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Effects of Hydrostatic Pressure on Myofibrillar Protein Extracted from Bovine *Semitendinosus*

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Introduction

Hydrostatic pressure (HP) is receiving considerable attention and could be useful application for a wide range of meat products[1)]. Although HP is receiving considerable attention as a non-thermal processing technique, the effects of pressure on the structural components of muscle are limited. So far, studies for denaturations of myosin, F-actin, and actomyosin by HP have been investigated to demonstrate the changes of muscle induced by HP.

Myosin consists of two heads and tail of helix structure and is known as playing an important role in gelation[2)]. Yamamoto et al. demonstrated that HP induced gelation of myosin filaments involved interfilamentous head to head interaction, however, tail to tail interaction induced by HP was not observed[3)]. F-actin consisted of G-actin, tropomyosin, toponin-I, troponin-C, and troponin-T is also important as a cofactor reinforcing the gel structure of myosin. A reversible F-G transformation of actin is caused by HP in the condition without ATP[4)]. At 0.6 M KCl concentrations, a large part of actin in actomyosin is found to exist as the depolymerized form (native G-actin) after HP treatment. However, most of the actin in actomyosin in buffer containing 0.6 M KCl is denatured by 150 MPa HP treatment[5)]. Actomyosin dissociates into actin and myosin with HP treatment, and myosin in actomyosin protects denaturation of F-actin against HP[6)].

Though the study of interaction between myosin and actin in Mf would be a key reaction in muscle contraction and the effects on the Mf induced by HP are more complex, studies about changes of Mf induced by HP have been performed at a limited condition of pressure intensity and duration time.

Our objective of the present research was to correlate biochemical changes of myosin and actin in pressurized Mf by measurements of Ca- and Mg-ATPase activities and changes in soluble protein. Also inactivation rate of myosin and actin was studied to evaluate the effect of HP on Mf.

Materials and methods

Muscles

Bovine skeletal muscles (*Semitendinosus*) were obtained from the carcasses (Holstein) after 48 hr slaughter.

Extraction of Myofibrillar protein

The myofibrillar protein prepared according to the procedure of Busch *et al.*(1972)[7].

Pressure treatment

The isostatic pressure unit (Mitsubishi heavy Industries. LTD, MFP-7000, Japan) fitted with a thermo-regulated system (Cooling pump CH-750AFH, TAITEC, Japan). Mf suspensions were pressurized at 100, 200, 300, 400, and 500 MPa for various duration times. The temperature condition was 15 ± 3 ℃. Control samples were maintained in atmospheric pressure at 4 ℃ while the samples were being treated.

Measurement of Ca- and Mg-ATPase activity

Ca- and Mg-ATPase activities was determined according to a modification of Chen and others(1989)[8]. The quantity of inorganic phosphate liberated was caculated by the method of Fiske and SubbaRow (1925)[9].

Soluble protein concentration

Protein concentration of supernatant was determined using the Biuret method[10]. Amount of soluble protein was calculated as a percentage of the protein present in the suspension of myofibrils.

Sodium dedocyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on gels of 10 % polyacrylamide containing 1% SDS (Laemmli, 1970)[11].

Results and Discussion

Changes of Ca- and Mg-ATPase activities

Ca- and Mg-ATPase activities of Mf treated by HP at 100, 200, 300, 400, and 500 MPa as a function of time were measured. There was no change depending on increasing duration time in Ca- and Mg-ATPase activities of Mf treated with 100 MPa. Ca- and Mg-ATPase activities of Mf treated with 200 MPa were gradually decreased as increasing duration time, Ca- and Mg-ATPase activities of Mf treated with 200 MPa for 25 min were reduced to 73 and 66% of control, respectively. Ca- and Mg-ATPase activities of Mf treated with 300 MPa for 15 min were reduced to 30 and 3% of control, respectively. Ca- and Mg-ATPase activities of Mf treated with 400 MPa for 5 min and 500 MPa for 1 min were almost disappeared.

Changes of inactivation rate of Ca- and Mg-ATPase activities

The inactivation rate of Ca- and Mg-ATPase of Mf treated HP (K_P) was calculated using the following formula (Matsumoto et al.)[12].

$$K_P=(\ln C_0-\ln C_P)/t$$

where and C_0 denote C_P the relative activity of the myoifibrillar ATPase before and after pressure treatment, respectively. Moreover, when the inactivation of the ATPase proceeded in two stages, K_P was recalculated for the 1st stage (K_{P1}) and the 2nd stage (K_{P2}), and t was assumed as an arbitrary number

The increase of the inactivation rate was started at 200 MPa treated Mf, and inactivation rates were dramatically increased above 300 MPa. The inactvation rate of both the 1st and 2nd stage increased with pressure size. The 1st inactivation rates of Ca- and Mg-ATPase activities were similar, while the 2nd inactivation rate of Mg-ATPase activity was faster than that of Ca-ATPase activity. The 1st inactivation rates appeared the fast phases, and the 2nd inactivation rates were the slow phases. Mainly, inactivations of Ca- and Mg-ATPase activity were occurred at the 1st stage.

Changes of protein solubility

Protein solubility in 0.1 and 0.6 M NaCl buffer (pH 7.0) induced by HP were measured. Protein solubility was expressed as the percent of protein that failed to sediment at 10,000 ×g for 30 min.

Protein solubility in 0.1 M NaCl buffer (pH 7.0) of Mf treated with HP were 4.39±0.18, 6.04±0.11, 9.49±0.29, 12.81±1.05, and 15.12±0.39% at control, 100, 200, 300, and 400 MPa for 5 min treatments, respectively. However, protein solubility in 0.1 M NaCl buffer (pH 7.0) of Mf treated with 500 MPa (12.60±0.20%) was decreased comparison with 400 MPa treatment. From the SDS-PAGE of these soluble proteins, increase of soluble protein in 0.1 M NaCl buffer (pH 7.0) was due to the release of low molecular weight proteins (15~35 KDa). The patterns of low molecular weight proteins (15~35 KDa) were not different between 400 MPa and 500 MPa treatment. However, high molecular weight proteins (above 97 KDa) of 500 MPa treatment were lower than that of 400 MPa treatment.

At high ionic strength (0.6 M NaCl), change of protein solubility induced by 100 MPa (66.07±0.59%) was slightly increased compared with control (61.98±1.50%). In pressure treatments above 200 MPa, protein solubility was gradually decreased.

The percents of soluble protein were 58.58±0.35, 55.73±0.08, 45.83±0.93, and 28.84±0.08% at 200, 300, 400, and 500 MPa, respectively. The changes of contents of soluble proteins observed by SDS-PAGE indicated that the amount of myosin heavy chain (MHC) and actin of soluble protein eluted in 0.6 M KCl buffer (pH 7.0) were decreased by increasing pressure size.

Summary

To investigate hydrostatic pressure (HP) effect on myofibrillar protein (Mf) extracted from bovine *Semitendinosus* muscle, Ca- and Mg-ATPase activities to evaluate denaturation of myosin and actin, and soluble protein contents were observed. In Mf treated with 100 MPa for 5 min was not observed denaturation of myosin and actin. In Mf treated with 200 MPa for 5 min, denaturation of myosin and actin were observed but inactivation rate was low (0.0136 min^{-1}). Inactivation rate of myosin and actin was dramatically increased above 300 MPa treatment. However denaturation of myosin and actin was not that critical with duration time. By increasing pressure size, the amount of myosin and actin in soluble protein eluted in 20 mM potassium phosphate buffer (pH 7.0) containing 0.6 M NaCl were decreased. SDS-PAGE of soluble protein released from Mf suspension in 0.1 M NaCl buffer (pH 7.0) showed that low molecular weight proteins (15~36 KDa) were released by HP treatment above 200 MPa. From the results, denaturation of myosin and actin, and release of light molecule proteins of Mf were observed by HP treatment over 200 MPa.

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