FEBS Letters 588 (2014) 308–317

journal homepage: www.FEBSLetters.org

Review

Mass spectrometry for the biophysical characterization of therapeutic monoclonal antibodies

Hao Zhang, Weidong Cui, Michael L. Gross *

Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130, USA

ARTICLE INFO

Article history:
Received 30 October 2013
Revised 16 November 2013
Accepted 18 November 2013
Available online 26 November 2013

Edited by Wilhelm Just

Keywords:
Monoclonal antibody
Mass spectrometry
Native ESI
Top-down and bottom-up
Protein footprinting
Hydrogen/deuterium exchange
FPOP
Ion mobility

ABSTRACT

Monoclonal antibodies (mAbs) are powerful therapeutics, and their characterization has drawn considerable attention and urgency. Unlike small-molecule drugs (150–600 Da) that have rigid structures, mAbs (~150 kDa) are engineered proteins that undergo complicated folding and can exist in a number of low-energy structures, posing a challenge for traditional methods in structural biology. Mass spectrometry (MS)-based biophysical characterization approaches can provide structural information, bringing high sensitivity, fast turnaround, and small sample consumption. This review outlines various MS-based strategies for protein biophysical characterization and then reviews how these strategies provide structural information of mAbs at the protein level (intact or top-down approaches), peptide, and residue level (bottom-up approaches), affording information on higher order structure, aggregation, and the nature of antibody complexes.

1. Therapeutic monoclonal antibodies (mAbs)

Therapeutic mAbs may have become the most popular drug candidates following their introduction into the clinic in the late 1980s [1]. Their high specificity and low side effects make mAbs powerful human therapeutics for oncology, autoimmunity/inflammation, infectious diseases, and metabolic disorders [2]. At present, approximately 30 therapeutic mAbs are being marketed. The sales contributed approximately $18.5 billion to the US economy in 2010 [1]. The high demands for new therapeutic mAbs have trigged a burst of mAb-based drug development. For example, 16 human mAbs entered the clinic during 1985–1996, whereas during 1997–2008, 131 human mAbs became available [3]. In 2011, more than 300 mAb-based thereapeutics were in clinical trials [2]. As older mAbs come off patent and go into production as generic drugs, the need for characterizing their higher order structure in quality control becomes even more important, motivating this review.

1.1. Introduction to mAbs

Therapeutic mAbs are glycoproteins that belong to the immunoglobulin (Ig) family. Ig's are used by the immune system to identify and neutralize foreign organisms or antigens [4,5]. Ig's are classified in five groups, IgA, IgD, IgE, IgG and IgM (as α, δ, ε, γ and μ), based on the structure of their constant regions [6]. At present, most approved mAbs are from IgG's (γ-immunoglobulin). IgG's have the typical "Y"-shaped structure comprised of two identical heavy and light chains (H and L chains) (Fig. 1). All heavy and light chains are covalently linked by disulfide bonds. IgGs can be further classified into four groups, IgG1, 2, 3, and IgG4 (as γ-1, γ-2, γ-3 and γ-4) on the basis of different patterns of inter-chain disulfide bonds and heavy-chain sequences. IgG1, 2 and 4 are widely used in therapeutics, whereas IgG3, which has a shorter serum half-life, is rarely used.

Each heavy chain contains one variable (VH) and three constant domains (CH1, CH2 and CH3), whereas each light chain contains one variable (VL) and one constant domain (CL). In the heavy chain, CH1 and CH2 are linked by a hinge region that contains inter-heavy chain disulfide bonds (IgG1 and IgG4 have two disulfide bonds in hinge region, whereas IgG2 has four). Antigen binding is mediated by the variable region, mainly by three loops connecting individual β-strands, which are called the complementarity determining regions (CDRs), from both heavy and light chains. Upstream of the heavy chain (VH and CH1) is the disulfide-bond-linked light chain (VL and CL), known as the fragment antigen-binding (Fab) region. The downstream constant regions (CH2 and CH3) of the heavy

* Corresponding author. Address: CB 1134, One Brookings Dr., St. Louis, MO 63130, USA. Fax: +1 314 935 7484.
E-mail address: mgross@wustl.edu (M.L. Gross).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.11.027

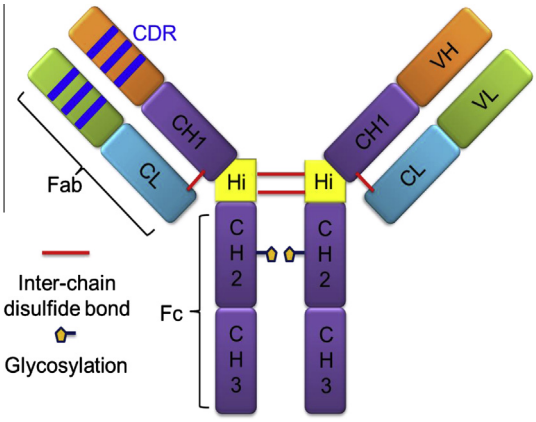

Fig. 1. IgG structure (IgG1). The global structure of IgG1 has two identical heavy chains and light chains. Four chains are attached covalently with inter-chain disulfide bonds and also by non-covalent interactions. The two constant regions from heavy chains (CH2 and CH3, Fc regions) respond to the binding to Fc gamma and FcRn receptors. The variable region from both light and heavy chains contains antigen-binding regions (CDRs). Variable regions with the close constant region together are called the Fab region. The Fab and Fc region are linked by the hinge region in the heavy chain. In the IgG1, there is a glycosylation site on the second constant region (CH2).

chain are called the fragment crystallizable (Fc) region, which is responsible for effector function during recycling [7].

A milestone in the development of therapeutic engineered mAbs was the introduction of murine mAbs from hybridomas [8]. Clinical applications of murine mAbs (suffix: -omab) began in the late 1980s [9]. Dissimilarities between murine and human immune systems led to clinical failure of those antibodies. Murine antibodies are engineered to generate chimeric mouse-human mAbs (~65% human in molecules, suffix: -ximab) by fusing the murine-variable regions onto human-constant regions [10]. The humanized mAbs (~95% human in molecules, suffix: -zumab) are produced by grafting murine hyper-variable regions on amino acid domains of human antibodies [11]. Both chimeric mouse-human and humanized mAbs have reduced immunogenicity and increased serum half-life [12]. With the development of phage-display technology and various transgenic mouse strains expressing human variable domains [13,14], fully human mAbs (suffix: -umab) with significantly reduced immunogenic potential and high similarity to human endogenous IgGs, have become rich sources of new therapeutics [1,3,15].

1.2. The challenge of verifying higher order structure of therapeutic mAbs

Unlike traditional small molecular drugs (150–600 Da), mAbs are large macromolecules (~150 kDa) with four polypeptide chains held in place by tens of inter- or intra-disulfide bonds as well as by non-covalent interactions [6]. For example, one approved therapeutic mAb, trastuzumab, has 6560 carbon atoms, 10132 hydrogens, 2090 oxygens, 1728 nitrogens and 44 sulfur atoms [16]. The functional form of the protein depends on its higher order structure (HOS), referring to the tertiary 3-D architecture determined by the secondary alpha-helices and beta-sheets, building upon the primary structure, and the quaternary complex formed by interacting/binding with other entities. Sources affecting the HOS of mAbs are not limited to primary structures. Variations in PTMs (post-translational modifications), mutations and modifications can trigger changes in HOS to affect binding to an antigen or to Fc-gamma and Fc-Rn receptors. Production and storage of therapeutic mAbs can introduce significant changes of HOS. From the view of patient health, HOS variations of these proteins can pose serious safety issues [17,18]. HOS can be fleeting for proteins; HOS is certainly more dynamic than primary structure. Although strategies to determine the primary structure of mAbs, including mutations, PTMs, and other modifications, have been available for decades, approaches to verify HOS are still needed. Although circular dichroism (CD), fluorescence and related optical spectroscopic methods are used for rapid HOS characterization [19], many regional but important structural changes are missed [20]. In the recently published draft guidelines for quality control of biosimilars (copies of therapeutic mAbs that are coming off patent), the US Food and Drug Administration (FDA) acknowledged that "a protein's three-dimensional conformation can often be difficult to define precisely using current physicochemical analytical technology". New approaches are under development to meet this challenge [16,20]. In this review, we focus on the new and promising MS-based biophysical approaches as means for characterization of mAb HOS.

2. Mass spectrometry based protein biophysics

2.1. Overview

The advantages of MS in biology are attracting the attention of structural biologists who address the biophysical properties of proteins [21]. Modern MS instrumentation and proteomics methods offer two major approaches to interrogate protein biophysics. One is an intact or top-down approach that employs native electrospray ionization (ESI), ion-mobility measurements, and fragmentation, usually by interaction with electrons, to provide a global view of the protein of interest [22]. The other is a bottom-up approach combining either protein footprinting [23] or cross-linking [24] that provide detailed peptide and even amino-acid-residue information. These terms "top down" and "bottom-up" first appeared in the MS-based proteomics literature [25]. Compared to bottom-up, top-down is less mature, requiring the invention of electron capture dissociation (ECD) [26] in 1998. Both top-down and bottom-up MS approaches have the advantages of small sample consumption, nearly no limit to protein size, and ability to determine in the gas phase the native or near-native protein properties. Furthermore, MS can be combined with protein footprinting to give an approach that is tolerant to solution media containing MS-unfriendly small molecules. Native ESI and top-down sequencing offer high throughput and unique specificity for oligomeric states and stoichiometry of native protein samples. By combining these complementary MS methods, important structural information can be discovered with intermediate structural resolution. We will review the principles of both approaches in the following subsections.

2.2. Intact and top-down based approach

At present, proteins and protein complexes with MW even at the mega-Dalton range can be directly analyzed by MS [27]. Top-down protocols provide information without requiring proteolytic digestion of protein samples prior to MS analysis [28]. Removing the digestion step should significantly reduce analysis time. Intact proteins and protein complexes are then interrogated close to their functional forms, even as protein assemblies [29]. Species existing in different oligomeric states or with PTMs can be analyzed separately. Although targeted analysis (e.g., oligomer specific analysis) can also be accomplished by bottom-up approaches by adding a pre-separation step, top-down approaches are more efficient. Using an approach targeting intact proteins, we can directly monitor the charge-state distribution and obtain stoichiometry