Using Ion Mobility to Measure Changes in Protein Structure upon Substrate-Ligand Binding

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OVERVIEW

- **An increase in mass can be accurately measured upon the binding of substrate to BCL-XL**
- **The change in the gas phase shape of BCL-XL is consistent to those made by NMR structural characterization techniques.**
- **Binding of the substrate indicates a collapse from an larger unfolded structure to a more compact structure.**

INTRODUCTION

Nanoelectrospray is an ionization technique which can efficiently generate large biological gas-phase ions. Transfer of non-covalently associated protein-protein complexes from solution to the gas phase generally results in the formation of ions possessing relatively few charges, and retaining their native folded structure

Additional biological structural determination techniques can be used to gain insight into protein structure, such as nuclear magnetic resonance (NMR) and X-ray crystallography. Both of which have the capability of providing a 3-dimensional structure.

Here we present a method providing rapid determination of substrates binding to the protein BCL-XL and also the ability to measure change in protein shape, as a consequence of substrate binding. The gas phase shape to the protein, in the presence and absence of the substrate is compared to and demonstrated to be consistent to the solution phase NMR structure.

METHODS

Instrumentation

The instrument used in these studies was a Synapt HDMS System (Waters Corporation), shown in **Figure 1,** which has a hybrid quadrupole/IMS/oa-ToF geometry. Briefly, samples were introduced by a borosilcate glass nanoelectrospray-spray tip and sampled into the vacuum system through a Z-Spray source. The ions pass through a quadrupole mass filter to the IMS section of the instrument. This section comprises three travelling wave (T-Wave) ion guides. The trap T-Wave accumulates ions whilst the previous mobility separation is occurring, then these ions are released in a packet into the IMS T-Wave in which the mobility separation is performed. The transfer T-Wave is used to deliver the mobility separated ions into the oa-ToF analyser.

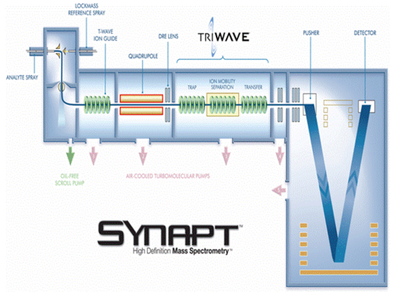


Figure 1. *Diagram of the Synapt HDMS System instrument.*

Samples and Gases

BCL-XL (24.5kDa) was buffer exchanged into an aqueous solution of 100mM ammonium acetate, to a final working protein concentration of 1.0µM. The peptide substrates BAD and BAK were added in stoichiometric amounts. Sulphur Hexafluoride (SF6) was obtained from BOC Gases LTD.

Experimental

SF6 was used as the trap/transfer gas. Nitrogen was used as the ion mobility gas. All samples were introduced into the SYNAPT HDMS System using a borosilicate nano-vial and a nanoflow Z-Spray ion source.

The *m/z* scale was calibrated with a solution of caesium iodode over the *m/z* range 600-8000.

The protein standards myoglobin, cytochrome-C and lysozyme were dissolved in acetonitrile 50% (v/v), formic acid (0.1% v/v) and 1,8-diazabicycloundec-7ene (DBU)[1] 0.1% (v/v). For example, the presence of DBU 0.1% (v/v) reduced the average charge state of myoglobin from +18 to +10.

T-Wave ion mobility calibration was carried out using a modification of an existing protocol, utilising charge reduced protein standards. The protein multiply charged ions, of known collisional cross-section[2], used for IMS calibration were; myoglobin +20 to +4 and cytochrome-C +16 to +3. The IMS calibration was validated using the multiply charged ions of the protein lysozyme whose CCSs have previously been determined on a standard IMS drift tube[2].

RESULTS

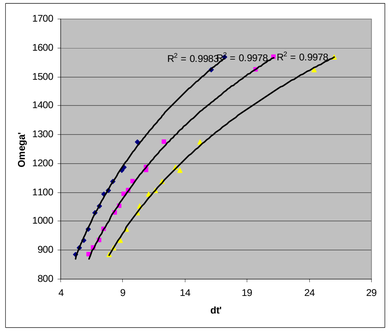


Figure 2. *T-Wave ion mobility calibration curves for myoglobin and cytochrome-C. T-Wave pulse height: 5.0, 5.5 and 6.0V. Charge and reduced mass corrected CCS plotted against corrected drift-time.*

By utilising solution phase charge reduction, one can extend the ion mobility calibration, therefore reducing the need for linear extrapolation. As shown in **Figure 2**, using a solution of myoglobin and cytochrome-C, containing 0.1% (v/v) DBU, one can produce and therefore, measure by ion mobility, the +5 and +4 charge states of myoglobin and cytochrome-C. Thus extending the drift-time function of the IMS calibration from 15msec to 26msec (T-Wave pulse height: 5.0V). Also note that the shape of the IMS calibration is no longer a power relationship, but is now a logarithmic relationship.

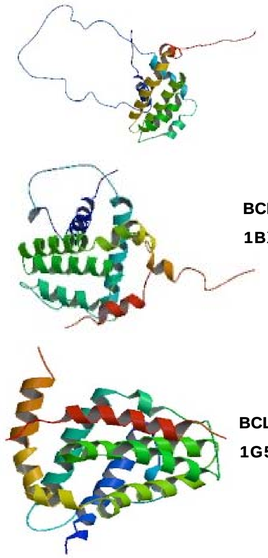
The extended IMS calibration was validated against the known CCSs of lysozyme[2] and the results are shown in **Table 2**.

PDB	PA (Å²)	EHSS (Å²)
1LXL	2549	3180
1BXL	1897	2422
1G5J	1811	2327

Table 1. *MOBCAL calculation for PDB files 1LXL, 1BXL and 1G5J. Collisional cross-section values displayed are derived from the Projection Approximation and the Exact Hard Sphere Scattering calculation[3].*

z	CCS (Å²)	T-Wave CCS (Å²)	% Difference
18	2989	2984	0.14
17	2894	2902	0.27
16	2823	2842	0.69
15	2733	2740	0.28
14	2672	2692	0.77
13	2598	2622	0.92
12	2525	2523	0.04
6	1355	1353	0.12
5	1313	1339	1.99

Table 2. *Comparison of T-Wave derived CCSs for lysozyme and the literature CCS values[2]. T-Wave CCSs calculated using extended IMS calibration.*



BCL-XL
1LXL

BCL-XL & BAK
1BXL

BCL-XL & BAD
1G5J

Figure 3. *Solution NMR structures of the protein BCL-XL, BCL-XL + BAK and BCL-XL + BAD obtained from RSCB Protein Data Bank.*

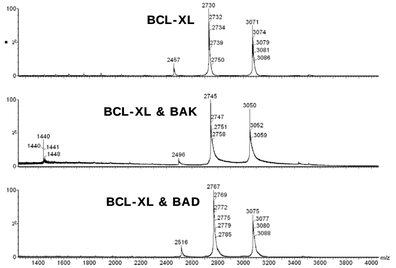


Figure 4. *Non-denatured mass spectra of the protein BCL-XL, BCL-XL & substrate BAK and BCL-XL & substrate BAD.*

Analysing the protein BCL-XL under native conditions, the deconvoluted mass for the multiply charged ions *m/z* 2457, 2730 and 3071 is 24,562Da. Upon addition of ligand BAK the deconvoluted mass increases to 27,441Da (BCL-XL & BAD). Upon addition of ligand BAD, the deconvoluted mass is 27,667Da (BCL-XL & BAD). Here we can demonstrate that a protein ligand complex can be maintained during it's transit through the mass spectrometer (**Figure 4**).

We also have the ability to measure the ions collisional cross-section. This measurement was carried out on the protein BCL-XL in the presence and absence of substrates BAK and BAD (**Table 3**). Ion mobility measurements were made over a 3 different T-Wave pulse heights: 5.0V, 5.5V and 6.0V. Nitrogen was used as the IMS gas at a pressure of 0.5mbar. Ion mobility drift times were in the order of 5 to 14msec, depending on ion of interest. **Figure 5** shows the arrival time distributions for selected charge states of the native BCL-XL in the absence of any substrate. What is clear is that the higher charge states (+15 to +11, *m/z* 1400-2200) show a large distribution of CCS ranging from 2000Å² to 3500Å², where as the +9 and +8 charge states show a single arrival time distribution.

Protein	T-Wave CCS Å²
BCL-XL	1995 +/- 22
BCL-XL & BAK	2166 +/- 14
BCL-XL & BAD	2134 +/- 24

Table 3. *T-Wave derived CCSs for the protein BCL-XL, BCL-XL & BAK and BCL-XL & BAD.*

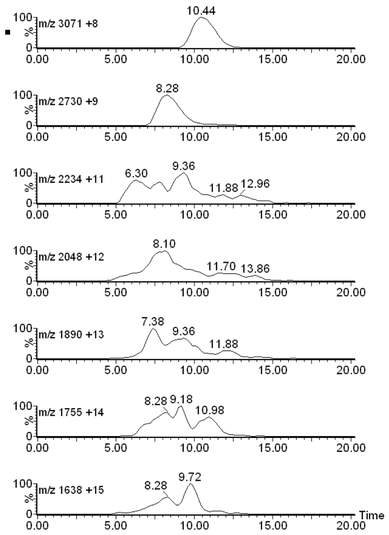


Figure 5. *Arrival Time Distribution (ATD, msec) for selected charge states of the protein BCL-XL in the absence of any substrates.*

CONCLUSION

- It would appear that BCL-XL, in the gas phase exists as a mixture of folded and unstructured protein. This is consistent to what has been observed by solution NMR (PDB 1LXL) where a large portion of the protein is unresolved due to it's unstructured nature. The CCS for charge states +9 and +8, showing a single ATD, can be measured accurately.
- Upon binding of the substrates BAK and BAD, the unstructured nature of BCL-XL disappears.
- Upon binding of the substrates BAK and BAD, there is an increase in collisional cross-section of BCL-XL of 8%.

1. Bagal, Zhang & Schnier; *Anal. Chem.* 2008 **80** (7) 2408-2418
2. http://www.indiana.edu/~clemmer/Research/research.htm
3. Mesleh, Hunter, Schvartsburg, Schatz & Jarrold; *J. Phys. Chem.* 1996, **100**, 16082-16086.

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