Development of Native and Denaturing LC-MS Methods for Characterization of Cysteine-Linked Antibody Drug Conjugates Using a Non-Toxic Mimic

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Introduction

Here we utilize a cysteine-linked antibody fluorophore conjugate to show how LC-MS methods may be optimized using a non-toxic ADC-mimic. The ADC-mimic was used to develop native and reduced SEC-MS conditions. The reduced mimic's heavy and light chains, as well as its IdeS-proteolysis fragments, were used to develop reversed-phase LC-MS methods. A tryptic digest of the ADC mimic was used to develop a peptide mapping LC-MS method.

Methods

Native SEC-MS

Column:	Tosoh TSK Gel SW3000XL, 2.0 mm x 300 mm, 4 µm; 25 °C
Isocratic flow:	100 mM ammonium acetate, pH 7, 70 µL/min
Handling:	PNGase F 37 °C, 18 h

Reduced SEC-MS

Column:	Tosoh TSK Gel SW3000XL, 2.0 mm x 300 mm, 4 µm; 25 °C
Isocratic flow:	30% ACN 0.1% TFA, 70 µL/min
Handling:	PNGase F 37 °C 18 h; 50 mM DTT, 37 °C, 1 h

Antibody Subunit RPLC-MS

Column:	BIOshell A400 Protein C4, 300 µm x 150 mm, 3.4 µm; 65 °C
Phases:	Water, 0.1% TFA; ACN, 0.1% TFA
Gradient:	Desalt 5% organic; 31%-32.5% organic / 4 min; 35%-38% organic / 10 min, 40 µL/min
Handling:	7.4 M GuHCl / 100 mM DTT 50 °C 1 h ; optionally IdeS 37 °C 3 h

Peptide Mapping

Column:	BIOshell A160 C18, 300 µm x 300 mm, 2.7 µm; 40 °C
Phases:	Water, 0.1% FA; ACN, 0.1% FA
Gradient:	3%-57% organic / 120 min; 10 µL/min
Handling:	Urea/DOC; TCEP/IAA; FASP digestion with Trypsin/LysC, 37 °C 16 h

An M-Class Acquity-Xevo G2 QToF (Waters) was used in all methods.

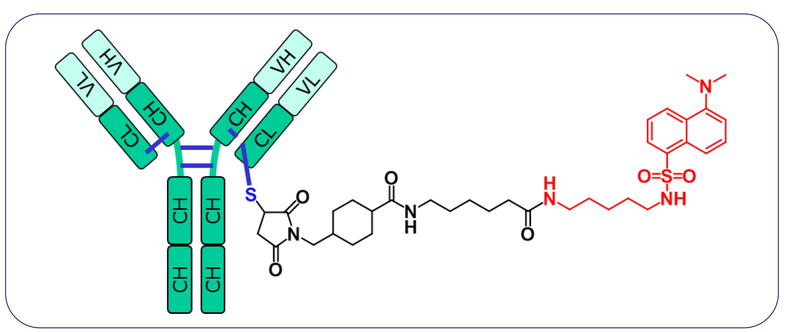

Figure 1. Structure of the SigmaMAb Antibody Drug Conjugate (ADC) Mimic, a human IgG1 monoclonal antibody with 0-8 dansyl fluorophores conjugated via an LC-SMCC crosslinker. Each dansyl-LC-SMCC attachment imparts a mass shift of 668 Da.

Results

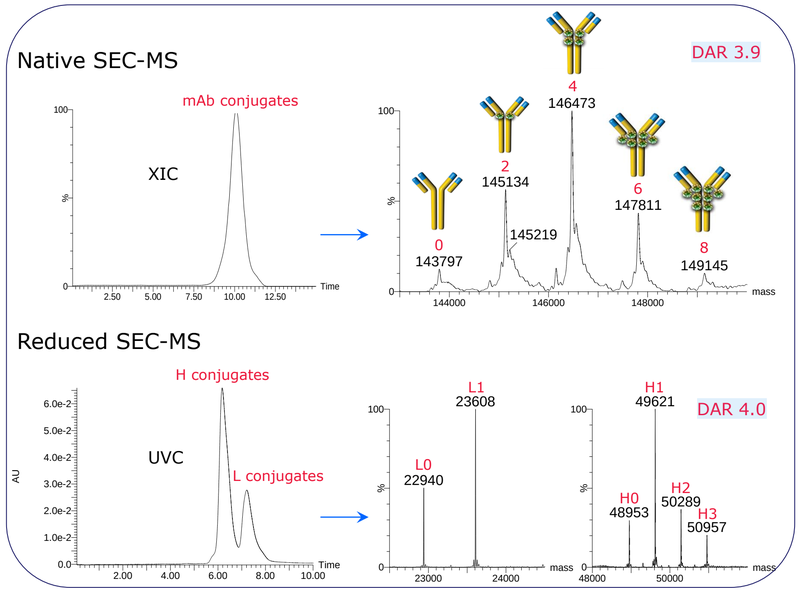

Figure 2. Deglycosylated ADC-mimic characterization by native and reduced SEC-MS methods. Each annotation counts the number of mimic-linkers conjugated to the antibody or subunit.

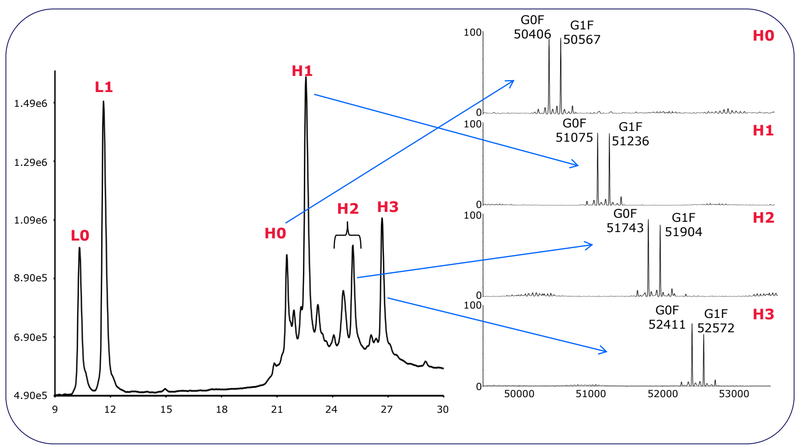

Figure 3. RPLC total ion chromatogram and deconvoluted MS spectra of reduced ADC-mimic heavy chain species. Annotation indicates glycoform and number of mimic-linker attachments.

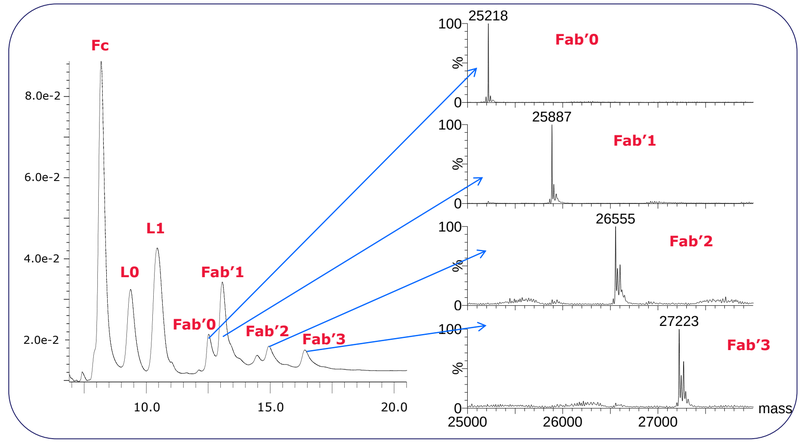

Figure 4. RPLC UV chromatogram and deconvoluted MS spectra of IdeS/reduced ADC-mimic Fab' species. Each annotation counts the number of mimic-linkers conjugated to the antibody subunit.

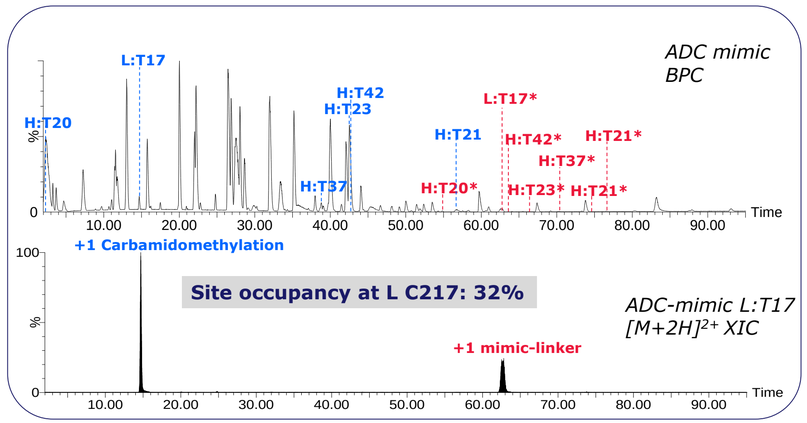

Figure 5. Tryptic peptide map of the ADC-mimic. Red and blue annotations are of conjugated and unconjugated peptides, respectively. Site occupancy was determined from XIC peak area ratio.

Conclusions

We have utilized a non-toxic ADC-mimic, with Cys-conjugation to dansyl-cadaverine-SMCC, to develop native and reduced SEC-MS methods, subunit RPLC-MS methods, and a peptide mapping method.

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