Tackling the increasing complexity of therapeutic monoclonal antibodies with mass spectrometry

Sara Rosati, Natalie J. Thompson, Albert J.R. Heck

Mass spectrometry (MS) is emerging as an efficient method for the structural analysis of various new antibody-based therapeutic products. Here, we review the current trends and describe recent applications that highlight the use of MS to tackle the increasing complexity of monoclonal antibodies.

Keywords: Analytical method; Antibody (Ab); Antibody-drug conjugate (ADC); Biopharmaceutical; Biosimilar; Bispecific antibody; Glycosylation; Mass spectrometry; Native mass spectrometry; Sequence variant

Sara Rosati, Natalie J. Thompson, Albert J.R. Heck*
Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Sara Rosati, Natalie J. Thompson, Albert J.R. Heck
Netherlands Proteomics Centre, Padualaan 8, 3584 CH Utrecht, The Netherlands

*Corresponding author.
Tel.: +31 (0)30 2536797;
Fax: +31 (0)30 2536919.;
E-mail: a.j.r.heck@uu.nl

1. Introduction

The use of macromolecular therapeutics has expanded very much in recent decades [1]. A great contribution to this growth comes from the introduction of therapeutic monoclonal antibodies (mAbs) [2]. Around 30 different Ab-based biopharmaceuticals have been approved by regulatory authorities for clinical use, predominantly of the IgG class, which is also naturally the most abundant class in humans. Mostly, they are used in cancer therapy, immunological diseases and infectious diseases [3]. A particular feature of these mAbs is their high specificity to their targets that generally leads to high patient tolerance, though many efforts are currently made to decrease immunogeneity issues [4].

IgG Abs consist of four polypeptide chains: two light and two heavy chains fused together to give rise to the characteristic Y shape (Fig. 1). Both intermolecular and intramolecular disulfide bridges contribute to the stability of the Ab. Complementarity-determining regions (CDRs) (i.e. the regions where the antigen binds) are situated at the extremity of each of the two antigen-binding fragments (Fabs).

Since the introduction of mAbs in the clinic about 30 years ago, major advancements have been made to optimize properties (e.g., immunogenicity, efficacy, and pharmacokinetics). From the early murine-based mAbs, through chimeric and humanized mAbs, we currently see mostly fully human mAbs entering the clinic. Furthermore, new entities are gaining popularity beside the conventional mAb products. Amongst them, Fab fragments, Ab-drug conjugates (ADCs), and bispecific Abs, have appeared and are already penetrating the market [1]. As the increasing molecular complexity of these new, improved therapeutic products puts a strain on their analysis, a number of different analytical techniques [e.g., separation techniques, nuclear magnetic resonance (NMR), circular dichroism (CD)] are currently used in the industry to cover multiple aspects of Ab analysis. [5,6]

In this review, we focus on mass spectrometry (MS) as one of the most versatile technologies for the in-depth structural analysis of mAbs. MS can be used for the characterization of small and large alterations in the mAbs [e.g., sequence variants, diversity in glycosylation profiles, and other post-translational modifications (PTMs), including disulfide shuffling]. It may also be used to analyze highly complex products [e.g., mixtures of (bispecific) Abs or ADCs]. Based on these characteristics, MS has become an essential tool in the biopharmaceutical industry, for not only the analysis of new

0165-9936/$ - see front matter © 2013 Elsevier Ltd. All rights reserved. doi:http://dx.doi.org/10.1016/j.trac.2013.02.013

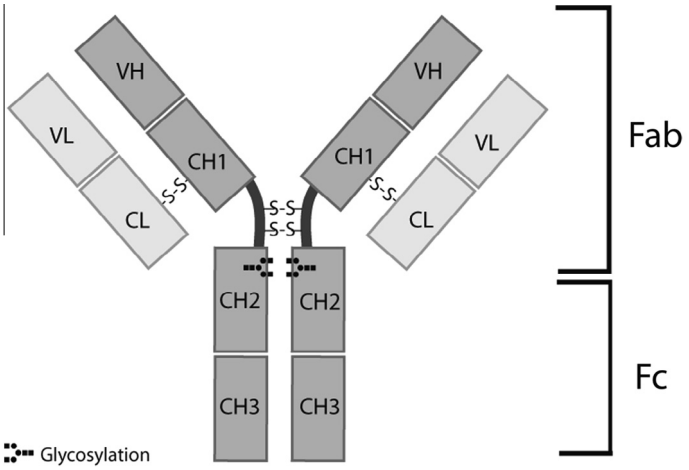

Figure 1. Structure of an IgG1 antibody. VH, Heavy chain variable domain; VL, Light chain variable domain; CH1, Heavy chain constant domain1; CH2, Heavy chain constant domain2; CH3, Heavy chain constant domain3; CL, Light chain constant domain; Fab, Antigen binding fragment; Fc, Crystallizable fragment.

Ab-based products, but also the evaluation of their biosimilars.

2. Discussion

2.1. Mass-spectrometry instrumentation used for the structural analysis of antibodies

The complex heterogeneity of mAbs is an analytically challenging system to characterize in depth. Traditionally, various techniques have been used in industry and academia alike to analyze mAbs. More recently, MS has been emerging as a versatile analytical technique that can address and elucidate many aspects of mAbs and be complementary to investigations by other approaches. MS can handle, with relative ease, single amino-acid substitutions and PTMs, but also binding interactions and global structural changes [7–11]. Until relatively recently, MS was relegated to small-molecule analysis because of the inability to transfer large biomolecules to the gas phase efficiently. However, in the late 1980s, the development of the ''soft'' ionization techniques, matrix-assisted laser desorption ionization (MALDI) [9] and electrospray ionization (ESI) [12], opened MS to the world of macromolecules and thus also to intact protein analysis.

Indeed, one of the first impressive demonstrations of the power of MALDI in 1988 was in the analysis of intact mAbs [13–15]. Initial ESI-based experiments analyzed mAbs under denaturing conditions in which acidic and organic solutions were used to transfer unfolded proteins to the gas phase. ESI was then coupled with time-of-flight (ToF) and triple-quadrupole mass analyzers, which possess a suitable mass range for studying denatured proteins (i.e. up to m/z 4000) [7]. With this type of instrumentation, the precise molecular weight can be determined and, therefore, the purity and the heterogeneity of mAbs.

However, under these denaturing conditions intramolecular and intermolecular interactions become disrupted, meaning that Ab tertiary structure and possible non-covalent complexes formed by these interactions cannot be studied. Around 2000, an alternative method emerged circumventing this issue, termed native MS, through which the non-covalent interactions that define protein tertiary and quaternary structure are largely preserved after ionization, thereby making MS compatible with the world of structural biology [16,17]. In native MS, a volatile buffer at neutral pH (e.g., aqueous ammonium acetate) is used with ''gentle'' nanoESI elution to transfer structurally intact proteins into the gas phase. Under these conditions, the proteins acquire fewer charges, thus pushing the detection window to a mass range over m/z 4000. To detect these high m/z ions successfully, ToF mass analyzers are typically used. The mass accuracy of ESI-ToF instruments for a protein the size of an intact Ab is typically 10–100 ppm, and the instrument mass resolution is 5000–10 000 (full width at half maximum resolution, FWHM) for state-of-the-art ToF and quadrupole-ToF (Q-ToF) instruments. With respect to intact Abs, the width of the isotopic distribution is approximately 25 Da making a resolution higher than 10 000 not particularly advantageous for mass measurement of mAbs [7]. Using native MS in conjunction with ToF mass analysis, possessing a theoretically infinite mass range, protein complexes up to megaDalton molecular weight (e.g., intact virus capsids) can now be analyzed