カジキおよびイワシ・ミオシンとアクチンの凝集相互作用

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Short Paper

Aggregation-Interaction between Myosin and Actin from Striped Marlin and Sardine Muscles

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It has been widely accepted that the gel-forming ability of fish meat pastes is dependent upon myosin present in them. Recently, it has been reported that the rod portion of myosin plays a significant role in the thermal gelation of mysoin.[1-4] Since the solubility or the aggregation (assembly) of myosin depends strongly on rod portion, information on the aggregation of myosin seems to be useful for the elucidation of the mechanism of the thermal gelation. In our experimental results of the desalting-aggregation, no appreciable difference between striped marlin and sardine myosins was observed. The present paper deals with the aggregation-interaction between both myosins and actins.

Myosin was prepared from striped marlin *Makaira mitsukurii* and sardine *Sardinops melanostictus* muscles by the method of Mackie and Connell[5] with some modifications. The myosin solution containing 0.5 M KCl, 5 mM ATP, and 20 mM Tris-maleate buffer (pH 7.5) was clarified by centrifugation at 100,000×g for 3 h, and then myosin in the supernatant was collected by ammonium sulfate fractionation (giving 40–55% saturation). After dialyzing against 0.6 M KCl-10 mM phosphate buffer (pH 6.8), myosin solution was centrifuged at 20,000×g for 30 min. G-actin was prepared from both muscles according to the procedure of Seki *et al.*[6] The F-actin was purified according to the method of Spudich and Watt.[7] These preparations were homogeneous in SDS gel electrophoresis. The protein solutions (8–10 mg/m*l*) in 0.6 M KCl and 10 mM phosphate buffer (pH 6.8) consisting of the various ratios of myosin and F-actin were stirred gently at 2–4°C for 1 h. The aggregation-interaction reaction was allowed to proceed for 16–18 h at 2–4°C by means of dialysis against 50 vol. of 0.1 M KCl containing the same buffer. Each dialyzate was centrifuged at 10,000×g for 20 min. In case of myosin aggregation, myosin in 0.1 M KCl–0.45 M KCl containing 10 mM phosphate buffer (pH 6.6) was centrifuged at 30,000×g for 30 min. The degree of aggregation was evaluated by the ratio of protein in the centrifugal supernatant to total protein.

Fig. 1 shows the aggregation-interaction between myosins and actins from striped marlin and sardine muscles. Though distinct difference with regard to the aggregation curve could not be observed between both myosins (insert), the presence of F-actin showed

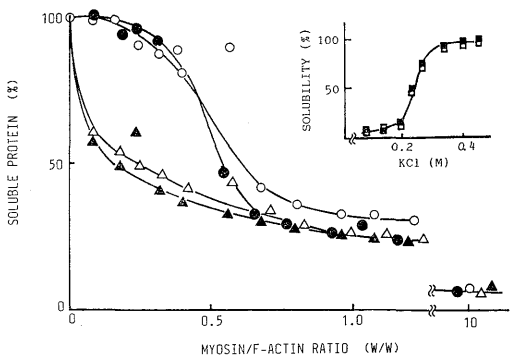

Fig. 1. Aggregation-interaction between myosin and actin from striped marlin and sardine muscles.
▲: Striped marlin myosin+Striped marlin F-actin,
△: Striped myosin+Sardine F-actin,
○: Sardine myosin+Sardine F-actin,
◉: Sardine myosin+Striped marlin F-actin.
Insert: The solubilities of myosin were polted as a function of KCl concentration.
■: Striped marlin, □: Sardine.

marked differences in extent of aggregation-interaction between striped marlin and sardine myosins. The protein amounts recovered by aggregation-interaction were high for striped marlin, but low for sardine, that is when myosin/actin (w/w)<1.0, the degree of aggregation was in the following order: striped marlin myosin+actin and striped marlin myosin+sardine actin<sardine myosin+striped marlin actin and sardine myosin+actin. It was suggested that striped marlin myosin in the presence of actin was stronger in aggregating ability than sardine myosin. It is not clear from this experiment but this difference may be due to the difference in binding ability of myosin with actin or in the aggregating ability of the rod portion. At this point further study will be necessary.

References

1) K. Samejima, M. Ishioroshi, and T. Yasui: *J. Food Sci.*, **46**, 1412–1418 (1981).
2) M. Ishioroshi, K. Samejima, and T. Yasui: *J. Food Sci.*, **47**, 114–120 (1981).
3) T. Yasui, M. Ishioroshi, and K. Samejima: *Agr. Biol. Chem.*, **46**, 1049–1059 (1982).
4) T. Taguchi, M. Tanaka, Y. Nagashima, and K. Amano: *J. Food Sci.*, **51**, 1407–1410 (1986).
5) J. A. Makie and J. J. Connell: *Biochim. Biophys. Acta*, **93**, 544–552 (1964).
6) N. Seki, M. Kitao, K. Konno, and K. Arai: *Nippon Suisan Gakkaishi*, **39**, 1211–1219 (1973).
7) J. A. Spudich and S. Watt: *J. Biol. Chem.*, **246**, 4866–4871 (1971).

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