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## Morphological Changes in Carp Crude F-actin and Reconstituted Actomyosin during Frozen Storage

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### Abstract

Morphological changes in carp crude F-actin and reconstituted actomyosin during frozen storage were observed under an electron microscope. In the presence of 0.6 M KCl at  $-8^{\circ}\text{C}$ , no morphological changes in crude F-actin filaments occurred after a 2-day storage. In the case of reconstituted actomyosin, however, the arrowhead filamentous structure was drastically deformed after a one-day storage under the same conditions. Such results therefore suggest that the filamentous structure of actomyosin (in the presence of both F-actin and myosin) is more unstable than crude F-actin in the absence of myosin under the conditions in the high KCl concentration.

### Introduction

Connell<sup>1)</sup> reported that actin in cod muscle remained stable after 30 weeks of frozen storage at  $-14^{\circ}\text{C}$ . On the other hand, Matsumoto<sup>2)</sup> showed that both G- and F-actins underwent denaturation during frozen storage. Matsumoto<sup>3)</sup> attributed this disagreement in the results of the two groups to differences in experimental conditions. The actin in Connell's study was *in situ*, whilst in Matsumoto's study, isolated actin was used. We<sup>4,5)</sup> reported that in model experiments using natural actomyosin (myosin B) prepared from carp, denaturation of the actin component was faster than that seen in myosin, and morphological deformation of the actomyosin filaments was simultaneously observed under an electron microscope. Since these changes were very similar to those observed in concentrated KCl solution<sup>5)</sup>, we presumed that one of the causes of this freeze denaturation was related to the concentrated unfrozen KCl solution produced under frozen conditions. Okada et al.<sup>6)</sup> showed that the structure of carp F-actin was easily changed from its filamentous state to small-sized filaments or aggregations under higher KCl concentrations. From the above results, a possible explanation of F-actin denaturation during frozen storage was thought to be due to the effects of salt concentration. Therefore, it seems that the freeze denaturation of actomyosin filaments could be ascribed to the weaker binding interaction of myosin and actin when in contact with the concentrated unfrozen KCl solution.

We now report the difference in deformation of the filamentous structure between crude F-actin only and reconstituted actomyosin by mixing myosin and

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crude F-actin during frozen storage by observing morphological changes using an electron microscope.

### Materials and Methods

The extraction procedure of G-actin from carp muscle was the same as described by Kitao et al.<sup>7)</sup> G-actin solution (36 ml, 3.55-4 mg/ml) extracted from acetone powder by 0.5 mM ATP solution (pH 8.0) was mixed with 4 ml of 1 M KCl solution (finally 0.1 M KCl). This was stood for 30 min at room temperature, and was followed by dialysis against 0.1 M KCl solution overnight at 4°C (0.1 M KCl-crude F-actin solution). A part of the solution was dialyzed against 0.6 M KCl solution to prepare a 0.6 M KCl-crude F-actin solution. Five ml of each solution was placed in a test tube and frozen at -8°C. Myosin was prepared using the method of Takashi et al.<sup>8)</sup> Myosin and 0.6 M KCl-crude F-actin solution were dialyzed against 0.6 M KCl-5 mM Tris · maleate buffer (pH 6.8) overnight at 4°C. The protein concentration of both solutions was adjusted to about 4 mg/ml. Reconstituted actomyosin was made by mixing 1 part F-actin to 3 parts myosin by weight<sup>9)</sup>. The solution was diluted in a 10-fold volume of distilled water, and the suspension was centrifuged at 1,500×g for 30 min. The precipitate was dissolved in a 0.6 M KCl-5 mM Tris · maleate buffer (pH 6.8) and the protein concentration was adjusted to about 3 mg/ml. Five ml placed in test tubes and stored at 0°C, -8°C, and -20°C. Samples were removed from the freezer at appropriate intervals and thawed in a water bath at 20°C. The solution was centrifuged at 20,000×g for 60 min and the supernatant was submitted to electron microscopic observation.

Diluted sample solutions (0.1-0.5 mg/ml) of thawed F-actin and reconstituted actomyosin solutions were prepared 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 either 75 or 100 kV<sup>4)</sup>.

Protein concentration was determined by the biuret method<sup>10)</sup>.

### Results and Discussion

In both KCl concentrations of 0.1 M and 0.6 M, on the 2nd day of storage at -8°C, F-actin filaments were observed and were found to be similar to those samples both before freezing and at 0°C-storage after 2 days (Fig. 1A-D). Moreover, after the 7th day in 0.6 M KCl, F-actin filaments were observed (Fig. 1E). In the case of frozen reconstituted actomyosin stored at -8°C for one day, the arrowhead structure of actomyosin filaments was drastically deformed as compared to the sample before freezing (Fig. 2A and B). No morphological changes at -20°C were detectable after a 1-day storage, and the deformation of the arrowhead structure were recognizable on the 6th day (Fig. 2C and D). It has already been reported that the deformation of myosin B filaments was observed after 5-7 h storage at -8°C<sup>4)</sup>, and after 9 h at -5°C<sup>5)</sup>. The above drastic changes in reconstituted actomyosin at -8°C were very similar to those seen in myosin B during frozen storage. After F-actin solution which had been stored (in the presence of 0.6 M KCl) for 1- or 7-days at -8°C was mixed with freshly prepared myosin solution (in the presence of 0.6 M KCl), the arrowhead filamentous structure of actomyosin was observed (Fig. 2E and F).

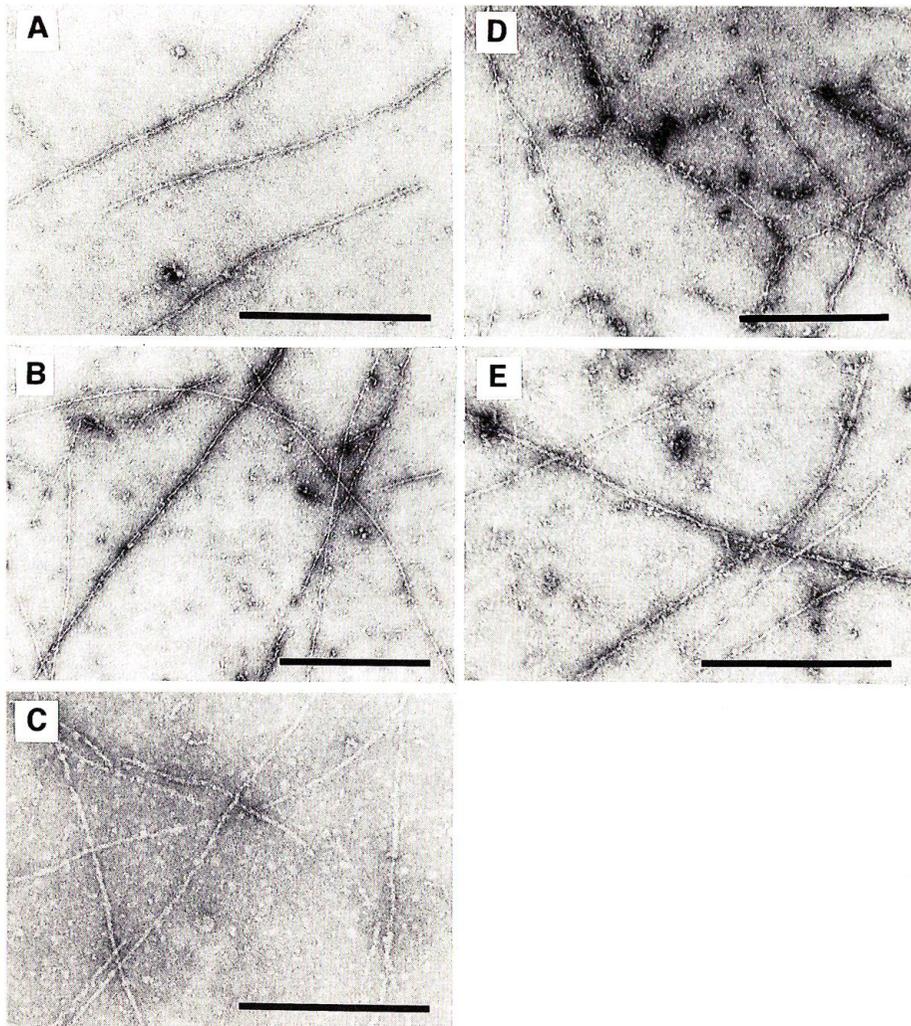


Fig. 1. Electron micrographs of carp F-actin stored in 0.1 and 0.6 M KCl solution at  $-8^{\circ}\text{C}$ . Storage conditions are A, before freezing in 0.1 M KCl; B, after 2 days in 0.1 M KCl at  $0^{\circ}\text{C}$ ; C, after 2 days in 0.1 M KCl at  $-8^{\circ}\text{C}$ ; D, after 2 days in 0.6 M KCl at  $-8^{\circ}\text{C}$ ; E, after 7 days in 0.6 M KCl at  $-8^{\circ}\text{C}$ . In all electron micrographs, the bars represent 500 nm.

Consequently, it can be assumed that when stored under frozen conditions, the structural stability of F-actin filaments in the absence of myosin was different from that of actomyosin filaments in the presence of both myosin and F-actin. Furthermore, it is suggested that the deformation of F-actin filaments in the presence of myosin (actomyosin filaments) occurred more easily than that in the absence of myosin under the conditions in high KCl concentration.

Okada et al.<sup>6)</sup> reported that the structure of carp F-actin was easily changed

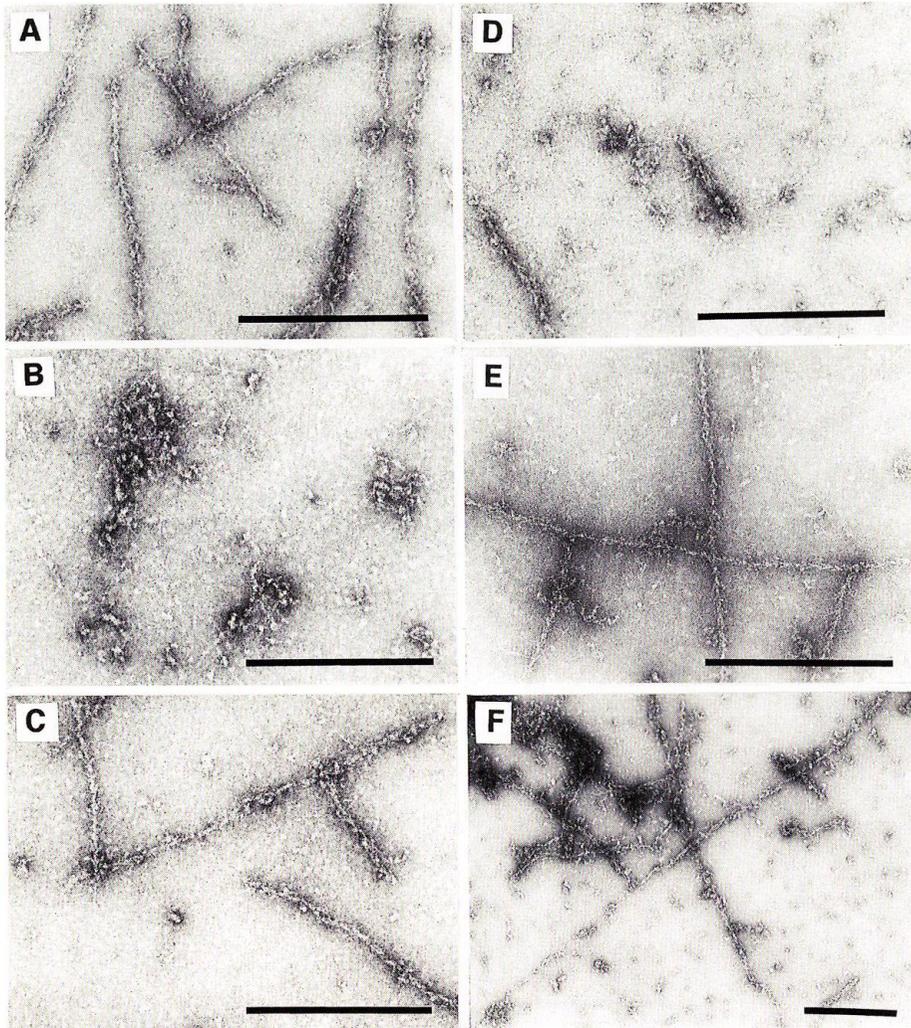


Fig. 2. Electron micrographs of carp reconstituted actomyosin during frozen storage and reconstituted actomyosin by mixing freshly prepared myosin and frozen stored F-actin. Storage conditions are A, reconstituted actomyosin (RAM) before storage; B, RAM after 1 day in 0.6 M KCl at  $-8^{\circ}\text{C}$ ; C, RAM after 1 day in 0.6 M KCl at  $-20^{\circ}\text{C}$ ; D, RAM after 6 days in 0.6 M KCl at  $-20^{\circ}\text{C}$ ; E, reconstituted actomyosin from freshly prepared myosin and 1 day-storage F-actin (in the presence of 0.6 M KCl) at  $-8^{\circ}\text{C}$ ; F, reconstituted actomyosin from freshly prepared myosin and 7 day-storage F-actin (in the presence of 0.6 M KCl) at  $-8^{\circ}\text{C}$ . In all electron micrographs, the bars represent 500 nm.

under higher KCl concentrations. No clear explanation can be given at this stage regarding the disagreement between their results and the present results. However, as we ourselves prepared the crude F-actin for this study, it is therefore possible that the extent of purification of the actin is related to the structural stability of F-actin.

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