イカ筋トロポミオシンの物理・化学的性質

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Physico-Chemical Properties of Squid Tropomyosin*1

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Tropomyosin preparations from obliquely striated mantle muscle of squid were obtained from low ionic strength extracts of natural actomyosin and their physico-chemical properties were studied. The sedimentation coefficient, 3.0 S, and the intrinsic viscosity, 0.31 dl/g, of squid tropomyosin were very similar to those of skeletal tropomyosin. The relative viscosity decreased with increasing ionic strength, becoming constant over 0.1 M KCl. The molecular weight of the squid tropomyosin subunits, $3.5\times$ and 3.7×10^4, obtained by SDS disc electrophoresis was slightly higher than those of other species. There are some differences between squid and vertebrates in the contents of some amino acids, such as Pro, Trp and Arg. Moreover, the squid tropomyosin was poorer than the skeletal and the non-muscle tropomyosins in easily forming the paracrystals and did not form any crystals.

Tropomyosin was first prepared from rabbit muscle by BAILEY[1] and was described as crystalline and rod shape protein. Tropomyosin is present in all muscle and has been prepared from a wide variety of muscles including the invertebrates[2,3].

In muscle, tropomyosin is observed by the fluorescent antibody staining method[4] as associated with actin of the thin filaments. However, its functional properties have not been known until 1963, when the role of tropomyosin in the contraction-relaxation system was discovered by EBASHI *et al.*[5]

Tropomyosin has been isolated from the muscles of various vertebrates and invertebrates, and recently it was isolated from some non-muscle cells such as platelet[6], *Physarum*[7] and fibroblast[8]. However, there are much less information of the invertebrate tropomyosin.

From the squid muscle which is characterised with its obliquely striated structure, tropomyosin has been isolated by YOSHIMURA[9] and TSAO[10]. Nevertheless, no further study has been reported thenceforth.

The present paper deals with the isolation of tropomyosin from obliquely striated muscle of the squid mantle and the characterization of its physico-chemical properties.

Material and Methods

Material

Fresh squid, *Ommastrephes sloani pacificus*, was purchased at the Tokyo Central Wholesale Fish Market.

Squid Tropomyosin

Tropomyosin preparations from squid mantle were obtained from the low ionic strength extracts of natural actomyosin.

The extracts were brought to 45% saturation of $(NH_4)_2SO_4$. After centrifugation, the supernatant was adjusted to 75% saturation with $(NH_4)_2SO_4$. The precipitate was collected by centrifugation and dissolved in 20 mM Tris-HCl buffer (pH 7.0). After adding LiCl to 0.4 M, the pH of the solution was adjusted to 4.5 and the precipitate was collected by centrifugation and dissolved in 20 mM Tris-HCl buffer (pH 7.0), and the obtained tropomyosin solution was clarified by centrifuging at 15,000 rpm for 1 h.

SDS Disc Electrophoresis

Sodium dodecylsulfate polyacrylamide gel disc electrophoresis was carried out according to the procedure of WEBER and OSBORN[11] with 10% polyacrylamide gel cencentration.

Ultracentrifugal Analysis

Analysis was done on 0.6 M KCl solution of the protein at 50,000 rpm and 20°C by a Phywe UL 50 analytical ultracentrifuge.

Viscometry

An Ostwald type viscometer with flow time of

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ca. 30 s per m*l* was used at 25°C.

Amino Acid Analysis

A Hitachi KLA amino acid analyzer was employed.

Other methods employed were the same as described in the previous papers[12,13].

Results

SDS Disc Electrophoresis

SDS disc electrophoresis of the squid tropomyosin showed a pattern as shown in Fig. 1 where no bands relating with actin, paramyosin and myosin were detected. Tropomyosin is found as two separate bands which are very similar to each other (molecular weights, $3.5\times$ and 3.7×10^4). The two bands should correspond with α- and β-chains of tropomyosin of rabbit skeletal muscle under the reducing reagent.

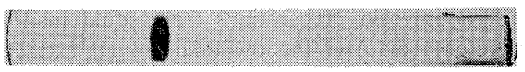

Fig. 1. SDS disc electrophoretic pattern of squid tropomyosin obtained from washing of natural actomyosin.

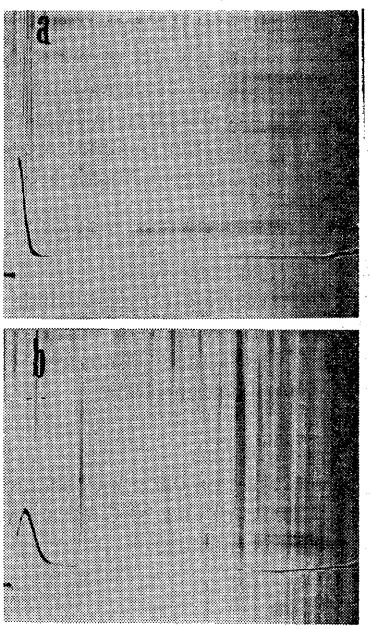

Fig. 2. Ultracentrifugal patterns of squid tropomyosin. Speed, 50,000 rpm; temperature, 20°C; protein concentration, 4 mg/m*l*; bar angle 60°. Pictures were taken at 10 (a) and 23 (b) min after reaching top speed.

Ultracentrifugal Analysis

On ultracentrifugation, the squid tropomyosin in 0.6 M KCl illustrated a monodisperse pattern as shown in Fig. 2, where the sedimentation velocity of the peak was estimated to be 2.6 S at 4.0mg/m*l*. The sedimentation coefficients obtained at various protein concentrations were plotted as in Fig. 3, and an extrapolated value, $S^0_{20,w}=3.0$ S, was obtained. This figure is close to those of the skeletal muscle of rabbit (2.6 S)[14] and the uterus of sheep (2.8 S)[15].

Viscometry

The intrinsic viscosity value was found as 0.31 d*l*/g in 1.0 M KCl from the slope of Fig. 4 and was in good agreement with those of rabbit (0.34 d*l*/g)[14] and scallop (0.34 d*l*/g)[16]. Also, the results of viscosity measurements as a function of the ionic strength was displayed in Fig. 5. The relative viscosity decreased with increasing ionic strength, becoming constant after 0.1 M KCl. These properties of squid tropomyosin revealed the features characteristic of the vertebrate's tropomyosin[1].

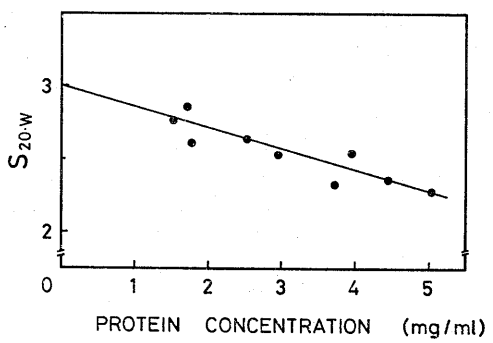

Fig. 3. Sedimentation coefficient of squid tropomyosin in function of protein concentration.

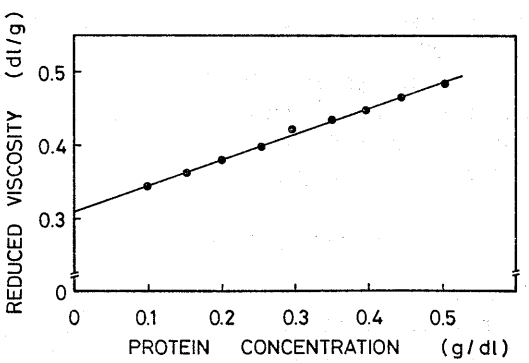

Fig. 4. Reduced viscosity of squid tropomyosin in function of protein concentration. Temperature, 25°C.

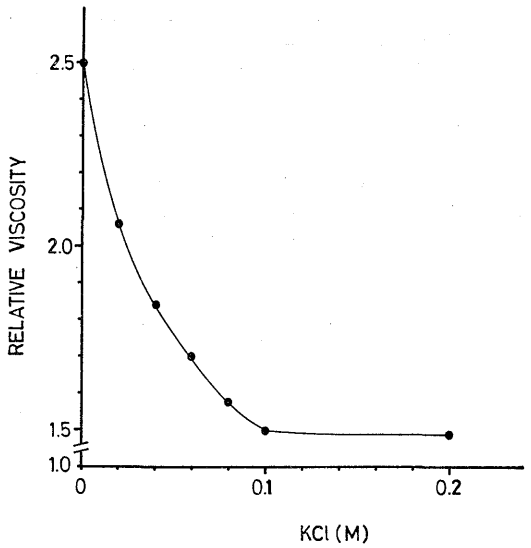

Fig. 5. Effect of the KCl concentration on the viscosity of squid tropomyosin.

Table 1. Amino acid composition of squid tropomyosin compared with those of rabbit and carp tropomyosin (mol/10^5 g protein)

	Squid	Rabbit[17]	Carp[18]
Ala	94	106	104
Gly	15	11	14
Val	31	30	32
Leu	102	96	105
Ile	32	34	27
Pro	3	1	0
Phe	9	4	3
Tyr	11	16	17
Trp	3	0	0
Ser	49	39	31
Thr	38	24	30
Cys/2	5	4	3
Met	12	19	19
Arg	68	42	45
His	4	6	7
Lys	81	116	130
Asp	107	89	89
Glu	210	216	214

Amino Acid Analysis

The results of amino acid analysis of the squid tropomyosin were shown in Table 1 with those of other origins for reference[17,18]. As compared with tropomyosin of the vertebrate, the squid tropomyosin showed some differences in the contents of Tyr, Ser, Met, Arg, His and Lys, by about 30%. The differences were also found in the ratio of Lys and Arg. Although all the known vertebrate tropomyosins are characterized by the absence of Trp and Pro[19], squid tropomyosin contained about 3 of Trp and 3 of Pro, respectively. However, the number of the SH groups in the squid tropomyosin, 3.2 mol/10^5 g tropomyosin, was similar to that of rabbit[20].

Discussion

The protein isolated here from the squid mantle muscle revealed various attributes of tropomyosin and has been identified as tropomyosin. The molecular weights of the squid tropomyosin subunits, $3.5\times$ and 3.7×10^4, obtained by SDS disc electrophoresis are slightly higher than those of rabbit ($3.4\times$ and 3.6×10^4)[21], scallop (3.5×10^4)[22] and carp (3.4×10^4)[23], respectively. The subunits of squid tropomyosin are probably corresponding to α- and β-chains of rabbit tropomyosin. However, the subunit ratio of the squid tropomyosin (1: 1) differs from that of rabbit (4: 1). The difference in subunit ratio between the squid tropomyosin and rabbit tropomyosin might be due to a differences in structure of muscle. Actually, the effectiveness of this viewpoint was suggested by PERRY[20] who studied tropomyosins obtained from various origins.

The ultracentrifugal patterns of the squid tropomyosin showed its homogeneity and its sedimentation coefficient was little defferent from those of other tropomyosins[14,15].

In the intrinsic viscosity value, the squid tropomyosin was comparable to rabbit's one[14]. Also, the effect of ionic strength on the relative viscosity of squid tropomyosin was of the same feature with that of rabbit tropomyosin[1]. However, there are some differences between squid and vertebrates in the contents of some amino acids, such as Pro, Trp and Arg. The squid tropomyosin contained Pro and Trp, though tropomyosins of other species have very little Pro and no Trp. Moreover, the squid tropomyosin was poorer than the skeletal[24] and the non-muscle[6] tropomyosins in the ease to form the paracrystals and did not form any crystal. These results suggested that the secondary structure of squid tropomyosin, such as α-helix, undergoes interference by the presence of Pro.

Study on the functional properties of the squid tropomyosin will be reported in the succeeding paper.

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