IM-MS of a Circadian Clock Protein Complex Reveals a Ligand-Dependent Conformational Switch

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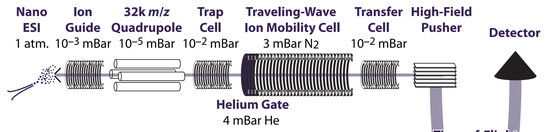
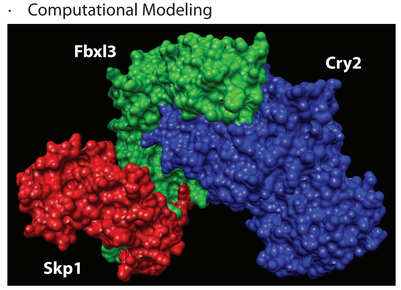
Introduction

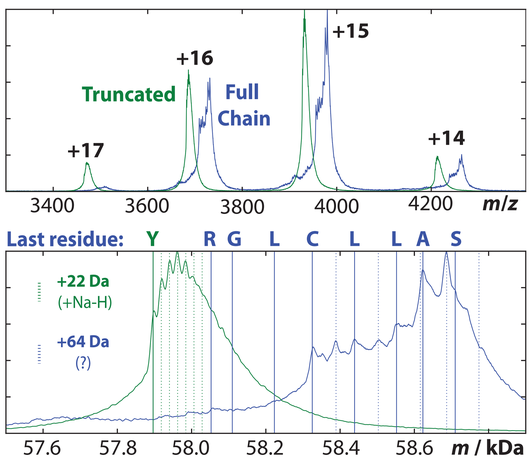
The circadian rhythm in mammals is regulated by various circadian clock proteins (CCPs), whose activities are controlled by protein degradation and metabolism. Recent studies have shown that ubiquitin E3 ligases and metabolites such as FAD directly bind certain CCPs and regulate their functions. The CCP-E3 complex of interest, Fbxl3-Cry2-Skp1, is most stable in solutions that have high ionic strengths and no organic content. Additionally, the constituent proteins in isolation have poor solubility under most solution conditions, making typical condensed-phase and mass spectrometry experiments extremely challenging. Native mass spectrometry is extremely advantageous for such systems because the complexes can be introduced intact into the gas phase, where they are available for mass measurement, ion mobility analysis, and tandem mass spectrometry.



- High-Resolution Native Mass Spectrometry
- Tandem Mass Spectrometry
- Ion Mobility (IM)
- Computational Modeling

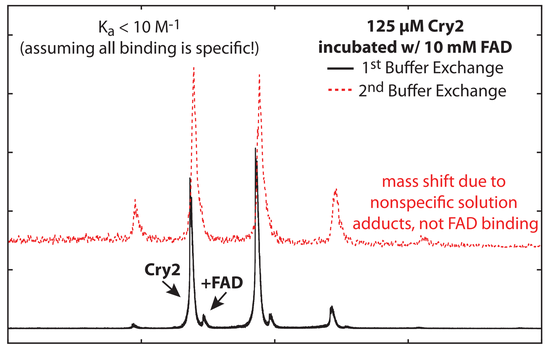


Isolated Cry2



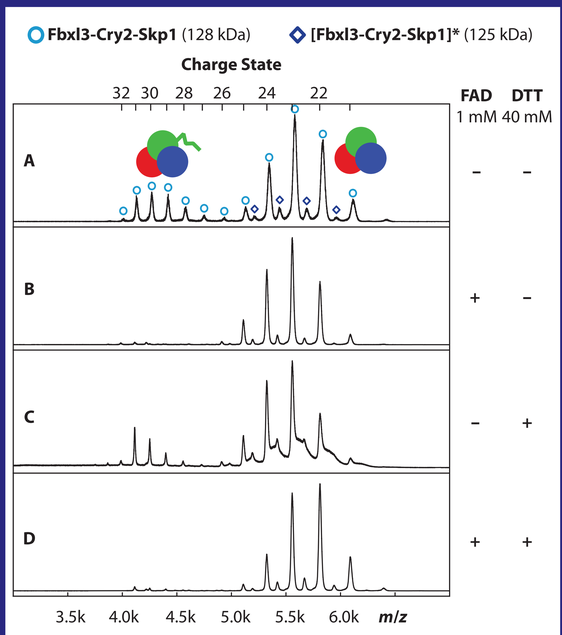
High-resolution native mass spectra reveal extensive degradation of the C-terminus of isolated WT Cry2. A truncated form of Cry2 (green) was prepared for FAD binding experiments to circumvent degradation.

FAD Binding to Cry2



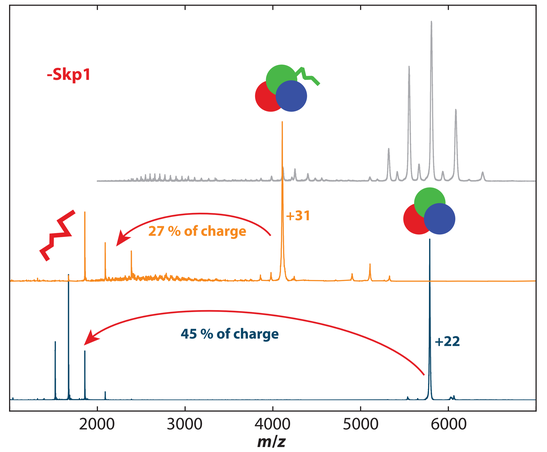
Even under conditions that cause nonspecific FAD binding during ESI, only minimal FAD association was observed. Additionally, all signal for Cry2·FAD was lost after a second buffer exchange. These results suggest that Cry2 binds FAD weakly compared to other cryptochromes ($K_a \sim 10^4$ M^{-1}) and flavoproteins, such as DNA photolyases ($K_a \sim 10^5$ M^{-1}).

FAD-Dependent Conformational Switch



Native mass spectra of the Fbxl3-Cry2-Skp1 complex (128 kDa) exhibit a bimodal charge state distribution, which suggests the presence of two conformations or conformational families. Analogous spectra measured for complexes incubated in the presence of FAD exhibit extremely weak intensities for the higher charge state distribution, even after all FAD is exchanged out of the buffer. Interestingly, a small population of a truncated form of the complex (125 kDa) is also observed with low charge states, but the corresponding higher-charge species are not observed.

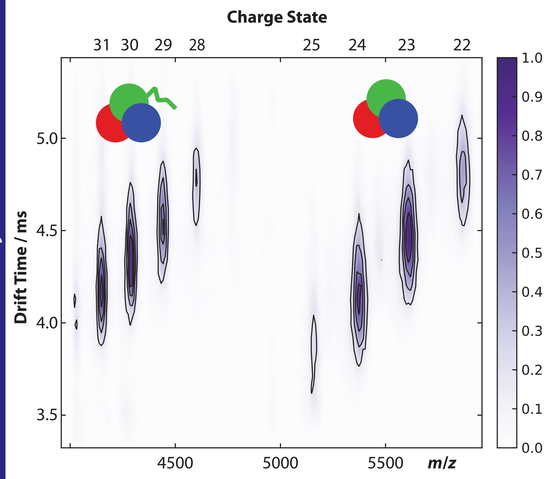
Tandem MS



Tandem mass spectrometry of Fbxl3-Cry2-Skp1 reveals loss of full-length Skp1 from all species. The higher-charged, full-length species, $[Fbxl3\text{-}Cry2\text{-}Skp1]^{+31}$, loses ~27 % of its charge to Skp1 during CID. In contrast, $[Fbxl3\text{-}Cry2\text{-}Skp1]^{+22}$ loses ~45 % of its charge to Skp1. Note that CID of full-length and truncated Fbxl3-Cry2-Skp1 both result in the loss of full-length Skp1.

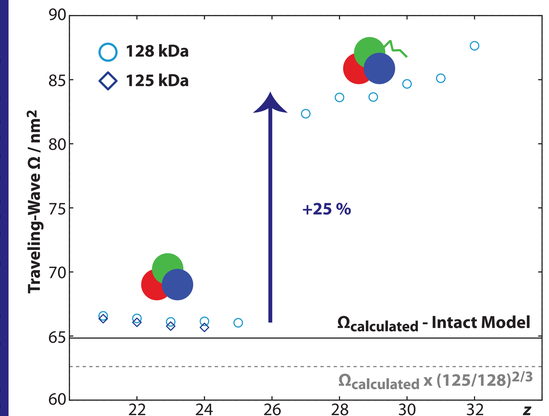
These results contrast recent results for protein complexes showing increasingly asymmetric charge partitioning with increasing precursor charge state (Pagel et al. *Anal. Chem.* **2010**, *82*, 5363). However, similar trends were observed for nonspecific cytochrome *c* dimers (Jurchen and Williams. *J. Am. Chem. Soc.* **2003**, *125*, 2817).

Ion Mobility MS



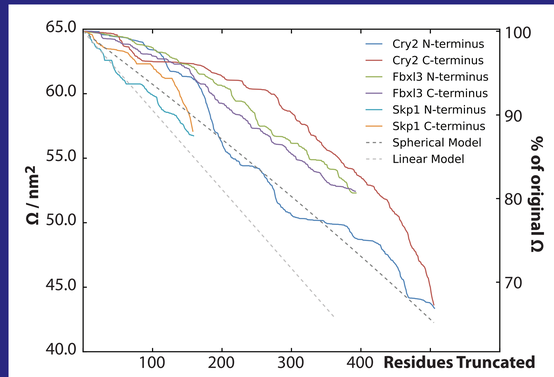
Arrival times for the Fbxl3-Cry2-Skp1 complex acquired using a Synapt G2 HDMS with a wave velocity of 200 m/s and a wave height of 25 V. Arrival times were acquired as a function of wave height and clearly show the presence of two distinct classes of structures.

Collision Cross Sections



TWIMS drift times were calibrated (Ruotolo et al. *Nat. Protoc.* **2008**, *3*, 1139) using native-like ions (Bush et al. *Anal. Chem.* **2010**, *82*, 9557).

Effect of Truncations



Linear combinations of the projection (PA) and exact hard-spheres scattering (EHSS) approximations were used to calculate Ω for every possible single truncation product of the complex. These results indicate that the truncated complex contains full-length Skp1.

Conclusions

Native mass spectrometry enabled the discovery of an FAD-dependent conformational change in a mammalian cryptochrome complex, even though interactions between FAD and Cry2 protein appear to be very weak. Interestingly, a truncated form of the complex (~3 kDa) only adopts compact structures, suggesting that the truncation is critical for the additional conformation observed for the full-length complex. Tandem MS, IM, and computational modeling provide insights into the location of the truncation and the associated structures.

Thanks

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