基質の結合によるタンパク質コンフォメーション変化における イオンモビリティ測定

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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

ナノエレクトロスプレーは生体高分子の気相イオンを効率よく生成することのできるイオン化法である。非共有結合により相互作用しているタンパク-タンパク複合体の溶液中の構造を維持したまま、ほとんど構造に変化のない気相イオンを生成させることが可能である。

タンパク質の三次元構造の情報を得るための手法として、核磁気共鳴(NMR)やX線結晶構造解析などが用いられているが、ここでは、BCL-XLタンパク質への基質の結合および、基質の結合による蛋白質の変化を迅速に測定するための手法を提案する。基質の有無によるタンパク質の気相での形状を比較し、NMRによって決定された溶液中における構造と一致することを実証した。

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.

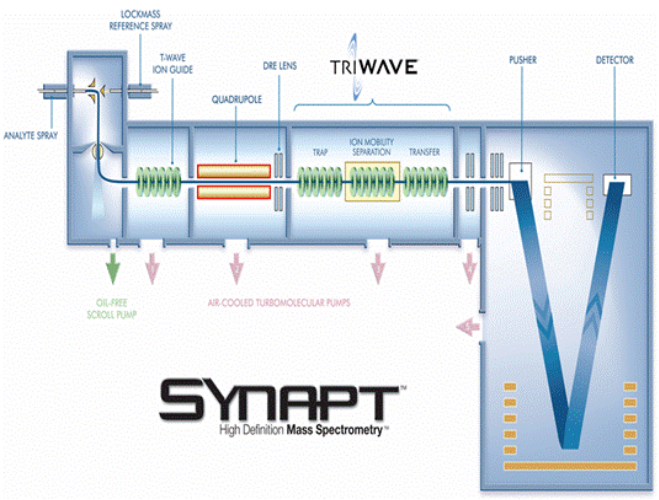

Figure 1. *Synapt HDMS System instrument.*

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

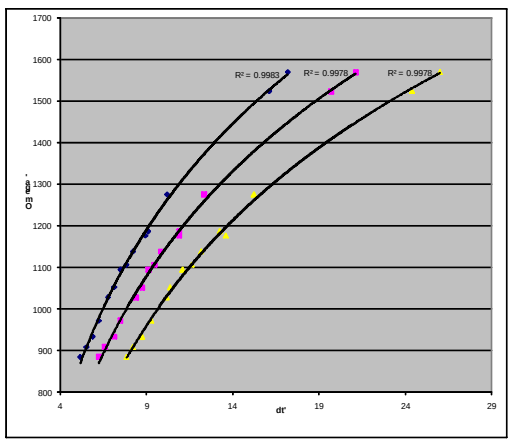

Figure 2. *ミオグロビンおよびシトクロム C を用いた T-Wave 検量線.*
T-Wave pulse height: 5.0, 5.5 and 6.0V. Charge and reduced mass corrected CCS plotted against corrected drift-time.

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. *1LXL, 1BXL および1G5J のPDB ファイルからMOBCAL を用いて計算した値*
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. *T-Wave を用いて測定されたリゾチームのCCS と文献のCCS[2] との比較*
T-Wave CCSs calculated using extended IMS calibration.

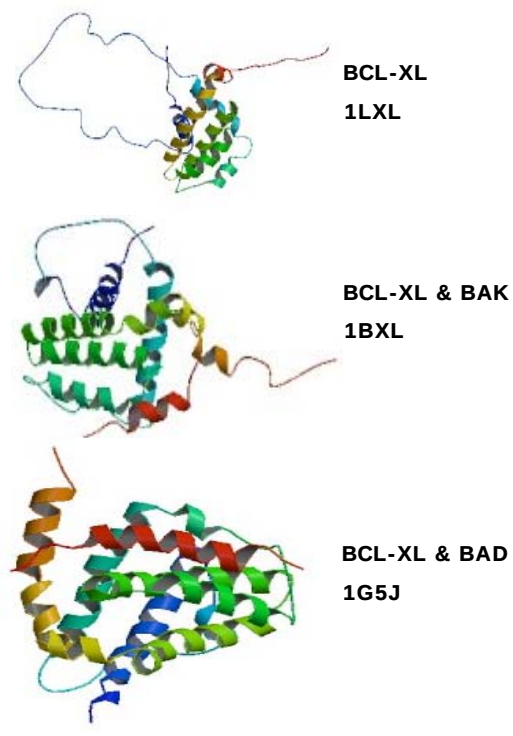

Figure 3. *RSCB Protein Data Bank から得た、NMR によって決定されたBCL-XL, BCL-XL + BAK およびBCL-XL + BAD の溶液中における構造*

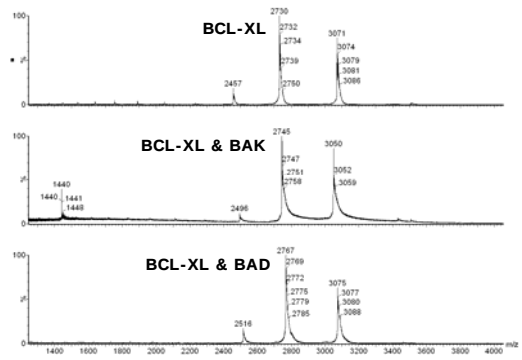

Figure 4. *重水素置換していないBCL-XL, BCL-XL & BAK およびBCL-XL & BAD のマススペクトル*

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. *BCL-XL, BCL-XL & BAK およびBCL-XL & BAD のT-Wave を用いて測定されたCCS*

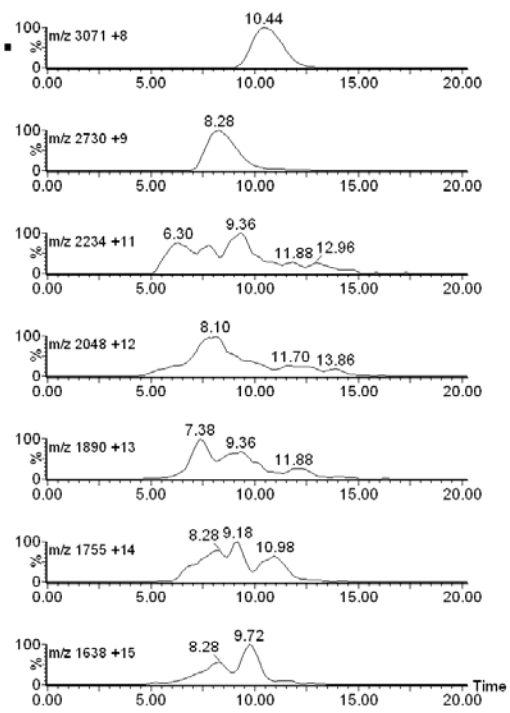

Figure 5. *基質の非存在下におけるBCL-XL の各電荷のイオンにおける到着時間の分布 (ATD, msec)*

CONCLUSION

- BCL-XL は気相状態において、フォールディングした状態と構造を形成していない状態とが混在していると考えられる。これは、構造形成していないことによるタンパクの大部分が未分離な状態である、という溶液 NMR によって観測された結果(PDB 1LXL) と一致する。
- 基質である BAK および BAD の結合により、BCL-XL の構造を形成していない成分由来のピークが消失した。
- 基質である BAK および BAD の結合により、BCL-XL の衝突断面積が 8%増加した。

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.