Top-down fragmentation of the native tetrameric 102 kDa Concanavalin A complex using ECD on a QTOF system

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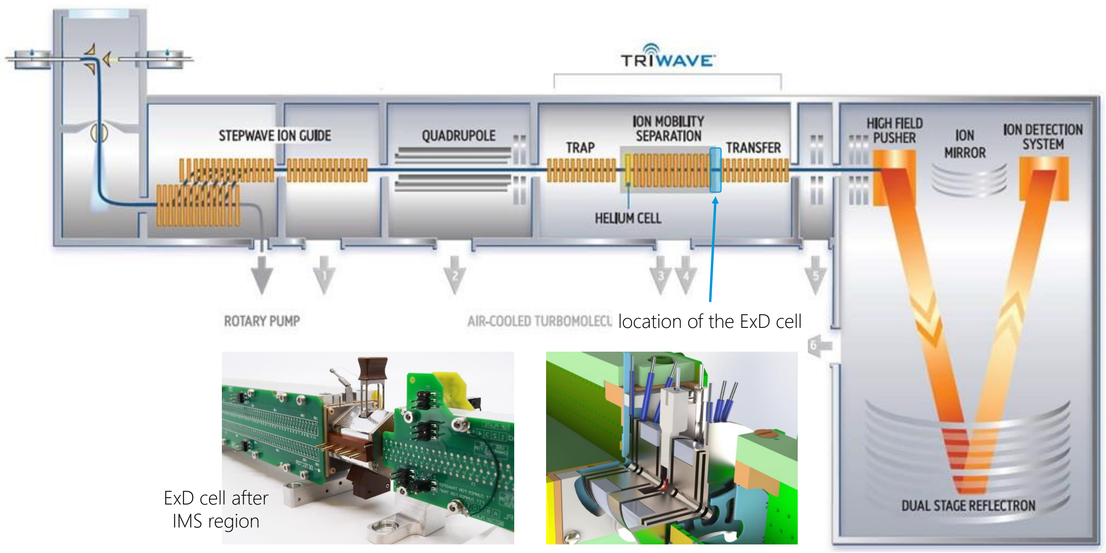
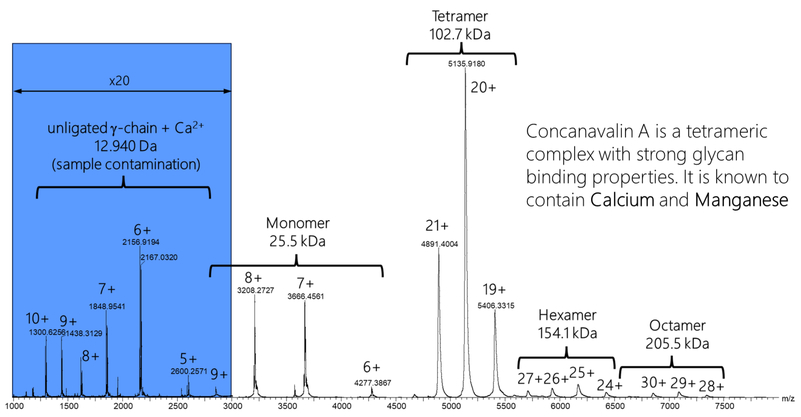
Electron capture dissociation (ECD) is well known to be effective for middle- and top-down fragmentation of proteins. The combination of ECD with native MS enables the investigation of non-covalent complexes to study differences in the behaviour upon binding of ligands or complex partners or structural changes.

Here we present data from the analysis of the tetrameric Concanavalin A (Con A) complex. Con A is a commercially available 25.5 kDa lectin from *Canavalia ensiformis* which forms a tetrameric complex known to bind carbohydrates with a preference to internal mannosyl and glucosyl residues. It is widely used in glycoprotein analysis as well as for glycoprotein purification.

The ECD was performed using a Waters Synapt G2-S QTOF system equipped with an e-MSion Inc. ECD cell being located between the ion mobility and the transfer region of the TriWave module. Ion generation was performed using static nanospray with the Waters offline nanoESI source. The Stepwave ion guide was additionally modified with a device changing the pressure in the Stepwave region to improve desolvation and transfer of big non-covalent complexes (MS Vision).

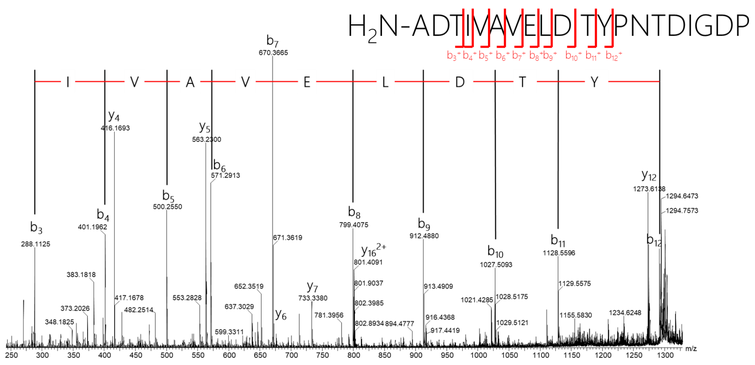




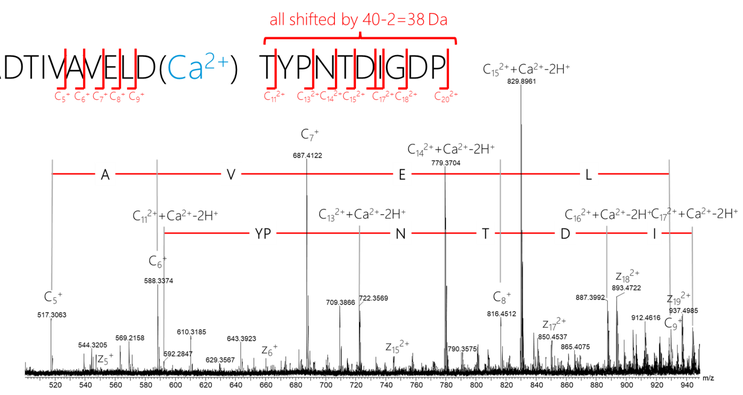
Concanavalin A is a tetrameric complex with strong glycan binding properties. It is known to contain **Calcium** and **Manganese**

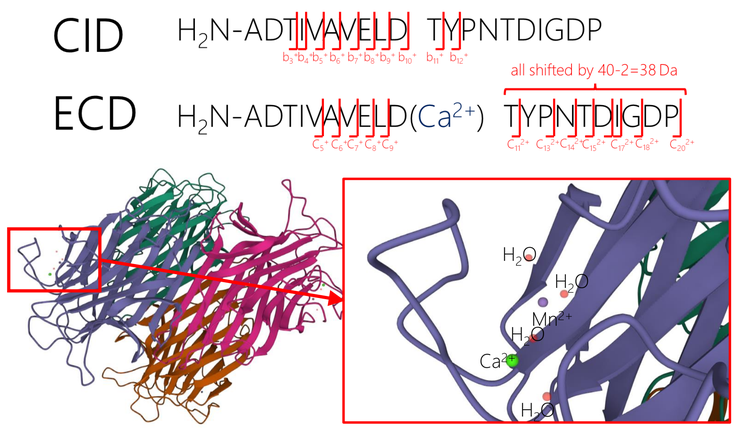
Native mass spectrum of Concanavalin A. Besides the native tetrameric complex, the monomer, higher hexa- and octamer assemblies as well as the unligated gamma-chain was observed. The latter being a minor sample contamination only (the intensity scale is expanded by a factor of 20 between m/z 1000 and 3000).

CID mass spectrum of the native tetrameric complex. Quad mass was set to 3900 to remove the unligated gamma-chain as well as the majority of the monomer. The CID spectrum shows the fragmentation from the N-terminus of the protein sequence which is typical for globular proteins.



ECD mass spectrum of the native tetrameric complex. Quad mass was also set to 3900. The ECD spectrum also mainly shows the fragmentation from the N-terminus of the protein sequence. All fragment ions above c_9 are observed as doubly charged Calcium adducts.

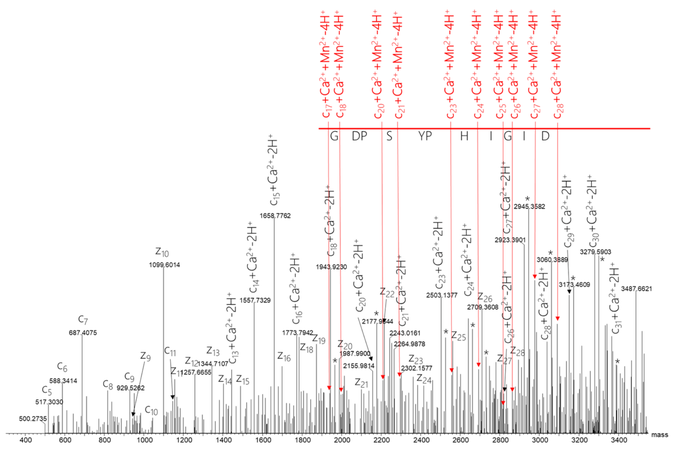




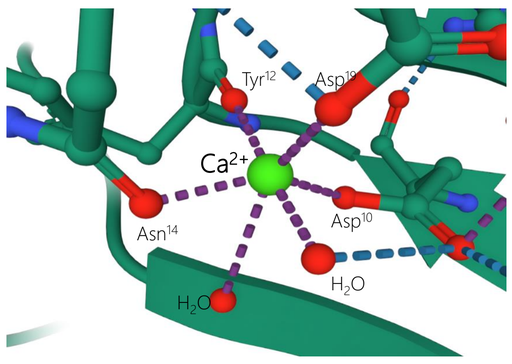
Hardman, K.D., Ainsworth, C.F. (1972) Biochemistry 11: 4910-4919; PDB 3CNA

Comparison of CID and ETD fragmentation data. Both fragmentation types cover (at least partially) the same sequence region. However, Calcium adducts can only be observed on the ECD-generated fragment ions.

The X-ray structure shows the localization of the Calcium and Manganese ions in the native protein as part of the N-terminal protein structure.



Deconvolution of ECD data also revealed a consecutive series of Calcium AND Manganese adducts to the ECD fragment ions. Whereas Calcium adducts were observed starting from residue 11, Manganese adducts were only observed on fragments including residue 17 or higher. This is in line with the fact that Calcium binding involves residues 10, 12 and 14 whereas Manganese binding involves residues 8, 10, 19, 24 and 34. Asp_{19} seems to be critical for Manganese binding.

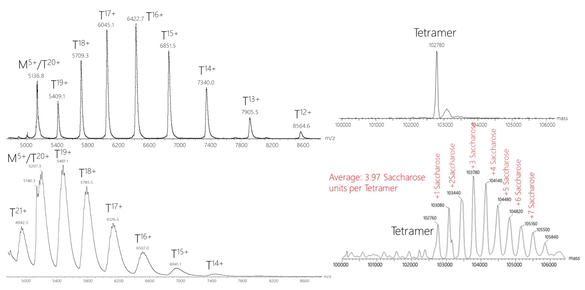


Adducts of Calcium AND Manganese detected as ions of the type

$$c_x^{2+} + Ca^{2+} + Mn^{2+} - 4H^+$$
$$\text{with } 17 < x < 28$$

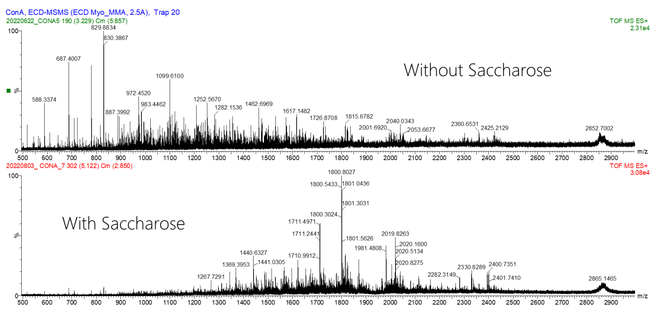
Residues known to be involved in Manganese binding: Glu_8, Asp_{10}, Asp_{19}, His_{24} and Ser_{34}. Asp_{19} seems to be critical for Manganese binding.

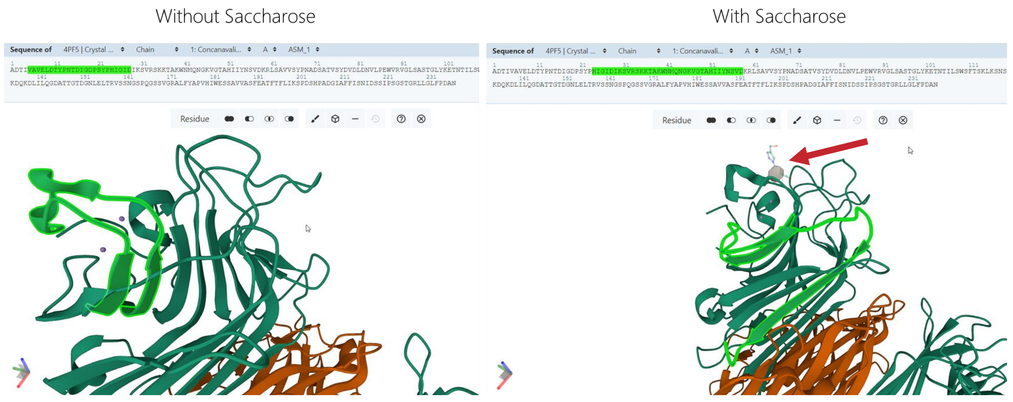
Concanavalin A glycan binding



Addition of Saccharose resulted in a spectrum showing a distribution of bound glycans with an average load of 3.97 Saccharose molecules per tetramer, almost exactly the expected value.
Where the excess sugar molecules are located is unclear.

ECD fragmentation of the sugar loaded tetrameric Con A shows significantly different fragmentation patterns from the sugar-free tetrameric complex. Unfortunately, unlike for the metal-adducts, so far no fragment ions with bound sugar could be observed so far.





The X-ray structure shows the location of the sugar molecule bound to Con A (red arrow). The green highlighted sequences have been observed as fragments. Without sugar, fragments are observed in the N-terminal region of residues 5-30. Upon sugar binding, the observed fragmentation shifts into the C-terminal direction starting only at residue 24, indicating some sort of stabilization or shielding of the more N-terminal residues by the bound sugar.

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