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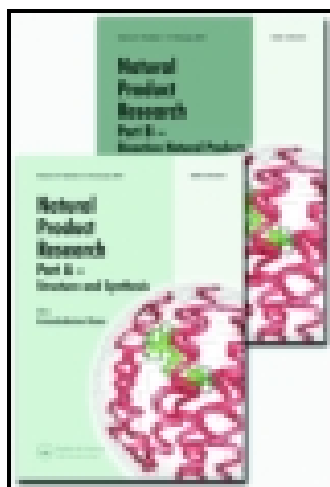
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Isolation and characterisation of tropomyosin from shrimp (*Penaeus vannamei* Boone) and its association property at high ionic strength

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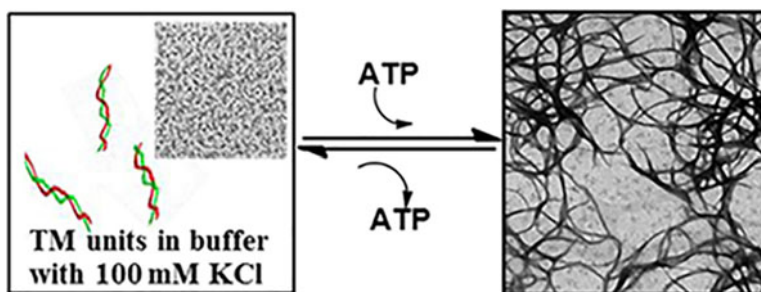
SHORT COMMUNICATION

Isolation and characterisation of tropomyosin from shrimp (*Penaeus vannamei* Boone) and its association property at high ionic strength

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Shrimps are important and highly demanded seafood, but they have been reported as a cause of food hypersensitive reaction. The major allergen of shrimp is tropomyosin (TM). However, so far, there has been few report on such purification procedure. In this study, we developed a strategy for the purification of TM from shrimp (*Penaeus vannamei* Boone). Subsequently, we demonstrated that the apparent MW of this protein is about 66 kDa, and this protein naturally contains two subunits (38.5 and 36.6 kDa) with a ratio of 1 to 1. Interestingly, different from other known TMs from vertebrates, shrimp TM can self-assemble into nanofibres at high ionic strength induced by ATP. These findings help to understand the structure and polymerisation property of TM from shrimps.

Keywords: shrimp; tropomyosin; purification; self-assembly; nanofibres

1. Introduction

Seafood is popular around the globe not only as a delicacy but also as a highly nutritious source of animal proteins. Shrimp is an important type of seafood that is consumed worldwide. Unfortunately, shrimp allergy is a common cause of allergic reaction worldwide (Lopata & Lehrer 2009). It may induce mild to severe allergic reactions (Albrecht et al. 2008) and usually persists throughout life. The major allergen of shrimp is tropomyosin (TM). Although TM is present in all the animals, the protein from invertebrates such as shrimps is only recognised as a food allergen. Such difference might derive from their difference in structure, so it is important to develop a procedure through which TM is isolated from shrimp in order to understand the

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structure and function of this protein, especially its allergic activity. However, so far, there has been few report on the isolation and characterisation of shrimp TM.

TM is a broad family of striated muscle regulatory proteins, which is found in virtually all eukaryotic cells. They control the stability of actin filaments through switching on/off of actin filaments in muscle cells by the interaction with actin and troponin (Wegner 1979). TM polymerisation in a head-to-tail manner automatically only occurs at low ionic strength, and this phenomenon was first reported in 1948 (Astbury et al. 1948). Consistent with this finding, a similar observation was obtained by other group (Sousa & Farah 2002). Thus, it appears that the above polymerisation cannot occur at high ionic strength. However, physiological condition requires high ionic strength, and whether TM could polymerise under physiological condition remains unknown.

In this study, we developed a strategy to purify TM from shrimp (*Penaeus vannamei* Boone) based on previous reported procedures with major modifications (Astbury et al. 1948; Rao et al. 2009). After characterisation, we found that ATP alone over the range of 10 to 30 mM can induce this TM at high ionic strength to polymerise, and such polymerisation is reversible under current condition. This is the first report which focuses on TM polymerisation at high ionic strength.

2. Results and discussion

2.1. Isolation and purification of TM

Shrimp muscle was chosen as materials for TM purification. The procedure is composed of three major steps based on the reported methods with major modifications (Astbury et al. 1948; Rao et al. 2009): cold acetone precipitation, removal of heat-sensitive proteins, and ion exchange chromatography on a DEAE-Sepharose Fast Flow column. Since TM is a heat stable coiled-coil protein, most of heat-sensitive proteins can be removed by heating samples in a boiling water bath for 5 min. This step is very effective for the preparation of TM from shrimp. Proteins fractionated in the purification steps were resolved by a polymerisation property assay and native PAGE. After protein fraction was eluted with NaCl (0.15 M) in the Tris-HCl buffer (pH 8.0) at a flow rate of 0.4 mL/min, the peak with a polymerisation property was collected for native PAGE analysis. Native PAGE exhibits a single band (Figure S1(A), lane 2), indicating that the protein is purified to homogeneity. It has nearly the same migrating rate as a marker with its apparent MW of ~66 kDa (Figure S1(A)), suggesting that this protein also has an apparent MW of 66 kDa, consistent with previous observation (Stafford et al. 2012). The purified protein exhibited a double-band pattern on SDS polyacrylamide gel electrophoresis (Figure S1(B)), the faster moving component is designated as α and the slower β subunit with a ratio of nearly 1 to 1. The apparent molecular weights of the two subunits were estimated to be 38.5 and 36.6 kDa, respectively. This value consisted with the isoforms of the TM components found in rabbit strained muscle (Sodek et al. 1972). The final protein yield was $0.29\% \pm 0.5\%$ (m/m). Additionally, the above purification method is different from a recently reported one from shrimp (*Litopenaeus vannamei*) in that uncooked shrimps were used as raw materials for protein purification in the present study, while cooked ones were used in another study. As a result, TM from shrimp (*Litopenaeus vannamei*) is only composed of one subunit (Liu et al. 2010).

2.2. Characterisation of TM

To elucidate the relationship between these two gel bands, the peptide mass fingerprintings (PMF) were compared by MALDI-TOF mass spectrometry using CHCA as the matrix. The MALDI-TOF-MS spectrum of the gel band 38.5 kDa generated from in-gel trypsin digestion is shown in Figure S2(A). In parallel, the MALDI-TOF spectrum of a control background-only

band was also obtained, by following the same in-gel trypsin digestion procedure. The PMF of gel band '38.5 kDa' is summarised in Table S1, after excluding the trypsin autolysis products from the control spectrum. It had twenty abundant peptide ions of m/z . Likewise, the MALDI-TOF spectrum of the tryptic peptides of the gel band 36.6 kDa was obtained as shown in Figure S2(B), and it also contains all of the above-mentioned peptide ions. These results suggest that the PMF of the 38.5 kDa subunit is very similar to that of the 36.6 kDa subunit. Thus, it is possible that the two subunits are expressed by the same gene or they may stem from the same precursor as suggested by previous findings (Helfman et al. 1986).

2.3. Protein assembly property

The TMs had been reported to have the head-to-tail polymerisation property and presented high viscosity at low ionic strength, but it depolymerised at high ionic strength (Sousa et al. 2010). To shed light on whether ATP can induce TM assembly under high ionic condition, stopped-flow light scattering experiments were conducted. The stopped-flow experiments were performed in which ATP (freshly prepared) in the buffer was rapidly mixed with TM solution in the same buffer containing 100 mM KCl (Figure 1(A)). We found that TM polymerised in an ATP concentration dependent manner. The intensity of light scattering remained unchanged upon mixing of TM in the buffer containing 50 mM Tris-HCl, pH 8.0, 100 mM KCl, with ATP in the same buffer, to give final concentration of TM and ATP are 5 μ M and 9 mM, respectively. It indicated that no protein assembly occurred at this ATP concentration. However, the intensity increased with an initial lag phase and reached a plateau 10 s after mixing TM solution with ATP at a final concentration of 12 mM. In contrast, the light scattering intensity increased faster with no distinct lag phase and reached a plateau 0.5 s when the final concentration of ATP was 15 mM. A similar phenomenon was found in the buffer containing 50 mM MOPS and 100 mM KCl at pH 7.0 as shown in Figure 1(B), but the polymerisation required a higher ATP concentration. Thus, it appears that such ATP-induced polymerisation constantly occurs at different buffer systems *in vitro*.

To obtain the information on the size and shape of the TM formed during polymerisation, TEM experiments were performed. Figure S3(A) shows that micrometer-sized, semiflexible fibrils can be formed from TM units in the presence of ATP. The fibrils were associated in large entangled network and it was difficult to follow a single fibril along its entire length because of overlapping with other fibrils. In addition to long semi-flexible fibrils, a small number of small

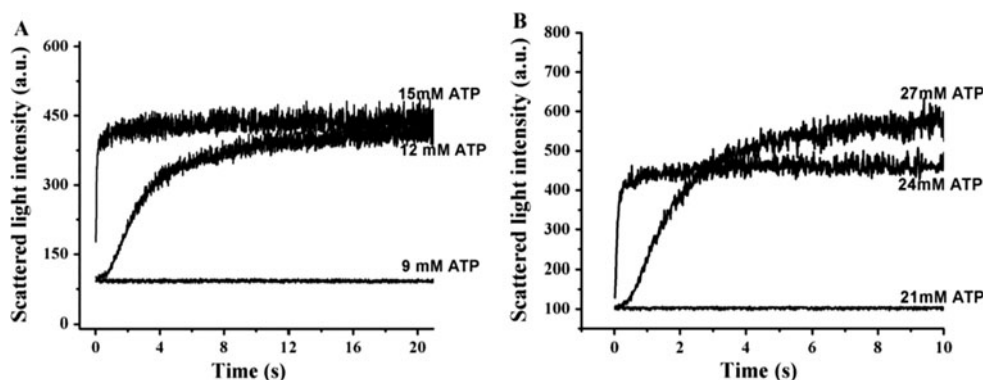


Figure 1. Time course of native TM self-assembly induced by different concentrations of ATP in two different buffers monitored by stopped-flow light scattering. Conditions: [TM] = 5 μ M, both ATP and TM are in the same buffer, 25°C.

aggregates with diameter of 5–20 nm were seen (Figure S3(B)). These results again demonstrated that protein polymerisation occurred in the presence of ATP under high ionic strength condition, being in good agreement with the stopped-flow results (Figure 1). TEM image presented herein is very similar to spontaneous polymerisation of TM under low ionic strength condition (Sousa et al. 2010), again indicative of the function of ATP. Additionally, these eco-friendly and organic fibrils is somewhat similar to the reported whey protein (Loveday et al. 2011) and β -lactoglobulin (Akkermans et al. 2008) fibrils induced by heat and low pH, demonstrating a potential use as biomaterials.

2.4. TM polymerisation induced by ATP is a reversible process

The observations above raise a question of whether the TM polymerisation induced by ATP is a reversible process. To answer this question, three samples were prepared, and their corresponding scattering light intensity was measured. The first is 5 μ M of TM alone in the buffer, whose scattering light intensity is 88.3 ± 5.2 (Figure S4). Upon treatment of the TM with 12 mM of ATP, which corresponded to the second sample, the scattering light intensity of this mixture increased to 449.4 ± 10.5 (Figure S4), indicating that TM polymerisation occurred. However, after the above solution was dialysed against the buffer containing 100 mM KCl, 50 mM Tris-HCl, pH 8.0 (which represents the third sample), the scattering light intensity of resulting solution decreased to 89.2 ± 6.5 (Figure S4), demonstrating that polymerised TM fibrils became dissociated back into free TM units. Thus, it appears that the above observed protein association is a reversible process.

3. Conclusions

We have developed a strategy for the purification of a new TM from *Penaeus vannamei* Boone, which contains two similar subunits in a ratio of 1 to 1. We found that ATP can induce this protein to self-assemble into nanofibres at high ionic strength, and the polymerisation appears to be a reversible process. The linear self-assembly of TM in the presence of ATP may also have the potential use as a template in the preparation of inorganic nanomaterials.

Supplementary material

Detailed experimental procedures relating to this article are available online, alongside Table S1 and Figures S1–S4.

Disclosure statement

No potential conflict of interest was reported by the authors.

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