

### Dissociation effect of chicken actomyosin by inosinic acid and its related compounds

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**Introduction:** Sodium pyrophosphate (PP) is widely used as a food additive to solubilize myosin from myofibrillar proteins of meat. PP-treated meat products shows better water-holding capacity and binding property that contribute to be preferable texture. One of its underlying mechanism is suggested to be a facilitation of actomyosin (AM) dissociation into myosin and actin. Our previous studies reported that inosinic acid (IMP) and adenylic acid (AMP) can also dissociate meat actomyosin and IMP improve the texture profiles of heat-induced meat gels (Okitani, A. *et al*, 2008; Nakamura, Y. *et al*, 2014). Here, we evaluated the dissociation effect of AM by inosinic acid and its related compounds, in order to elucidate structural requirements for AM dissociation.

**Materials and methods:** Raw chicken breasts were purchased from retail shops and immediately used. AM solution was prepared according to our previous method (Okitani, A. *et al*, 2008). Briefly, minced meat was incubated with 6 volumes of Weber-Edsall solution for 24 h at 4 °C. The supernatant was diluted to a concentration of 0.2 mol/L KCl with distilled water and centrifuged. The precipitate was collected and re-dissolved in 0.6 mol/L KCl. The protein was refined by repeating this dilution-centrifugation step two more times. The concentration of protein was determined by a Biuret method.

The prepared AM solution (2 mg protein/mL) was mixed with a sample in reaction buffer (0.6 mol/L KCl, 20 mmol/L Tris-HCl, pH 7.2) and incubated for 10 min on ice. After centrifugation, the supernatant was collected and subjected to SDS-PAGE analysis. The intensities of band were quantified using Image Studio Lite (LI-COR Biosciences).

**Results:** The AM solution was incubated with PP at 0, 1, 2, 4, 8 or 16 mM. The band densities of liberated myosin and actin were elevated with the increasing of PP concentration. In the negative control (0 mM), small proportion (ca.10%) of myosin was detected here, indicating the purity of AM was considered to be enough high (ca. 90%). At 4 mM PP, the liberated myosin reached 65% of total myosin. Next, IMP and AMP were also incubated with the AM and its dissociation effect was evaluated. IMP and AMP exhibited a dissociation effect in a dose-dependent manner. At 4 mM IMP, the liberated myosin reached 18%. AM dissociation activity of AMP is weaker than that of IMP. In addition, inosine and hypoxanthine, which is a component of IMP, did not show an apparent effect of AM dissociation at the range of tested concentration. Therefore, these data suggest that the whole structure of ribonucleotide involve in the AM dissociation. The degree of its activity might depend on the moiety of base in ribonucleosides.

**Conclusions:** The present study suggests that the whole structure of IMP might be necessary to dissociate chicken AM into myosin and actin.

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#### Literature:

1. Okitani, 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(8), 2005-2011.
2. Nakamura, Y., Migita, K., Okitani, A. and Matsuishi, M. (2014), IMP improves heat-induced meat gel. *Animal Science Journal*, 85, 595-601.