Fourier−Transform Ion Cyclotron Resonance Mass Spectrometry Studies of the Homophilic Non−Covalent Interactions of Shiga−Like Toxin subunits, as well as their Interactions with Carbohydrate Ligands

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Introduction

Recent advances in electrospray (ES) mass−spectrometry have provided a technique that allows the observation of non−covalent complexes of biomolecules formed in aqueous solutions and transfered intact into the gas phase (1). This has prompted the application of mass−spectrometry for binding studies of biologically relevant interactions. Soft ionization conditions, sufficient m/z range and high resolution which is neseccary for unambiguous assignment of all peaks in the spectra are three important factors that allow the extraction of the maximum information content from such experiments. To achieve these objectives Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT−ICR) combined with a nano−electrospray source was chosen because of its unrivaled resolution, which is critical in the case of interactions between a large protein receptor and a relatively small oligosaccharide ligand. The stoichiometry of a complex, its solution binding characteristics and relative stability in the gas phase can be obtained from a single FTICR experiment.

Shiga−like holotoxins SLT−1 and SLT−2 are hexameric AB_5 proteins, where the enzymatic A subunit (32 kDa) is non−covalently associated with a homopentamer of the receptor−binding B subunits (Fig. 1). The crystal structure of the SLT−1 B−pentamer complexed with a Gb_3 trisaccharide analogue has been solved at 2.8 Å resolution (2). Three saccharide binding sites are found in each 7.7 kDalton B subunit. The subunits associate non−covalently to form a doughnut−shaped B−pentamer with 15 saccharide binding sites aligned on one face of the toxin. The A−subunit is anchored by its C−terminal α−helix, which protrudes through the central pore of the B−pentamer, on the face opposite to binding domains, thereby allowing all 15 sites to engage cell surface receptors. Here, we report the application of ES, in conjunction with FT−ICR MS, to delineate several aspects of the chemistry of self−association of the Shiga−like toxin and its association with P^k ligands.

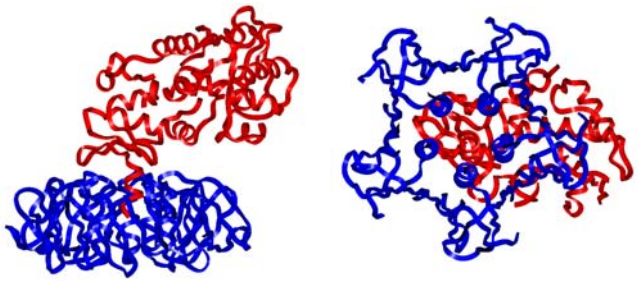

Figure 1. Crystal structure of Structure of Shiga toxins.

Mass−Spectrometry

Mass spectra were obtained using a Bruker 47e ApexII FT−ICR MS. The glass sampling capillary on the electrospray source (Analytica, Branford) was replaced with a stainless steel capillary which was heated to ~150º C. The electrospray needle was constructed from an aluminosilicate capillary (0.68 mm i.d.), with one end pulled to approximately 50 μm (i.d.). The solution flow rate ranged from 10 to 60 nL/min. All proteins have been dialysed against millipore water, lyophilized and redissolved in aqueous 50 μM ammonia bicarbonate (pH 7.2) at a concentration of 0.1 mg/ml. Acetic acid was used for pH adjustment when needed.

References

1. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M., Science, 1989, 246, 64−71.

2. Ling, H.; Boodhoo, A.; Hazes, B.; Cummings, M. D.; Armstrong, G. D.; Brunton, J. L.; Read, R. J., *Biochemistry*., 1998, 37, 1777−1788.

Self−Association of Shiga−like Toxin

The signal for intact holotoxin was obtained by reducing the pH of the solution to ~3. A representative spectrum of SLT−1 holotoxin is shown on Fig. 2. However, partial decomposition of the holotoxin is observed under these conditions. This result indicates that the holotoxin is readily transferable from solution to the gas−phase, however, acidic conditions are required so that sufficient charge is generated on the protein so that the mass−to−charge ratio falls within the mass range of our instrument (m/z < 5000) Mass−spectra of SLT−1B confirmed that the B5 pentamer is stable in the absence of the A subunit (Fig. 3). For cloned SLT−2B the homopentameric structure was not observed.

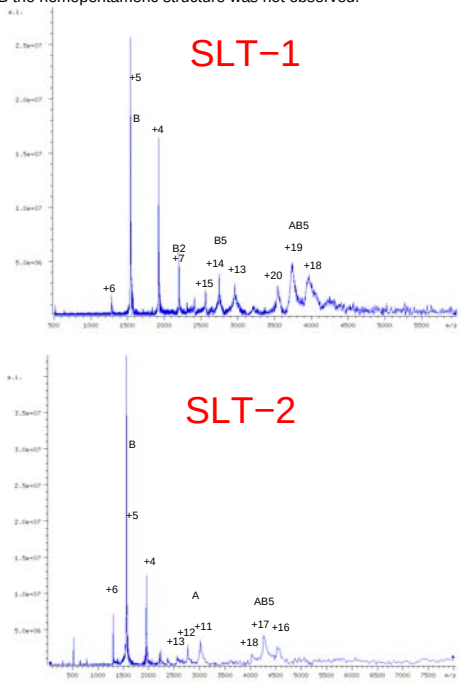

Figure 2. Representative spectra of holotoxins.

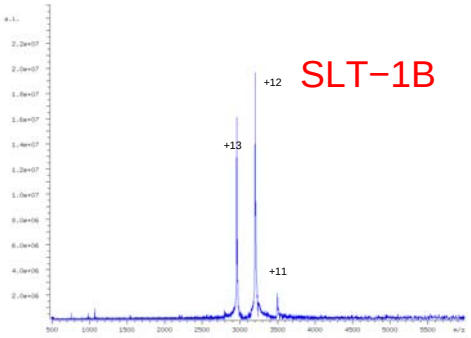

Figure 3. Homopentamer of SLT−1B.

Complex between SLT−1 and Pk−Trisaccharide

We found experimental conditions under which homopentameric SLT−1B can be observed in one spectrum together with it's complexes with P^k−trisaccharide (Fig. 4). Resolution of the peaks allowed calculation of binding constants from complex−to−free−receptor ratio, assuming the binding of P^k−trisaccharide to SLT−1B occurs non−cooperatively. The data are presented in the Table. Values of calculated K_D fall into a narrow region and confirm the validity of assumptions used in a mathematical model that predicts the distribution of bound species based on a given association constant and non−cooperative binding. The dissociation constant for P^k−trisaccharide glycoside **1** obtained in this study (0.5 mM) is in good agreement with values determined by microcalorimetry (0.5−1 mM). Competitive solid phase assay (Bundle, unpublished results) also gave a comparable value of 2 mM.

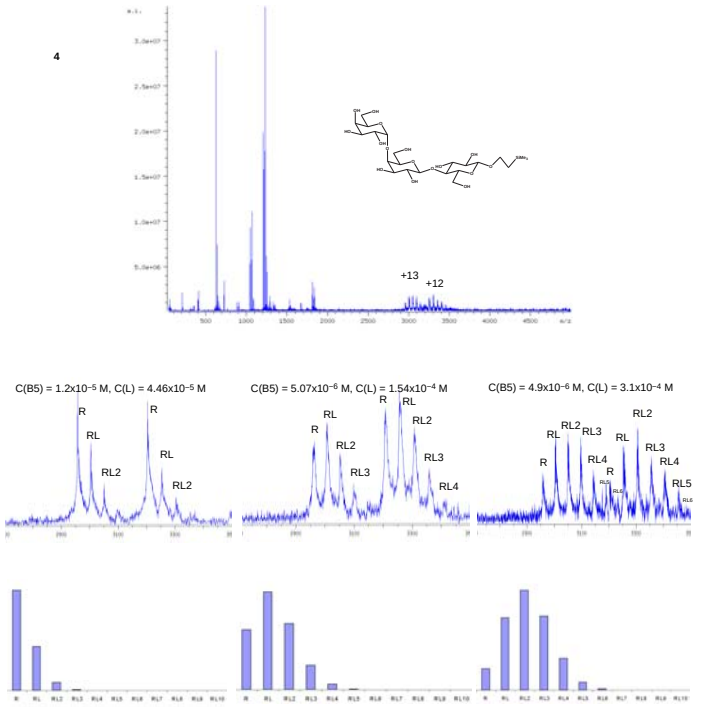

Figure 4. Different binding states of SLT−1B in complex with P^k−trisaccharide. Actual spectra and 10 binding sites model (K_{D1} = 5x10^{-4} M, K_{D2} = 5x 10^{-5} M.

Conclusions

- The assembly of multimeric forms of both natural and recombinant forms of the toxin molecule are readily assessed by this technique.
- Specific non−covalent complexes between the lectin part of the toxin and carbohydrate ligands were observed by FT−ICR MS.
- The distribution of signal intensities of complexed and uncomplexed forms suggests that abundances of ions in the gas phase corresponds to the distribution of bound and unbound receptor species in solution.
- A dissociation constant for complex SLT−1B with P^k trisaccharide has been obtained from the mass spectra.