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Original article

Stability and orientation of cecropin P1 on maleimide self-assembled monolayer (SAM) surfaces and suggested functional mutations

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ABSTRACT

One of the main challenges of biosensor design is to understand the protein or peptide stability on the chip in high resolution structural detail. Since conventional experimental methods are limited by the resolution for their applications on surface tethered peptides/proteins, a recently developed coarse grained simulation method is employed to explore the peptide/surface interaction in residue-level resolution. This work shows how the coarse grained model successfully describes peptide–surface interactions by evaluating thermal stability of the peptide cecropin P1 in bulk solution and on surfaces by physical adsorption and chemical tethering. The simulation also reproduces observations of peptide orientations on the self-assembled monolayer surface from earlier experimental work. Additionally, using knowledge obtained from the simulations, specific mutations are suggested and the desired structure and pose on the surface is obtained. In summary, this work sheds a light on the reasonable biosensor design that is guided by simulations.

1. Introduction

The behavior and stability of proteins at solid interfaces are key factors influencing the performance of many technologies such as protein-based biosensors, protein microarrays, and medical implants [1–7]. Many protein-based biosensors have been developed because of their potential advantages including high cost-efficiency, good portability, and rapid-response potency. However, the optimal performance of a number of current biosensors is not fully achieved for practical use due to the lack of predictable behavior of proteins on the chip surface [8]. The activity of a protein on a chip is highly dependent upon its stability change induced by the surface potential and its orientation affected by surface chemistry. Based on knowledge of such surface–protein interactions, several efforts have been reported to achieve improved performance of protein-based biosensors, including solid surface coating [9,10], specific tethering [11,12], and solvent adjustment [13]. Despite the success in such examples, fundamental knowledge of protein–surface interactions at residue-level resolution is still lacking, so that reasonable design of protein-based biosensors is far from routine application.

As shown by previous research on protein–surface interactions, it is difficult to capture high resolution structural details of proteins and peptides on surfaces using experimental methods such as circular dichroism (CD), attenuated total reflection Fourier transform IR (ATR-FTIR), 2-dimentional nuclear magnetic resonance (NMR), and atomic force microscopy (AFM) [4,14–19], due to either low resolution or limit in ability for applications to surface attached proteins [20]. Therefore, alternative methods are needed to capture the behavior of proteins on surfaces in residue-level detail. Molecular simulation may be able to bridge this gap due to its inherent competence in investigating detailed structures. Such approaches have been successfully applied to studies of protein–surface interactions [21,22]. Atomistic simulations provide atomic-level structural details of the peptide, but it is difficult to measure the stability of peptides due to large sampling requirements in both folded and unfolded structures. Therefore, coarse grained simulation methods are more favorable to meet the sampling needs by reducing the number of degrees of freedom. Many studies have shown success in understanding protein orientation on charged or non-charged self-assembled monolayer surfaces with different hydrophobicity [23–29]. Coarse grained force field was further developed for protein–membrane interactions and solvent conditions [30,31].

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However, to capture the mechanism of protein–surface interactions requires a sophisticated protein model that can reproduce the protein folding mechanism and a well parameterized and validated surface potential with which to couple it. Such a coarse grained model has been previously built based on the Karanicolas and Brooks' Go-like protein model and extensions implementing a well parameterized surface force field [32]. This model can capture the stability and structure of a protein when it is either physically adsorbed on or chemically tethered to a surface with a specific hydrophobic character.

By using such a coarse grained simulation method, we would like to understand the detailed structures of a peptide that interacts with a surface and how the simulation can guide the design of the peptide for a desired behavior on the surface. To achieve this goal, a simple peptide–surface model system is studied. Specifically, cecropin P1, a small antimicrobial peptide, is used. This peptide has been widely used both experimentally and computationally as a model peptide for biosensor design due to its small size, simple secondary structure, and clear application for activity detection [13]. The sequence of this peptide is composed of 32 residues with various hydrophobicities. Basically, hydrophobic residues are dilutely scattered along the sequence, which provide potential sites for hydrophobic interactions with membranes when a helix structure formed. There is one region near the C-terminus formed by 5 continuous hydrophobic residues (residue 22–26), which is identified as the part that penetrates the bacterial cell membrane. The formation of secondary structure in cecropin P1 is dependent on the solvent and surface conditions. In pure water or phosphate buffer solution (PBS), cecropin P1 has a random-coil structure, but when interacting with membranes or in hydrophobic solution [33,34] it forms an α-helix. Previous experimental work using sum frequency generation (SFG) vibrational spectroscopy by Chen *et al.* [13,35,36] has shown the interesting results that cecropin P1 forms different secondary structures or employs different surface orientations under various solvent conditions and on maleimide terminated self-assembled monolayer (SAM) surfaces.

This work examines the interaction between cecropin P1 and a maleimide self-assembled monolayer surface, which is consistent with the experimental set-up of Han *et al.* [13] In their work, two main findings were: (a) the peptide forms a better helical structure on the surface than in the bulk solution; (b) the peptide has different orientations on the maleimide SAM surface by tethering the peptide with different terminal residues. By using coarse grained simulation methods, in this work, we explore the thermal stability of cecropin P1 in the bulk and on surfaces. The surface tethered peptides show largely improved stability compared with peptides in bulk solution. The peptide orientations with different termini tethered on the surface is also measured in a molecular dynamics simulation, which is similar to the experimental data [13]. Furthermore, informed by the structural detail obtained from the simulation, specific mutations are suggested to obtain a desired orientation of the peptide on the surface. Simulations of the redesigned peptide show that it may adopt a standing-up pose on the surface with either end tethered.

In summary, this work shows how the coarse grained model successfully reproduces the experimentally observed peptide structure and orientation information which leads to a better understanding of residue-level peptide–surface interactions. Furthermore, this success in describing peptide–surface interactions further suggests its potential implementation to understand and predict the behavior of large proteins on SAM surfaces. This work also presents a scenario where a coarse grained model could reasonably suggest mutations for a peptide for desired stability or orientation at the interface, which is a significant step forward in improving the performance of protein-based biosensors.

2. Method

2.1. Peptide and surface

As described in Chen's experimental study [13,35,36], a cysteine residue is added to the desired end of the peptide in each simulation to provide a tether site to the SAM surface. A perfect α-helical structure is used as the initial template for the cecropin P1 peptide. Each initial structure of cecropin P1 (for either wild-type or the mutated peptides) is relaxed with energy minimization using CHARMM in implicit solvent. The structure obtained is then submitted to the Go model builder on the MMTSB website (http://www.mmtsb.org) to generate input files for the coarse grained simulations. A moderate surface hydrophobic parameter is chosen to represent the maleimide terminated SAM surface as used in the reference experimental work [13,36]. Details for setting up the surface in each simulation will be discussed in the next section.

2.2. Simulation

The protein model used in this work is the Karanicolas and Brooks's (KB) Go-like model [37–39]. This model describes each residue by one site placed at the C_α position of the residue. Native contacts are defined in this model based on the hydrogen bonding between backbone atoms or side-chain–side-chain interactions, which have been shown as the main factors contributing to protein folding transition states [37]. By using this model, it has shown that the protein folding energy surface and the protein folding mechanisms can be reproduced [37–41].

A coarse grained potential for the protein–surface interactions was recently developed [32] based on and to be utilized with the KB Go-like protein model [37–39] which is shown as

$$V_{surface} = \sum_{i}^{N} \left\{ \pi\rho\sigma_i^3 \in_i \left[\theta_1 \left(\frac{\sigma_t}{z_{is}}\right)^9 - \theta_2 \left(\frac{\sigma_t}{z_{is}}\right)^7 + \theta_3 \left(\frac{\sigma_t}{z_{is}}\right)^3 - (\theta_s(\chi_s - 4.5) + \theta_p \chi_p) \left(\frac{\sigma_t}{z_{is}}\right)^3 \right] \right\} \quad (1)$$

where the summation is over all of residues in the protein (N), z_{is} is the distance between residue i and the surface, σ_i and ε_i are residue specific van der Waals parameters. Both σ_i and ε_i are residue specific Lenard-Jones parameters calculated with Lorentz–Berthelot combining rules between residue i in the protein and the sites comprising the surface with σ_s = 3.0 Å and ε_i = 1.0 kcal/mol. The parameters (θ's as shown in Table 1) [32] were determined by the benchmark experimental data from Latour's lab [42], which was further validated by other experimental data.

As shown in Eq. (1), the first three terms of the potential function between the protein and the surface successfully capture the adsorption well and the energy barrier features as observed in many other experimental studies [42,43]. Furthermore, the two third power terms were also added to the function to account for hydrophobic effects of different SAM surfaces and different residues in a protein or peptide by using the hydrophobic index of surfaces χ_s [33] and amino acids χ_p [44].

The hydrophobicity of the maleimide SAM surface, which is used in this work, is set to be moderately-hydrophilic with a χ_s

Table 1
Parameters for the surface model [32].

θ_1	θ_2	θ_3	θ_s	θ_p
0.2340	0.4936	0.1333	0.0067	0.0333