PROTEIN-LIGAND INTERACTIONS

The sensation of astringency in the VUV rays of the DESIRS

Astringency is the feeling of dryness and puckeriness inside the mouth that accompanies the consumption of plant products, such as wine or tea, or unripe fruit. Tannins, secondary plant metabolites, are at the origin of this feeling, which is still poorly characterized. A new approach using mass spectrometry coupled with VUV radiation, developed on the DESIRS and DISCO beamlines has helped to better understand the molecular mechanisms involved in this astringency phenomenon.

The ion trap on its mobile frame (on the right-hand side), installed here on the DISCO beamline, as it was coupled to the DESIRS beamline for the experiments on the IB5 protein.

One of the players involved in this feeling of astringency is a family of salivary proteins, called proline-rich proteins (PRPs), one of the «intrinsically disordered proteins» and therefore flexible. PRPs have a high affinity for tannins, molecules produced by bark, roots, leaves or fruit. These PRPs trap and bind tannin molecules (ref. 1). They are particularly abundant in the saliva of mammals with diets rich in tannins, this function having been selected to protect the body from the anti-nutritional effects of tannins. Indeed, the tannins will also bind to enzymes involved in digestion, rendering them inactive and therefore with repercussions on digestion.

Studying PRPs: a real headache

CAs with other intrinsically disordered proteins, the absence of a well-defined 3D structure and the many repeat PRP sequences makes them and their interactions difficult to study using standard structural biology techniques, such as crystallography or nuclear magnetic resonance.

However, the use of alternative approaches has provided a certain amount of information about these interactions. Thus, using small angle X-ray scattering (SAXS) technique on the SWING beamline has

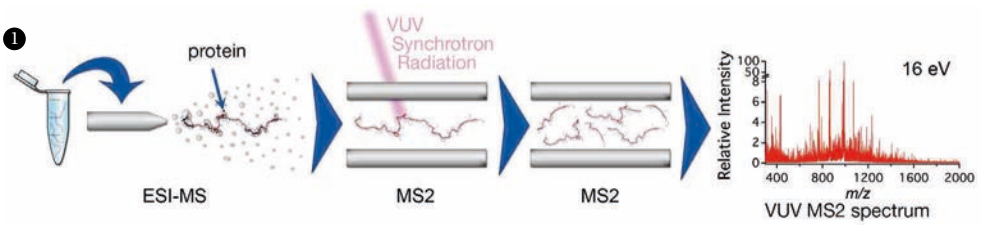

Figure 1 : Diagram of the MS/MS technique based on activation by VUV synchrotron radiation.

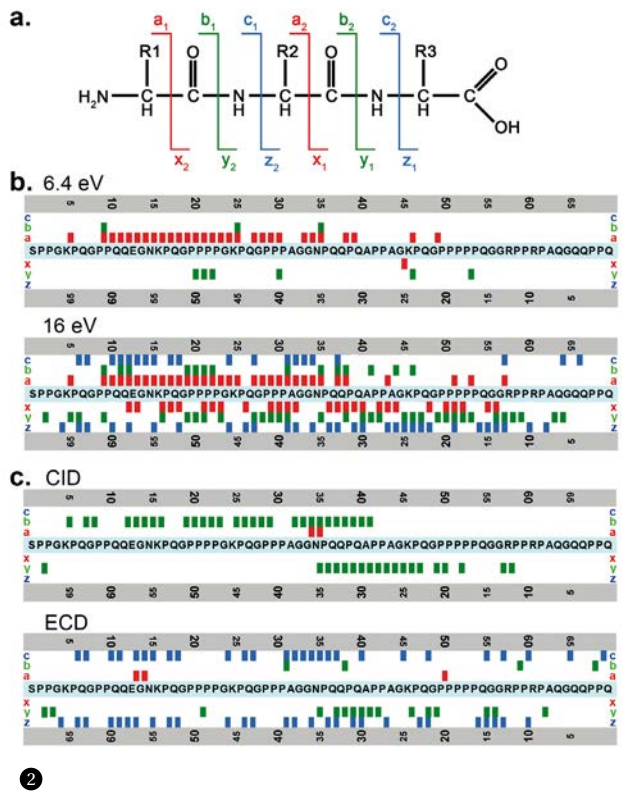

Figure 2 : Nomenclature for MS/MS peptide fragmentation (a). Patterns of $IB5^{7+}$ protein fragmentation after activation at 6.4 and 16 eV (b), by CID and ECD (c).

helped characterize the conformation of these proteins (ref. 2) as well as the supramolecular objects formed during their interactions with tannins (ref. 3).

Using mass spectrometry, the stoichiometries of PRP•tannin (ref. 4) complexes have been determined, and this technique, when coupled with ion mobility, showed that PRP-tannin binding induces a conformational change in PRPs (ref. 5). However, the localization of the tannin-PRP interaction sites had not yet been precisely determined. A new approach using mass spectrometry coupled with VUV radiation, developed on the DESIRS and DISCO beamlines, has answered this question (ref. 6).

Mass spectrometry and reasoned fragmentation

This technique is based on the coupling of a commercial ion-trap mass spectrometer with synchrotron radiation (ref. 7) (Figure 1). The development of sensitive electrospray ionization (ESI) sources, which make it possible to ionize and desolvate molecular and supramolecular biological objects without fragmenting them, now makes mass spectrometry (MS) a powerful tool for the study of biological objects. Indeed, MS is a fast, sensitive and specific technique, but also able to provide information on the structure of molecules. This information can be generated by tandem mass spectrometry experiments (MS/MS) in which an ionized target molecule is selected, then activated and dissociated into fragments, which then are analyzed and identified in a final step. The standard technique for activating ions, called «collision induced dissociation» (CID) and provided on most commercial mass spectrometers, involves slow heating of ions through collisions with a gas. This method is not applicable to locating non-covalent interactions between a protein and another object. To locate interaction sites between a ligand and a protein an activation method is required to fragment the polypeptide chain while maintaining the ligand linked to its interaction site. The technique that has been developed activates the target molecule using active radiation in the ultraviolet range under vacuum (VUV) (Figure 1).

How do VUVs may produce fragments

The relevance of the MS/MS technique is related to the wealth of information it can generate and therefore the number of fragments produced, as well as the quality of

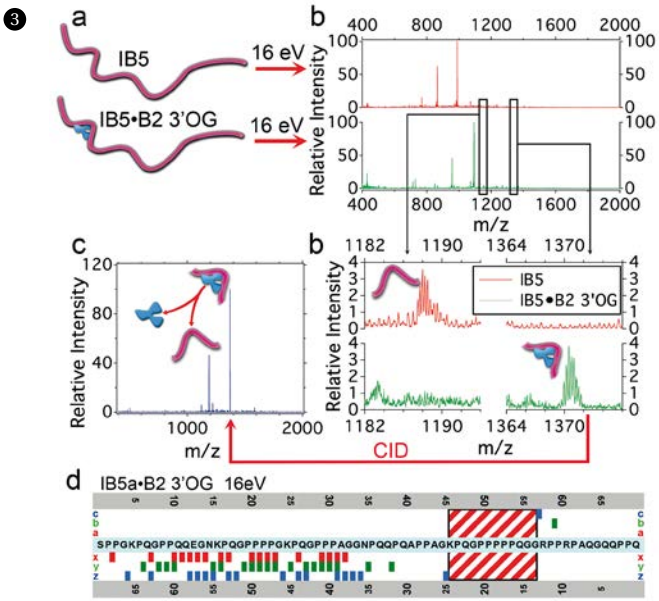

Figure 3 : Localisation of the B2 3'OG binding site on IB5. The objects $IB5^{7+}$ and $IB5•B2\ 3'OG^{7+}$ were selected and irradiated with 16 eV photons (a). Comparison of the MS/MS 16 eV VUV spectra identified specific fragments in the $IB5•B2\ 3'OG^{7+}$ fragmentation spectrum in which the m/z ratio had a mass difference corresponding to that of $B2\ 3'OG^{7+}$ compared with fragments from the fragmentation spectrum of $IB5^{7+}$ (b). CID activation of identified fragments confirmed the presence of the ligand (c). The map of B2 3'OG-carrying fragments identified the 'KPQGPPPPPQGG' sequence as a B2 3'OG binding site on IB5.

the information provided. The first step in validating this method focused on comparing the fragmentation patterns obtained by VUV irradiation with those obtained with standard techniques such as collision-induced dissociation (CID) or electron-capture dissociation (ECD). For this, the human salivary protein IB5 with a +7 charge state ($IB5^{+7}$), was activated by four different radiation wavelengths, and two laboratory techniques (CID and ECD). Figure 2 shows the fragmentation patterns obtained for two photon energies (6.4 and 16 eV), as well as for the CID and ECD experiments. Comparison of fragmentation patterns shows that below the ionization threshold of the protein (6.4 eV) the majority of fragments are those formed by breaking the C-C bond (α-bond type), while above the ionization threshold (16 eV), all types of fragments are present. This observation confirms the existence of two different fragmentation regimes. Below the ionization threshold, the fragments generated result from the photodissociation of the ion precursor, whereas the appearance of radical cations $[M+7H]^{\bullet 8+}$ and $[M+7H]^{\bullet\bullet 9+}$ lead predominantly to a dissociative photoionization mechanism. This mode of fragmentation is the most informative and requires the use of photon energy in the VUV. Thus, thanks to synchrotron radiation, a 91% sequence coverage was obtained while it was only 58% by CID and 68% by ECD.

Tannin and PRP remain linked

In a second step, the study focused on the interactions between the IB5 protein and a model tannin, B2 3'OG. The non-covalent complex $IB5•B2\ 3'OG^{+7}$ was preserved during the ionization step and was then selected and irradiated by the synchrotron beam. Figure 3b shows the comparison of the MS/MS 16 eV VUV spectra of $IB5^{+7}$ and $IB5•B2\ 3'OG^{+7}$. This comparison made it possible to identify about forty peaks present only on the fragmentation spectrum of $IB5•B2\ 3'OG^{+7}$ and therefore potentially the ligand carrier. This analysis also verified the presence of fragments without the corresponding tannin on the fragmentation spectrum of the protein alone. An additional MS/MS step (MS^3) targeted ligand carrier fragments, but this time they were activated using CID, to separate the fixed tannin and confirm the identity of the fragments. Figure 3c shows the MS^3 spectrum of a tannin-carrying fragment. In this figure, two major peaks are present, corresponding to the selected parent ion and to an ion fragment with a lower mass, corresponding to the lost tannin. The identification of these fragments finally showed that the 'KPQGPPPPPQGG' sequence contains the preferred tannin interaction site on the IB5 protein (Figure 3d). Within this sequence, the 5-proline cluster probably adopts the start of a secondary type I or II polyproline helix structure, forming a rigid element, thus providing an initial anchorage point for tannin (ref. 8).

This study demonstrated the potential of coupling mass spectrometry with the VUV radiations for the structural study of flexible proteins such as IDPs and their supramolecular assemblies. The technique could be applied to other types of protein, but can also be adapted to the study of many other biological structures such as sugars, lipids, DNA and RNA... at the molecular and supramolecular level. Indeed, this technique could also generate specific fragmentation with regard to the structure of the object studied, depending on whether it is folded or completely denatured and thus reveal the tertiary or quaternary structure of proteins in addition to their primary sequence.

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