P-10-24

ADPase activity of myofibrils and actomyosin prepared from chicken muscle (#593)

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Introduction

Actomyosin (AM) is a well-known complex of actin and myosin, which hydrolyzed ATP into ADP to acquire energy of muscle contraction [E. D., Forrest, J. C., Gerrard, D. E. & Mills, E. W. (2001). Principles of Meat Science, 4th ed., Kendal/Hunt Publishing Company, Iowa, USA, pp. 73-75]. This activity remains in vitro and develops even when AM is associated with other proteins to form myofibrils (MF). We found that Inosine-5′-monophosphate (IMP) breaks the interaction between actin and myosin to liberate those proteins from MF with assistance of ADP likely to break restraints (probable to be Z line) against actin filament in MF [Matsuishi, M., Tsuji, M., Yamaguchi, M., Kitamura, N., Tanaka, S., Nakamura, Y., and Okitani, A. (2016). Anim. Sci. J., 87, 1407-1412]. However, sometimes ADP could independently release actin and myosin from myofibrils. We hypothesized that actomyosin or a contaminant had ADPase activity to produce AMP (adenosine-5′-monophosphate) which can dissociate AM into actin and myosin as well as IMP [Okitani, A., Ichinose, N., Koza, M., Yamanaka, K., Migita, K., Matsuishi, M. (2008). Biosci. Biotechnol. Biochem., 72, 2005-2011]. The aim of this study is to clarify whether this hypothesis is true or not.

Methods

Breast meats (M. pectoralis superficials) of chicken was obtained from retail shops as chilled meats and used immediately. MF was prepared from minced meats according to the method of Matsuishi and Okitani [Matsuishi, M., Okitani, A. (2000). Meat Sci., 56, 369-377]. AM was extracted from meats by Weber-Edsall solution and refined by 3 times precipitation-solubilization cycle according to the method of Szent-Györgyi [Szent-Györgyi (1951). Chemistry of Muscular Contraction, Academic Press, New York, pp. 146-152[]. ADPase and ATPase activity of MF and AM were measured by the absorbance of pigment developed by the reaction between released phosphate and the mixture of ammonium molybdate and Amidol reagent according to the method of Matsuishi and Okitani (2000). ADP, AMP and ATP were analyzed with Waters M600 HPLC system (Nihon Waters K.K., Tokyo, Japan) equipped with Shimpack CLC-ODS (M) column (4.6x150 mm, Shimazu Co. Ltd., Kyoto, Japan) according to the methods of Watanabe et al. [Watanabe, A., Tsuneishi, E., Takimoto, Y. (1989). J. Food Sci., 54, 1169-1172].

Results

When MF (0.25 mg/mL) and AM (0.25 mg/mL) were incubated with 1 mM ADP in the presence of 0.2 M KCl/2 mM $MgCl_2$/20 mM Tris-HCl (pH8)/2 mM NaN_3 at 30°C, about 0.01 micromole of phosphate was liberated by 1 mg proteins for 1 min, indicating that AM as well as MF has ADPase activity. Four mg/mL of AM was incubated with 0-4 mM ADP or ATP in the presence of 20 mM Tris-HCl (pH 7.2)/2 mM NaN_3/0.6 M KCl/8 mM $MgCl_2$/1 mM $CaCl_2$at room temperature for 30 min. As shown in Fig. 1, remarkable superprecipitation was observed in the presence of 1 and 2 mM ADP as well as 2 and 4 mM ATP, suggesting that ATP may be produced in the mixture of AM and ADP.

AM sample was gently stirred in the presence of 10 mM Tris-HCl (pH7.2)/1 mM NaN_3 /0.02 % TritonX-100/0.2 M KCl at 0°C for 15 min and then centrifuged at 9,900 x *g* and 4°C for 10 min. ADPase and ATPase activities of AM before fractionation, the obtained supernatant and precipitate were measured. As shown in Fig. 2, ADPase activity was not found in the supernatant or precipitate, although the activity was found in the mixture of the supernatant and precipitate. On the other hand, ATPase activity was found in the precipitate as well as the mixture of the supernatant and precipitate. These results suggested that some components necessary for ADPase activity were separated into the supernatant and the precipitate, while actomyosin which was precipitated in these conditions would be responsible for ATPase activity.

The supernatant or the precipitate prepared from AM was incubated with 1 mM ADP in the presence of 0.2 M KCl/2 mM MgCl2/20 mM Tris-HCl (pH8)/2 mM NaN3 at 30°C for 0-20 min, and then trichloroacetic acid was added to the incubated solution to the final concentration of 4%. The mixture was centrifuged at 1,400 x g for 8 min and the obtained supernatant was applied to the analysis by HPLC. As shown in Fig. 3 (A), when the supernatant was incubated with ADP, the concentration of ADP decreased and that of AMP increased with the elapse of time, while ATP increased. Changes of these compounds suggested that the supernatant may contain adenylate kinase converting 2ADP to ATP and AMP [Kishi, F., Maruyama, M., Tanizawa, Y., Kanazawa, A. (1986) J. Biol. Chem., 261, 2942-2945]. On the other hand, when the precipitate was incubated with ADP, the concentration of ADP, AMP and ATP were not changed (Fig. 3 (B)). All above results suggest that actomyosin was bound with adenylate kinase, which converts 2ADP into ATP and AMP, and actomyosin ATPase hydrolyzed ATP into ADP. Phosphate and AMP was likely to be generated through this reaction cycle.

Notes

Conclusion

MF and AM had ADPase activity shown by liberation of phosphate. This activity would be given by the reaction cycle of adenylate kinase converting ADP into ATP and AMP, and actomyosin ATPase hydrolyzing ATP into ADP and phosphate.

Notes

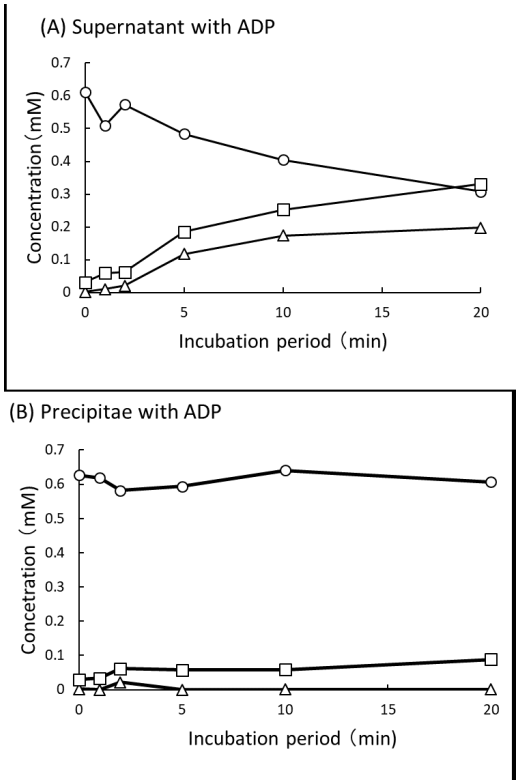

Fig. 3. Changes in the concentration of ATP, ADP and AMP in the mixtures of ADP and the fractions.
The fractions were prepared from AM as described in the text. Circle, ADP; square, AMP; triangle, ATP.

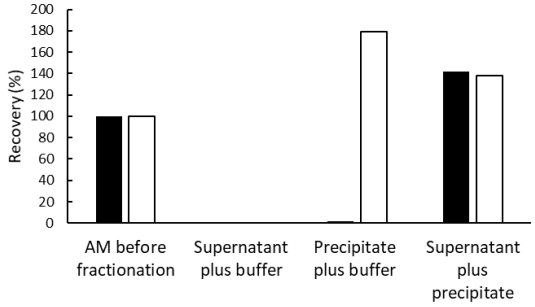

Fig. 2. Recovery of ADPase and ATPase activities of fractions obtained from AM.
Closed rectangle, ADPase activity; open rectangle, ATPase.

Notes

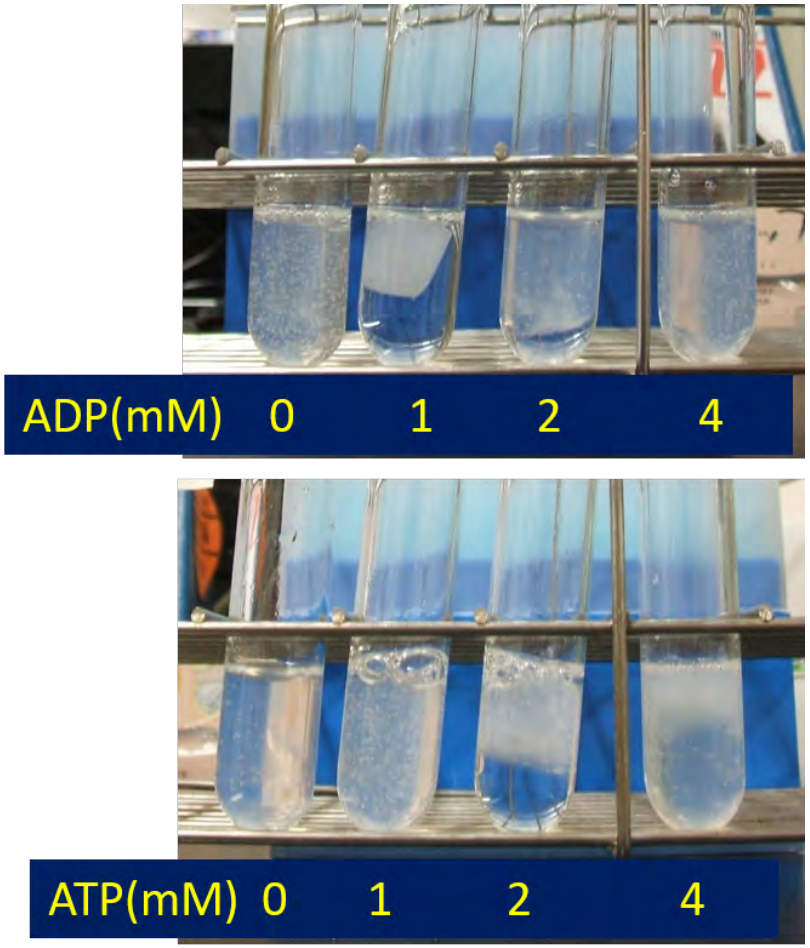

Fig. 1.
Superprecipitation of AM caused by various concentrations of ADP and ATP.

Notes