Characterization of Monoclonal Antibody Drug Products Using High Resolution NMR

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Purpose

High-resolution 2D ^{1}H-^{13}C and ^{1}H-^{15}N correlated nuclear magnetic resonance spectroscopy (NMR) provides a robust approach for producing unique spectral signatures of the higher order structure of protein therapeutics, including monoclonal antibodies (mAbs) at atomic resolution in solution. Such signatures can be used as a tool to establish consistency of protein folding for drug quality assessment as well as for structural comparability of related drug products.

Methods

Using the IgG1κ NIST monoclonal antibody (NISTmAb), we demonstrate the acquisition of ^{1}H-^{13}C and ^{1}H-^{15}N correlated 2D NMR spectra at natural isotopic abundance using both conventional and state-of-the art rapid acquisition techniques. Furthermore, we demonstrate their use for generating unbiased statistical comparisons of mAb structure. Techniques are demonstrated on intact antibodies and protease-cleaved Fab and Fc fragments as well as intact and released mAb glycans.

Results

Results from this study indicate that 2D NMR methods are capable of statistically discriminating between dissimilar species, such as between the Fab domains of from different mAbs or between the glycosylated and variably deglycosylated Fc domains. Furthermore, statistical analysis suggests that, within the limit of detection, no significant structural differences are observed between the Fab and Fc domains of intact mAbs and their corresponding fragments, validating a domain fragment based approach for mAb HOS characterization.

Conclusion

This study demonstrates the precision and resolution with which 2D NMR techniques can be used characterize the higher order structure of protein therapeutics, including mAbs, at atomic resolution within reasonable experimental time frames and how these methods can be used to establish statistical structural comparability between drug samples.