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LETTER

Mutation of the critical pH-gating residues histidine 231 to glutamate increase open probability of outer membrane protein G in planar lipid bilayer

Dear Editor,

Outer-membrane protein G (OmpG) is a nonspecific β-barrel porin in the outer membrane of *Escherichia coli* (*E. coli*), allowing the passage of ions and molecules up to 900 Da (Fajardo et al., 1998). It comprises of 280 amino acids that form 14-stranded β-sheets with seven long loops (L1–L7) on the extracellular side and six short turns on the periplasmic side (Subbarao and van den Berg, 2006; Yildiz et al., 2006; Liang and Tamm, 2007). Despite that the OmpG gene exists in the genome of several *E. coli* strains (Nikaido, 1999), expression of OmpG was only observed in *E. coli* mutants lacking OmpF and LamB (Fajardo et al., 1998) to enable the diffusion of maltodextrins across the bacterial outer membrane. Very interestingly, unlike usual trimeric channel-forming porins, OmpG exhibits fascinating characteristics of a functional monomer in physiological and structural studies (Conlan and Bayley, 2003; Mari et al., 2010). Recent atomic force microscopy (AFM) studies showed the dimeric OmpG in lipid, but no evidence shows the physiological relevance of oligomeric forms of OmpG (Mari et al., 2010). The functional monomeric channel porin enables OmpG to be engineered (Bayley et al., 2008) as a single-molecule biosensor (Chen et al., 2008a).

The *in vitro* studies of OmpG in planar lipid bilayers revealed that channel properties of OmpG were pH-dependent, voltage-dependent, and spontaneous gating (Conlan et al., 2000; Conlan and Bayley, 2003). High resolution crystallography structure (Yildiz et al., 2006), AFM (Mari et al., 2010) and fourier-transform infrared (FTIR) (Korkmaz-Ozkan et al., 2010) studies of OmpG demonstrated that the extracellular loop L6 was responsible for pH-dependent gating of OmpG channels (Cowan et al., 1992; Schirmer, 1998). The mobilization of L6 led to the spontaneous gating behavior of OmpG, resulting in very few observed NMR signals from L6 (Liang and Tamm, 2007).

The apparent pK_a for the open-to-closed transition of OmpG was found to be 6.0 (Conlan and Bayley, 2003), which is similar as pK_a of histidine side chain. Interestingly, two histidine residues (His 231, His 261) were found to be essential for pH-gating of OmpG (Yildiz et al., 2006) (Fig. S1). Replacing both histidines with alanines or cysteines in FTIR spectroscopy studies showed pH-independent mutant channels (Korkmaz-Ozkan et al., 2010), but detail channel conductance of these mutants was not analyzed yet. Interactions between positively charged arginine and negatively charged glutamate in β11–β13 were reported to bring the channel closure at acidic pH in FTIR studies (Korkmaz et al., 2012). Therefore, manipulations of interactions between essential residues can make OmpG proteins with different channel properties, which provided a route to engineer OmpG protein to be an effective molecular biosensor.

Over the last decade, stochastic sensing, a potentially important means of single molecule detection, was developed based primarily on single-channel recording of channel proteins. The functional monomeric OmpG is naturally the best candidate for stochastic sensing, despite of its pH-dependent gating. Several mutations were engineered to weaken or abolish the pH gating properties of OmpG, such as reducing loop L6 mobility (introducing a disulfide bond between the extracellular ends of strands β12 and β13) or optimizing intra-strand hydrogen bonding between β11 and β12 (deletion of Asp 215) (Chen et al., 2008a, 2008b). To further reduce the pH-dependent gating of the OmpG channel for a quiet channel biosensor, the pH sensitive His 231 residue was replaced by a negatively charged residue glutamate (OmpG-H231E) in this work. The charge interaction between Glu 231 and His 261 was proposed to stabilize the upright fold of L6 (especially at acidic pH).

Using the bacterial expression system, both OmpG-WT and OmpG-H231E were over-expressed as inclusion bodies. The purified OmpG proteins were well solubilized using high concentration of urea and purified in DM micelles (Fig. S1). The detergent mediated reconstitution of OmpG proteins in POPE/POPG (3:1) liposomes was conducted using the dialysis methods. After the proteoliposome was diffused to the pre-painted aperture of planar lipid bilayer apparatus, the observed channel current demonstrated that the OmpG proteins were well folded in liposome (Fig. 1), indicating a reliable method of lipid mediated protein refolding for channel conductance studies.

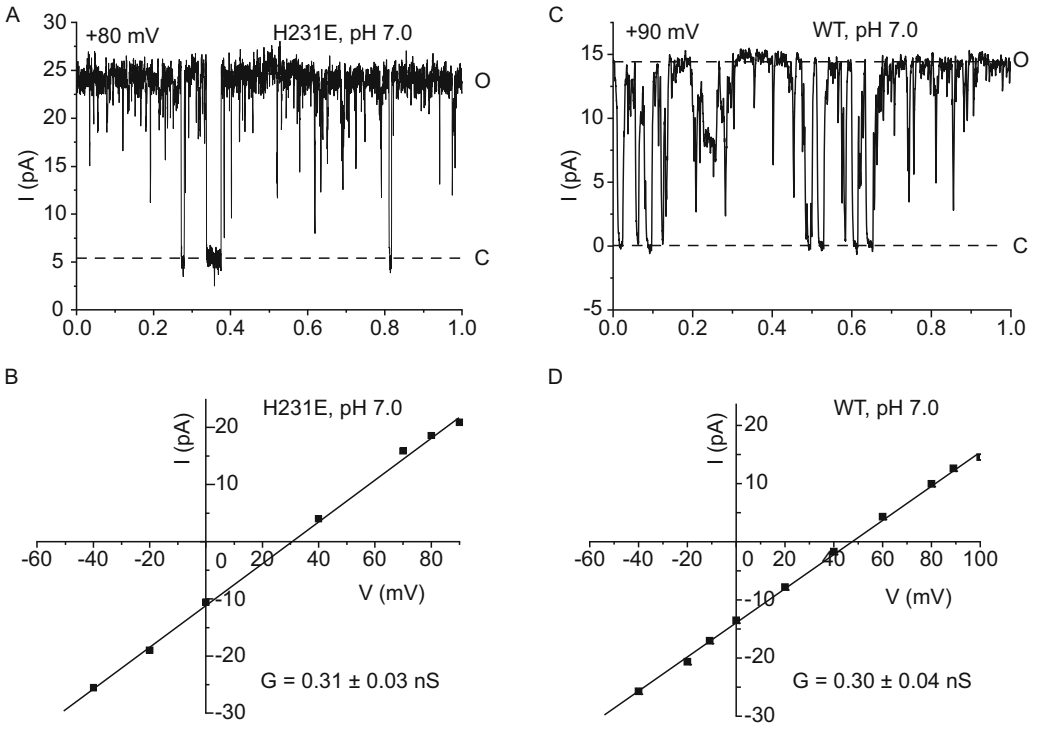

Figure 1. Single channel recording and unitary conductance derivations of OmpG-H231E and OmpG-WT in planar lipid bilayer. (A) Representative current traces of single OmpG-H231E channel at pH 7.0 and in the voltage of +80 mV (upper panel) and −20 mV (lower panel). O: open state; C: closed state. (B) The current-voltage (I/V) plot and unitary conductance derivation of the OmpG-H231E channel shown as in (A) in lipid planar lipid from series of current recordings at different voltages. (C) Representative current traces of single OmpG-WT channel at pH 7.0 and in the voltage of +90 mV (upper panel) and −10 mV (lower panel). (D) The current-voltage (I/V) plot and unitary conductance derivation of the OmpG-WT channel shown as in (C) in lipid planar lipid from series of current recordings at different voltages.

In the recorded current of OmpG-H231E (Fig. 1A) and OmpG-WT (Fig. 1C) at pH 7.0, statistics analysis of current fluxes for the two proteins revealed stable current recording, and single-step currents were observed (Fig. S2), indicating that only single OmpG channel existed in the POPE/POPG planar bilayer (Conlan et al., 2000). Stable current observations at positive or negative potentials were consistent with the current recording with more KCl concentration in the *trans*-chamber: 15 mmol/L KCl in *cis*-chamber and 150 mmol/L KCl in *trans*-chamber.

With accumulations of current traces at different potentials (Fig. S2), the linear regression of current-voltage plot (I/V plot) can be calculated (Fig. 1B and 1D). During the single channel current recording, the reversal potential of OmpG-H231E was 35.3 ± 1.6 mV (n = 10). According to the GHK equation, this measurement showed a low anion/cation permeability ratio of OmpG-H231E, which is also shown in the measurement of OmpG-WT reverse potential (44.3 ± 3.2 mV, n = 3), suggesting that the mutation did not affect the weak cation-selective of OmpG (Conlan et al., 2000). In addition, OmpG-H231E exhibited average unitary conductance value of about 0.31 ± 0.03 nS (n = 6), which is similar as OmpG WT (0.30 ± 0.04 nS, n = 3) under our experimental condition.

Since the conformation transition of OmpG was reported to be around 6.0, similar as pK_a of histidine side-chain (Conlan and Bayley, 2003). Single channel current recordings and unitary conductances of OmpG-H231E and OmpG-WT were also obtained at three different pH conditions (pH 6.0, pH 7.0 and pH 8.5). Then, the gating activity of OmpG-H231E and OmpG-WT under weak acidic (Fig. S3A), neutral (Fig. S3B) and alkaline (Fig. S3C) conditions were measured. With current recordings of OmpG-H231E and OmpG-WT at series of potentials, unitary conductance of the two proteins at different pH conditions were derived (Fig. S3D). The derived conductance values were observed to increase with pH increments, indicating that the various pH conditions might influence charge distributions in hydrophilic pore inner surface of OmpG, leading to more cation flux through the channel at elevated pH conditions. Whether conductance of anion or other neutral compounds fluxing through the channels were in the similar pH-dependent manner await further investigations. Despite of the pH-dependent ion fluxes for both OmpG-H231E and OmpG-WT, no significant conductance differences were observed between OmpG-H231E and OmpG-WT, at a specific pH condition (Fig. S3D). The observed similar

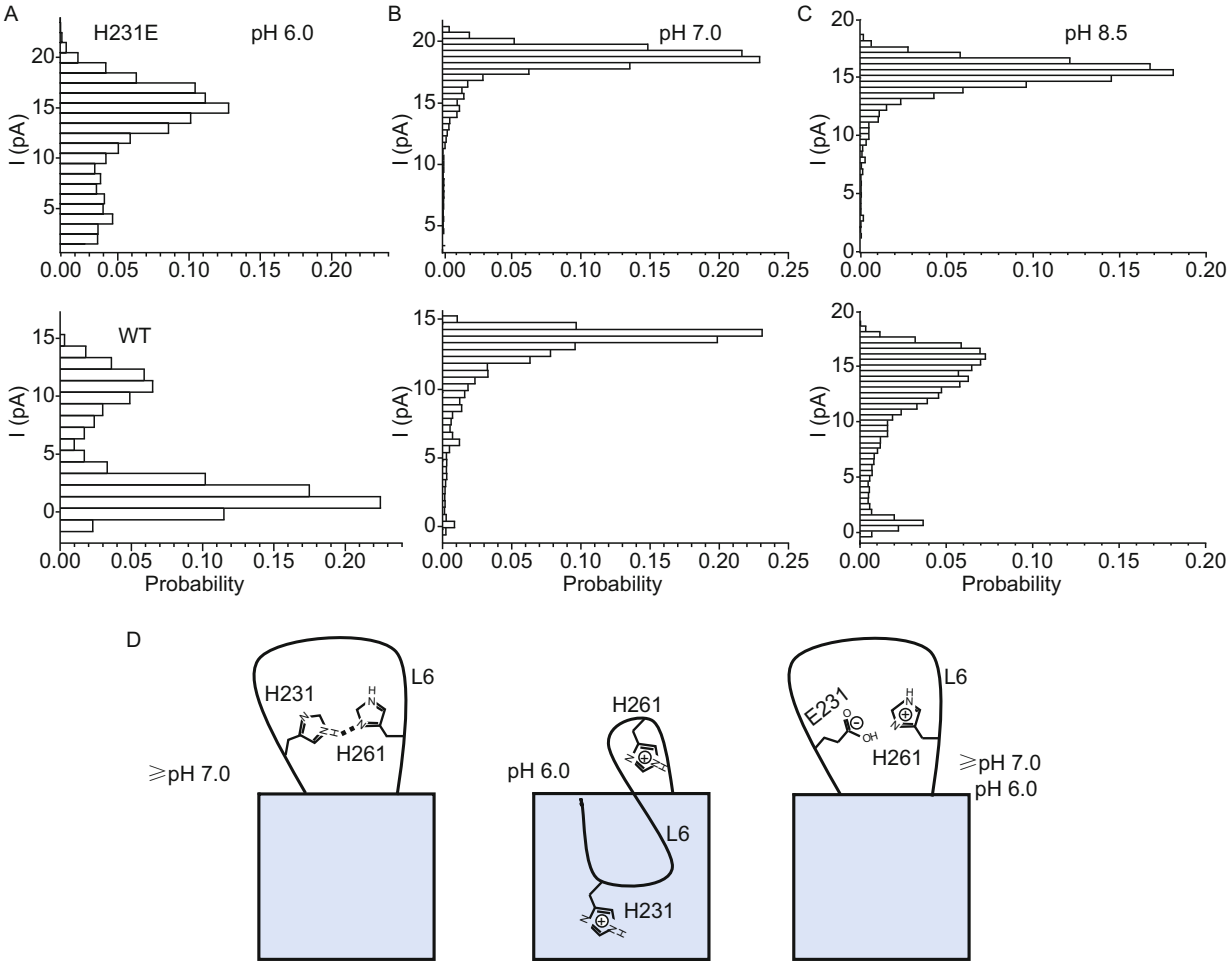

Figure 2. Comparisons of event amplitude histogram derived from current traces of OmpG-H231E and OmpG-WT channels, and channel open/close model at different pH. Histogram plot of current amplitude of OmpG-H231E (upper panel) or OmpG-WT (lower panel) versus probability at transmembrane potential of +80 mV, at (A) pH 6.0; (B) pH 7.0 and (C) pH 8.5. (D) Models of essential residues (H231, H261, H231E) maintaining channel open/close at different pH.

unitary conductance of OmpG proteins with the mutation of His 231 to a negatively charged glutamate, indicated that the His 231 plays dispensable role in affecting channel conductance or ion flux in the OmpG protein.

OmpG current recordings at different pH also provided clues to analyze channel gating properties. Statistical analysis of the channel current recording illustrated the probabilities of different current amplitudes for OmpG-H231E or OmpG-WT at three different pH conditions (Fig. 2). At pH 6.0, the OmpG-WT was reported to have high close probability (highest at 23% and total of 61.5%) at around 0 pA (closed state) and relative low open probability (highest at 6.5% and total of 24.5%) at around 11 pA (open states), while the OmpG-H231E was reported to have low close probability (highest at 3%, and total of 6%) at around 0 pA (closed state) and quite high open probability (highest at 13% and total of 53%) at around 15 pA (open state) (Fig. 2A), strongly showing the higher open probability of OmpG-H231E than OmpG-WT at pH 6.0. At pH 7.0, both OmpG-H231E and OmpG-WT were reported to have high open probability (highest at 23% and total of about 80%) at around 18 pA (OmpG-H231E) or around 14 pA (OmpG-WT) (Fig. 2B). At pH 8.5, the OmpG-WT was reported to have low close probability (highest at 4% and total of 8%) at around 0 pA (closed state) and relative high open probability despite of wide distributions (highest at 7% and total of 78%) at around 15 pA (open states), while the OmpG-H231E was reported to have almost zero close probability at around 0 pA (closed state) and quite high open probability (highest at 18% and total of 92%) at around 15 pA (open state) (Fig. 2C), suggesting the higher open probability of OmpG-H231E than OmpG-WT at the weak alkaline condition.

Obviously, both OmpG-H231E and OmpG-WT were reported to have similar gating properties at neutral pH (Fig. 2B), indicating that both the proposed charge interaction between His 261 and Glu 231, and the previously observed hydro-

gen bond interactions between Ser 218 and His 231, between His 231 and His 261 at neutral pH are playing positive roles in maintaining open conformation of OmpG-WT proteins (Fig. 2D, left panel). At weak alkaline pH (8.5), both OmpG-H231E and OmpG-WT were observed to have high gating probabilities. But broad distribution of the open probability for OmpG-WT (Fig. 2C) indicated that the OmpG-H231E had better pH stability at pH 8.5. At weak acidic pH (6.0), almost half of OmpG-WT was observed to be in closed state, which is consistent with the previously proposed hydrogen bond breaking at acidic pH and consequent L6 blockage in the pore region (Fig. 2D, middle panel) (Yildiz et al., 2006). Replacing the His 231 to negatively charged glutamate, immediately abolished the proposed repulsive interaction between two protonated histidines, while the resulting interactions between positive charged His 261 and negatively-charged Glu 231 might form attractive charge interactions, maintaining the correct upright fold of L6, without further blockage in the OmpG lumen (Fig. 2D, right panel).

It is known that channel gating is closely correlated with protein dynamics. The reported less pH-dependent gating might reflect relatively rigidity of the protein, especially the L6 of OmpG. Therefore, the H231E mutation is believed to have more stable interactions with ambient residues, very possibly due to spatial proximities between the H231E and His 261. At the same time, the unitary channel conductance data demonstrated similar behaviors of OmpG-H231E and OmpG-WT at different pH (Fig. S3D), indicating that H231E mutant does not affect channel conductance too much. Combining together, the H231E mutation is effective to decrease pH-dependent gating properties of OmpG at acidic pH and maintain more stable charge-interactions between Glu 231–His 261 at weak alkaline pH. The unchanged channel conductance and decreased pH-dependent gating properties of OmpG-H231E provide good potential to engineer a constant-open channel for further biosensing applications using the monomeric beta-barrel porin channel.

FOOTNOTES

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Mu Yu[1,2], Peibei Sun[2], Yao He[2], Liang Xiao[2], Demeng Sun[2], Longhua Zhang[2]✉, Changlin Tian[1,2]✉

[1] High Magnetic Field Laboratory, Chinese Academic of Sciences, Hefei 230031, China
[2] Hefei National Laboratory for Physical Science at Microscale and School of Life Science, University of Science and Technology of China, Hefei 230027, China

✉ Correspondence: zlhustc@ustc.edu.cn (L. Zhang), cltian@ustc.edu.cn (C. Tian)

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