LOCALIZATION OF NON-COVALENT PROTEIN-LIGAND BINDING SITES BY TOP-DOWN MASS SPECTROMETRY BASED ON VACUUM ULTRA-VIOLET (VUV) ACTIVATION

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Since the last decade, numerous researches have shown that many proteins are completely or partially disordered in their functional state. These observations question the usual paradigm in biology establishing that a well-defined three-dimensional structure is a prerequisite for the function of a protein. The high flexibility of these proteins, called Intrinsically Disordered Proteins (IDPs), gives them advantages to carry out their function and in particular to interact with their partners. Therefore, these proteins are able to interact with different and/or several partners leading to complex mixture. The structural study of such mixtures and flexible objects with classical bio-structural techniques is still challenging and time consuming. Therefore, the study of these proteins requires additional techniques. Top-down approaches using electron capture dissociation have demonstrated their ability to provide information on the primary structure of the full protein. They are also able to identify the protein binding site with its ligand. However, the sequences of IDP are particularly rich in proline residues whose cyclic ring precludes the formation of *c*- and *z*-type ions. Here, we present a method based on the coupling of VUV-synchrotron radiation and mass spectrometry in order to localize the noncovalent binding site of B2 3'O-gallate, a ligand tannin, on IB5, a human basic salivary proline-rich proteins (PRPs). PRPs belong to IDPs and their main function is to scavenge tannins. Tannins are harmful substances with anti-nutritional effects, which are widespread in plant-based foods.

Firstly, we have compared the effect of four wavelengths 193 (6.4 eV), 157 (7.8 eV), 94 (13.2 eV) and 77.5 nm (16 eV) with classical MS/MS techniques such as CID and ECD on the fragmentation of IB5. Secondly, we have studied the noncovalent binding site of B2 3'O-gallate on IB5 using VUV-radiation at 77.5 nm.

Regarding the fragmentation of IB5, our results show two different regimes of fragmentation as a function of the wavelengths. Below the threshold of the IB5 photoionization, we observed only *a*- and *x*-type fragment, while above, all types of fragments are generated. The best sequence coverage from all experiments has been obtained with VUV-excitation at 77.5 nm. Regarding the localization of the binding site on IB5, we have identified almost fifty fragments still linked to the ligand. Finally, our results allow to localize the noncovalent binding site of B2 3'OG on IB5.