Elucidation of Factors Inducing Changes in Mg-ATPase Activity of Myofibrils During Meat Conditioning

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INTRODUCTION

It is known that the Mg-ATPase activity of myofibrils changes chracteristically during meat conditioning. For example, Yang et al. (1) reported that the maximum value of the Mg-ATPase activity of myofibrils (MVAA) existing at 0-0.1 M KCl increased by ca. 200% and the dependency of the Mg-ATPase activity of myofibrils on KCl concentrations (DAAK) in the range of 0-0.2M KCl increased remarkably during postmortem storage of rabbit muscle. A few reports showed that endogenous proteinases, e.g., calpain, could induce similar but not equal changes *in vitro* (2, 3, 4).

Therefore, the aim of this work is to elucidate factors inducing the changes of Mg-ATPase activity of myofibrils during meat conditioning.

MATERIALS AND METHODS

Myofibrils were prepared from rabbit skeletal muscles at 1 day postmortem according to the method of Yang et al(1). Proteasome was prepared from rabbit muscles as our report(5). Calpain II was extracted from muscles with a neutral buffer and purified by ammonium sulfate fractionation and DEAE-cellulose column chromatography. Cathepsin mixtures were obtained from the extracts of the muscle at pH3.7 by precipitation with 25-65% saturated ammonium sulfate. Mg-ATPase activity of myofibrils was measured as described by Yang et al.(1). Myofibrils (0.25 mg/ml) were incubated with 1 mM ATP in 20 mM Tris-HCl(pH 8.0)/2 mM $MgCl_2$ /2 mM NaN_3 /0-0.2M KCl at 25℃ for 5min. SDS polyacrylamide gel electrophoresis(SDS-PAGE) was performed by the Laemmli's(6) method. Isoelectric focusing was performed with Rotofor cell, free solution isoelectric focusing apparatus (Bio-Rad Laboratories), according to the manufacturer's instructions.

RESULTS AND DISCUSSIONS

Myofibrils were incubated with 1 mM Ca^{2+}, which was reported to weaken Z disk structure(7), at 25 ℃ and pH 5.5 or 7.0, and their Mg-ATPase activity at 0-0.05M KCl was measured. There was no change in the activity on the treatment at either pH. Furthermore, the treatment of myofibrils with proteasome at either pH 5.5 and 7.0 induced no change in the Mg-ATPase activity. There was no change in the Mg-ATPase activity on the treatment of myofibrils with calpain II at pH 5.5 and 25℃ for 10hr. However, the treatment at pH 7.2 for 1hr resulted in an increase by ca.10% in the activity at 0-0.05 M KCl and decrease in the activity at 0.1 M KCl. After that time the activity at 0.05 and 0.1 M KCl decreased with the elapse of the time up to 10hr, resulting in the increase of DAAK. The cathepsin mixture altered the Mg-ATPase activity at both pHs 5.5 and 7.0. Especially, the treatment at pH 5.5 caused a gradual increase in DAAK for up to 33hr as shown in Fig. 1. The activity at 0.004 M KCl incresed by ca. 20% after 12hr incubation and then decreased gradually.

As mentioned above, calpain II and the cathepsin mixture raised markedly DAAK, but did not cause a remarkable increase in MVAA, suggesting the existence of other unkown factors increasing MVAA. In order to find such factors, the muscle extract was prepared with the buffer of pH 7.2. Myofibrils were incubated with the extract in 50 mM sodium acetate buffer (pH 5.5)/ 3 mM dithiothreitol/ 1 mM EDTA/ 0.08 M KCl/ 7.5 mM NaN_3 at 25℃ for 38hr, and then washed with 0.16 M KCl/ 5 mM NaN_3. Through this treatment MVAA amounted time-dependently up to 150%. The factors in the muscle extract were assumed to be some proteins, because the ability to change MVAA was lost by a trypsin-treatment.

In order to isolate the factors, the muscle extract was fractionated with isoelectric focusing. Myofibrils were incubated with the obtained five fractions, R1(pI 1.2-4.3), R2(pI 4.8-6.5), R3(pI 7.0-8.0), R4(pI 8.3-9.6), and R5(pI 10.3-14.0), respectively, at pH 5.5 and 25℃ for 18hr, resulting in the increase of Mg-ATPase activity at 0-0.05 M KCl by R4. The treatment of myosibrils with R4 at 25℃ for 3hr induced 30% increase of MVAA at 0.05 M KCl, but there was no more change on the prolonged treatment(Fig. 2). SDS-PAGE of the treated myofibrils revealed the appearance of 35,000 and 48,000 Da components, assumed to originate from R4, corresponding to the increase of MVAA. They were suggested to adhere to myofibrils during incubation. On the treatment of myofibrils with R4 at pH 5.7-7.2, the lower pH gave the larger increase of MVAA and the more remarkable adhesion of those proteins. Therefore, the time-dependent adhesion of those basic proteins through a denaturation at around pH 5.5 to myofibrils was assumed to raise the Mg-ATPase activity.

In order to confirm whether or not such phenomena as *in vitro* occurs during meat conditioning, rabbit muscles were stored at 0℃ for 12 days postmortem and myofibrils were analyzed with SDS-PAGE. As shown in Fig.3, the 35,000 Da component emerged above the band of tropomyosin and increased gradually, while the 48,000 Da component was

not observed. Therefore, the 35,000 Da component was considered to be the factor increasing the Mg-ATPase activity *in situ*.

However, R4 increased the Mg-ATPase activity not only at low KCl concentrations but also at high KCl concentrations (see Fig. 2), resulting in no change in DAAK. Thus, myofibrils which had been treated with R4 at pH 5.5 and 25℃ for 3hr were incubated with different concentrations of calpain or the cathepsins mixture. The successive treatment with R4 and calpain increased the Mg-ATPase activity at all KCl concentrations. On the other hand, the successive treatment with R4 and a cathepsin mixture increased both MVAA and DAAK (Fig. 4), which was quite similar to the change during meat conditioning .

Therefore, it is possible that the coordinate action of a basic 35,000 Da protein in R4(assumed to be glyceraldehyde 3-phosphate dehydrogenase) and cathepsins induce the changes in Mg-ATPase activity of myofibrils during meat conditioning.

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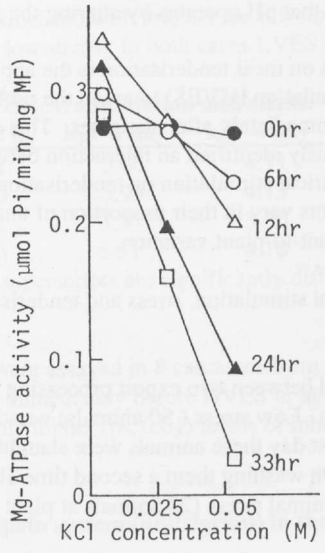

Fig.1. Mg-ATPase activity of myofibrils treated with the cathepsin mixture at pH 5.5 for varying periods. Myofibrils(MF, 5mg/ml) were incubated with the cathepsin mixture(specific activity against Z-Phe-Arg-MCA, 1.78 mU/ml) in 50mM sodium acetate buffer (pH5.5)/3mM dithiothreitol/0.08M KCl/ 7.5mM NaN_3 at 25°C for 0-33hr. After incubation the Mg-ATPase activity of the myofibrils diluted 5 times was measured.

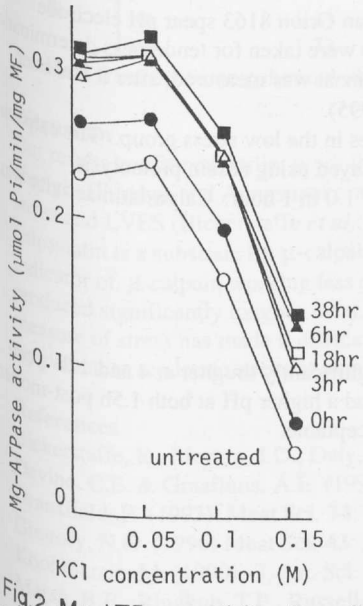

Fig.2. Mg-ATPase activity of myofibrils incubated with R4 for varying periods. Myofibrils(MF, 5mg/ml) were incubated with R4(1.9mg/ml) in 75mM sodium acetate buffer (pH5.5)/3mM dithiothreitol/1mM EDTA/0.08M KCl /10mM NaN_3 at 25°C for 0-38hr. The Mg-ATPase activity of the myofibrils washed with 0.16M KCl/5mM NaN_3 were measured.

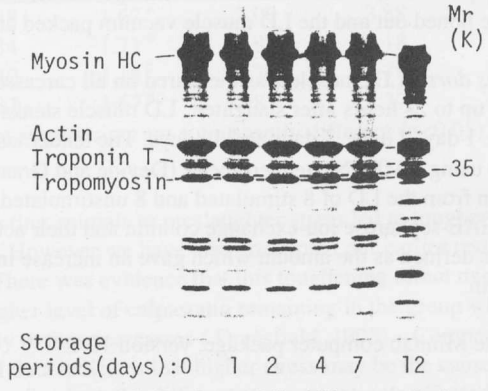

Fig.3. SDS-PAGE of myofibrillar proteins prepared from rabbit muscles stored at 0°C

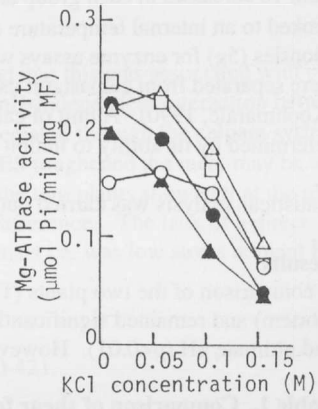

Fig.4. Effects of cathepsins treatment on Mg-ATPase activity of myofibrils pretreated with R4. Myofibrils(MF, 5mg/ml) were pretreated with R4 for 3hr as described in Fig.2. After the pretreatment, myofibrils were washed with 0.16M KCl/5mM NaN_3 and then incubated with the cathepsin mixture (specific activity against Z-Phe-Arg-MCA, 0(△), 0.83(□), 1.7(●) and 2.5 (▲) mU/ml) in 50mM sodium acetate buffer (pH5.5)/3mM dithiothreitol/1mM EDTA/0.08M KCl/7.5 mM NaN_3 at 25°C for 18hr. ○, treated without R4 and the cathepsin mixture.