Protein Complex Analysis by chemical cross-linking and High-Mass MALDI Mass Spectrometry

Alexis Nazabal and Ryan Wenzel
CovalX research laboratories, CovalX AG, Technoparkstrasse, 1; CH-8005; Zürich; Switzerland
alexis.nazabal@covalx.com, www.covalx.com

Introduction
The development of new methodology for the direct analysis of protein-protein interactions is of high interest as protein complexes are involved in all cellular processes. Here we present the use of chemical cross-linking combined with high-mass MALDI ToF mass spectrometry for different applications: Characterization of protein complexes, analysis of drugs targeting protein-protein interactions and analysis of therapeutic proteins aggregates.

Method
To analyze protein complexes with MALDI ToF mass spectrometry we have used cross-linking chemistry and a High-Mass detector (HM1, CovalX, Zürich, Switzerland) retrofitted on a MALDI ToF Bruker Reflex III.

Results

Analysis of Protein complexes
Using the combination of chemical cross-linking and high-mass MALDI mass spectrometry it is possible to analyze with high-sensitivity (nM range) and high accuracy protein complexes. In Figure 1 we present the direct analysis of different non covalent complexes: Immunocomplex analysis, complexes formed by effectors of salmonella, the AMP Kinase complex and an example of supershift complex (Figure 1).

Immunocomplex analysis

A. Immunocomplex formed between the monoclonal antibody 5C4 and the bovine prion Protein bPrP. B. Sandwich assay between 1E5, 5C4 and bPrP

Effectors of Salmonella

Analysis of protein complexes formed by effectors of Salmonella. A. SopE and CDC42 before cross-linking. B. Detection of the complex [SopE•CDC42]. C. InvB before cross-linking. D. Detection of dimer and trimmer of InvB.

AMPk complex

Analysis of the AMP Kinase complex [α•β•γ] before cross-linking. Detection of dimer and trimmer of InvB.

Super shift complexes

Analysis of protein complexes formed by CDC42 and GSTSopE. A. A mixture of CDC42 and GSTSopE has been cross-linked and analyzed by high-mass MALDI ToF MS. B. Same experience after adding a monoclonal antibody Anti-GST.

Analysis of Therapeutic Proteins aggregates
Aggregation of therapeutic proteins can become a major problem when application to a patient requires a high-concentration to achieve therapeutic efficacy. The major con-sequence of therapeutic proteins aggregation is also an increasing immunogenicity that can have consequences for the patient treated (Figure 3).

Acknowledgments
The author gratefully acknowledge Prof. Renato Zenobi (Chemistry, ETHZ) Prof. Amt and Dr. Schlumberger (Microbiology, ETHZ), Lukas Brändli (Chemistry ETHZ).

Figure 1

Figure 2

Figure 3