STRUCTURAL CHANGES IN RABBIT SKELETAL MUSCLE G-ACTIN AFTER HIGH HYDROSTATIC PRESSURE

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Abstract: The effects were assessed of high hydrostatic pressure on the structure of rabbit skeletal muscle G-actin. The pressure effects on the structure were measured by spectroscopic measurements such as circular dichroism (CD) spectroscopy, nuclear magnetic resonance (NMR) after releasing the pressure, and surface hydrophobicity was also achieved after releasing the pressure. The decrease in α-helix content was significant exposed to pressure of 300 MPa. The surface hydrophobicity increased with the increase of high pressure applied, and the fluorescence intensity of G-actin showed the maximum increase in the surface aromatic hydrophobicity exposed to pressure of 300 MPa. The signal intensity at 2.055 ppm began to decrease above 300 MPa, and the signal disappeared completely at 400 MPa. Also the singnal near 2.035 ppm originate from methy proton disappeared above 300 MPa. These results indicated that high pressure-induced structural change of G-actin is irreversible.

Key words: **High hydrastatic pressure; G-actin; Circular dichroism (CD); Nuclear magnetic resonance (NMR)**

I. INTRODUCTION

High hydrostatic pressure is a fundamental thermodynamic parameter that can be used to alter the biophysical properties of biomolecules, such as protein and nucleic acids. High hydrostatic pressure also be used to exquisitely control biomolecular interactions. The adoption of various high hydrostatic pressure applications in the life sciences and food sciences have grown recently. One of potential high-pressure applications on food is the modification of proteins (or enzymes) in food materials, including restructuring and texturization (for example gelation, tenderization). In order to promote the use of pressure in food engineering more efficiently, we may turn our attention to microscopic world of protein structure, from which the macroscopic pressure effects on food materials originate. Pressure induces the depolymerization of polymeric structures into subunits, the partial unfolding (or denaturation) of monomeric structures by the weakening of hydrophobic interactions and the splitting of intermolecular electrostatic interactions or hydrogen bonds. In the field of food technology, the rearrangement and /or destruction of those noncovalent bondings in the tertiary structure of proteins under pressure may result in generating new food products which we had not experience in eating. The physicochemical and biochemical properties of actin, the major protein in muscle, are also influenced by high pressure. Actin is composed of two domains that are separated by a cleft, in which one molecule of ATP or ADP and one divalent cation are present. It undergoes transformation from a monomeric form (G-actin) to a long, helical polymer (F-actin). Ikkai and Ooi (1966) first did a study on the effect of pressure on actin. According to their report, actin is irreversibly denatured above 150 MPa without ATP, but above 250 MPa with ATP; The amount of protein denatured by pressure was dependent on the initial protein concentration; ATP pervented actin from pessure-induced denaturation; In the presence of ATP under pressure, the reversible F-G transformation liberated both ADP and Pi. Ikeuchi et al. (2002) recently succeeded in monitoring the behavior of G- and F-actins under pressure and found that the denaturation of actin under pressure is coupled with the loss in exchangeability of bound ATP with external ATP. Fourier-Transform INfraRed (FT-IR) spectrum measurement of Borbardier et al. (1997) showed that ATP protected G-actin against denaturation under pressure. These facts indicate that ATP plays an important functional role in the stabilization of actin under pressure.

In the present experiments, the pressure effect on the structure of G-actin was studied. Spectroscopic measurements such as circular dichroism (CD), nuclear magnetic resonance (NMR) were performed after pressure-release, and surface hydrophobicity was also achieved after pressure release.

II. MATERIALS AND METHODS

Protein preparation

Actin preparations from rabbit skeletal muscle were obtained from acetone dried powder according to the procedure of Pardee and Spudich (1982). Unless used immediately, G-actin with ATP was stored at -20C after lyophilization. The purity of the protein was tested by SDS-PAGE as shown in Figure1. The lyophilized G-actin was dissolved in the

solution containing 2 mM Tris-HCl (pH 8.0), 0.2 mM ATP, 0.2 mM CaCl2 , 0.5 mM 2-mercaptoethanol and 1mM NaN3.

High pressure apparatus

The high-pressure device was basically the same as a cold isostatic pressing (CIP) used conventionally for the molding of ceramics. The pressure is generated with a hydraulic pump and the pressurized water is enclosed in a steel cylinder of great thickness and resistance (Nikkiso Co. Ltd., Tokyo). Each sample solution was vacuum-sealed in a polyethylene bag, transferred to a large polyethylene bag and then the bag was placed in the pressure vessel.

SDS-PAGE (polyacrylamide gel electrophoresis)

SDS-PAGE was performed by the method described by Laemmli (1970). The gels were stained with Coomassie Brilliant Blue R-250. Molecular weights of proteins were determined using myofibrillar protein.

Measurement of the secondary structure of G-actin.

The circular dichroism (CD) spectra of pressurized G-actin solutions (0.1 mg/ml) in 2 mM Tris-HCl, 0.2 mM ATP, 0.5 mM 2-mercaptoethanol and 0.2 mM CaCl2 (pH 8.0) were recorded by a Jasco J-725 spectropolarimeter at 20ºC using a quartz cell with a 1 mm light path for far UV (200-260 nm). The mean residual ellipticity [θ] is expressed in degrees·cm2·d mol-1. [θ] was calculated by using a molecular weight of 42,300 and 375 residues. Devolution of the CD spectrum for the sample was performed using program, J-700 for Windows optional software, provided by JASCO (Yang et al., 1986).

Measurement of the surface (aromatic) hydrophobicity of G-actin

The surface hydrophobicity of pressurized G-actin solutions (0.05 mg/ml, in 2 mM Tris-HCl, 0.2 mM ATP, 0.5 mM 2-mercaptoethanol and 0.2 mM $CaCl_2$ (pH 8.0) was measured according to the method of Boyer *et al*. (1996). 8µl of 5 mM 8-anilino-1-naphthalene-sulphonic acid (ANSA) was added to 2.5 ml of the G-actin solution. The fluorescence intensity of the ANSA-protein conjugates was measured at 380 nm with excitation at 475 nm.

Nuclear magnetic resonance (NMR) measurement

All 1D NMR spectra were recorded on a Bruker DPX-400 (400 MHz) spectrometer at 298 K using a 5 mm ^{1}H probe (Sigemi Co. Japan). 3-(Trimethylsilyl)-1-propanesulfonic acid (DSS) was used as an internal reference. The presaturation procedure was adopted to suppress the solvent signals. ID spectra were recorded with data points of 64K, and 512 scans. Heavy water (99.9 atomic % D) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

III. RESULTS AND DISCUSSION

Measurement of the secondary structure of G-actin

Far UV-CD spectra of G-actin with ATP after release of pressure are illustrated in Figure 2. G-actin was exposed to pressures of 0.1, 100, 200, 300 and 400 MPa for 5 min. The decrease in α-helix content was significant exposed to pressure of 300 MPa. The values of [θ]222 obtained for Native G-actin was -8400+/- 200 deg (cm2.dmol), which corresponds to about 26% alpha helix structure (Yang et al. 1986). The signals at 222 nm of G-actin pressurized at 300 MPa decreased to -7100 deg/(cm2.dmol), which corresponds to 21.9% alpha-helix structure. The decrease in the ellipicity at 220-232 nm probably reflected the collapse of the structure of G-actin which was attributed to the irreversible pressure-induced denaturation.

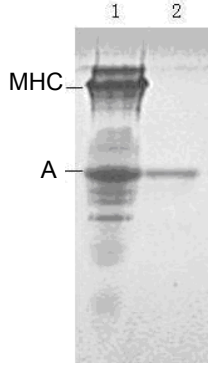

Figure 1. SDS-PAGE profile of G-actin monomer
1, Myofibrillar protein; 2, G-actin monomer. MHC, Myosin Heavy chain; A, Actin

Measurement of the surface hydrophobicity of G-actin

The change in surface hydrophobicity measured by ANSA is shown in Figure 3. The fluorescence intensity increased with the increase of high pressure applied, and the fluorescence intensity of G-actin showed the maximum increase in the surface aromatic hydrophobicity exposed to pressure of 300 MPa. These facts suggest that high pressure-induced structural change of G-actin is irreversible.

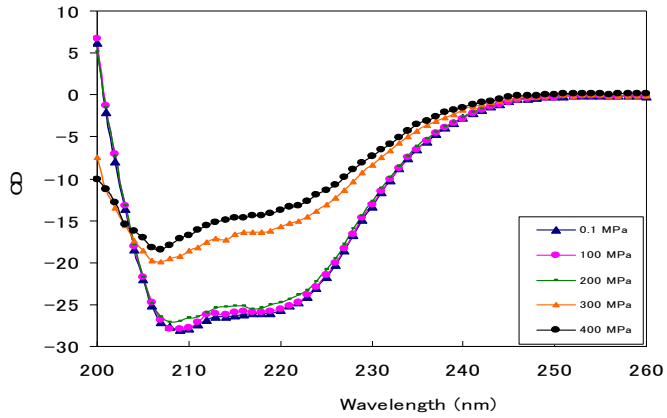

Figure 2. Ultraviolet circular dichroism (CD) spectra of G-actin with ATP after release of pressure.
G-actin was exposed to the designated pressure for 5 min. (1) 0.1 MPa, (2) 100 MPa, (3) 200 MPa, (4) 300 MPa, (5) 400 MPa. The protein concentration was 0.1 mg/ml in 2 mM Tris-HCl, 0.2 mM ATP, 0.5mM 2-mercaptoethanol and 0.2 mM CaCl2 (pH8.0)

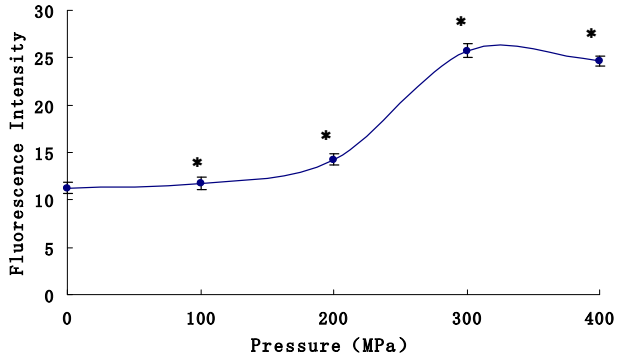

Figure 3. Effect of pressure treatment on the Aromatic Hydrophobicity of G-actin with ATP.
Excitation wavelength 380 nm, Emission wavelength 475 nm. Each value is expressed as the mean ± standard devition (n=6); $p < 0.05$

NMR measurement

We have recorded the 1H NMR spectra of G-actin with ATP immediately after pressure release. Figure 4 shows the 1H NMR spectrum of G-actin with ATP at an aliphatic region after release of pressure. The mark × shows the impurities (foreign matter) or buffer composites. The sharp NMR signals at 0.9 ppm, 2.035 ppm and 2.055 ppm pointed to in this figure are considered to originate from the exterior methyl group of protein, N-acetyl group at the N terminal amino acid and the methyl proton of methionine (Met44 and Met47) in G-actin molecule, respectively (Heintz et al., 1996). These methionine groups are located in a highly flexible region in G-actin and are part of the DNase l binding loop. As shown as Figure 4, almost no changes of singnals have found until pessure 200 MPa. The signal intensity at 2.055 ppm began to decrease above 300 MPa, and the signal disappeared completely at 400 MPa. Also the singnal near 2.035 ppm originate from methy proton disappeared above 300 MPa. The phenomenon of signal disappear

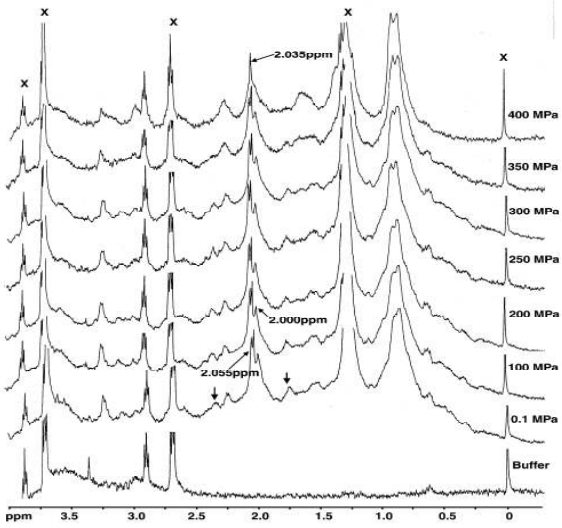

Figure 4. 1H NMR spectra of G-actin with ATP after release of pressure. G-actin was exposed to the designated pressure for 5. The protein concentration was 2 mg/ml in 1 mM Na2HPO4 (pH 8.0), 0.2 mM ATP, 0.2 CaCl2, 0.2 mM 2-mercaptoethanol; 90% H2O/10% D2O

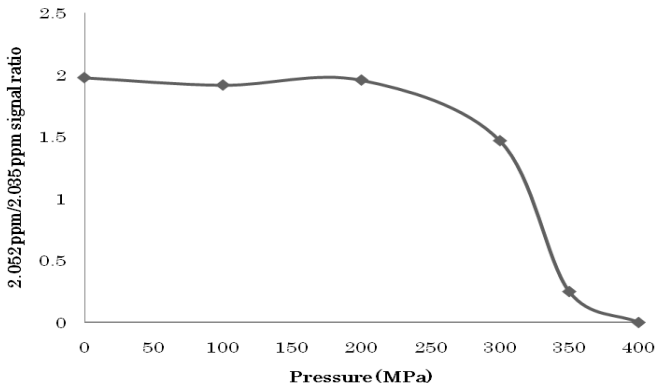

Figure 5. Time dependence of the relative ratio of NMR signal (2.055 ppm/2.035 ppm) of G-actin with ATP

originate from Met is to suggeted that the region of Met44 and Met47 is buried deeply into the inside of the molecule in concomitant with irreversible release of bound ATP in the refolding process after decompression.This can be seen from the inset of Figure 5, which shows the pressure dependence of the relative integral ratio (2.055ppm/2.035 ppm) when H(1') ribose of ATP was employed as the reference signal intensity (6.18 ppm). The G-actin with ATP exposed to relatively low pressure (below 200 MPa) induced no reduction in the relative ratio of NMR signal, but further increase in pressure the sigmoid curve was decreased steeply.

IV. CONCLUSION

The disappearance of the characterized 1H NMR signal at 2.055 ppm, which is considered to originate from the methyl proton of methionine (Met 43 and 47) in actin, and the loss in biochemical activities (DNase I inhibition capacity) at 300 MPa were almost identical. These methionine residues are located at the surface of the actin molecule and near the DNase I binding site. Furthermore, the loss in the exchangeability of ATP bound to G-actin under pressure may lead to its irreversible denaturation (Ikeuchi et al. 2001). These suggested that the rapid collapse of the three dimensional structure around the upper region (e. g. the vicinity of DNase I binding site) of the actin molecule could be caused under pressure, following the irreversible dissociation of the bound ATP which is essential for the stabilization of actin.

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