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Rapid report

Theoretical conformation of the closed and open states of the acetylcholine receptor channel

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Abstract

We conduct a theoretical analysis to show the recently imaged structure of the acetylcholine receptor pore is in a non-conducting state. A hypothesised open state consistent with a lower resolution image is created and shown to have high conductance.

Keywords: Acetylcholine receptor; Pore; Ion channel; Gating; Brownian dynamic

The acetylcholine receptor pore, responsible for mediating the electrical signaling between nerve and muscle cells, was recently imaged to high resolution using electron microscopy [1]. The structure obtained was hypothesised to be in a closed, non-conducting state, and the structural changes required to open the channel were suggested. Here we conduct a theoretical analysis to support these hypotheses. We find that no current will flow through the imaged structure. However, a set of minor conformational changes consistent with those suggested and with a lower resolution electron microscope image of an open channel [2] do create a highly conductive sodium channel.

In Fig. 1A we show the imaged and hypothesised open structure of the transmembrane pore. We examine the conductance properties of the imaged structure by incorporating the coordinates of the ligand binding domain [3] (PDB code 1I9B) into the recently imaged membrane spanning structure to build a complete picture of the protein. Next we trace the molecular surface of the pore, and create a dielectric model of the channel. Dielectric constants of 80, 60 and 2 are assigned to the bulk water, channel and protein. The radius of the pore boundary for the imaged structure and two alternative open structures (described below) is shown in Fig. 1B.

The extracellular domain is strongly electronegative, and strongly attracts cations. We show the energy well seen by a single Na^+ ion passing through the channel in Fig. 2A. Our calculations show that the extracellular domain can hold up to 11 sodium ions in a stable equilibrium (this is not surprising given that the structure has a net charge of $-45e$), while the intracellular section of the channel provides an unfavourable home for ions. In Fig. 2B we show the energy required for a twelfth ion to cross the intracellular end of the channel while the other ions find their minimum energy positions, calculated using Poisson's equation solved with a boundary element method [4]. A large energy barrier of 30 kT is seen in the narrow hydrophobic section of the channel. The location of this barrier can also be seen by a bump in the single ion profile. This barrier is large enough to prevent conduction and confirms the conjecture that this section of the channel forms an electrostatic channel gate [1].

Next we carry out Brownian dynamics simulations on the channel to determine its likely conductance. In these simulations we trace the motions of ions in and around the channel under the influence of electric and random forces using the Langevin equation [5,6], a technique that has successfully been used to describe ion permeation in potassium, calcium and other channels [7,8]. In these simulations, a number of ions are added to reservoirs 60 Å in diameter attached to each end of the protein. The height of the reservoirs is adjusted to bring the concentration in each reservoir to 150 mM. As ions fill the electronegative protein, more are added to the appropriate reservoir to keep the concentration in the reservoir constant. Once an equilibrium state is reached, no more ions are added to the

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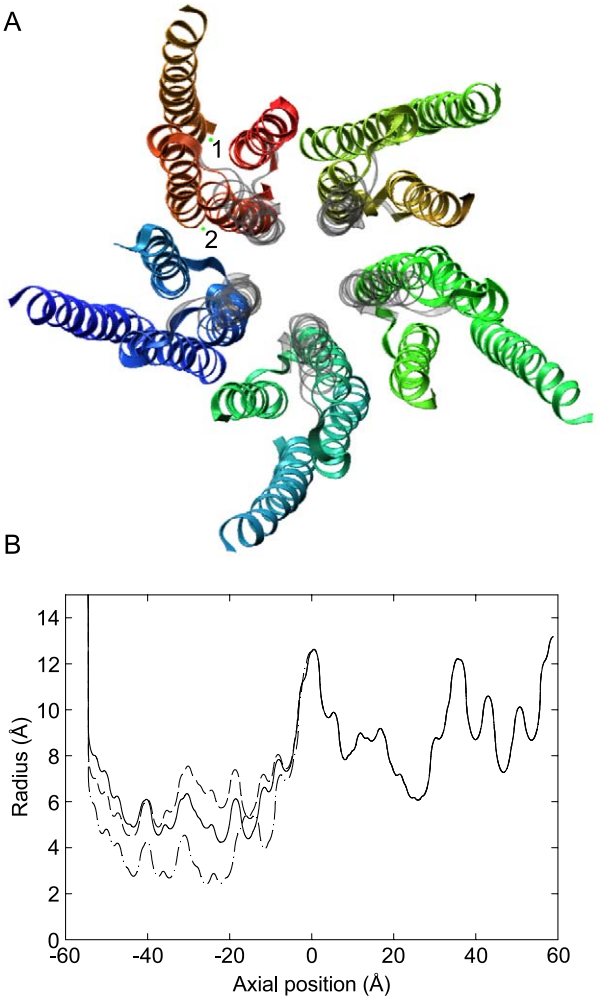

Fig. 1. (A) The imaged (grey) and hypothesised open (coloured, opaque) structures of the acetylcholine receptor channel, viewed from outside the cell looking at the plane of the membrane. Each of the five subunits contains four transmembrane helices and are arranged symmetrically about the channel axis. Only the conformation of the pore lining M2 helices differ between the imaged and open structures. To create the open structure, the M2 helix is rotated by 15° clockwise about the axis of the disulfide bridge in the binding domain (point 1). The lower section of the helix is then rotated by 30° clockwise about axis 2. (B) The minimum pore radius of the imaged structure (dash-dot line) and two hypothesised open structures (solid and dashed lines). One open structure (dashed line) is 1 Å wider in the center of the channel than the other.

system. Water molecules are not modelled explicitly, but rather are replaced by a continuum exerting random and frictional forces on the ions. The protein atoms are held at fixed positions. The total electric force acting on each ion is then calculated from Poisson's equation as described above. This electric force is added to the random and frictional forces exerted by the aqueous solution, and short range forces that describe the interaction of ions with the protein walls. The trajectories of the ions are calculated using 2-fs timesteps, recalculating the forces at every step. Further details of the simulations can be found in the references noted above.

We find that, on average, 11.2 ions are resident in the transmembrane pore and binding domain during the simulation (at −60 mV). However, ions will not pass the narrowest hydrophobic section of the pore in either direction under an applied potential of up to ±160 mV, confirming that the imaged structure represents a non-conducting state as a consequence of the electrostatic interactions between the ion and protein.

As cations cannot pass the narrow hydrophobic section of the channel, that section must either become wider, or alter so as to present polar residues to the permeating ion, to create an open structure. For this channel, Unwin [2] has shown that activation causes a rotation of the inner β sheets of the binding domain by 15° about the axis of the disulfide bridge joining the two β sheets of each subunit. As the bottom of each inner sheet contacts the end of the pore lining M2 transmembrane helix, it is suspected that this motion is transferred to the pore. In fact, the lower-resolution electron microscope image of the open channel [2]

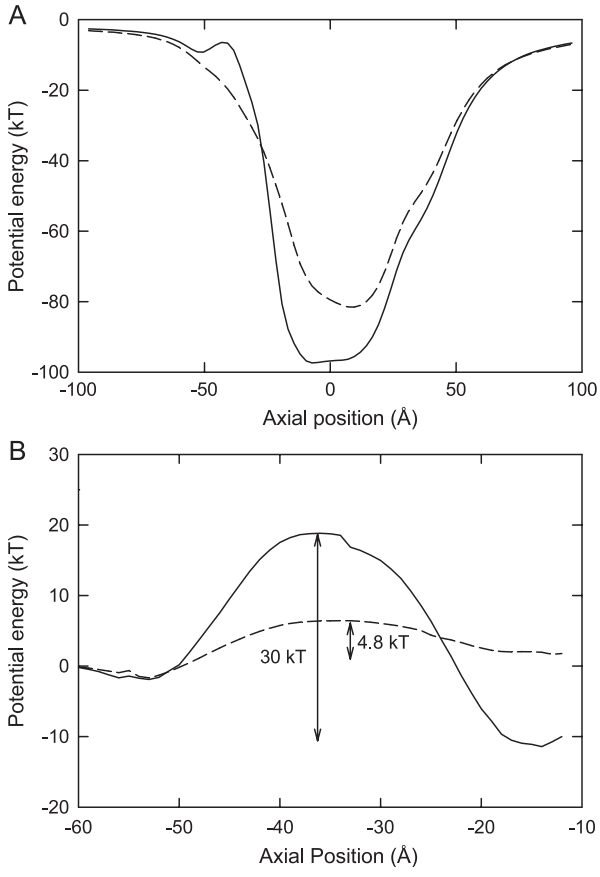

Fig. 2. Electrostatic energy profiles. (A) The energy of a single sodium ion calculated as it moves along the axis of the transmembrane channel and extracellular binding domain of the imaged structure (solid line) and hypothesised open structure (dashed line). (B) The energy required for an ion to cross the intracellular end of the channel while 11 other ions are resident in the channel and binding domain, obtained from the imaged structure (solid line) and postulated open structure (dashed line). The channel and binding domain extends roughly between $z = \pm 58$ Å.

shows that only the pore lining M2 helix undergoes movement whereas the rest of the protein remains unchanged. This leaves only limited space in which the M2 helix can move, and thus limits the diameter of the open channel to be not much more than 9 Å. The inner helix is shown to gain a kink about midway through the membrane and this suggests a rotation of the helix that moves the hydrophobic residues further from the pore axis.

This open configuration is shown in Fig. 1A and B and was modelled by rotating the M2 helix about two different axes shown. First, the entire helix (that is, the coordinates of all the atoms in the helix) is rotated clockwise by 15° about the axis passing perpendicular to the membrane through the disulfide bridge of the binding domain (marked 1 in the figure). Next the intracellular end of the helix is rotated by 30° in the opposite direction about the axis running near the top of the helix perpendicular to the plane of the membrane (axis 2). To ensure a smooth structure, the degree of rotation is scaled by the distance along the helix, such that the intracellular end rotates by 30°, the middle 15° and the top not at all. Helices 1, 3 and 4 are kept in the same position throughout. This motion creates a pore of around 9.5-Å minimum diameter (near the intracellular end) presenting polar residues to a permeating ion in a configuration similar to that suggested by Unwin [2]. In particular, the hydrophobic residues L251 and V255 are moved away from the pore while polar residues S252 and S248 are moved closer. The radius of the hypothesised open configuration is indicated by the solid line in Fig. 1B.

In the open configuration, the energy barrier seen by a single ion at the internal end of the channel is eradicated and the depth of the energy well reduced as seen by the dashed line in Fig. 2A. Importantly, the energy barrier preventing an ion from conducting when the channel contains 11 other ions is largely removed as seen in Fig. 2B. Using Brownian dynamics simulations, we find that this configuration is highly conductive, having a straight I–V curve in the range 0 to −220 mV as shown in Fig. 3A. Single channel patch clamp recordings have found the channel to have multiple conductance states of 15, 25 and 35 pS [9]. Our simulation results have a conductance in this range of about 20 pS.

Finally we examine how the conductance of the open configuration changes as we alter the radius of the intracellular end of the channel. In Fig. 3B we plot the current passing through the channel against the intracellular channel radius (measured at $z = -45$ Å). It can be seen that the current increases as the channel radius is increased as this has the effect of lowering the intracellular energy barrier seen in Fig. 2B. We also consider an alternative open configuration in which we expand the central hydrophobic section of the pore by a further 1 Å (which is sterically possible if the other helices remain fixed). The pore radius for this alternative configuration is shown by the dashed line in Fig. 1B. Not surprisingly, this configuration has a higher conductance. This suggests that the different conductance states of the channel could be created by stable open

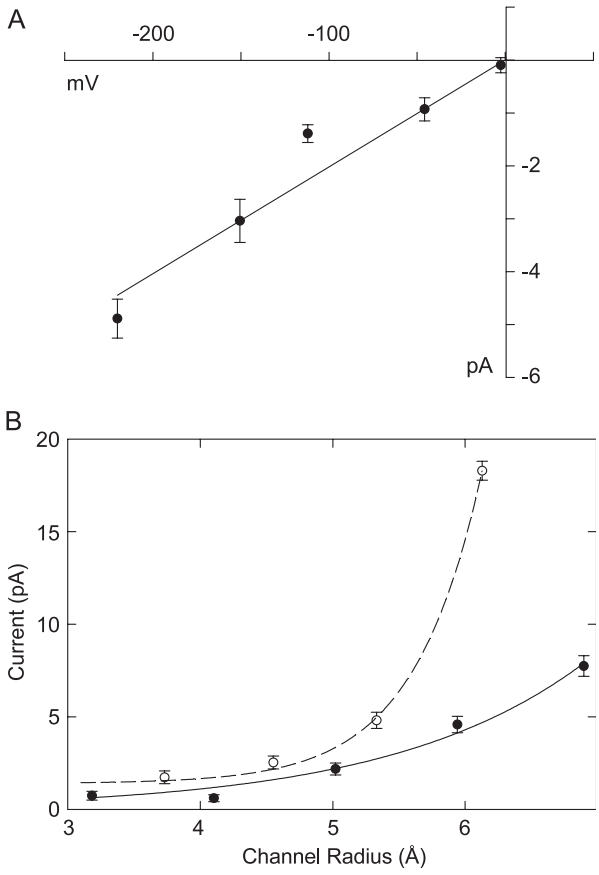

Fig. 3. (A) Current–voltage curve of the hypothesised open channel. (B) Current versus minimum channel radius for two different hypothesised open states under a −150 mV driving potential. The dashed curve represents a state in which the central hydrophobic section of the channel is widened by 1 Å. The radius is measured near the intracellular end of the channel ($z = -45$ Å). Both curves are obtained under symmetrical 150 mM solution of NaCl.

structures of different radius. We also find that the current passing through the channel increases more quickly in this configuration as we widen the intracellular end of the channel, as shown by the dashed line in Fig. 3B. This is due to the central hydrophobic section of the channel contributing less to the intracellular energy barrier.

The conductance of the pore is highly sensitive to its exact shape. For example, as seen, a slight widening of the hydrophobic section of the pore significantly increases its conductance. Thus, it is hard to specify an exact open structure using the crude approach adopted here. Although more detailed models are possible in which, for example, atoms are allowed to relax to equilibrium positions in molecular simulations, their value would be limited due to the small amount of structural information available on the open channel to guide such simulations. Rather, we only confirm the plausibility of two important hypotheses put forward by Miyazawa et al. First, the imaged acetylcholine pore structure does indeed represent a non-conductive state. Second, only a minor conformational change of the inner

helices is required to open the channel, and the motions suggested by Miyazawa et al., involving a rotation of the pore lining helix and a widening of the pore, lead to a highly conductive state of the channel. The approach adopted here provides a general framework for understanding the gating and conductance properties of ion channels.

References

[1] A. Miyazawa, Y. Fujiyoshi, N. Unwin, Structure and gating mechanisms of the acetylcholine receptor pore, Nature 423 (2003) 949–955.
[2] N. Unwin, Acetylcholine receptor channel imaged in the open state, Nature 373 (1995) 37–43.
[3] K. Brejc, Crystal structure of an ACh-binding protein reveals the ligand binding domain of nicotinic receptors, Nature 411 (2001) 269–276.
[4] G. Moy, B. Corry, S. Kuyucak, S.H. Chung, Tests of continuum theories of ion channels: I Poisson–Boltzmann theory versus Brownian dynamics, Biophys. J. 78 (2000) 2349–2363.
[5] S.C. Li, M. Hoyles, S. Kuyucak, S.H. Chung, Brownian dynamics study of ion transport in the vestibule of membrane channels, Biophys. J. 74 (1998) 37–47.
[6] S.H. Chung, T.W. Allen, S. Kuyucak, Conducting state properties of the KcsA potassium channel from molecular and Brownian dynamics simulations, Biophys. J. 82 (2002) 628–645.
[7] B. Corry, T.W. Allen, S. Kuyucak, S.H. Chung, Mechanisms of permeation and selectivity in calcium channels, Biophys. J. 80 (2001) 195–214.
[8] T.W. Allen, S.H. Chung, Brownian dynamics study of an open-state KcsA potassium channel, Biochim. Biophys. Acta 1515 (2001) 83–91.
[9] O.P. Hamill, B. Sakmann, Multiple conductance states of single acetylcholine receptor channels in embryonic muscle cells, Nature 294 (1981) 462–464.