4-P10

CONTINUOUS OBSERVATION OF CHANGES IN MYOSIN DURING HYDROSTATIC PRESSURE TREATMENT

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Keywords: myosin, hydrostatic pressure, gelation

Introduction

In the course of pressure treatment, there are two particular phases, which are raising and releasing of pressure. These two phases cause volume change of the solvent and the protein as well, so it is expected that conformational changes in myosin is much larger than those under a constant pressure. We reported that pressure-induced gelation of myosin was observed in 0.1 M KCl [1], while monomeric myosins in 0.5 M KCl formed aggregates instead of gel formation [2, 3]. These our previous observations were performed with myosin after pressure release, and it was not clear in which phase of pressure treatment caused these changes in myosin.

Tumminia *et al.* [4, 5] and Davis [6] reported turbidity decrease of myosin filaments with pressure application. Their studies were aimed to elucidate myosin filamentogenesis in physiological conditions, and the applied pressure was below 100 MPa. In the standpoint of food science, much higher pressure and acidic condition are required for expression of functional properties of myosin, such as gelation. In the present study, we examined the time course of pressure-induced changes of myosin in various KCl concentrations at pH 6.0.

Materials and Methods

Myosin was prepared from rabbit skeletal muscle and purified by DEAE-Sephadex column chromatography. Myosin dissolved in 0.1—0.5 M KCl and 10 mM Tris-maleate (pH 6.0) at a protein concentration of 2 mg/ml was put into a cylindrical quartz cuvette, which has a free piston at one end, and transferred into a pressure vessel equipped sapphire windows. The pressure vessel was set in a spectrophotometer. Pressure was applied by a hand pump and distilled water was used as a pressure transfer medium. Pressure was hold for 10 minutes, then released. Turbidity as an absorbance at 350 nm was monitored from beginning of pressure application to 20 minutes after pressure release. Some of pressurized myosins were negatively stained and observed by a transmission electron microscope.

Results and Discussion

Pressure-induced turbidity change of myosin in 0.1—0.5 M KCl and at 2 mg/ml is shown in Fig. 1. The turbidity of myosin in 0.1 M KCl before pressure application was 1.2, and this high turbidity was characteristic of filaments. Turbidity immediately decreased from 1.2 to about 1.0 by pressure application. During pressure elevation to the desired value, turbidities of myosins sharply increased when the applied pressures were 250—400 MPa. The turbidities of each myosins under pressure at 250—400 MPa remained at constant levels, and the each of them was higher than that of the unpressurized. When the applied pressure was below 200 MPa, the turbidities under pressure showed gradual and slight increase. As soon as pressure was released, turbidities increased. Gelation was observed above 250 MPa after the pressure treatment, while myosin filaments pressurized below 200 MPa remained as suspensions. Turbidities of myosin in 0.2 and 0.3 M KCl quickly dropped below 0.2 by pressure application, except 0.2 M KCl at 400 MPa, and they remained at low values under pressure. These low turbidities under pressure suggest dissociation of myosin filaments. Turbidity sharply increased concomitant with pressure release. No gelation was observed in the pressurized myosin in 0.2 and 0.3 M KCl. There were no substantial turbidimetric changes in monomeric myosins in 0.4 M and 0.5 M KCl under pressure below 150 MPa. Turbidity levels under a constant pressure above 250 MPa were higher than those at atmospheric pressure, and they increased further after release of pressure. These turbidimetric changes in monomeric myosins during pressure application indicate that aggregation of myosin monomers under pressure takes place when the pressure is above 250 MPa and aggregation proceeds even after release of pressure.

Electron microscopic observation of myosin filaments in 0.3 M KCl at pH 6.0 indicated that myosin formed filaments at atmospheric pressure (Fig. 2). After pressure application, irregular filamentous structure was observed

in the 200 MPa sample, and granular structure as well as short filaments with knots was observed in the myosin pressurized at 300 and 400 MPa. These turbidimetric and morphological results indicate that myosin filament dissociates under pressure, and the dissociated species reassociate by pressure release. However, no regular association through tail to tail interaction as seen at atmospheric pressure occurred in pressurized myosins.

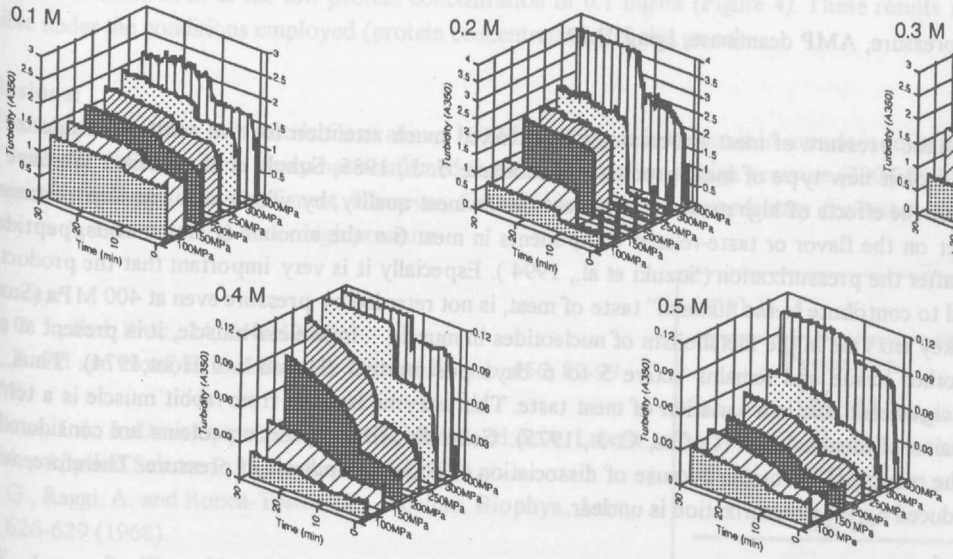

Fig. 1 Turbidimetric changes of myosin at 0.1— 0.5 M KCl during pressure treatment. Myosin in 0.1— 0.5 M KCl and 10 mM Tris-maleate (pH 6.0) at 2 mg/ml was pressurized at the indicated pressure. Turbidity was monitored during pressure application for 10 min and after pressure release for 20 min. Time 0 means the time at which pressure reached the designated pressure, and the pressure was retained for 10 minutes, then the pressure was released.

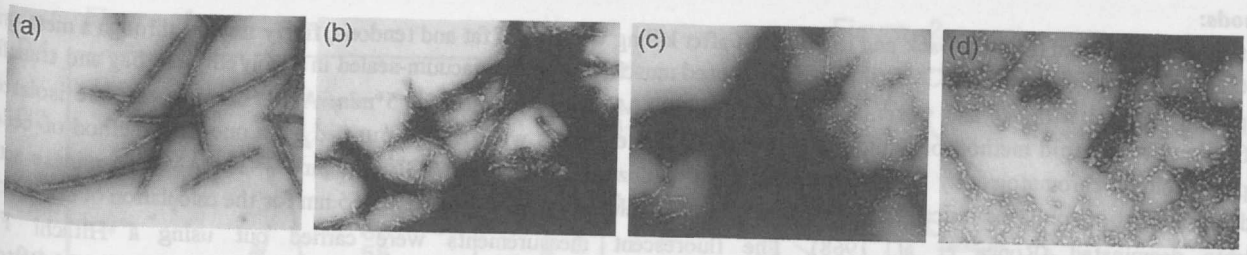

Fig. 2 Electron micrographs of myosin after pressure application in 0.3 M KCl. Myosin was negatively stained by 2% uranyl acetate. Scale bar indicates 1 μm. (a); control, (b); 200 MPa, (c); 300 MPa, and (d); 400 MPa.

Conclusion

Myosins in 0.1—0.3M KCl at pH 6.0 exits as filaments. The filaments in 0.1 M KCl did not dissociate under pressure, while those in 0.2 and 0.3 M KCl dissociated during pressure application. Gelation of myosin only occurred at 0.1 M KCl and above 250 MPa. The present study indicates that filamentous structure should be retained under pressure for pressure induced gelation of myosin. Once filaments dissociate by pressure application, gel is no longer formed because of irregular reassociation of myosin after pressure release.

References

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