ACS central science

FIRST REACTIONS

Structure from Splatter

Julie A. Leary

Department of Molecular and Cellular Biology, University of California, Davis, 1 Shields Road, Briggs 130, Davis, California 95616, United States

The use of mass spectrometry (MS) to determine native protein structure has become an invaluable tool for protein biochemists. Although initially met with extreme skepticism, it is now very clear that the complementary use of "native condition" MS with dissociative MS techniques and molecular modeling has provided specific structural information for multimeric protein assemblies and subunit interaction sites.[1−5]

Early pioneering studies by the groups of Carol Robinson and Albert Heck helped pave the way for more recent studies by Wysocki and co-workers who introduced surface induced dissociation (SID) in combination with ion mobility MS for native protein structure determination.[6,7] The refinement of SID and its development in conjunction with ion mobility mass spectrometry (IMS) by the Wysocki group have brought this particular dissociation technique into the mainstream of MS for biomolecule structure determination.[8−10]

Early pioneering studies by the groups of Carol Robinson and Albert Heck helped pave the way for more recent studies by Wysocki and co-workers who introduced surface induced dissociation (SID) in combination with ion mobility MS for native protein structure determination.

The methodology of SID simply described involves accelerating an analyte ion toward a specific surface under high vacuum conditions.[11,12] During impact, the ions fragment (small organic molecule such as a peptide) or dissociate (multiprotein assemblies). In many instances, the results of SID are far more specific than collision induced dissociation (CID) given the higher energies involved with large surfaces, and therefore it can often provide unambiguous information on amino acid modifications, peptide sequence, and noncovalent interaction sites. In the *ACS Central Science* article by the Wysocki group, the

Will mass spectrometry join other common methods of protein structure elucidation? New developments from the Wysocki group show how surface induced dissociation can help toward this goal.

authors show that the protein assembly toyocamycin nitrile hydratase (TNH) is a protein dimer of two identical trimers each containing alpha, beta, and gamma subunits (Figure 1). The general approach used for analyzing such quarternary multisubunit protein assemblies is to first buffer exchange the sample into a mass spectrometer appropriate buffer such as ammonium acetate and obtain the molecular mass of the intact complex. Once this is established, a variety of organic solvents at different concentrations can be used to gently disrupt the complex, thus allowing one to determine the number and mass of the subunits. This solution disruption methodology is also used to probe which subunits might be more easily lost from the complex, thus giving some information on location of the subunits relative to each other. It can also be used to identify which subunits may be interacting with each other.

SID of the disrupted complexes, in combination with IMS, generates maps of drift time vs mass to charge ratio, which then allows the investigator to place the subunits in space relative to each other and determine if and how they are interacting with each other. The collision cross sections generated from the drift times of the SID generated species are probed with MOBCAL and various homology modeling and molecular dynamics calculations. In cases where further refinement is required to determine the exact locations of interactions sites between subunits, chemical cross-linking and site specific labeling techniques are used.

The TNH hexamer complex is quite challenging given the fact that the assembly is composed of two identical dimers made up of three different subunits, alpha beta and gamma.

Published: December 15, 2015

DOI: 10.1021/acscentsci.5b00390
ACS Cent. Sci. 2015, 1, 475−476

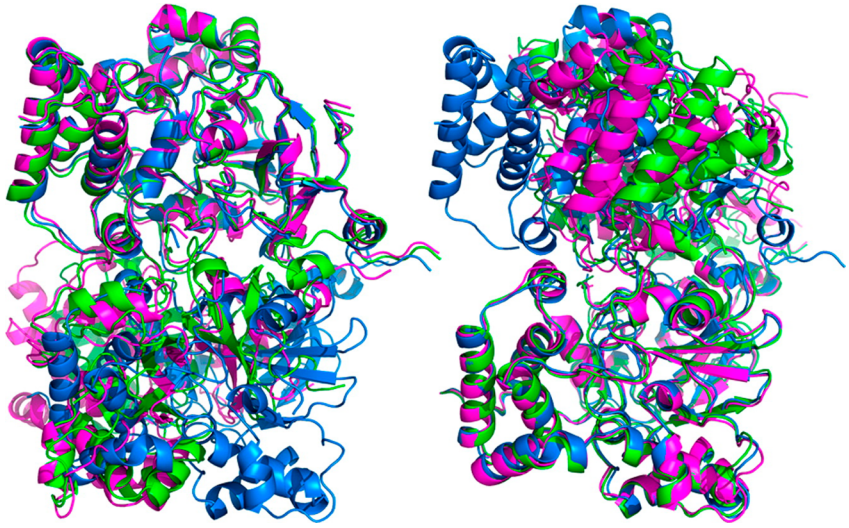

Figure 1. Two possible hetero-hexomeric structures of TNH. Figure reproduced with permission from ref 6. Copyright 2015 American Chemical Society.

This is not a case of making a better mouse trap, but rather taking the initiative and convincing the chemical community that dissociating these biomolecular complexes via a surface rather than a gas is much more efficient.

Unless the nanoelectrospray conditions, buffer, solvents system, and voltages are carefully chosen and calibrated with known quarternary structure protein assemblies, the complex might incorrectly be measured as consisting of only one heterologous trimer. Equally important is the fact that the authors carefully and thoroughly conduct both chemical cross-linking and site specific modifications in order to validate the specific interaction sites between the subunits.

Linking SID to an ion mobility instrument was exceptionally forward-looking and brave. This is not a case of making a better mouse trap, but rather taking the initiative and convincing the chemical community that dissociating these biomolecular complexes via a surface rather than a gas is much more efficient.

Author Information

E-mail: jaleary@ucdavis.edu.

REFERENCES

(1) Heck, A. J. R.; van den Heuvel, R. H. H. Investigation of intact protein complexes by mass spectrometry. *Mass Spectrom. Rev.* **2004**, *23*, 368−369.

(2) Sharon, M.; Robinson, C. V. The Role of Mass Spectrometry in Structure Elucidation of Dynamic Protein Complexes. *Annu. Rev. Biochem.* **2007**, *76*, 167−193.

(3) Ruotolo, B. T.; Benesch, J. L.; Sandercock, A. M.; Hyung, S. J.; Robinson, C. V. Ion mobility-Mass Spectrometry Analysis of Large Protein Complexes. *Nat. Protoc.* **2008**, *3*, 1139−1152.

(4) Leary, J. A.; Schenauer, M. R.; Stefanescu, R.; Andaya, A.; Ruotolo, B. T.; Robinson, C. V.; Thalassinos, K.; Scrivens, J. H.; Sokabe, M.; Hershey, J. W. Methodology for Measuring Conformation of Solvent-Disrupted Protein Subunits using T-Wave Ion Mobility MS; An Investigation into Eukaryotic Initiation Factors. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1699−1706.

(5) Zhou, M.; Sandercock, A. M.; Fraser, C. S.; Ridlova, G.; Stephens, E.; Schenauer, M. R.; Yokoi-Fong, T.; Barsky, D.; Leary, J. A.; Hershey, J. W.; Doudna, J. A.; Robinson, C. V. Mass Spectrometry Reveals modularity and a complete subunit interaction map of the eukaryotic translation factor eIF3. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 18139−18144.

(6) Song, Y.; Nelp, M. T.; Bandarian, V.; Wysocki, V. W. Refining the Structural Model of Heterohexameric Protein Complex: Surface Induced Dissociation and Ion Mobility Provide Key Connectivity and Topology Information. *ACS Cent. Sci.* **2015**, DOI: 10.1021/acscentsci.5b00251.

(7) Blackwell, A. E.; Dodds, E. D.; Bandarian, V.; Wysocki, V. H. Revealing the quaternary structure of a heterogeneous noncovalent protein complex through surface-induced dissociation. *Anal. Chem.* **2011**, *83*, 2862−2865.

(8) Jones, C. M.; Beardsley, R. L.; Galhena, A. S.; Dagan, S.; Cheng, G.; Wysocki, V. H. Synmmetrical gas-phase dissociation of non-covalent protein complexes via surface collisions. *J. Am. Chem. Soc.* **2006**, *128*, 15044−15045.

(9) Zhou, M.; Wysocki, V. H. Surface Induced Dissociation; Dissecting Noncovalent Protein Complexes in the Gas Phase. *Acc. Chem. Res.* **2014**, *47*, 1010−1018.

(10) Zhou, M.; Huang, C.; Wysocki, V. H. Surface-induced dissociation of ion mobility-separated noncovalent complexes in a quadrupole/time of flight mass spectrometer. *Anal. Chem.* **2012**, *84*, 6016−6023.

(11) Grill, V.; Shen, J.; Evans, C.; Cooks, R. G. Collisions of ions with surfaces and chemically relevant energies. *Rev. Sci. Instrum.* **2001**, *72* (8), 3149.

(12) Wysocki, V. H.; Joyce, K. E.; Jones, C. M.; Beardsley, R. L. Surface Identification of Small Molecules, Peptides and Non-Covalent Protein Complexes. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 190−208.

DOI: 10.1021/acscentsci.5b00390
ACS Cent. Sci. 2015, 1, 475−476