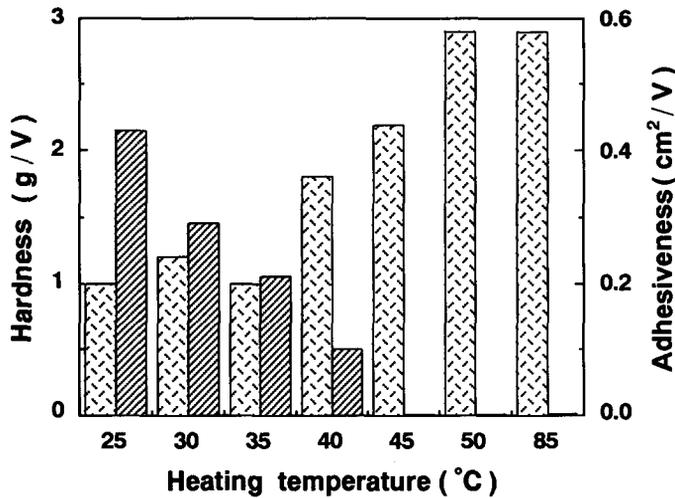


Fig. 2. Hardness and adhesiveness of carp actomyosin sol heated for 5 min at different temperatures.

its maximum in the range of 50–85°C, the aggregated masses changed to coagulated masses, and at the same time, the naked F-actin filaments also disappeared. From the above results, it can be assumed that the transformation of actomyosin filaments with arrowhead structure and F-actin filaments seems to be related to gel formation. Since the arrowhead structure of actomyosin filaments was transformed in preference to the loss of straight filamentous structures of F-actin, the aggregated masses may have likely formed by the aggregation of released myosin. Therefore, it seems that gel hardness can mainly be ascribed to the aggregated masses of myosin. This explanation is compatible with the interpretation reported previously¹⁾.

Morphological changes of actomyosin solution were observed during 1–48 h at 30°C. No morphological changes were observed and intact arrowheaded actomyosin filaments were found after treatments over 1–2 h at 30°C (Fig. 3A and B). After 3–4 h at 30°C, aggregated masses and arrowhead-poor actomyosin filaments appeared (Fig. 3C and D). The aggregated masses and straight F-actin filaments with little myosin attached were abundant after 6 h (Fig. 3E). After 12 h, no arrowhead filaments of actomyosin were apparent and only a few F-actin filaments were found (Fig. 3F). This morphological observation was almost the same after 24–48 h (Fig. 3G).

When the actomyosin solution was kept at 30°C, morphological transformation of arrowheaded actomyosin occurred and at the same time aggregated masses were formed by the aggregation of myosin released from actomyosin filaments. The structure of straight F-actin filaments was then lost. From the above results, it is assumed that the myosin component denatured faster than the F-actin component. This explanation is supported by the first-order mode of thermal inactivation of actomyosin reported by Arai et al.¹⁰⁾. It has been reported that in the case of freeze denaturation of actomyosin due to the effects of salt concentration, granular matters were apparent, and at the same time, actomyosin (myosin B) filaments were shor-

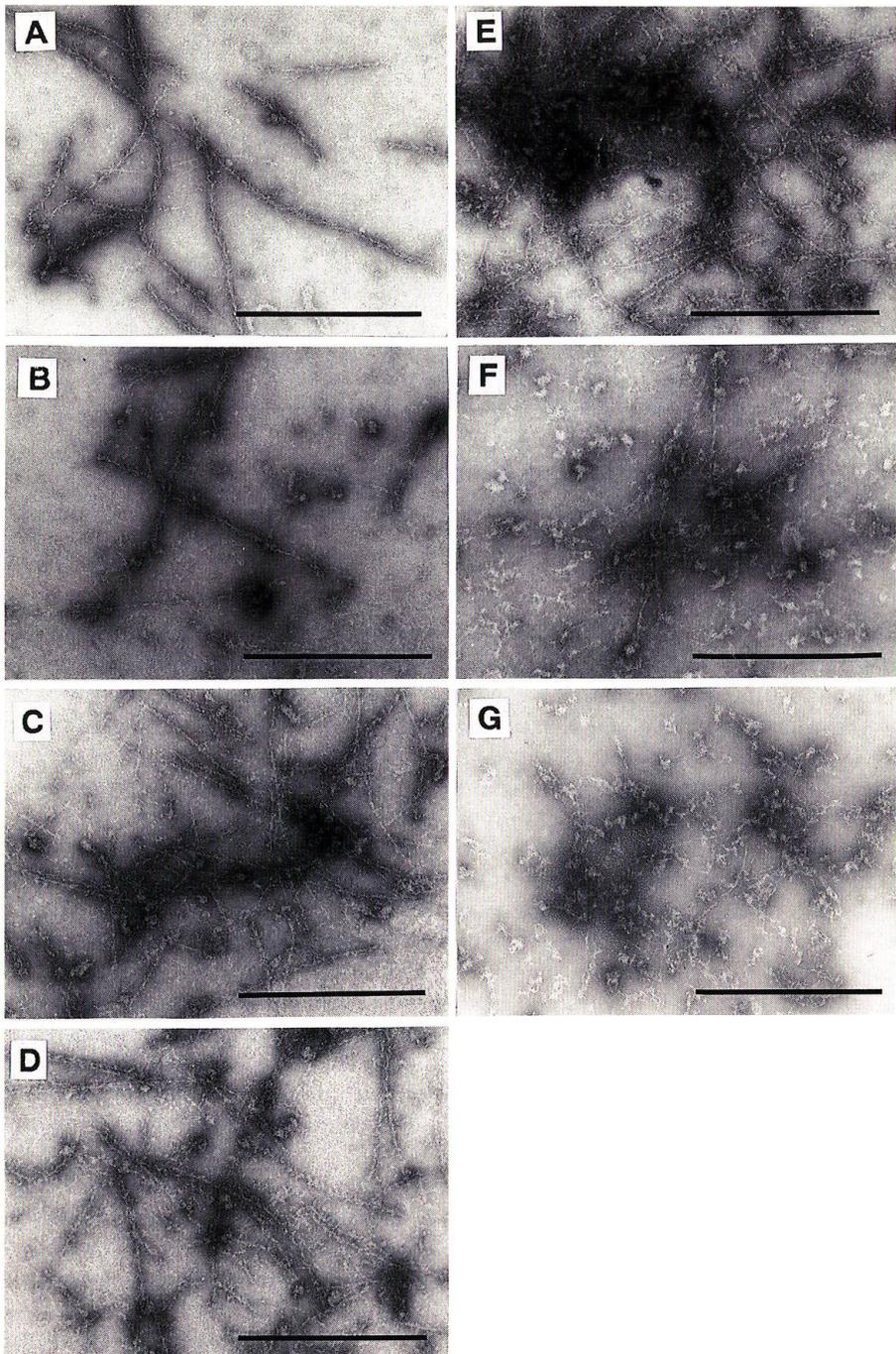


Fig. 3. Electron micrographs of carp actomyosin solutions heated for 1-48 h at 30°C. Heating hours are A, 1 h; B, 2h; C, 3 h; D, 4 h; E, 6 h; F, 12 h; and G, 48 h. In all electron micrographs, the bars represent 500 nm.

tened to a smaller size⁹⁾. In this case, it has been explained that the F-actin component denatured faster than the myosin component on the basis of biphasic first-order mode of thermal inactivation¹¹⁾. Thus, morphological changes in thermal treatments differed from those seen in freeze denaturation.

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