Conformational state of the MscS mechanosensitive channel in solution revealed by pulsed electron–electron double resonance (PELDOR) spectroscopy

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AUTHOR SUMMARY

Membranes provide an impermeable barrier to the flow of polar molecules and are a defining characteristic of cellular organisms. All organisms must permit passage of polar molecules into and out of the cell. Membrane proteins, such as channels and transporters, mediate this process. For example, mechanosensitive ion channels (MscS) open and close (gating) and act as safety valves to protect bacterial cells against hypotonic shock by gating in response to intracellular pressure (1). How this happens is controversial. Here, we used pulsed electron double resonance (PELDOR, also known as DEER) to study the mechanism of gating by a bacterial mechanosensitive channel (MscS) and found, unexpectedly, that the structure of MscS is consistent with an open form.

Tools to study in atomic detail the gating movements of a large membrane protein such as MscS do not exist. Therefore, MscS has been investigated at lower resolution using techniques such as protein crystallography (at 3.45 and 3.8 Å) (1, 2), continuous wave electron paramagnetic resonance (EPR) (3), and extended motion dynamics (EMD) (4). Unfortunately, the models of gating derived by these techniques are mutually incompatible; each shows very different arrangements of the transmembrane helices. All three techniques have strengths and weaknesses; crystallography in particular is often cited as producing artifacts in helical arrangements due to packing in the lattice. All three techniques are widely used (but individually, thus preventing robust comparison of their predictions). Our goal was to resolve the arrangement of the transmembrane helices of MscS to serve as a guide to other systems and to help understand channel-gating mechanisms (Fig. P1).

We used PELDOR (or DEER) to study the arrangement of the transmembrane helices in detergent solution, because PELDOR is free from crystal packing constraints, and its distance measurements are precise. In order to apply PELDOR, we covalently attached spin labels (nitroxide molecules with an unpaired electron) to seven site-directed MscS mutants. We characterized the electrophysiology, function in vivo, and solution oligomerization state of each mutant to identify any confounding effects. PELDOR measures the coupling between electron spins, which

Fig. P1. PELDOR data accurately measure the separation of the transmembrane helices in MscS, allowing the solution species to be identified. Different models of MscS for both the closed and open conformations, derived from X-ray crystallography, cwEPR, and EMD, give rise to different distance distributions that can be tested experimentally. Inset: The PELDOR time trace shows an oscillation, which can be transformed into a distance distribution that can then be used to evaluate different models. Here, the experimentally derived distance distribution (green shape) is compared to that of the open crystal structure (red line), which was found to be the predominant conformation of MscS in solution (highlighted with black box). Data interpretation relies only on the first peak.


The authors declare no conflict of interest.

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Data deposition: The atomic coordinates and structure factors reported in this paper have been deposited in the Protein Data Bank, www.pdb.org [PDB ID codes 4AGE (D67R1) and 4AGF (L124R1)].

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reveals itself as an oscillation in the time domain due to coherent spin evolution. This oscillation is transformed from the time domain into the distance-distance domain to give the spin-to-spin distances (Fig. P1). For multimeric protein systems, only data with such an oscillation visible in the time traces give reliable distance information. We measured the spatial distribution of the three transmembrane helices of MscS and found that the distances were reproducible in two different detergents [n-dodecyl-β-D-maltopyranoside (DDM) and N-tetradecyl phosphocholine (Fos-14)].

The distances can be compared with the six structures for MscS and thus provide an independent assessment of the different models. The distribution of the helices is consistent with the open crystal structure of MscS. This was surprising, because we expected the closed form, which the wild-type protein adopts when crystallized from detergent solution (1). The surprising finding that the protein exists in solution predominantly in the open form was tested by successfully crystallizing two mutants from DDM solution. The structures of both spin-labeled mutant proteins show the open crystal structure and are in excellent agreement with the PELDOR experiments.

The power of employing PELDOR has been predicted (5). Our present work demonstrates that PELDOR can be indeed applied to highly complex systems, including the membrane-embedded portion of ion channel transmembrane helices and is sufficiently accurate to distinguish between competing models. Thus, PELDOR can be applied to probe function, impose experimental restraints on models, and maximize information from protein crystallography by monitoring changes in secondary structure of proteins in their active state. This may be very useful in the study of other important ion channels where the structural basis of their gating is of interest.