LIBERATION OF ACTIN AND MYOSIN FROM MYOFIBRILS INDUCED BY POLYPHOSPHATES

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Abstract – The aim of this study is to demonstrate the detail of the effects of polyphosphates (5'-adenosine diphosphate (ADP), potassium triphosphate (TRP) and etc.) except sodium pyrophosphate (PYP) on myofibrils. Among ADP derivatives, adenine base was needed for releasing thin and thick filaments from the restraints of myofibrils. Commercial TRP is known to be contaminated with about 10% pyrophosphate. Four, 8 and 16 mM TRP induced the liberation of actin and myosin from myofibrils incubated with 20 mM Tris-HCl (pH 7.2)/2 mM NaN_3/0.2 M KCl at 0°C for 22 h, while 0.4, 0.8 and 1.6 mM PYP did not. Furthermore, TRP was not hydrolyzed under these conditions. Thus, it was clarified that TRP itself, not pyrophosphate contaminating TRP or produced by hydrolyzation of TRP, liberated the both proteins. Four mM TRP could liberate a larger amont of actin and myosin in the presence of 8 mM IMP than in the absence, suggesting that the low concentration of TRP released only thin and thick filaments from the restraints of myofibrils. Other polyphosphates seemed to be the same as TRP. About 1 h was necessary for the complete liberation of actin and myosin from myofibrils by TRP and other polyphosphates. Phase-contrast microscopic observation showed that myofibrils incubated with 4 mM ADP plus 8 mM IMP and 8 mM TRP for 24 h swelled immediately after the addition of those, and then were melted.

Key Words – myofibril, polyphosphate, triphosphate, ADP

I. INTRODUCTION

Polyphosphates used in the manufacture of sausage and ham have been known to improve binding properties and water-holding capacity of meat. Pyrophasphate was reported to dissociate actomyosin to actin and myosin in meat and to solubilize the both proteins to improve binding properties and water-holding capacity of meat [1, 2]. Pyrophosphate have been also known to lower NaCl concentration necessary to solubilize myofibrillar proteins and swell myofibrils [3, 4, 5]. Triphosphate and hexametaphosphate were shown to increase the solubility of actomyosin and extractability of proteins from myofibrils [1]. Their work also suggested that triphosphate is effective only after it is hydrolyzed into pyrophosphate in the presence of 0.6 M KCl and 2 mM $MgCl_2$. Furthermore, we demonstrated that 5'-inosine monophosphate (IMP) dissociated actomyosin to actin and myosin [6].

The objective of this study is to reveal the detail of the effects of polyphosphates except pyrophosphate on myofibrils.

II. MATERIALS AND METHODS

Breast meats (*M. pectoralis superficials*) of chicken (a cross of White Plymouth Rock x White Cornish) were obtained from retail shops as chilled meats and minced immediately after trimming off visible fat. IMP (disodium salt), ADP (disodium salt), 5'-guanosine diphosphate (GDP, sodium salt), 5'-uridine diphosphate (UDP, disodium salt), PYP and TRP were purchased from Wako Pure Chemical Industries (Osaka). Sodium tetrapolyphosphate (TEP) and sodium hexametaphosphate (HEP) were obtained from Nacalai Tesque, Inc. (Kyoto). Sodium ultraphosphate (ULP) and sodium metaphosphate (MEP) (food additive grade) were purchased form Mitejima Chemical Inc. (Osaka). All other chemicals were of analytical grade. Myofibril solution (0.5 mL of 10 mg protein/mL in 0.16 M KCl/5 mM NaN_3) was mixed with various reagents to prepare 1.2 mL of an incubation

mixture containing 4.17 mg/mL of myofibrils/0.2 M KCl/20 mM Tris-HCl buffer (pH 7.2)/6 mM NaN_3/8 mM IMP, 1-4 mM ADP, 0.1-8 mM PYP, 1-32 mM other polyphosphates or nothing. The mixture was incubated at 0°C for the specified period and then centrifuged at 25,000x*g* for 10 min. The supernatants obtained were subjected to SDS-PAGE analysis carried out by the method of Laemmli [7] using a 10 % gel to ascertain liberation of actin and myosin from myofibrils into supernatants. This method is based on the properties of free actin or free myosin that is solubilized in 0.2 M KCl and actomyosin or myofibrils that are precipitated in 0.2 M KCl [8, 9]. Small portions of mixture after incubation were observed by phase-contrast microscopy at a magnification of 1,000 x.

III. RESULTS AND DISCUSSION

We found that ADP released thin and thick filaments from the restraints of myofibrils (details are submitted to a journal). In order to reveal the structure of nucleoside-5'-diphosphates needed to release the restraints, myofibrils were incubated with ADP, GDP that has a purine ring same as ADP or UDP that has a pyrimidine ring, in the presence of 8 mM IMP. Fig. 1 shows that only ADP liberated actin and myosin from myofibrils at both 1 mM and 4 mM. Thus, an adenine-based structure was found to be needed to release thin and thick filaments from the restraints of myofibrils. On the contrary, nucleoside-5'-monophosphates such as IMP were reported to need a purine ring to dissociate actin and myosin [12]. These differences are likely to be due to structural differences of binding sites of ADP and nucleoside-5'-monophosphates in myofibrils.

Commercial triphosphate is known to contain about 10 % pyrophosphate. Moreover, it was reported that triphosphate itself did not solubilize muscle proteins, but pyrophosphate produced by hydrolysis of triphosphate did [10, 11]. Fig.2 shows SDS-PAGE pattern of supernatants obtained from myofibrils which were incubated with 1-32 mM TRP or 0.1-3.2 mM PYP. Although 0.4 mM PYP (i in Fig. 2) did not liberate actin and myosin, 4 mM TRP (d) liberated those proteins. This indicates that pyrophosphate likely to contaminate triphosphate with 10 % could not liberate actin and myosin but triphosphate itself could do it. Moreover, under this condition orthophosphate was not released from TRP (data

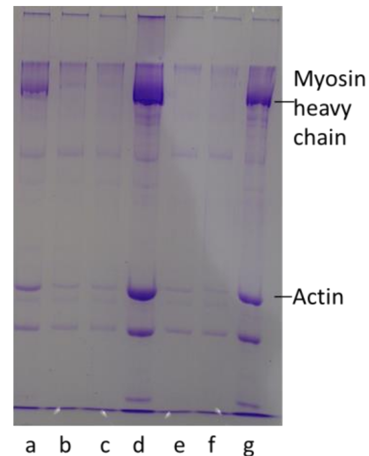

Fig. 1. SDS-PAGE of the supernatants of myofibrils(MF) incubated with ADP, GDP and UDP in the presence of IMP.
Myofibrils were incubated with 1 mM ADP (a), 1 mM GDP (b), 1 mM UDP (c), 4 mM ADP (d), 4 mM GDP (e) or 4 mM UDP (f) at 0°C and pH 7.2 in the presence of 8 mM IMP for 22h, and then centrifuged in 0.2 M KCl . The supernatant obtained was subjected to SDS-PAGE. g, whole myofibrils incubated without ADP, GDP and UDP at 0°C and pH 7.2 for 22h.

not shown), indicating that triphosphate was not hydrolyzed into pyrophosphate. Therefore, it is clarified that triphosphate itself instead of pyrophosphate contaminating or produced through hydrolysis acted on myofibrils. This is different from the results of Yasui et al. [10] and Hamm and Nerral [11], which might be ascribable to the KCl concentration (0.2 M vs 0.6 M) and temperature (0°C vs 20°C).

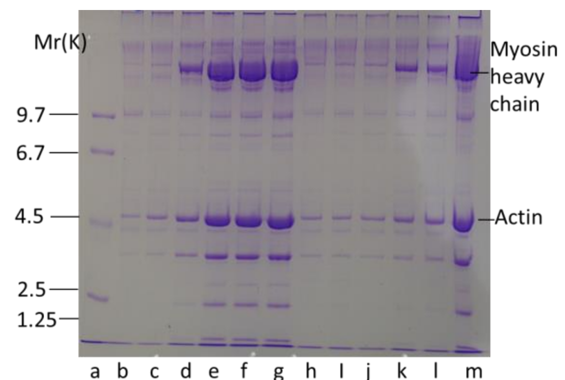

Fig. 2. SDS-PAGE of the supernatants of myofibrils incubated with TRP and PYP. Myofibrils were incubated with TRP of (b, 0 mM; c, 1 mM; d, 4 mM; e, 8 mM; f, 16 mM; g, 32 mM) or PYP of (h, 0.1 mM; i, 0.4 mM; j, 0.8 mM; k, 1.6 mM; l, 3.2 mM) at 0°C and pH 7.2 for 22h , and then centrifuged in 0.2 M KCl. The supernatant obtained was subjected to SDS-PAGE. a, molecular weight marker; m, whole myofibrils incubated without TRP and PYP.

Fig. 3 shows SDS-PAGE pattern of supernatants obtained from myofibrils incubated with 1-32 mM

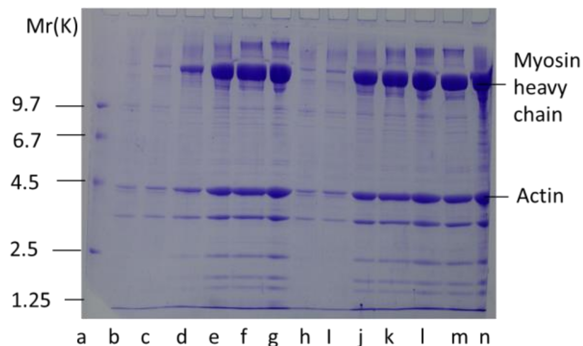

Fig. 3. SDS-PAGE of the supernatants of myofibrils incubated with TRP in the absence or presence of IMP. Myofibrils were incubated with with TRP of (b and h, 0 mM; c and i, 1 mM; d and j, 4 mM; e and k, 8 mM; f and l, 16 mM; g and m, 32 mM) in the absence (b-g) or presence (h-m) of 8 mM IMP at 0°C and pH 7.2 for 22h, and then centrifuged in 0.2 M KCl. The supernatant obtained was subjected to SDS-PAGE. a, molecular weight marker; n, whole myofibrils without TRP and IMP.

TRP in the presence or absence of 8 mM IMP. Eight-32 mM TRP (e, f, g, k, l, m in Fig. 3) liberated large amounts of actin and myosin irrespective of the presence or absence of IMP. This result indicates that such a high concentration of TRP not only dissociates actin and myosin but also releases thin and thick filaments from the restraints of myofibrils. Four mM TRP liberated a little amount of actin and myosin in the absence of IMP (d), while it liberated a large amount of those proteins in the presence of 8 mM IMP (j), indicating that such a low concentration of TRP has only a power to release thin and thick filaments from the restraints of myofibrils. This action is the same as the function of ADP and low concentrations (1-1.5 mM) of PYP (details are submitted to a journal). Similar results were obtained in the case of TEP, HEP, ULP and MEP. Thus, all these polyphosphates act specifically against myofibrillar structures.

The duration of incubation necessary for the liberation of actin and myosin from myofibrils by triphosphate was examined and the result is shown in Fig. 4. When myofibrils were incubated with 8 mM TRP, the liberation of the both proteins occurred partially after 15 min incubation (e in Fig. 4) and occurred almost completely after 1 h (g) and 22h of incubation (j). Thus, incubation time for a period of 1h was found to be necessary for the both proteins to be liberated fully. Similar results were obtained in the case of TEP, HEP, ULP and MEP.

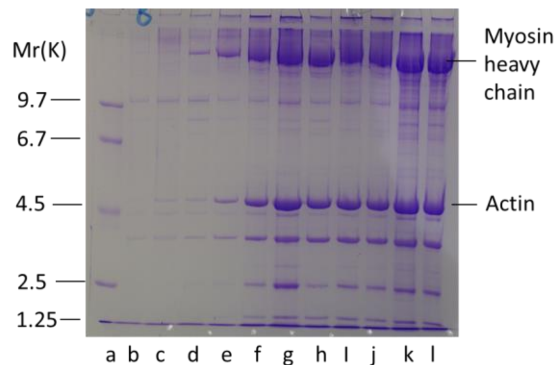

Fig. 4. SDS-PAGE of the supernatants of myofibrils(MF) incubated with TRP for various periods.
Myofibrils were incubated with (d-j) or without (b and c) 8 mM TRP at 0°C and pH 7.2 for: b and d, 0 min; e, 15 min; f, 30 min; g, 1 h; h, 2 h; i, 4 h,; c and j, 22 h, and then centrifuged in 0.2 M KCl. The supernatant obtained was subjected to SDS-PAGE. a, molecular weight marker; k and l are whole myofibrils incubated without TRP at 0°C for 0 min and 22h, respectively.

When myofibrils were incubated with polyphosphates, structural changes of the whole myofibrils were examined by phase-contrast microscopy. Fig. 5 shows that 4 mM ADP plus 8 mM IMP induced no changes after 0 h incubation but narrowing of A-band and swelling of myofibrils after 24 h incubation. Eight mM TRP induced swelling of myofibrils immediately after mixing (0 h) and myofibrils were completely melted after 24 h incubation, which were similar with the effect of PYP (data not shown). These results revealed that 4 mM ADP plus 8 mM IMP and 8 mM TRP in the presence of 0.2 M KCl at pH 7.2 induced the similar changes of myofibrils with that induced by 10 mM PYP in the presence of 0.4 M KCl at pH5.5 [3].

IV. CONCLUSION

TRP itself but not contaminating pyrophosphate was clarified to liberate myosin and actin from myofibrils in the presence of 0.2 M KCl at pH 7.2. Polyphosphates such as TRP were likely to have specific action against myofibrillar structure, i.e., to dissociate actin and myosin and to release thin and thick filaments from the restraints of myofibrils, resulting in swelling and melting of myofibrils.

REFERENCES

1. Yasui, T., Sakanishi, M. & Hashimoto, Y. (1964). Effects of inorganic polyphosphates on the solubitily and extractability of myosin B. Journal of Agricultural and Food Chemistry 12: 392-398.

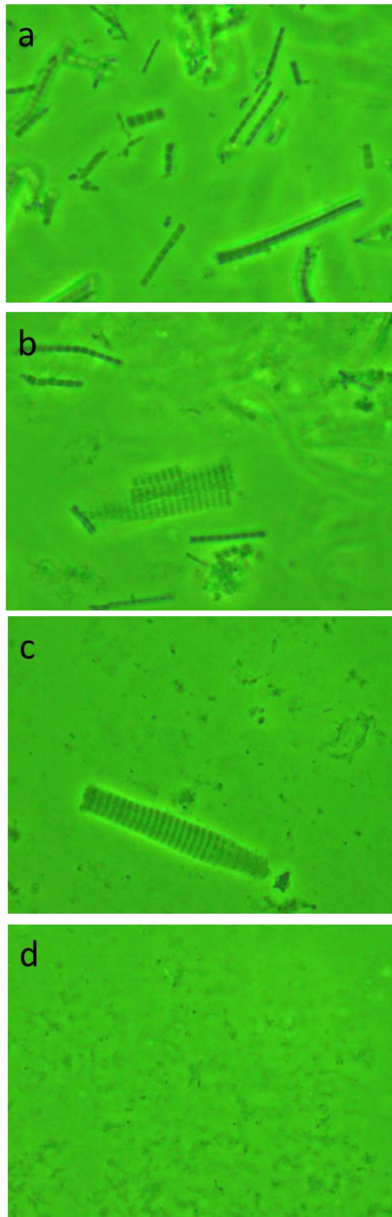

Fig. 5. Phase-contrast microscopic observation of myofibrils incubated with ADP plus IMP or TRP.
Myofibrils were incubated with 4 mM ADP plus 8 mM IMP (a and b) or 8 mM TRP (c and d) in 0.2 M KCl at 0°C and pH 7.2 for 0 h (a and c) or 24h (b and d). Magnification , 1,000 x.

2. Bechtel, P. J. (1986). Muscle as food. Orland: Academic Press, Inc., pp. 178-181.

3. Offer, G. & Trinick, J. (1983). On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Science 8: 245-281.

4. Xiong, Y. L., Xingqiu, L., Harmon, R. J., Wang, C. & Moody, W. G. (2000). Salt- and pyrophosphate-induced structural changes in myofibrils from chicken red and white muscles. Journal of Science of Food and Agriculture 80: 1176-1182.

5. Paterson, B. C., Parrish, JR. F. C. & Stromer, M. H. (1988). Effects of salt and pyrophosphate on the physical and chemical properties of beef muscle. Journal of Food Science 53: 1258-1265.

6. Okiani, A., Ichinose, N., Koza, M., Yamanaka, K., Migita, K. & Matsuishi, M. (2008). AMP and IMP dissociate actomyosin into actin and myosin. Bioscience Biotechnology and Biochemistry 72: 2005-2011.

7. Laemmli, U. K. (1970). Cleavage of structural poteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685

8. Szent-Györgyi, A. (1951). Chemistry of Muscular Contraction. New York: Academic Press, pp. 143-155.

9. Briskey, E. J. & Fukazawa, T. (1971). Myofibrillar proteins of skeletal muscle. Advanced in Food Research 19: 279-360.

10. Yasui T., Fukazawa, T., Takahashi, K., Sakanishi, M. & Hashimoto, Y. (1964). Specific interaction of inorganic polyphosphates with myosin B. Journal of Agricultural and Food Chemistry 12: 399-404.

11. Hamm, R. and Neraal, R. (1977). On the enzymic breakdown of tripolyphosphate and diphosphate in comminuted meat. Zeitschrift für Lebensmittel-Untersuchung und –Forschung. 164: 243-246.

12. Nakamura, Y., Migita, K., Okitani, A. & Matsuishi, M. (2012). Enhancing effect of IMP on myosin and actin extraction from porcine meat. Bioscience Biotechnology and Biochemistry 76: 1611-1615.