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Fish fast skeletal muscle tropomyosins show species-specific thermal stability

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Abstract

Tropomyosin (TM) was isolated from the fast skeletal muscle of six fish species, whose amino acid sequences of this protein have already been revealed. The thermal stability of these TMs was measured by differential scanning calorimetry (DSC) and circular dichroism (CD), while the molecular weights were measured by mass spectrometry. The results showed clear differences in thermostability among these fish TMs, though the identity of amino acid sequences was more than 93.3%. Therefore, only a few amino acid substitutions could affect the overall stability of the TM molecule. Especially, several residues located on the molecular surface were considered to be responsible for such stability difference. In contrast, the molecular weights of these TMs as measured by mass spectrometry were higher than those calculated from amino acid composition, suggesting the presence of post-translational modification(s) which could also affect their thermal stability.
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Keywords: Tropomyosin; Fish; Skeletal muscle; Thermal stability; Primary structure; α-Helix; Differential scanning calorimetry; CD spectrum; Coiled-coil

1. Introduction

Tropomyosin (TM), one of the striated muscle regulatory proteins, forms a family of highly conserved actin binding proteins. TM molecule is composed of two subunits (approximately 33 000 Da), forms a coiled-coil structure consisting of two parallel α-helical polypeptides (Crick, 1953; Fraser et al., 1965), and, in striated muscle, associates with one troponin complex and seven actin monomers (McLachlan and Stewart, 1976; Hitchcock-DeGregori and Varnell, 1990; Bernstein et al., 1993). TMs are also found in non-muscle cells of eukaryotes (Smillie, 1979; Lees-Miller and Helfman, 1991) and yeast (Drees et al., 1995). The structural information on vertebrate and invertebrate TMs accumulated to date prompts this protein a suitable model for the relationship between sequence, structure, and function (Cho et al., 1990; Kluwe et al., 1995; Perry, 2001; Palm et al., 2003; Miura-Yokota et al., 2005).

During the past few years, numbers of reports on vertebrate TM sequences have been increasing (Perry, 2001). Recently, attention has also been drawn to the isoform multiplicity, function–structure relationships, and involvement in some diseases (Ruiz-Opazo and Nadal-Ginard, 1987; Ishimoda-Takagi and Kobayashi, 1987; Ishimoda-Takagi et al., 1986, 1990; Forry-Schaudies and Hughes, 1991; Lemonnier et al., 1991; Gimona et al., 1995). However, the molecular basis of these findings is only poorly understood, probably because of the still limited number of amino acid sequences and structural data available. In this regard, data concerning ectotherm animal TMs are still fragmentary, and, in particular, studies pertaining to the thermostability of fish TMs need to be addressed at structural levels.

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As far as teleostean TMs are concerned, Hamoir (1951) following Bailey's method (1946), isolated TM from carp muscle for the first time. Saad et al. (1958) compared TMs from frog, carp, and lamprey, and found that amino acid compositions of TMs were similar to each other. Odense et al. (1969) applied heat treatment to the isolation of TM from cod muscle. Later, the presence of isoforms in fish skeletal muscle had been reported (Seki and Iwabuchi, 1978; Heeley and Hong, 1994). Seki and Iwabuchi (1978) electrophoretically analyzed TMs from 25 species of fish, and found that mackerel, yellowfin tuna, and bigeye tuna TMs gave rise to two bands on one-dimensional gels. Heeley and Hong (1994) revealed that fast skeletal muscle of salmonids and herring TMs were noticeably heterogeneous by two-dimensional polyacrylamide gel electrophoresis.

The deduced primary structures have already been reported for several teleostean species such as zebrafish (Ohara et al., 1989), Atlantic salmon (Heeley et al., 1995; Jackman et al., 1996), white croaker (Ochiai et al., 2001), walleye pollack (Ochiai et al., 2003), pufferfish (Ikeda et al., 2003), and bluefin tuna (Huang et al., 2004). However, little information is thus far available on the relationship between stability and structure of fish muscle TMs (Ochiai and Watabe, 2002).

Fish are ectotherms, and thus their muscle function is expected to be greatly affected by environmental temperatures. In the present study, we tried to purify the protein from the fast skeletal muscle of the above six fish species, whose amino acid sequences of TM have been already deduced, in order to identify the amino acid substitutions that may be critical for the stability difference of TMs. These species are also of interest from the viewpoint of adaptation, because they not only inhabit quite different ambient temperatures, but also differ in locomotory activity. Namely, white croaker and walleye pollack inhabit temperate and cold waters, respectively, while zebrafish is a tropical freshwater fish. Salmon migrate a long distance for reproduction, while bluefin tuna can swim at high speed, and their body temperature can exceed ambient temperatures by as much as 10 °C (Carey and Teal, 1966). Pufferfish swims very slowly, using mainly dorsal and ventral fins. Generally, habitat environments force organisms to tune the structures of proteins so that they maximize the functions under their respective conditions.

The present study was thus concerned with (1) purification of TMs from six fish species (and rabbit as a control), and investigation of their basic properties; (2) comparison of thermostability among these TMs, and relationship between the sequence variation and the stability differences; and (3) determination of molecular mass by MALDI-TOF mass spectrometry to detect the presence of post-translational modifications. The observed differences in properties among the fish TMs are discussed with regards to amino acid substitutions and post-translational modifications.

2. Materials and methods

2.1. Materials

Fast skeletal muscles of white croaker (*Pennahia argentata*), pufferfish (*Takifugu rubripes*), walleye pollack (*Theragra chalcogramma*), Atlantic salmon (*Salmo salar*), bluefin tuna (*Thunnus thynnus*), zebrafish (*Danio rerio*), and rabbit (*Oryctolagus cuniculus*) were used. Bluefin tuna and Atlantic salmon were purchased at the Tokyo Central Wholesale Market, transferred to the laboratory in dry ice, and stored at −80 °C. Live specimens of white croaker, pufferfish, and zebrafish were obtained by angling (off the coast of Tokyo), by harvesting from a fish tank (located in our university campus), or by purchasing from a local pet dealer, respectively. Frozen leached meat of walleye pollack was kindly supplied from Nippon Suisan Kaisha Co. Ltd. (Tokyo, Japan). The rabbit, purchased from Nippon Biosupply Center (Tokyo, Japan), was slaughtered under anaesthesia with ethyl ether, and fast skeletal muscle was dissected promptly from the dorsal part of the animal.

2.2. Isolation of TM

All purification procedures were carried out at 0–4 °C unless otherwise stated. First, acetone dried powder was prepared as follows. Fast skeletal muscles were minced and mixed with 10 vol. of 50 mM KCl containing 2 mM $NaHCO_3$, and washed with the same solution three times. The mixture was centrifuged at $3000 \times g$ for 5 min. The precipitate was treated with absolute acetone three times, and dried overnight.

The completely dried powder was extracted with 10 vol. (v/w) of 20 mM Tris–HCl (pH 7.5) containing 1 M KCl and 10 mM 2-mercaptoethanol overnight. The mixture was then centrifuged at $20\,000 \times g$ for 20 min. The supernatant was subjected to isoelectric precipitation at pH 4.5 with 1 N HCl. The pellet after centrifugation at $20\,000 \times g$ for 20 min was brought to pH 7.6 with 1 N NaOH, and subjected to ammonium sulfate fractionation, and a 50–60% saturated fraction was obtained. These procedures were repeated twice, and the final TM fraction was dialyzed against 1 mM $NaHCO_3$. The protein concentration was determined by the biuret method (Gornall et al., 1949).

2.3. SDS-PAGE

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was performed according to Laemmli (1970) using 15% polyacrylamide slab gels. After the run, gels were stained with 0.05% Coomassie Brilliant Blue R-250 (Sigma-Aldrich Chemical, St. Louis, MO, USA). Protein molecular weight markers were also purchased from Sigma (SDS-6H).

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