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PRESSURE EFFECT ON THE STABILITY OF AMP DEAMINASE FROM RABBIT SKELETAL MUSCLE

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Background:

The application of high hydrostatic pressure of meat processing has attracted much attention for the merits to tenderize meat, accelerate the aging of meat, and exploit new type of meat products (Macfarlane, J. J., 1985, Suzuki et al., 1996). We have so far performed a series of studies about the effects of high pressure treatment on the meat quality, by which high pressure treatment was found to have little adverse effect on the flavor or taste-related components in meat (i.e. the amount of amino acids, peptides and nucleotides) during conditioning after the pressurization (Suzuki et al., 1994). Especially it is very important that the production of IMP in meat, which is considered to contribute to the "umami" taste of meat, is not retarded by pressure even at 400 MPa (Suzuki et al., 1994). AMP deaminase is a key enzyme in the metabolism of nucleotides in muscle. In skeletal muscle, it is present at a much higher concentration than any other tissue and remains active 5 to 6 days postmortem (Hamm and Hoot,1974). Thus, AMP deaminase is assumed to play a significant role in a variation of meat taste. This enzyme isolated from rabbit muscle is a tetrameric structure having polypeptide chains of identical size (Coffee, C. J., 1975). Generally, the polymeric proteins are considered to be pressure-labile compared with the monomeric proteins because of dissociation of subunits under high pressure. Therefore, why the content of IMP in meat is not reduced by the pressurization is unclear.

Objectives:

The utilization of high pressure technology for the meat tenderization or acceleration of meat conditioning has been well documented. However, little research was found on the effect of high pressure treatment on the taste-related components, in particular, IMP in meat. AMP is converted to IMP by deaminase action. Then, this study was conducted to investigate the effects of high pressure on the stability of AMP deaminases isolated from muscle and in muscle system.

Methods:

White muscle dissected from the back and leg of rabbit after killing was free of fat and tendons, finely minced through a meat grinder and stored in a deep freezer at -80℃ until use. Each minced muscle (50 g) was vacuum-sealed in a polyethylene bag and transferred to a pressure vessel with ice and water. Pressure at 100-400 MPa was applied for 5 min. AMP deaminases were isolated by modification of the rapid method of Smiley et al. (1967). The enzymes were almost purified by a one-step method of cellulose phosphate column chromatography without carrying out crystallization (see SDS-PAGE in Figure 1). The AMP deaminase activity was determined spectrophotometrically at 265 nm; $\Delta\ \varepsilon$ (mM) values of 8.86 was used at 265 nm for the calculation of the μmole of substrate deaminated (Ronca et al, 1968). The fluorescent measurements were carried out using a Hitachi F2000 fluorespectrophotometer, which was modified in our laboratory to measure fluorescence in the pressure range from 0.1 M Pa to 400 MPa (pressure device). Fluorescence spectra of enzyme samples were measured in a 1000 μl quartz cuvette between 300-400 nm after excitation at 295 nm, and spectral shifts of fluorescence emission were quantified by specifying the center of spectral mass : $\langle\nu_i\rangle=\Sigma(\nu_i \times F_i) / \Sigma F_i$ where ν_i is the wavenumber and F_i the fluorescence intensity at ν_i (Ruan et al., 1998). A turbidity in the solution was also measured to make sure whether protein aggregation was caused after pressure release.

Results and discussions:

Figure 1 shows the elution profile of AMP deaminase of the unpressurized muscle from cellulose phosphate. The enzyme was eluted with a linear gradient of 0.45-1.0 M KCl containing 1 mM mercaptoethanol at pH 8. The preparations of AMP deaminase from the pressurized muscles were conducted in the same manner (data not shown in this paper). As shown in this Figure, the specific activity almost remained constant over the peak, indicating little contamination of other proteins. The total activities of each sample were calculated from specific activities between Fr. No. 20 and Fr. No. 40. The results are shown in Table I. The total activities in the muscle were decreased with increasing pressure intensity. The total activity from pressurized muscle at 300 MPa was less than one -half of unpressurized muscle. However, the question arises in the pressurized muscle whether the enzymatic activity itself was lowered, or the extractability decreased. Then, fractions 28-34 of unpressurized muscle showing constant specific activity (Figure 1) were pooled, and concentrated to confirm the pressure effect on the AMP deaminase itself. Red shifts in spectra and decreases in fluorescence efficiency were observed, suggesting a considerable increase in polarity of tryptophan environment accompanying dissociation of subunits under high pressure (data not shown). Figure 2 shows the pressure dependence of the center spectral mass of the intrinsic fluorescence of AMP deaminase (0.1 mg/ml). The curve upon compression reached high pressure plateau at 300 MPa. It shows a progressive exposure of tryptophan residues followed by a breakdown of the signal corresponding to

the dissociation. On the other hand, the decompression curve did not return to the original position. Probably, aggregate after pressure release exists as a heterogeneous population of species due to the reduction of affinities among the subunits after dissociation under high pressure. The effect of pressure on the enzymatic activity of AMP deaminase is shown Figure 3. The activity measured after pressure release was drastically decreased between 100 and 200 MPa and was completely lost at 250 MPa. Besides, the delayed regain of the enzymatic properties on return to atmospheric pressure was not observed. The protein aggregation could not be avoided even at the low protein concentration of 0.1 mg/ml (Figure 4). These results indicate that the transition is not reversible under the conditions employed (protein concentration, pH and so on).

Conclusions:

AMP deaminase itself is pressure-labile at above 100 MPa (Figures 2-4), but it in muscle is resistant to even high pressure of 300 MPa (Table 1). Therefore, this study has confirmed that the application of high pressure (200-300 MPa) of meat processing does not work against the flavor or taste of meat. However, further investigations are necessary for better understanding of pressure effect on this enzyme (homotetramers) under high pressure.

Table 1

Pressure (MPa)	Total activity (μ mole/min)
0.1	203
200	164
300	89

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Figure 1

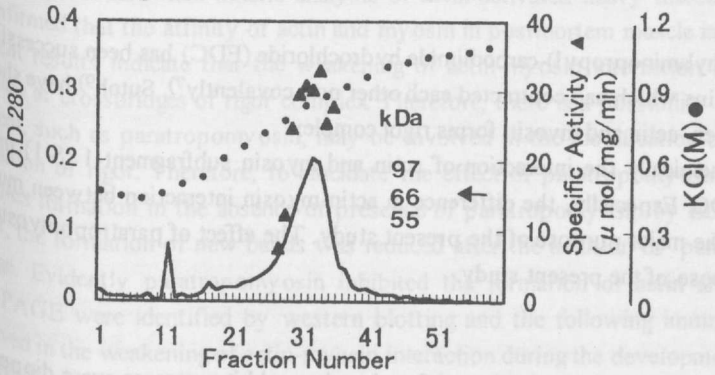

Figure 2

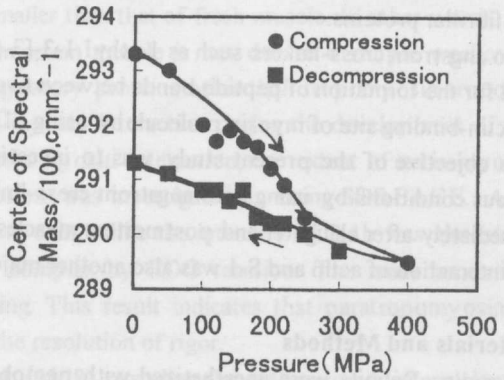

Figure 3

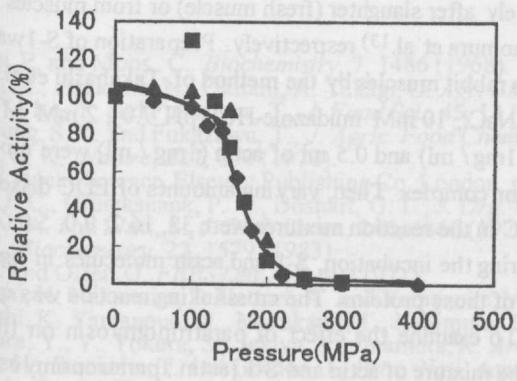

Figure 4