Effect of carnosine on ATP hydrolysis by actomyosin under acidic conditions

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Introduction: Skeletal muscle of livestock animals causes rigor mortis in the process of the conversion of muscle into meat. One of the factors which are involved in the progression of rigor mortis is ATP hydrolysis by myosin with physiological changes such as pH decline and increasing of Ca^{2+} concentration in muscle. Skeletal muscle contains a large amount of carnosine (CAR). CAR functions to maintain intracellular homeostasis, such as an antioxidant and a buffering property. However, the effect of CAR on the progression of rigor mortis is still unclear. Moreover, to clarify the effect of CAR on rigor mortis, ATP hydrolysis by myosin in the presence of actin should be examined since myosin binds to and dissociates from actin repeatedly during the progression of rigor mortis. Therefore, we investigated the effect of CAR on the ATP hydrolysis by actomyosin under the conditions assumed to be the environment in post-mortem muscle.

Materials and methods: Actomyosin was prepared from chicken breast muscle. The actomyosin ATP hydrolysis reactions were investigated at different pH (7.0/5.5) and pCa (9.0/5.0) considering the physiological changes in post-mortem muscle. The actomyosin ATPase assay was conducted in the presence of 1 mM $MgCl_2$ and 150 mM KCl. In the ATPase assay, ATP was added to the actomyosin suspension (1.0 mg/ml) to initiate the ATP hydrolysis, and the reaction was stopped by adding trichloroacetic acid. ATPase activity was determined by measuring the amount of inorganic phosphate (Pi) using the Fiske-Subbarow method. The superprecipitation of actomyosin was performed as *in vitro* model of muscle contraction. The change in appearance of the actomyosin suspension (3.0 mg/ml) after the addition of ATP was observed. In addition, to evaluate the superprecipitation reaction, the change in turbidity of the actomyosin suspension (0.7 mg/ml) after the addition of ATP was monitored by measuring the absorbance at 660 nm.

Results: In the absence of CAR, the ATPase activity of actomyosin at pH 5.5-pCa 9.0 was lower compared to that at pH 7.0. On the other hand, the activity at pH 5.5-pCa 9.0 was similar to that at pH 7.0 in the presence of CAR and was significantly higher than that in the absence of CAR. Therefore, actomyosin ATPase activity under acidic and low Ca^{2+} level conditions was activated by CAR. The superprecipitation reactions at pH 7.0 occurred rapidly both in the presence and the absence of CAR. Whereas, at pH 5.5, the compact precipitate was formed rapidly in the presence of CAR while the larger precipitate was observed in the absence of CAR. The turbidity of actomyosin solution in the presence of CAR increased rapidly after the addition of ATP compared to that in the absence of CAR. Therefore, superprecipitation reaction was accelerated in the presence of CAR under acidic conditions.

Conclusions: CAR activates the actomyosin ATP hydrolysis under acidic and low Ca^{2+} level conditions, suggesting that CAR could affect the progression of rigor mortis in the post-mortem skeletal muscle.