NATIVE MASS SPECTROMETRY DISTINGUISHES HOMO VS HETERO NON-COVALENT DIMERIC AGGREGATES OF MONOCLONAL ANTIBODIES

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AIM

The purpose of this study was to develop an assay to characterize intact soluble monoclonal antibody (mAb) dimers using native mass spectrometry.

INTRODUCTION

- Protein aggregation is a common degradation process which can affect the production, formulation and storage of therapeutic mAbs.
- Mab aggregation is an undesired effect that can be responsible for loss in biological activity, decreased mAb solubility or immunogenic response in patients.
- SEC (size exclusion chromatography) with UV detection is typically used for the separation and quantification of protein aggregates.
- While SEC-UV has the ability to separate different types of protein structures (e.g. monomers, Half-mAbs, dimers, trimers, tetramers, etc) it cannot provide the molecular weight information needed for the assignment of these species.
- ESI-MS can be coupled (on-line or off-line) with SEC for accurate assignment of these mAb species.
- In the case of a mixture of two therapeutic mAbs, two types of dimers can be formed by non-covalent interactions: *homodimers* (AA and BB dimer types) — formed between the same type of mAb monomers and *heterodimers* (AB type) formed when two different types of monomers are interacting to form aggregates.
- In this poster, native nanospray ESI-MS was used to characterize the mAb aggregates of Trastuzumab (TmAb), NIST mAb and the AA, BB and AB dimer types (homodimers and heterodimers) from a mixture of two BMS *stressed* mAbs (mAb1 and mAb4).

METHODS

Sample Preparation

The formulation buffers of Trastuzumab (TmAb, 20 mg/mL) and NIST mAb (10 mg/mL) were exchanged with 100 mM ammonium acetate (pH 6.5) using Micro Bio-Spin P6 size exclusion chromatographic columns (BioRad). Preparative scale SEC fractions of the monomeric and HMW species of the *individually stressed* BMS mAb1 and mAb4 samples, as well as a (mAb1+mAb4) *stressed mixture* were buffer exchanged using the same protocol.

SEC separations

Preparative scale SEC-UV separations were performed to isolate enriched fractions of the BMS mAb1 and mAb4 monomers and the HMW soluble aggregate species. Analytical scale SEC-MALS separations were performed to evaluate the purity of the enriched fractions as well as to estimate the monomer:HMW species ratios from the non-enriched mAb samples.

ESI- MS conditions

All buffered exchanged mAb samples (5 µL) were loaded into metal coated nanospray glass emitters and placed in front of the instrument inlet in a previously optimized XYZ position of the nanoESI source.

Full scan ESI-MS spectra were acquired in positive ion Sensitivity mode over a mass range of 3,000—20,000 on a Synapt G2-Si instrument equipped with a nanospray (nano-ESI) source. The optimized source parameters include: spray voltage (1.5 kV), cone voltage (150 V) and a source temperature set to room temperature. The Ar gas flow for the Trap collision cell was set to 7 mL/min, while the Trap CE and Transfer CE voltages were set to 30 V and 10 V respectively. Spectra were acquired in MassLynx SCN916 using 2 sec scans. Spectra deconvolution was performed using the MaxEnt1 algorithm to obtain the average molecular weight of all the mAb species investigated here.

Synapt G2-Si QTof mass spectrometer

RESULTS

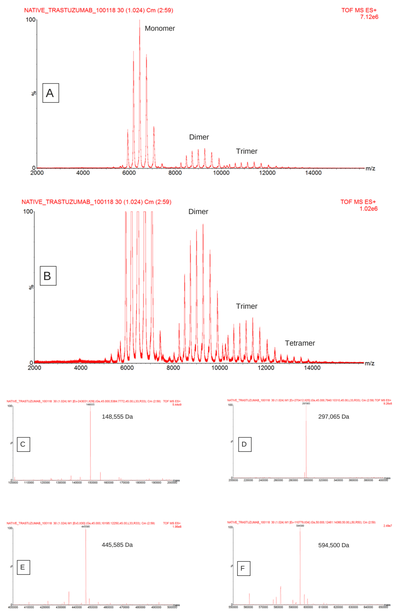

Figure 1. Native ESI-MS spectra of Trastuzumab (TmAb): (A) mAb monomer and its corresponding high molecular aggregates (dimers, trimers and tetramers) can be detected by nanoESI-MS; (B) detailed view of the native HMW soluble aggregate spectra; MaxEnt1 deconvoluted spectra of the TmAb monomer (C), dimer (D), trimer (E) and tetramer (F).

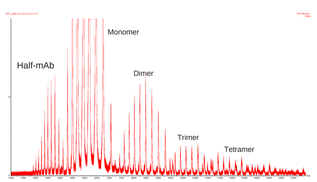

Figure 2. Native ESI-MS spectra of NIST mAb. In addition to the mAb monomer and the HMW soluble aggregates (dimers, trimers and tetramers), a half-mAb species is clearly detected in front of the monomer signal.

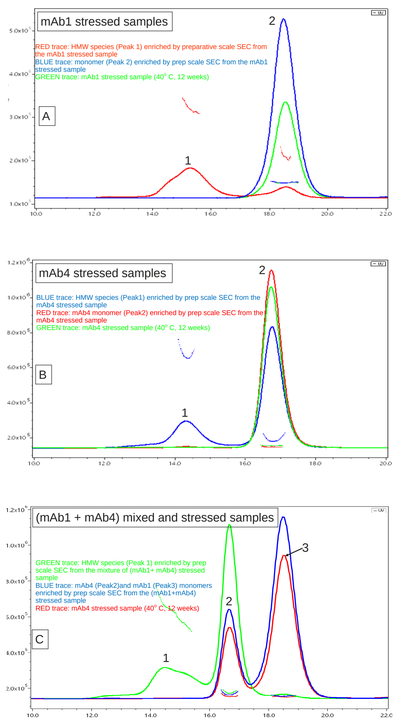

Figure 3. Analytical scale SEC-MALS chromatograms showing the separation of the monomeric and HMW species of the BMS mAb 1 and BMS mAb4 stressed samples:
(A) mAb1 stressed samples: the HMW species of mAb1 (Peak 1) cannot be detected in the non-enriched stressed sample (the green trace shows mostly a monomer peak labeled as Peak 2), but is clearly present in the enriched sample (red trace) obtained by preparative scale SEC;
(B) mAb4 stressed samples: the HMW species of mAb4 (Peak 1) cannot be detected in the non-enriched stressed sample (the green trace shows mostly a monomer peak labeled as Peak 2), but is clearly present in the enriched sample (blue trace) obtained by preparative scale SEC;
(C) Mixture of (mAb1+mAb4) stressed sample: the SEC HMW fraction (Peak 1 from the green trace) isolated by preparative scale SEC contains a heterogeneous mixture of HMW species clearly separated from the mAb1 monomer (Peak 2) and mAb4 monomer (Peak3).

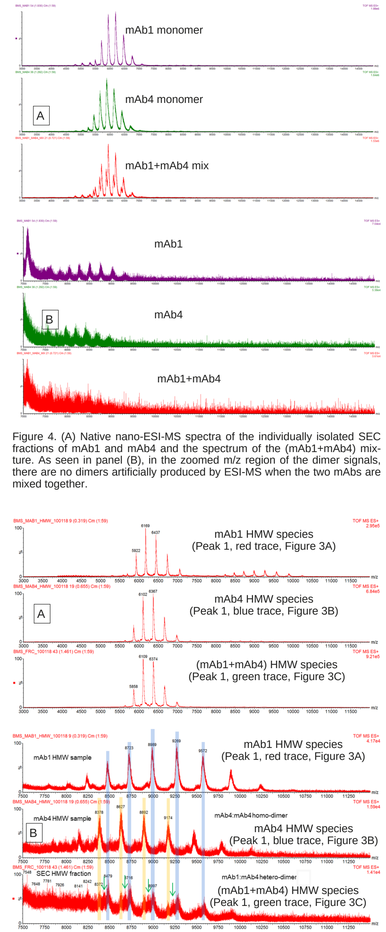

Figure 4. (A) Native nano-ESI-MS spectra of the individually isolated SEC fractions of mAb1 and mAb4 and the spectrum of the (mAb1+mAb4) mixture. As seen in panel (B), in the zoomed m/z region of the dimer signals, there are no dimers artificially produced by ESI-MS when the two mAbs are mixed together.

Figure 5. A) Native nano-ESI-MS spectra of the individually isolated SEC fractions of HMW mAb1 species and HMW mAb4 species and the spectrum of the SEC isolated (mAb1+mAb4) dimer. Panel (B) displays the m/z range of mAb dimers where the signals of (mAb1:mAb1) homo-dimers and (mAb4:mAb4) homodimers are separated from the (mAb1:mAb4) heterodimers. The SEC isolated fraction of the (mAb1+MAb4) stressed sample contains a mixture of all these three species as shown in the bottom panel of this figure.

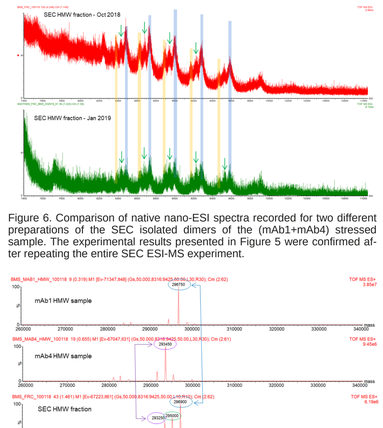

Figure 6. Comparison of native nano-ESI spectra recorded for two different preparations of the SEC isolated dimers of the (mAb1+mAb4) stressed sample. The experimental results presented in Figure 5 were confirmed after repeating the entire SEC ESI-MS experiment.

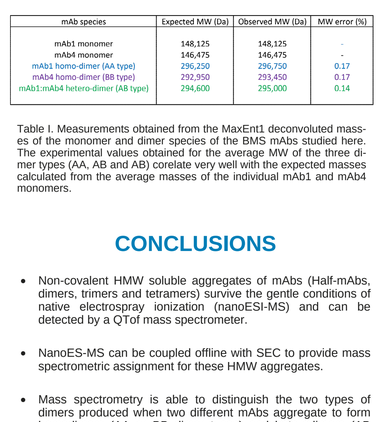

Figure 7. Comparison of MaxEnt1 deconvoluted dimer spectra of mAb1 homodimers, mAb4 homodimers and mAb1:mAb4 heterodimers. The last species is unique to a HMW mAb dimer species isolated by SEC.

mAb species	Expected MW (Da)	Observed MW (Da)	MW error (%)
mAb1 monomer	148,125	148,125	-
mAb4 monomer	146,475	146,475	-
mAb1 homo-dimer (AA type)	296,250	296,750	0.17
mAb4 homo-dimer (BB type)	292,950	293,450	0.17
mAb1:mAb4 hetero-dimer (AB type)	294,600	295,000	0.14

Table I. Measurements obtained from the MaxEnt1 deconvoluted masses of the monomer and dimer species of the BMS mAbs studied here. The experimental values obtained for the average MW of the three dimer types (AA, AB and AB) corelate very well with the expected masses calculated from the average masses of the individual mAb1 and mAb4 monomers.

CONCLUSIONS

- Non-covalent HMW soluble aggregates of mAbs (Half-mAbs, dimers, trimers and tetramers) survive the gentle conditions of native electrospray ionization (nanoESI-MS) and can be detected by a QTof mass spectrometer.
- NanoES-MS can be coupled offline with SEC to provide mass spectrometric assignment for these HMW aggregates.
- Mass spectrometry is able to distinguish the two types of dimers produced when two different mAbs aggregate to form homodimers (AA or BB dimer types) and heterodimers (AB dimer types).

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