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LETTER TO THE EDITOR

Conformational sampling on acid-sensing ion channel 1 (ASIC1): implication for a symmetric conformation

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Dear Editor,

Acid-sensing ion channel 1 (ASIC1) is an ion channel that is capable of transporting Na^+ through the cell membrane upon activation by extracellular (EC) protons. Owing to essential physiological and pharmacological functions in the central nervous system, ASIC1 has been appreciated as an important neuronal receptor and drug target [1]. The mechanic and dynamic fundamental of channel activation and ion permeation of ASIC1 and other members of ASICs has not been fully understood. The recent low-pH crystal structure of the chicken ASIC1 (cASIC1) at 1.9 Å resolution has revealed the overall organization of the channel [2]. Structurally, ASIC1 is a homotrimer, forming a chalice-like architecture. Each subunit is composed of two domains, a large EC domain and a transmembrane (TM) domain. The EC domain resembles a clenched hand, which can be further divided into finger, thumb, palm, knuckle and β-turn subdomains. The TM domain comprises two transmembrane helices, TM1 and TM2, in a "forearm" arrangement (Figure 1A) [2].

The crystal structure of cASIC1 provides a framework for probing the mechanism underlying the gating of ASICs. Recently, based on the crystal structure of cASIC1, we performed a study on the dynamics-function relationship for this channel using molecular simulation, mutagenesis and electrophysiological methods [3]. The results demonstrated that the current X-ray crystal structure of cASIC1 is a reasonable conformation that is biologically relevant and should be a good starting structure for both computational simulations and experimental studies. However, concerns regarding the validity of the crystal structure of ASIC1 still exist. One concern is why the entire architecture composed of three identical subunits is asymmetric, especially that of the TM domain (Figure 1A). Shaikh and Tajkhorshid [4] argued that this asymmetry of ASIC1 structure may be an artifact induced by crystal packing. Another concern is that the current crystal structure of cASIC1 reflects the desensitized/closed, state, which may hinder further insights into the behaviors of the channel. To clarify these concerns and to test the existence of other biologically relevant conformations of ASIC1, we performed a conformational sampling analysis on this channel on the basis of its crystal structure.

Similar to other ion channels such as the potassium channel and nicotinic acetylcholine receptor, ASIC1 should undergo large-scale (occasionally global) conformational deformations when eliciting cellular functions, such as gating [5, 6]. Simulating global conformational changes of proteins is beyond the scope of the conventional molecular dynamics and Monte Carlo simulation methods [6]. Normal mode analysis (NMA) is a powerful computational method for studying large-amplitude molecular deformational motions that are widely involved in biological functions of macromolecules [7].

Accordingly, NMA was used in this study to explore the conformational profile of ASIC1. NMA was performed by using the standard techniques [8] by the web server developed by Delarue *et al*. (http://lorentz.immstr.pasteur.fr/nomad-ref.php) [9]. The trajectories of lowest frequency modes, which give rise to the largest displacements and provide information on the important intra- and inter-domain motions [10], were used for the conformational sampling. Snapshots isolated from the NMA trajectories were subjected to energy minimization with C_α atoms fixed and the potential energy of each conformational model was calculated by using the MM-PBSA method encoded in the AMBER program (version 9.0) [11].

The NMA simulation on the crystal structure of cASIC1 revealed two interesting motions for the TM domain, rocking motion around the twist region (mode 2) and twisting rotation around a hinge located around Leu440 (modes 1 and 3) (Figure 1) [3]. From the rocking motion of the TM domain, we detected a more symmetric conformation of ASIC1. The symmetry of the structure of ASIC1 can be described by the inclination angle (θ) of the TM domain with respect to the EM domain. Thus, θ can be defined as the intersection angle between two lines, one line links the mass centers of the EC domain

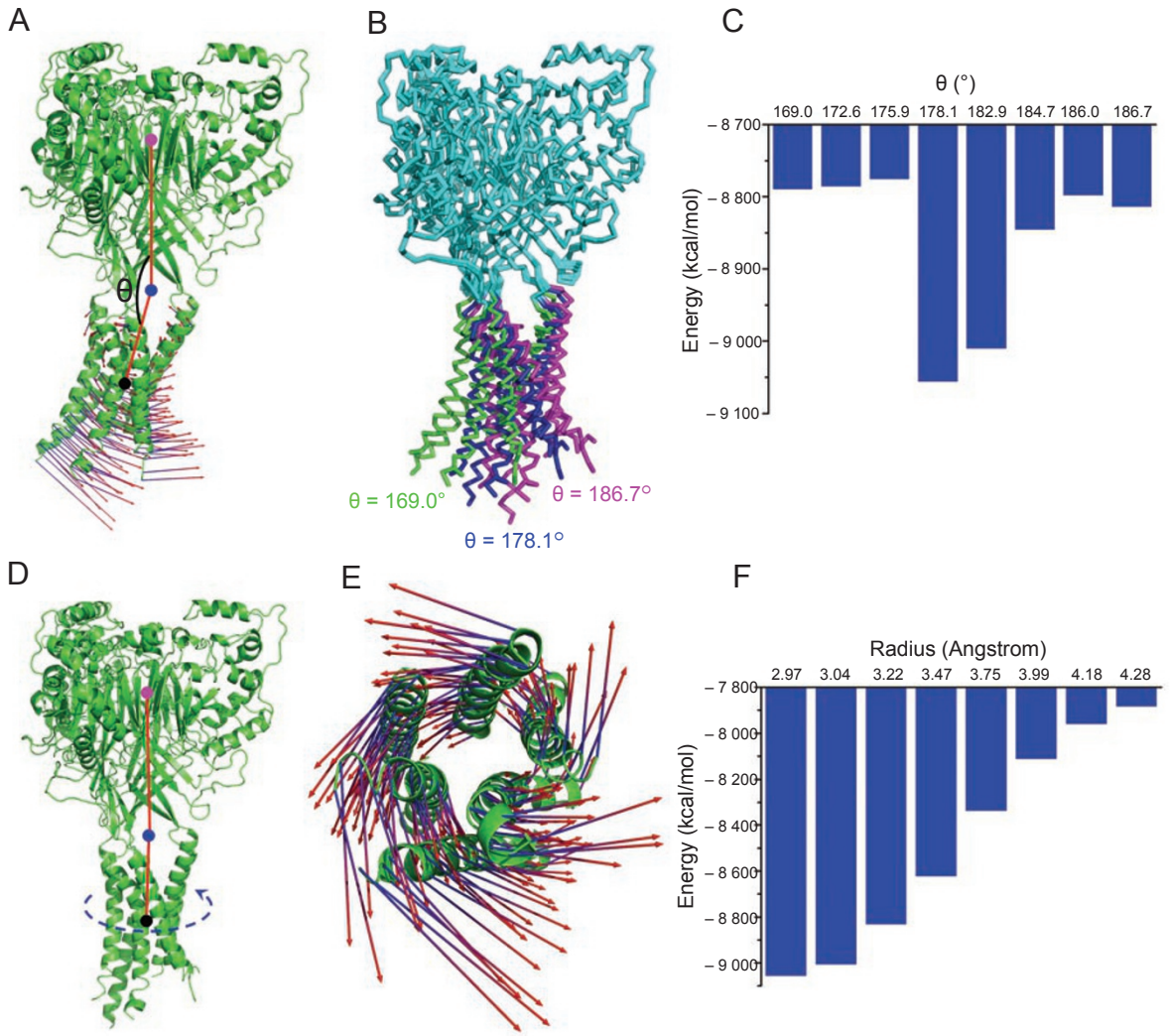

Figure 1 Conformational transition and potential energy evolution of ASIC1 analyzed by NMA. **(A)** Rocking motion of the TM domain produced by NMA on the crystal structure of ASIC1. The crystal structure is displayed in cartoon view. The mass centers of the EC domain, the wrist region and the TM domain are indicated by magenta, blue and black balls, respectively. The inclination angle of the TM domain with respect to the EC domain is indicated by θ. The vector arrows of mode 2, which show the direction of the rocking motions are mapped onto the TM domain of the ASIC1 crystal structure. **(B)** Structural superposition of three representative conformations isolated from the rocking trajectory. **(C)** The potential energy for the conformational transition corresponding to the motion of mode 2. The inclination angles (θ) of these conformations are displayed as abscissa. **(D)** Twisting rotation motion for the TM domain revealed by the second cycle of NMA on the relatively symmetric conformation with θ =178.1°. The cyclic arrow indicates the rotation motion of the TM domain. **(E)** Detailed display of the twist rotations of the TM domain in mode 3 from the second NMA. The vector arrows are mapped onto the TM domain of the symmetric conformation. The TM domain is shown in the intracellular view. **(F)** The potential energy for the conformational transition corresponding to the motion of mode 3 from the second NMA. The radius of the potential gate of the channel pore is displayed as abscissa.

and the wrist region, and the other line connects the mass centers of the wrist region and the TM domain (Figure 1A). The rocking motion revealed that the TM domain might swing around the wrist between θ = 169° and θ = 186.7° (Figure 1B). Thus, we isolated seven typical snapshots from the moving trajectory, and their potential energies were calculated after structural minimization (Figure 1C). As the receptor was simulated at a constant temperature (25 °C in this study), it is unlikely that the kinetic energy of the system would change with the channel conformational transition. Therefore, the potential energy profile can be used to describe the energetic landscape of ASIC1. The evolution of potential energy with respect to θ indicates that the lowest energy conformation is located at θ ≈ 180° (Figure 1C). Structurally, the conformation of ASIC1 with θ = 180° is almost a symmetric structure. From the point of view of energetic landscape, therefore, native ASIC1 channel should be ar-

ranged in a symmetric manner.

After obtaining a symmetric conformation from the NMA on the crystal structure of cASIC1, we performed a second cycle NMA on this conformation. The important motion modes, especially the rocking and twisting motions of the TM domain revealed by the crystal structure-based NMA, were also detected by this additional round of NMA simulation. We performed conformational analyses along the trajectory of modes 1 and 3, which produced similar result. These two modes revealed that the TM domain underwent a twisting rotation (Figure 1D) around a hinge. The hinge is located around the bottleneck of the channel pore formed by residues around Leu440 [3]. Notably, the twisting motion of TM domain directly modulates the size of the channel pore. For example, the twisting motion of mode 3 may maximally increase the diameter of the bottleneck by ~2.5 Å (Figure 1E). This is in good agreement with the notion that the TM domain can undergo a twisting-to-open motion, which is closely associated with the ASIC1 gating [3]. Interestingly, the twisting-to-open motion is an energetically unfavorable process, since the potential energy increases as the channel pore opens (Figure 1F). We suggest that the energy required for opening the channel can be largely complemented by the binding of protons (H^+s) to the acidic pocket in ASIC1 [2, 3].

Based on the crystal structure, we have performed a conformational sampling on ASIC1 by using the NMA method. Three lowest frequency modes revealed a deformation pathway that is possibly associated with the gating of ASIC1. The rocking motion of the TM domain around the wrist allows the channel to pass a more symmetric conformation in comparison with the known crystal structure [2]. This symmetric conformation is the lowest energy structure in the potential surface (Figure 1C and 1F) and may undergo a twisting rotation, which consequently opens the channel. However, this motion is energetically unfavorable (Figure 1F). Accordingly, the binding energy of protons to the acidic residues could be consumed by the twisting-to-open process during the channel gating. Thus, we conclude that ASIC1 may adopt different conformations and its lowest energy conformation should be symmetric (Figure 1D). In addition, we detected the presumed open conformations of ASIC1 (Figure 1F). These findings may shed new light on understanding the structural dynamics associated with ASIC1 functioning.

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