Dissociation of actomyosin into actin and myosin and liberation of actomyosin from myofibrils induced by various phosphates

Masanori Matsuishi*, Kiwamu Kanno, Masako Sawada, Ikumi Suzuki and Akihiro Okitani

School of Food Science and Technology, Nippon Veterinary and Life Science University, Tokyo 180-8602, Japan

*Corresponding author e-mail: matmas@nvlu.ac.jp

Abstract – Aim of this study is to examine whether various phosphates dissociate free actomyosin or actomyosin restrained in myofibrils by gel permeation chromatography and SDS-PAGE. Eight millimolar pyrophosphate (PYP), tripolyphosphate (TRP) or inosine-5'-monophophate (IMP) dissociated actomyosin in 0.6 M KCl into myosin and actin. PYP or IMP dissociated actomyosin in 0.2 M KCl into myosin and actin, while TRP solubilized whole actomyosin without dissociation. PYP or TRP liberated whole actomyosin without dissociation from myofibrils, although IMP did not. These results suggested that PYP and TRP release actomyosin from particular restraints in myofibrils, however released actomyosin might not be dissociated into actin and myosin because of another binding between both proteins which cannot be cleaved by PYP and TRP.

Key words: actin, **inosine-5'-monophosphate, myosin, pyrophosphate, tripolyphosphate,**

I. INTRODUCTION

Various inorganic polyphosphate used for manufacture of meat products dissociate actomyosin into myosin and actin, enhancing extraction of myosin from meat and allow to produce meat products which have high binding properties and water-holding capacity even at low salt concentrations. The authors reported that IMP, an organic phosphate, dissociates actomyosin insoluble at 0.2 M KCl into actin and myosin soluble at 0.2 M KCl [1]. Furthermore, we indicated that IMP improves binding properties and water-holding capacity of model sausages to the same extent as PYP [2] and is likely to be one of the candidate which resolve postmortem rigor during meat aging [3]. However, only ATP of these phosphates was indicated to dissociate actomyosin into actin and myosin apparently as changes of molecular mass [4].

In this study, actomyosin and myofibrils incubated with PYP, IMP and TRP were analyzed by gel permeation chromatography and SDS-PAGE to examine the dissociation of actomysin into actin and myosin and the liberation of actomyosin from myofibrils.

II. MATERIALS AND METHODS

Breast meats (*M. pectoralis superficials*) of chicken were purchased from retail shops and minced with a meat chopper. Actomyosin was extracted from minced meats with the Weber-Edsall solution (0.6 M KCl/0.04 M $NaHCO_3$/0.01 M Na_2CO_3) [5]. Myofibrils were prepared from meats according to the method of Yang et al. [6].

A Sepharose CL-2B (GE Healthcare Bio-Science AB, Sweden) column (⌀2 cm x 80 cm) was used to examine the changes of molecular size of proteins. Actomyosin (1.67 mg/mL) was incubated with or without 8 mM phosphates in 20 mM Tris-HCl (pH 7.2)/2 mM NaN_3/0.6 M KCl (0.6 K-solution) at 0°C for 1 h, and then centrifuged. The obtained supernatant was subjected to the column equilibrated with 0.6 K solution containing 8 mM phosphates or not. Actomyosin (1.67 mg/mL) or myofibrils (4.17 mg/mL) were incubated with 8 mM phosphates in 20 mM Tris-HCl (pH 7.2)/2 mM NaN_3/0.2 M KCl (0.2 K-solution) at 0°C for 1 h, and then the supernatant was subjected to the column equilibrated with 0.2 K-solution containing 8 mM phosphates.

Eluted solutions were collected in 6 mL. Each fraction was analyzed with SDS-PAGE [7].

III. RESULTS AND DISCUSSION

SDS-PAGE pattern of fractions eluted from a Sepharose CL-2B column to which supernatant of actomyosin treated with 0.6 M KCl and 8 mM PYP was subjected in the presence of 0.6 M KCl was shown in Fig. 1. Myosin heavy chains (MH) and actin monomers (AM) were detected separately in fraction No. 24 and 34. Treatment of actomyosin with IMP and TRP gave similar results as the case of PYP, while in the case of actomyosin treated without phosphates MH and AM were detected together in fraction No.16 (data not shown). Thus, these phosphates were indicated to dissociate actomyosin in 0.6 M KCl into myosin and actin regarding changes of molecular size.

PYP and IMP dissociated actomyosin into myosin and actin in the presence of 0.2 M KCl as well as 0.6 M KCl (data not shown). Figure 2 shows SDS-PAGE of fractions eluted from a column on which supernatant of actomyosin treated with 0.2 M KCl and 8 mM TRP was applied. Most of MH and AM were detected together in fraction No. 16, indicating that TRP solubilizes actomyosin without dissociation.

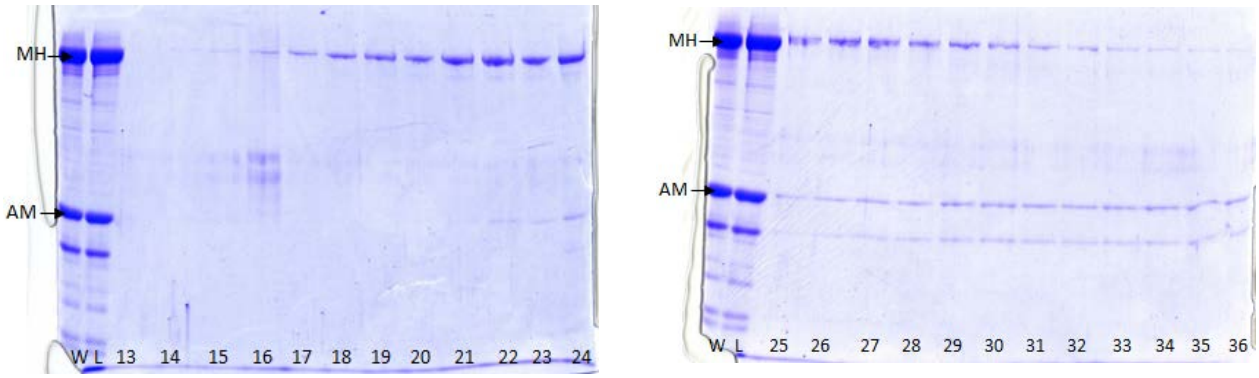

Fig. 1. SDS-PAGE of fractions eluted from a Sepharose CL-2B column on which supernatant of actomyosin treated with 0.6 M KCl and 8 mM PYP was applied. The column was equilibrated with 0.6K-solution containing 8 mM PYP. W, whole actomyosin (before centrifuge); L, loaded sample; MH, myosin heavy chain; AM, actin monomer.

When myofibrils treated with 8 mM PYP or TRP were centrifuged and the obtained supernatants were subjected to a column equilibrated with a 0.2-K solution containing 8 mM PYP or TRP, MH and AM were detected together in fraction No.16. However, no protein was detected in any fractions in the case of IMP (data not shown). These results suggest that PYP and TRP release actomyosin from particular restraints in myofibrils, however, actomyosin liberated from myofibrils might not be dissociated to actin and myosin because of another binding like a hoop which cannot be cleaved by PYP and TRP.

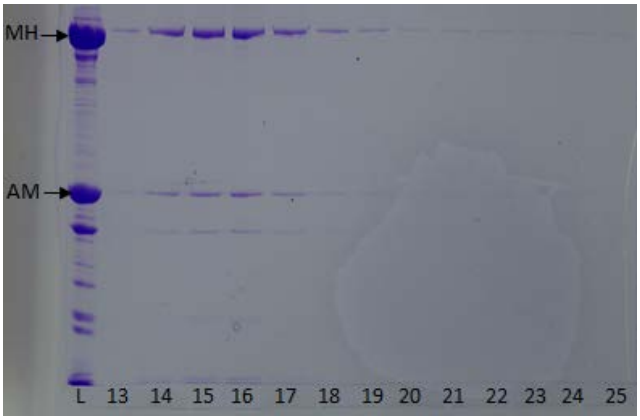

Fig. 2 SDS-PAGE of fractions eluted from a Sepharose CL-2B column on which supernatant of actomyosin treated with 0.2 M KCl and 8 mM TRP was applied. The column was equilibrated with 0.2K-solution containing 8 mM TRP. Abbreviations on the pictures of gels are the same as those in Fig. 1.

IV. CONCLUSION

PYP and IMP dissociate actomyosin in both of 0.6 and 0.2 M KCl, however, TRP dissociates actomyoisn only in 0.6 M KCl. Furthermore, although PYP and TRP release actomyosin from particular restraints in myofibrils, actomyosin liberated from myofibrils might not be dissociated to actin and myosin because of another binding like a hoop which cannot be cleaved by PYP and TRP.

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