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Research paper

Characterization of mechanical unfolding intermediates of membrane proteins by coarse grained molecular dynamics simulation

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ABSTRACT

Single-molecule force spectroscopy by atomic force microscopy allows us to get insight into the mechanical unfolding of membrane proteins, and a typical experiment exhibits characteristic patterns on the force distance curves. The origin of these patterns, however, has not been fully understood yet. We performed coarse-grained simulation of the forced unfolding of halorodopsin, reproduced the characteristic features of the experimental force distance curves. A further examination near the membrane-water interface indicated the existence of a motif for the force peak formation, i.e., the occurrence of hydrophobic residues in the upper interface region and hydrophilic residues below the lower interface region.

1. Introduction

Single molecule force spectroscopy by atomic force microscopy (AFM) has provided an important tool for studying biological molecules in detail. For instance, forced unfolding experiments of membrane proteins usually exhibit characteristic sawtooth like force (F) – distance (D) curves, where F is the force acting on the stylus tip of the cantilever and D is the distance between the membrane surface to the cantilever tip [1,2]. The patterns of F–D curves depend on membrane protein species [1–10], and the characteristic pattern of the F–D curve peculiar to each membrane protein is thought to carry important information regarding intramolecular interactions during the forced unfolding process of the protein. However, the origin of these sawtooth patterns have not been understood well in detail.

To address the issue, we have developed a coarse-grained model of a membrane protein and performed Brownian dynamics (BD) simulation [11,12] of bacteriorhodopsin (BR) [13] for the forced unfolding out of a purple membrane. As a result, the simulations reproduced essential features of experimentally obtained F–D curves [14]. In particular, the positions of force peaks (FPs) were in good agreement with the experiments in the literature [15,16]. Importantly, we observed that each peak position of the simulated F–D curve was associated with a specific unfolding intermediate. We, then, classified these unfolding intermediates into ten types, and discussed the forced unfolding mechanism of membrane proteins. Is this mechanism generally applicable to the other membrane proteins?

The purpose of this study is twofold. First, we performed coarse-grained molecular dynamics simulations for the forced unfolding of halorhodopsin (HR) [17], another membrane protein than BR, to demonstrate the general applicability of our method. As a result, the simulated F–D curves were in good agreement with the experimentally obtained ones [5]. Furthermore, we analyzed the unfolding intermediates of HR. Second, we compared the forced unfolding processes of BR and HR, and examined characteristic features of the unfolding intermediates that are associated with prominent peaks on the F–D curves.

2. Materials and methods

We have recently developed a coarse grained model of BR and performed BD simulation for the forced unfolding of BR [14]. In this study, we applied this method to HR, and performed the forced unfolding simulation for the extraction from the C-terminus of HR. The HR molecule was represented as a chain of $N = 254$ spherical rigid body particles, hereafter denoted as peptide bond particles (PBPs) or simply as particles, where i-th particle (PBP_i) represents the main chain segment, $C_i^{\alpha C}$–$C_iO_iN_{i+1}H_{i+1}$–$C_{i+1}^{\alpha N}$, between the i-th and the $(i + 1)$-th residues. In the local coordinate system (LCS) associated with PBP_i [18], the center of PBP_i was matched to the origin of LCS (Fig. 1(A)). To each PBP, we attached two extra points representing H-bond donor (hbh) and acceptor

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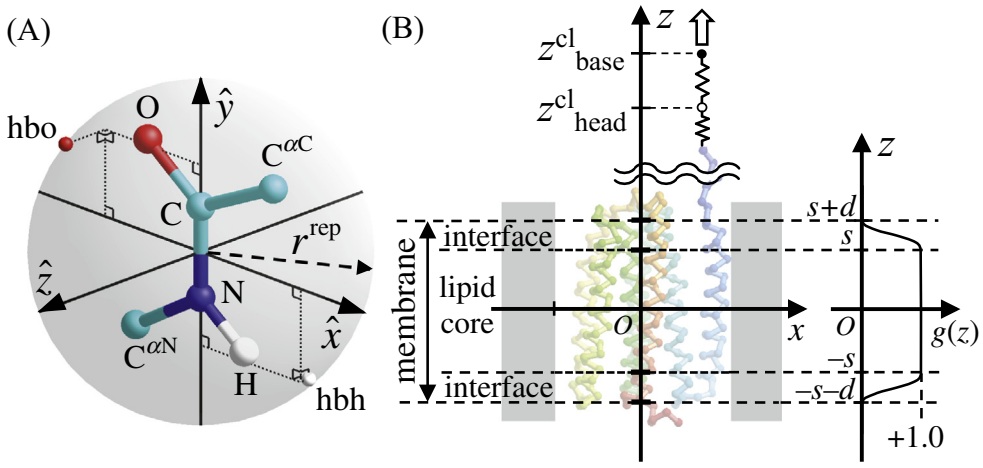

Fig. 1. Coarse grained model of halorhdopsin. Each amino acid residue was represented by a peptide bond particle (PBP) (A). With respect to LCS, the position vector, $\hat{\mathbf{r}}$, of the six constituent atoms are (139.8, 128.2, 0.0), (0.0, 66.0, 0.0), (−101.6, 137.1, 0.0), (0.0, −66.0, 0.0), (83.9, −120.5, 0.0) and (−123.3, −146.1, 0.0) in picometers, for $C^{\alpha C}$, C, O, N, H, and $C^{\alpha N}$, respectively [18]. To each PBP, we attached two extra points representing H-bond donor (hbh) and acceptor (hbo) to each PBP, and their position vectors are (189.1, −141.9, −7.6) and (−180.6, 130.3, 79.6) in picometers, respectively. The HR molecule was modeled as a one-dimensional chain of PBP (B). GSC was introduced so that the *xy*-plane became the middle plane of the membrane system, and the *z*-axis was set perpendicular to it. The polypeptide chain is excluded from the gray regions. $D: -2.5\text{nm} \leqslant z \leqslant 2.5\text{nm}$ and $(x^2+y^2)^{\frac{1}{2}} > 2.0\text{nm}$ .The shape of the smoothing function $g(z)$ is shown on the right, where $g(z) = \begin{cases} 0, & \text{for } s+d < |z| \\ \frac{1}{2} - \tilde{z} - \frac{1}{2\pi}\sin(2\pi\tilde{z}), & \text{for } s \leqslant |z| \leqslant s+d, \\ 1, & \text{for } |z| < s \end{cases}$

$\tilde{z} = (1/d)\{|z| - s - (d/2)\}$,$s$ and d were set to 1.4 and 0.7 nm, respectively.

(hbo). For each atom, the transformation from LCS to the global coordinate system (GCS) is expressed as $\mathbf{r} = \mathbf{A}_i\hat{\mathbf{r}} + \mathbf{r}_i$ where $\mathbf{r}_i$ represents the position vector of the center of PBP_i in GCS, and $\mathbf{A}_i$ is a 3 × 3 rotation matrix. Hereafter, a position vector with respect to LCS (GCS) is represented with (without) a hat. The state of the system was represented as $\boldsymbol{q}^{\text{sys}} = \{\boldsymbol{q}_0, \ldots, \boldsymbol{q}_{253}, \boldsymbol{r}^{\text{cl}}_{\text{head}}, \boldsymbol{r}^{\text{cl}}_{\text{base}}\}$, where $\boldsymbol{q}_i = \{\mathbf{r}_i, \mathbf{A}_i\}$ and $\boldsymbol{r}^{\text{cl}}_{\text{head}}$ ($\boldsymbol{r}^{\text{cl}}_{\text{base}}$) is the cantilever head (base) position.

The C-G model of HR was embedded into implicit membrane/water environment (Fig. 1(B)), where the polypeptide chain was confined in a cylindrical region. The starting structure was built based on the X-ray structure of the mature HR (PDB ID: 1E12 [19]), and the sequential residue numbers from Ala22 to Asp274 were renumbered from Ala1 to Asp253. The membrane affinity of each main- and side chain unit was reflected in the force field functions. The protonated Schiff-base of retinal was excluded from the model. Alpha helical H-bonds in the transmembrane (TM) helices were incorporated by the attractive forces between PBP_i and PBP_{i+3} (i = 1, 2,. . ., 249). The AFM forces were transmitted from the cantilever to the intra-membrane domain of HR through the stretched polypeptide chain (SPC), which spans from the cantilever tip to the membrane surface. In this study, the amino-acid residue length of SPC, L_{SPC}, was used as a reaction coordinate of the forced unfolding reaction. Note that the C^{α} atom of the residue at the lower end of SPC does not necessarily sit exactly on the membrane surface. Here we introduce the concept of the hypothetical surface residue [14], i_{surf}, whose C^{α} atom is exactly on the upper membrane surface, i.e., $z = d + s$ (d = 0.7 nm (thickness of the interface region), s = 1.4 nm (half-thickness of the lipid core region)). Thus the value of L_{SPC} is 253 − i_{surf}, where

$$i_{\text{surf}} = j + \frac{(d+s) - z^{\alpha}_j}{z^{\alpha}_{j+1} - z^{\alpha}_j},$$

and j is the largest integer that satisfies $z^{\alpha}_j \leqslant (d+s) < z^{\alpha}_{j+1}$. Thus, the value of i_{surf} can be either integer or non-integer.

The potential energy of the system is expressed as $U^{\text{sys}} = U^{\text{pep}} + U^{\text{mem}} + U^{\text{hb}} + U^{\text{cl}} + U^{\text{wall}}$. The main chain segment of the HR forms a linear polypeptide chain due to the first term on the right hand side,

$$U^{\text{pep}} = \sum_{i=1}^{N}\left\{\left(k^{\text{b}}/2\right)\left(\mathbf{r}_i^{\alpha C} - \mathbf{r}_i^{\alpha N}\right)^2 + \left(k^{\text{a}}/2\right)(\theta_i - 110.1^{\circ})^2 + \left(k^{\text{da}}/2\right)(\phi_i + 65.4^{\circ})^2\right\} + \sum_{i=0}^{N-3}\left(\sum_{j=i+3}^{N} U^{\text{rep}}_{ij}\right)$$

where θ_i is the bond angle $\angle N_i C^{\alpha}_i C_i$. Note that $U^{\text{da}}{}_i = (k^{\text{da}}/2)(\phi_i + 65.4^{\circ})^2$ is set for proline. Steric repulsion between a nearby pair of PBPs, i.e., PBP_i and PBP_j for which $|\mathbf{r}_j - \mathbf{r}_i| < 2r^{\text{rep}}$, arises from the last term of the above equation: $U^{\text{rep}}{}_{i,j} = (k^{\text{rep}}/2)(2r^{\text{rep}} - |\mathbf{r}_j - \mathbf{r}_i|)^2$, whereas $U^{\text{rep}}{}_{i,j}$ is set to 0 for the other pairs. The values of parameters k^{b}, k^{a}, k^{da}, k^{rep}, and r^{rep} were set to 125.02 [J/m^2], 4.618×10^{-22} [J/deg^2], 3.281×10^{-23} [J/deg^2], 100 [N/m], and 0.2366 [nm], respectively.

$U^{\text{mem}} (= \sum_{i=0}^{N}(\Delta U^{\text{mc}}_i \cdot g(z_i)) + \sum_{i=1}^{N}(\Delta U^{\text{sc}}_i \cdot g(z^{\alpha}_i)))$ represents the affinity of amino acid residues to the membrane (Fig. 1(B)), where z_i (z^{α}_i) represents the z coordinate of the origin (C^{α} atom) of the ith PBP (residue), and the values of $\Delta U^{\text{sc}}{}_i$ ($\Delta U^{\text{mc}}{}_i$) were set to −3.5, 21.0, 9.6, 23.9, −2.7, 6.2, 19.3, −1.3, 2.2, −11.7, −11.7, 11.8, −6.8, −14.3, −7.0, 5.8, 4.5, −13.4, −9.7, and −8.7 (9.8, 27.1, and 12.4) [kJ/mol] for A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, and V (N-terminus, C-terminus, and >CH–CONH–), respectively.

The main chain H-bonding interactions were represented by $U^{\text{hb}} = \sum_{i=1}^{N-4} U^{\text{hb}}_i$, where $U^{\text{hb}}{}_i$ was set to 0 if $(i+4)$th residue is proline and $\{(\Delta U^{\text{hb,m}} - \Delta U^{\text{hb,w}}) \cdot g(z^{\text{hb}}_i) + \Delta U^{\text{hb,w}}\} \cdot h(r^{\text{hb}}_i)$, otherwise. $r^{\text{hb}}{}_i$ and $z^{\text{hb}}{}_i$ stands for $|\boldsymbol{r}^{\text{hbo}}{}_i - \boldsymbol{r}^{\text{hbh}}{}_{i+4}|$ and the z component of $(\boldsymbol{r}^{\text{hbo}}{}_i + \boldsymbol{r}^{\text{hbh}}{}_{i+4})/2$, respectively. $h(r)$ is represented by $-3(r/r^{\text{hb}}_0)^4 + 8(r/r^{\text{hb}}_0)^3 - 6(r/r^{\text{hb}}_0)^2 + 1$, for $r < r^{\text{hb}}_0$, and 0 otherwise, where the value of r^{hb}_0 was set to 0.25 nm. H-bond energy parameters in the membrane ($\Delta U^{\text{hb, m}}$) and in the water ($\Delta U^{\text{hb,w}}$) were set to −27.26 kJ/mol and −8.726 kJ/mol [14], respectively. To determine the value of $\Delta U^{\text{hb,m}}$, we simulated the HR system with $U^{\text{cl}} = 0$ (no extraction condition) at different values of $\Delta U^{\text{hb,m}}$ at the absolute