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Original article

pH-dependence of coiled-coil structural parameters of shrimp tropomyosin studied by molecular dynamics simulation

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Introduction

Ingestion of marine invertebrates, especially shrimp, causes food allergy [1]. The major responsible allergen is tropomyosin (TM) [2]. TM is α-helical coiled-coil protein, binds to actin filaments and regulates muscle contraction [3]. Food allergens generally show stability against heat and resistance to digestive degradation [4]. Thus, intact or large fragments could be absorbed in the intestine and enter the blood stream [5]. The heat stability of invertebrate TMs is varied depending on species [6]. TM shows resistance to digestion in the stomach, but not in the small intestine, although it is not known why TM shows such organ-specific resistance.

Molecular dynamics (MD) simulation of biomolecules describes the behavior of proteins, nucleotides and nano-materials [7]. In MD simulation, the motion equation of the system, which contains biomolecules and usually small molecules such as water and ions, is solved numerically. In ordinary MD simulation, the protonation states of side-chains of Asp, Glu, His, Cys, Lys and Tyr are fixed. In MD simulation under constant pH conditions, the protonation state of side-chains is evaluated and the stable protonation state is chosen, according to the instantaneous structure and external pH. In the present study, to understand the mechanisms underlying the difference in digestibility and behaviors of TMs between the stomach and the small intestine, MD simulations were performed in explicit water molecule under constant pH conditions (pH 1 and 7) [8]. Then, the important parameters of coiled-coil, radius and phase per residue, were analyzed.

Materials and methods

For MD simulation, Amber 14 [7] was used. To obtain the initial structure of shrimp TM, SWISS-MODEL [9] was used. For the homology modeling, pig *Sus scrofa* TM structure (PDB ID: 1C1G) was used as a template, and the sequence of kuruma prawn *Marsupenaeus japonicus* TM (GenBank ID: BAF47263.1) as a target [10,11].

The default force field in Amber 14 for the simulation under constant pH was used. The system consisted of TM dimer, 231,078 TIP3P waters, 464 Na^+ and 418 Cl^-. Cutoff distance for real space was set to 8 Å on the particle mesh Ewald method. Before MD simulation, the energy minimization was performed under the constant volume condition. For the solvent relaxation, energy minimization consisting of 1,000 cycles was performed with the positional restraint for TM on the initial coordinate. Then, for the whole system relaxation, 2,500 steps of energy minimization were performed without any restraint. For MD simulation, the bond lengths involving hydrogen atoms were kept by SHAKE algorism to enable the time step of 2 fs, and Langevin dynamics was adopted with the collision frequency of 2 ps^{-1}. After energy minimization, MD simulation at constant volume was performed for 20 ps at 300 K with restraint of TM to the energy-minimized structure to relax the solvents. Following MD simulation was conducted under the constant pressure of 1 bar by Berendsen barostat with the relaxation time of 2 ps. To relax the whole system, MD simulation at the fixed protonation state was performed for 10 ns. Finally, MD simulation [8] was performed at pH 1 and pH 7 (10 ns × 3 for each pH).

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The protonation state was judged every 1 ps based on the protonation energy estimated by generalized Born implicit solvent model at a salt concentration of 0.1 M. The protonation states of Asp, Glu and His were evaluated and then changed to more stable ones if necessary. When the protonation state was changed, 100 steps in energy minimization of water molecules were performed. The simulation at last 5 ns under constant pHs, containing 2,500 snapshots, was performed employing TWISTER [12] to obtain the coiled-coil parameters, radius and phase per residue.

Results and discussion

TWISTER calculates the axis of α-helix, which is constituted by the axis point belonging to each residue. TM contains two α-helices, and thus, two axes of α-helix were obtained. The coiled-coil axis point was calculated as the mid-point of axis points of α-helices for each residue. The coiled-coil radius was calculated as the distance between α-helix and coiled-coil axis points. The residue-averaged coiled-coil radii at pH 1 and pH 7 were 4.83±0.03 Å (n = 3) and 4.80±0.01 Å (n = 3), respectively with no significant difference (Student's t-test, $p > 0.05$) (Fig. 1A). Thus, pH would not affect the averaged coiled-coil radius.

Although the difference in the coiled-coil radius between pH 1 and pH 7 was small, substantial difference in the averaged value was observed around Asp137 and Glu218. These two are called the acidic core residues [13]. Residues around the acidic core residues showed large standard deviations of radius (see vertical lines in Fig. 1A). Thus, the structure of TM around the acidic core residues was found to be fluctuated. The difference in the averaged coiled-coil radius between pH 1 and 7 was mainly attributed to the fluctuation in the acidic core, which would not have resulted from the difference in pH.

The coiled-coil phase per residue is the index for the twist of coiled-coil. When the coiled-coil phase is zero, the two helices become parallel coils. When the sum of coiled-coil phase becomes 360° or -360°, one helix winds around the other. The coiled-coil phases per residue at pH 1 and 7 were -3.38±0.16° (n = 3) and -3.18±0.21° (n = 3), respectively, with no significant difference (Student's t-test, $p > 0.05$) (Fig. 1B). As in the case of coiled-coil radius, the difference was mainly observed around the acidic core residues.

In the present study, there was no marked difference in the coiled-coil structure of TM between pH 1 and 7. Thus, the difference in the digestibility between the stomach and small intestine would not have resulted from the structural difference of TM, but rather from the different properties of proteinases. To understand the resistance of TM to pepsin, and the possible essential factor for its allergenicity, further studies *in vivo* and *in silico* are required.

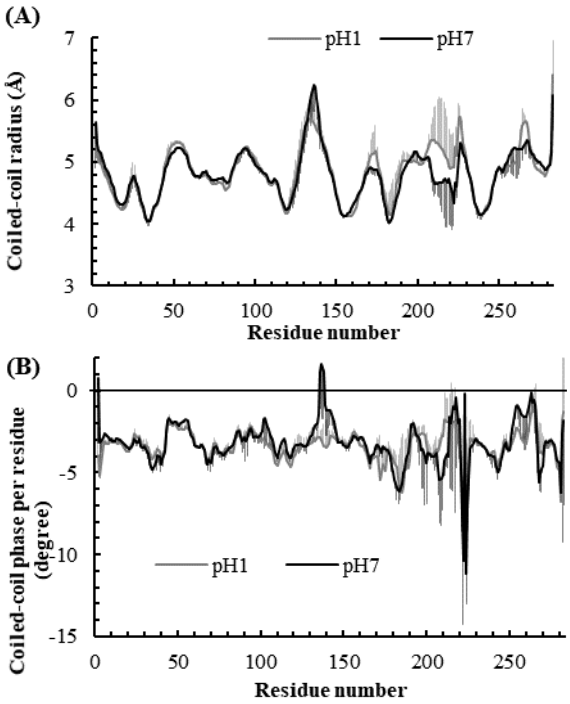

Fig. 1. The coiled-coil radius (A) and phase per residue (B) of shrimp tropomyosin at pH 1 and 7.

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