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The investigation of the secondary structures of various peptide sequences of β-casein by the multicanonical simulation method

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Abstract

The structural properties of Arginine-Glutamic acid-Leucine-Glutamic acid-Glutamic acid-Leucine-Asparagine-Valine-Proline-Glycine (RELEELNVPG, in one letter code), Glutamic acid-Glutamic acid-Glutamine-Glutamine-Glutamine-Threonine-Glutamic acid (EEQQQTE) and Glutamic acid-Aspartic acid-Glutamic acid-Leucine-Glutamine-Aspartic acid-Lysine-Isoleucine (EDELQDKI) peptide sequences of β-casein were studied by three-dimensional molecular modeling. In this work, the three-dimensional conformations of each peptide from their primary sequences were obtained by multicanonical simulations. With using major advantage of this simulation technique, Ramachandran plots were prepared and analysed to predict the relative occurrence probabilities of β-turn, γ-turn and helical structures. Structural predictions of these sequences of β-casein molecule indicate the presence of high level of helical structures and βIII-turns. The occurrence probabilities of inverse and classical β-turns were low. The probability of helical structure of each sequence significantly decreased when the temperature increased. Our results show these peptides have highly helical structure and better agreement with the results of spectroscopic techniques and other prediction methods.

Keywords: Molecular modeling; Multicanonical simulation; β-casein; Helical structures

1. Introduction

β-casein is a milk protein, which represents 36% of bovine casein. It is used quite often in the food industry due to its various functional properties. Moreover, many peptides of this protein are bioactive and directly influence numerous biological process evoking behavioral, gastrointestinal, hormonal, immunological, neurological, and nutritional responses. Hence, peptides of casein are of the interest as in vitro studies point to liberation upon digestion and possible physiological activity [1]. Although, recent studies intensified on the conformation of β-casein, information about its secondary and tertiary structure is still controversial [2,3]. The monomer β-casein molecule is constituted by 209 amino acids residues in a single chain with a large amount of proline residues distributed along the protein [4,5]. The N terminal portion of the β-casein molecule (residues

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1–40) contains the phosphoserine residues and the C terminal one third of the molecule (residues 136–209) contains many apolar residues [6].

Since β-casein cannot be crystallized, a direct observation of its secondary structure by X-ray crystallography is not possible. Therefore, the amount of various secondary structures has been estimated from measurements using various spectral techniques and using algorithms to predict secondary structure from its primary structure [3]. The spectroscopic techniques (Raman, Fourier-transform infrared and circular dichroism) indicate the presence of significant amount of α-helices (up to 29% of the residues), β-sheets (up to 34% of the residues) and turns (up to 35% of the residues) [7–12]. A model for the secondary structure of β-casein was then suggested by Kumosinski et al. [13] that predicted the possible secondary structural assignments for β-casein, using molecular modeling, modifying secondary structural algorithms of Garnier et al. [14] and Raman spectroscopy results. According to this work, α-helix was predicted for the residues 1–6, 35–40 and 43–48.

Although a number of techniques have been used to resolve the conformational structures of β-casein, alternative approaches such as computer molecular modeling from amino acid sequences can contribute to a better understanding of their three-dimensional structures. Determination of the three-dimensional structure of a protein or a peptide only from its primary structure is extremely difficult. The major difficulty in conventional protein simulations such as the Metropolis Monte Carlo (MC) method [15] or molecular dynamics (MD) [16] lies in the fact that simulations get trapped for a long simulation time in one of a huge number of energy local minima. This problem of the MC and MD methods can, to a large extent, be alleviated by the multicanonical (Muca) method [17] (for recent review, see Ref. [18]). The Muca method is characterized by the equiprobability of all energy states, so one can easily jump from a state to another. This method has been performed firstly to peptide and protein model by Hansmann et al. [19], and others [20,21]. Recently, the detailed translation of Muca method is also presented for the simulation of the pentapeptide Leu-enkephalin in previous work [22].

2. Aim

In this work, the Muca method was used to elucidate the structural properties and examined possible contribution of various peptide sequences of β-casein. Three different segments of this protein were selected based on secondary structural predictions of previous studies. The selected segments are RELEELNVPG (1–10), EEQQQTE (36–42) and EDELQDKI (43–49) of β-casein which are predicted to contain α-helix according to Kumosinski et al. [13]. This work is also intended to compare the prediction by the other molecular modeling and spectroskopic investigations. Thus, modeling of these peptides can provide important insight into the studying bioactive peptides which are chemically synthesized to confirm the biological properties with a specific amino acid sequence. The simulation method used in this study allowed us to estimate the levels of different secondary structures in the selected peptide sequences of β-casein at different temperatures.

3. Simulation model

For all these sequences, NH2 and COOH were chosen as N- and C-terminal groups, respectively. All molecules are modeled by potential energy function ECEPP/2 (Empirical Conformational Energy Program for Peptides), which assumes rigid geometry, and is based on electrostatic term, 12–6 Lenard–Jones and hydrogen-bond term for all pairs of atoms in the peptide together with torsion term for all torsion angles [23,24].

For the present sequences, the peptide bond angles ω are kept fixed at 180°, which leave dihedral angles ϕ, ψ in the backbone and χ in the side chain as independent degrees of freedom. Therefore, a conformation is defined by these variables. To avoid the complications of the electrostatic and hydrogen-bond interactions of side chains with solvent for non-polar amino-acids, explicit solvent molecules were neglected for simplicity. We use the standard dielectric constant $\varepsilon = 2$ of ECEPP. This force is implemented in the package FANTOM [25], which is used for the present simulations.