III. Ion mobility measurement – collision cross section

This example demonstrates the determination of stoichiometry by native MS and measurement of collision cross section by ion mobility of a photosynthetic switch protein - OCP. Cyanobacteria primarily collect light via the phycobilisome (PBS), a megadalton extramembrane antenna pigment−protein complex containing covalently bound bilin pigments. The orange carotenoid protein (OCP), a 35 kDa water-soluble protein, plays a photoprotective role in cyanobacterial photosynthesis similar to that of nonphotochemical quenching in higher plants by dissipating the excess absorbed energy as heat. Under high-light conditions, the OCP binds to the phycobilisome (PBS) and reduces the extent energy transfer to the photosystems. The protective cycle starts from a light-induced activation of the OCP. Detailed information about the molecular mechanism of this process as well as the subsequent recruitment of the active OCP to the phycobilisome is lacking.

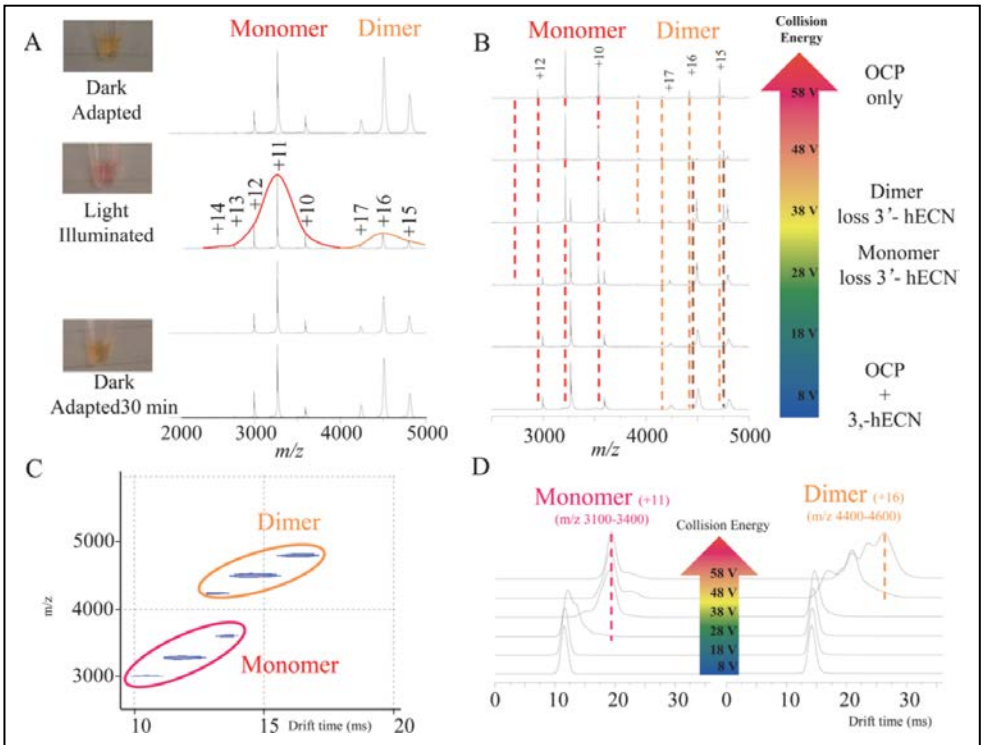

Figure 1. Native MS spectra of the OCP. (A) The dark-adapted (sample colored orange) OCP in 200 mM ammonium acetate was analyzed by native MS. Two charge envelopes were observed in the spectrum. The left envelope (labeled in red) corresponds to the MW of the monomeric OCP and one 3′ -hECN. The right envelope (labeled in orange) corresponds to the MW of the dimeric OCP and two 3′ -hECNs. The dark-adapted sample was illuminated with white light for 1.5 min. Two spectra were acquired when the OCP sample was restored to a dark environment for 5 and 30 min. (B) Native MS of the OCP with a collision energy ramp. (C) Two-dimensional IM-MS spectrum of the OCP sample. The Y axis shows the mass spectrometry measurement (mass to charge ratio, m/z), while the X axis shows the ion mobility measurement (drift time, milliseconds). Multiple charge states from two species were grouped into monomer (red) and dimer (orange). (D) Ion mobility measurement of the OCP. Two charge states, one for the OCP monomer (+11) and one for the OCP dimer (+16), were monitored by ion mobility with the collision energy ramp.

The OCP sample was buffer exchanged with 200 mM ammonium acetate and injected into a Water Synapt G2 hybrid ion mobility quadrupole TOF mass spectrometer. OCP monomer with one 3′ -hECN (35.5 kDa, charge states from +10 to +12), and OCP dimer with two 3′ -hECNs (71 kDa, charge states from +15 to +17) were observed. The overall monomer:dimer ratio is ~1:1. This ratio changes as a function of light illumination (Fig. 1A),

suggesting that the monomeric form of the OCP becomes the dominant species in the light-illuminated OCP sample, whereas the dark-adapted OCP sample is a mixture of the monomer and dimer. The shift to a higher charge state upon illumination of the monomeric OCP is indicative of the presence of an “open” conformation of the OCP monomer. This open conformation may correspond to the OCP^r that interacts with the PBS.

We also monitored the dissociation of 3′-hECN from the OCP with ion mobility (IM) upon introduction by native MS (Figure 1C,D). The OCP monomer and dimer, each containing three major charge states, can be separated by ion mobility. Comparing the drift time spectra of each oligomeric state, +11 for the monomer and +16 for the dimer, against the collision energy ramp, we found that single drift time distribution for each charge state at a low collision energy (8V) became broader, split, and shifted to longer times, which was caused by protein conformational changes. This result (Figure 1D) indicates that the cross section of the OCP for both monomer and dimer increased upon activation under the increased collision energy and adopted a more “unfolded” (larger size) conformational state. The drift time shift first occurred for the monomer at 38 V but occurred at 48 V for the dimer. Previous studies have indicated that conformational changes of the OCP take place during the transition between the OCP^o and the OCP^r as monitored by amide I and amide II vibrational changes. It was not known at that time whether transitions in the oligomerization state of the OCP are involved in this process. This is now resolved by native MS, which captures the interconversion between the monomeric and dimeric OCP. The CID and ion mobility experiments indicate that the pigment is more easily dissociated from the monomer than the dimer. The monomer has a more solvent-exposed 3′-hECN. The results from native MS and IM indicate that there is a lightinduced conversion between the dimeric and monomeric OCP. Moreover, they suggest that this conversion is required in the transition of the OCP^o to the OCP^r and that the relatively open form of the monomer (with the extended charge state in native MS) is the active OCP^r.

References

1. H. Zhang, H. Liu, D. M. Niedzwiedzki, M. Prado, J. Jiang, M. L. Gross, B. E. Blankenship, Molecular Mechanism of Photoactivation and Structural Location of the Cyanobacterial Orange Carotenoid Protein, *Biochem*. 2014, in print.