Ion Mobility Mass Spectrometry of Two Tetrameric Membrane Protein Complexes Reveals Compact Structures and Differences in Stability and Packing

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Abstract: Here we examined the gas-phase structures of two tetrameric membrane protein complexes by ion mobility mass spectrometry. The collision cross sections measured for the ion channel are in accord with a compact configuration of subunits, suggesting that the native-like structure can be preserved under the harsh activation conditions required to release it from the detergent micelle into the gas phase. We also found that the quaternary structure of the transporter, which has fewer transmembrane subunits than the ion channel, is less stable once stripped of detergents and bulk water. These results highlight the potential of ion mobility mass spectrometry for characterizing the overall topologies of membrane protein complexes and the structural changes associated with nucleotide, lipid, and drug binding.

Membrane proteins are crucial for mediation of the molecular traffic in and out of cells and organelles. Their hydrophobic nature makes them notoriously difficult to study by conventional structural biology approaches. Here we applied ion mobility mass spectrometry (IM-MS) to membrane protein complexes released after collisional activation from micelles in the gas phase. To assess their three-dimensional (3D) structures after their release, we compared collision cross sections (CCSs) of two 130 kDa tetrameric membrane assemblies, a transporter and an ion channel. The results showed that the CCS of the ion channel is consistent with a relatively compact structure. This implies that the native-like quaternary structure of membrane proteins can be protected from the harsh activation conditions required to release them in the gas phase. Our results also showed that the transporter, which is composed of two cytoplasmic and two transmembrane subunits, is less stable than the ion channel. This suggests that the ion channel, which has four subunits containing transmembrane regions, is protected in the micelle more than the transporter having two transmembrane subunits.

The potassium channel KirBac3.1 and the ATP-binding cassette (ABC) transporter BtuC2D2 were selected because both of them are tetramers and they have similar masses, volumes, and accessible surface areas (Table S1 in the Supporting Information). KirBac3.1 is a homotetramer that encloses a central permeation pathway. Each subunit has two transmembrane helices that form the transmembrane domain and intracellular N and C termini that form the cytoplasmic domain (Figure 1). The ABC transporter BtuC2D2 is a bacterial importer that mediates vitamin B12 uptake. BtuC2D2 is a tetramer composed of two ATP-binding cassettes (BtuD2) and two transmembrane subunits (BtuC2). Each subunit contacts its two immediate neighbors but has no interface with the diagonally positioned subunit.

Mass spectra were recorded for KirBac3.1 under accelerating voltages designed to disrupt the extensive association with detergent but maintain subunit interactions (Figure 1I). A series of peaks corresponding to the intact tetrameric complex (21+ to 28+) was observed. Peaks above and below were assigned to trimeric and monomeric species, respectively. The close agreement between the measured and theoretical masses (134960 and 134951 Da) indicated that the tetramer was largely devoid of detergent. Similarly, mass spectra of the BtuC2D2 complex showed charge states corresponding to the tetramer, BtuC monomer, and the BtuCD2 trimer (Figure 1II). The close agreement between the measured and theoretical masses of the tetramer (129901 and 129655 Da) confirmed the subunit stoichiometry and the absence of detergent. The average charge states of both KirBac3.1 and BtuC2D2 (25.3+ and 21.2+) correspond closely to the number of solvent-exposed basic residues on their soluble domains (27 and 22; Table S1 and Figure S1). Interestingly, BtuC2D2 has four additional basic residues on the surface of the transmembrane domain that may be shielded from charging by the detergent micelle.

To investigate the overall topology of the two membrane protein assemblies, we used IM-MS. By separation of ions according to their transit times through neutral gas molecules, under the influence of a weak electric field, an arrival time distribution (ATD) is recorded. Calculating this ATD against protein ions of known structure enables the CCS of an unknown to be determined. The experimental CCS is then compared with the theoretical one calculated from atomic coordinates. This approach has been used previously to examine the 3D structures and extent of unfolding of soluble protein complexes in the gas phase. However, it is not known whether membrane complexes retain native-like structures when subjected to the relatively high activation necessary to release them from micelles.

The ATDs of the 21+ to 23+ charge states of tetrameric KirBac3.1 are monomodal and relatively narrow (t/Δt = 8—10) with CCSs ranging from 6600—7200 Å2, implying that the ion structures are not distorted significantly (Figure S2). The average experimental and theoretical values of the CCS (6900 and 6969 Å2, respectively) are very similar. For BtuC2D2, however, while monomodal ATDs are observed for the 19+ to 21+ ions, the...
mobility resolutions ($t/\Delta t = 2$–$4$) with CCSs ranging from 6000–7200 Å$^2$ are $\sim$2-fold broader than those for KirBac3.1. This implies that BtuC$_2$D$_2$ populates more conformational states than KirBac3.1 (Figure S2). The average CCS measured (6600 Å$^2$) also agrees with that calculated from atomic coordinates of BtuC$_2$D$_2$ (6579 Å$^2$). Taken together, these data suggest that KirBac3.1 retains a compact, native-like structure while BtuC$_2$D$_2$ exists as a range of conformers with an average CCS that encompasses the native architecture.

To determine whether the different subunit architectures affect their gas-phase structures, we examined the CCSs of the two tetramers as a function of activation energy. Unlike soluble protein tetramers, for which significant unfolding has been observed, no change was detected for either membrane complex, even at maximum acceleration voltages (Figure S2). However, significant differences were observed upon activation of their respective trimers. The 10$^+$/ charge state of the KirBac3.1 trimer retained a single ATD that was essentially constant with increasing activation (Figure 1I inset). The CCS was 16% smaller than that calculated by simply removing a subunit from the native tetramer. This implies significant collapse of the trimer. In contrast, a bimodal ATD was observed for the 10$^+$ charge state of the BtuCD$_2$ trimer at higher voltages (Figure 1II inset). This is consistent with two populations of conformers for the BtuCD$_2$ trimer. Interestingly, removal of a BtuC subunit from tetrameric BtuC$_2$D$_2$ yielded an experimental CCS that is smaller than the theoretical one, again consistent with collapse of the trimer. Activation increases the CCS and implies partial unfolding of a single subunit, either BtuD or BtuC (the two yield similar CCSs). However, BtuC carries more charge during collision-induced dissociation and is therefore more likely to be unfolded than BtuD while still attached to the trimer. Removal of a single KirBac3.1 subunit from the tetrameric complex required a higher activation energy, in line with its 2-fold greater subunit interface in comparison with the BtuC/BtuD subunits within BtuC$_2$D$_2$ (Table S2 and Figure S3). Unfolding and dissociation of subunits from BtuC$_2$D$_2$ occurs more readily than in KirBac3.1 because of differences in stability and packing.

The CCSs measured for KirBac3.1 are in accord with the native states determined by X-ray crystallography. This was unexpected; we anticipated that membrane protein complexes would not retain compact configurations under the harsh conditions required to disrupt the detergent assembly. We speculate that the majority of collisions serve to disrupt the detergent assembly. Perturbation of the structure of the naked membrane protein complex is therefore minimal because of the protective effects of the encapsulating micelle. That the gas-phase structure of KirBac3.1 is more compact and resistant to dissociation and unfolding than that of BtuC$_2$D$_2$ is attributed not only to this protection but also to its larger subunit interfaces. The broader distribution of conformations for BtuC$_2$D$_2$ than for KirBac3.1 is also consistent with known functions, as the solution structures of active transporters are often more flexible than those of pore-forming channels.

In summary, the knowledge of stability, packing, and topology of subunits gained from experiments such as these highlights their potential to contribute to the understanding of membrane complex structure. For the tetramers studied, high-resolution structures are available. In the absence of atomic structures where sufficient homology exists, models could be proposed on the basis of the subunit stoichiometry and packing, constrained by CCSs from IM-MS. We also envisage that this method can serve as a means of probing the stability and conformational change associated with ligand/lipid binding in these important intractable complexes.

Figure 1. (bottom) MS and (top) IM contour plot of (left) the KirBac3.1 tetramer (PDB entry 1XL6)$^2$ and (right) the BtuC$_2$D$_2$ tetramer (PDB entry 1L7V)$^3$. The four subunits are colored pink, yellow (BtuD), blue, and green (BtuC). Black planes indicate the hydrophobic boundaries of the transmembrane regions.Insets: ATDs for the 10$^+$ charge states of trimeric (left) KirBac3.1 and (right) BtuCD$_2$ formed via gas-phase dissociation of the tetramers, superimposed on the CCS axis, recorded with acceleration voltages of (lower) 180 and (upper) 240 V. Also shown are representative structures of (A) a collapsed KirBac3.1 trimer, (B) a threefold-symmetric structure with collapsed N- and C-termini, (C) the BtuCD$_2$ trimer having a compact subunit arrangement with collapsed N-termini, (D) a structure similar to C but with partial unfolding of BtuC, and (E) the trimer derived from the tetrameric structure with collapsed N-termini.
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Supporting Information Available: Materials and methods, Tables S1 and S2, comparisons of Kir4.1 and BtuC3D2 (Figure S1), IM data (Figure S2), and subunit interfaces (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

References


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