Top-Down Structural Analysis of an Intact Monoclonal Antibody by Electron Capture Dissociation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Results and Discussion

Top-down electron capture dissociation (ECD) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was performed for structural analysis of an intact monoclonal antibody (IgG1-kappa (κ) isotype, ~148 kDa, see Figure 1). Simultaneous ECD for *all* charge states (42+ to 58+) generates more extensive cleavages than ECD for an isolated single charge state. The cleavages are mainly localized in the variable domains of both heavy and light chains, the respective regions between the variable and constant domains in both chains, the region between heavy chain constant domains C_H2 and C_H3, and the disulfide bond (S-S) linked heavy chain constant domain C_H3. The light chain yields mainly N-terminal fragment ions due to the protection of the inter-chain disulfide bond between light and heavy chain, and limited cleavage sites are observed in the variable domains for each chain where the S-S spans the polypeptide backbone. Only a few cleavages in the S-S linked light chain constant domain, hinge region, and heavy chain constant domains C_H1 and C_H2 are observed, leaving glycosylation uncharacterized. Top-down ECD with a custom-built 9.4 T FT-ICR MS for structural characterization of IgG1κ provides more extensive sequence coverage than top-down collision induced dissociation (CID) and electron transfer dissociation (ETD) for time-of-flight and comparable sequence coverage for top-down ETD with orbitrap mass analyzers.

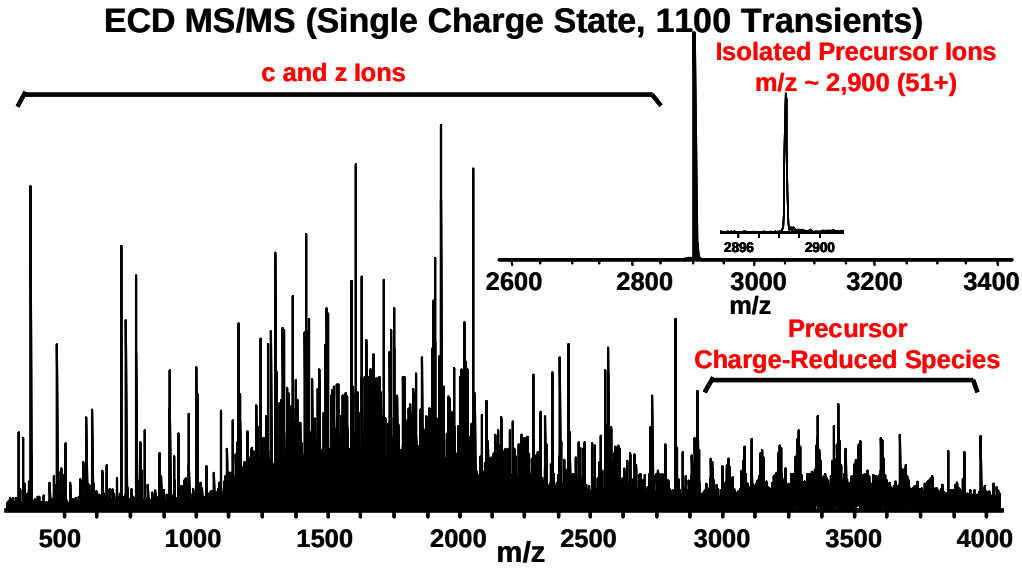

Figure 1. ECD product ion mass spectrum for the 51+ charge state ions from IgG1κ mAb. Inset: isolated precursor ions of *m/z* ~ 2900.

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References

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