Studies on the Properties of Fish Actomyosin—III. Lecithin found in the Actomyosin from Fish Muscle

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In the previous paper,¹⁾ we suggested existence of lipids in the actomyosin preparation from yellowtail, which account for the protective effect of α -tocopherol on the solubility of actomyosin, and identified the lipids to be lecithin.

The present study aims to examine whether the lecithin is contained ordinarily in the actomyosin preparations from other fishes as from yellowtail.

Materials and Methods

Preparation of actomyosin. The muscle of yellowtail, *Seriola quinqueradiata* TEMMINCK and SCHLEGEL, goby, *Acanthogobius flavimanus* (TEMMINCK and SCHLEGEL), young bass, *Lateolabrax japonicus* (CUVIER), gray mullet, *Mugil cephalus* LINNAEUS, flatfish, *Kareius bicoloratus* (BASILEWSKY), rainbow trout, *Salmo gairdneri iricleus* GIBBONS, and carp, *Cyprinus carpio* LINNAEUS, all in the prerigor state were used for the preparation of actomyosin. After removing the red muscle, 50 g of the back muscle was homogenized with 100 ml of cold distilled water. The sarcoplasmic proteins were then removed by washing the homogenate with 10 volumes of cold distilled water before dissolving the actomyosin. After centrifugation at $1,000\times g$ for 5 minutes, the actomyosin was isolated by extracting the precipitate with 3 volumes of 0.6 M KCl-WEBER EDSALL solution, followed by centrifugation at $18,000\times g$ for 30 minutes. The supernatant was diluted to an ionic strength of 0.12 with distilled water and kept for 5 hours in ice bath, followed by centrifugation at $1,000\times g$ for 5 minutes. After repeating three times the dissolution-precipitation the purified protein was used as actomyosin preparation.

Determination of phospholipids. The lipids of the actomyosin preparation (50-60 g) were extracted with 10 volumes of absolute ethanol-ether (3:1) at room temperature for 2 hours. After filtration of the supernatants, the solvent was removed under reduced pressure in a current of nitrogen. The lipids were dissolved in petroleum ether and chromatographed on silicic acid column by the method of LIS et al.²⁾ The phospholipids fraction was collected in a small volume of absolute methanol, and phosphorus was determined by ALLEN's method³⁾ after digestion in 9 N-sulphuric acid. The amount of phospholipids was calculated as mg of inorganic phosphate $\times 25$.

To identify the lipids, the ethanol-ether extract of the actomyosin preparation

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was chromatographed on the Tōyō Roshi No. 51 filter paper using the solvent of di-iso-butyl-ketone: acetic acid (30:7).⁴⁾ The spots on chromatogram were detected with Rōdamin B and phosphomolybdic acid.

Content of myosin. The content of myosin dissociated from the actomyosin was determined by the method of WEBER.⁵⁾

Protein concentration. Protein concentration was determined by semimicro-Kjeldahl method.

Results and Discussion

Lecithin content of the actomyosin preparation from yellowtail muscle. The lecithin content on each purification stage of the actomyosin from yellowtail (about 1 kg in body weight) was determined. As shown in Table 1, the lecithin content of

Table 1. Lecithin content in the different purification stages of actomyosin preparation.

Purification stage	Lecithin (μ moles/mg of protein-N)
First	—
Second	0.167
Third	0.126
Fourth	0.122

Values are the average of three determinations.

the actomyosin preparation was 0.126 and 0.122 μ moles per mg of protein-N on the third and fourth purification stage, respectively. From the above values, it may be assumed that the purified actomyosin contains a constant amount of lecithin per mg of protein-N.

When the extraction of lecithin from the actomyosin preparation was carried out by the organic solvents, as shown in Table 2, a small amount of lecithin was only extracted by treatment with ether at room temperature for 2 hours. In contrast, the most of lecithin was easily extracted from the preparation by the treatment with

Table 2. Extraction of lecithin from the actomyosin preparation.

Solvent	Lecithin extracted (μ moles/mg of protein-N)
Unfrozen actomyosin preparation	
Ether	0.028
Ethanol-ether(3:1)	0.120
Frozen actomyosin preparation	
Ether	0.088

The preparation was frozen at -5°C for 15 hours.

ethanol-ether (3:1). However, after freezing of the preparation a considerable amount of lecithin was removed from it by treatment with ether. From these results, it is suggested that the lecithin contained in the actomyosin preparation is possibly bound loosely with protein.

Lecithin contents of the actomyosin preparations from several species of fish.

To determine whether fish actomyosin contains lecithin ordinarily, the lipid of actomyosin preparations from several species of fish was identified by the method as described above. Typical examples are shown in Fig. 1. From the results that Rf value (0.7) of the lipids extracted from each preparation was identical with that of purified egg lecithin and one spot was revealed by Rödamin B and phosphomolybdic acid, it appears that the major component of the lipids contained in a fish actomyosin is lecithin.

The content of lecithin in fish actomyosin preparations was determined. As shown in Table 3, there was a significant difference in the lecithin content among the actomyosin preparations obtained from different species.

Fig. 1. Paper chromatograms of lipid in fish actomyosin preparation.

A: Purified egg lecithin.

B-E: Ethanolic extracts of the actomyosin preparations from flatfish (B), carp (C), gray mullet (D), and rainbow trout (E).

Table 3. Lecithin content of fish actomyosin preparation.

Species	Date	Mean body weight (g)	Lecithin (μ moles/mg of protein-N)*
Goby	Dec. 28	39	0.033 ± 0.003
Young bass	Dec. 19	151	0.054 ± 0.007
Gray mullet	Jan. 6	390	0.161 ± 0.018
Flatfish	Jan. 16	252	0.139 ± 0.020
Rainbow trout	Mar. 10	72	0.117 ± 0.011
Carp	Jan. 7	861	0.252 ± 0.052

* Standard deviation of the values obtained in the preparations from three fish bodies.

Amount of myosin dissociable from the actomyosin preparation. As shown in Table 3, the actomyosin preparation from carp muscle had a high value in lecithin

content, as compared with those from the other fish tested. These considerable differences in lecithin contents of the preparations might be due to a constituent component ratio of the actomyosin.

Therefore, the content of myosin dissociable from actomyosin in the presence of ATP was determined. The actomyosin dissolved in 0.6 M KCl solution containing 1 mM ATP and 1 mM $MgCl_2$ was centrifuged for 3 hours in an average gravitational field of $100,000 \times g$ at $5^\circ C$. The initial protein concentration ranged from 0.3 to 0.5% and pH between 6.8 and 7.0. After centrifugation, a clear solution of top layers (50 to 60% of the total volume) was separated from the rest of the solution and the pellet. The relative content of dissociable myosin was expressed as the ratio of protein in the supernatant to the initial protein present.

Table 4. Myosin content dissociated from the actomyosin in the presence of ATP.

Species	Protein-N mg		$\frac{(II)}{(I)} \times 100$
	Actomyosin (I)	Myosin (II)	
Flatfish	4.38 ± 0.05	1.88 ± 0.05	42.9 ± 1.7
Rainbow trout	5.43 ± 0.09	2.35 ± 0.05	43.3 ± 1.6
Carp	4.89 ± 0.03	2.27 ± 0.04	56.1 ± 1.2

Values are obtained by three determination.

Table 4 shows the approximate content ratios of myosin in the actomyosin preparations from carp, rainbow trout, and flatfish. From the result that carp actomyosin showed the highest value in the content ratio of myosin, it appears that the actomyosin preparation from fish muscle is in the tendency to exist with a high myosin content ratio to the actomyosin, corresponding to a high level of lecithin. The role of lecithin in the actomyosin is now being studied.

Summary

1. The lecithin content in different purification stages of actomyosin from yellow-tail was determined successively. From the result, it was assumed that the purified actomyosin contains a constant amount of lecithin (Table 1).
2. The amount of lecithin extracted from the actomyosin treated with organic solvents suggests that the lecithin is possibly bound loosely with protein (Table 2).
3. The lipids of actomyosin preparations from several different species of fish were identified. From the results, it appears that the major part of the lipids contained in a fish actomyosin is lecithin (Fig. 1).
4. There was a significant difference in the lecithin contents among the actomyosin preparation from several species of fish (Table 3).
5. The content of myosin dissociable from the actomyosin in the presence of

ATP was determined. The comparison of the myosin contents in the actomyosin preparations from several fish showed that the actomyosin having a high level of lecithin had a high value in the content ratio of myosin (Table 4).

References

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