Backbone $^1$H, $^{13}$C, and $^{15}$N resonance assignments of the Fc fragment of human immunoglobulin G glycoprotein

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Abstract The Fc portion of immunoglobulin G (IgG) recruits complements and its cognate receptors, thereby promoting defensive mechanisms in the humoral immune system. These effector functions critically depend on N-glycosylation at the Fc region, which is therefore regarded as a crucial factor in the design and production of therapeutic antibodies. NMR spectroscopy plays a unique role in the characterization of conformational dynamics and intermolecular interactions of IgG-Fc in solutions. Here, we report NMR assignments of the glycosylated Fc fragment (Mr 53 kDa), cleaved from a chimeric antibody with human IgG1 constant regions, which was produced in Chinese hamster ovary cells with uniform $^{13}$C- and $^{15}$N-labeling.

Keywords Immunoglobulin G · Fc · Glycoprotein · Mammalian expression system · NMR spectroscopy · Resonance assignment

Biological context

Immunoglobulin G (IgG) is a multifunctional glycoprotein composed of an Fc region and two Fab regions, which are connected through the hinge region (Yamaguchi et al. 2007). The Fab regions recognize and capture specific antigens, while the Fc region recruits complements and its cognate receptors, Fcγ receptors (FcγRs), and offers acceptor sites for bacterial proteins including protein A and protein G. The Fc region has a homodimeric structure comprising the C-terminal halves of the heavy chains, each composed of the C_H2 and C_H3 domains. The C_H2 domain possesses a conserved N-glycosylation site, Asn297, at which a biantennary complex-type oligosaccharide is expressed with microheterogenieties characterized by the presence and absence of the non-reducing terminal galactose, fucose, sialic acid, and bisecting N-acetylglucosamine residues.

The effector function of IgG critically depends on N-glycosylation in the Fc region. The outer carbohydrate moieties govern the structural integrity of the FcγR-binding site of IgG, while the core fucosylation impairs antibody-dependent cellular cytotoxicity because of its negative steric effect against IgG interaction with FcγRIII (Ferrara et al. 2011; Krapp et al. 2003; Mizushima et al. 2011; Yamaguchi et al. 2006). Hence, the Fc glycoforms are now considered as a crucial factor in the design and
production of therapeutic antibodies in biopharmaceutical fields (Berkowitz et al. 2012; Jiang et al. 2011).

NMR spectroscopy offers unique tools for characterizing the conformational dynamics and intermolecular interactions of IgG-Fc in solution (Kato et al. 1991a, 1993a, 1995; Kim et al. 1994a; Latyppov et al. 2012). We developed protocols for uniform and amino acid-selective stable isotope labeling of an IgG glycoprotein and its functional fragments, using mammalian expression systems (Kato et al. 2010; Yamaguchi and Kato 2010). Based on partially (approximately 66 %) achieved spectral assignments (Yamaguchi et al. 2006), we previously reported NMR analytical results to characterize the N-glycosylation-dependent conformational changes of human IgG1-Fc and its interaction with a specific RNA aptamer (Matsumiya et al. 2007; Miyakawa et al. 2008; Yamaguchi et al. 2006).

In an extension of these studies, we herein report NMR assignments of the glycosylated version of Fc fragment (Mr 53 kDa) cleaved from a chimeric antibody with human IgG1 constant regions that was expressed by Chinese hamster ovary (CHO) cells with uniform 13C- and 15N-labeling.

Methods and experiments

The CHO/DG44 cell line (Urlaub1980) was kindly provided by Dr. Lawrence Chasin (Columbia University, NY). An anti-CCR4 chimeric antibody (designated KM3060), with constant regions, was produced in a CHO cell line as described previously (Yamaguchi and Kato 2010; Yamaguchi et al. 2006). The CHO cells were cultivated using the Nissui NYSF 404 medium supplemented with 2 % dialyzed fetal bovine serum. The CHO cells were cultivated using the Nissui NYSF 404 medium supplemented with 2 % dialyzed fetal bovine serum. At the final stage of cell culture, the medium was replaced with an isotopically labeled one depending on temperature, i.e. 52 °C at 42 °C, 51 °C at 50 °C, 50 °C at 48 °C, 49 °C at 46 °C, and 48 °C at 44 °C. Hence, we established spectral assignments based on the triple resonance spectral dataset recorded at a higher temperature, i.e. 52 °C, complemented with HSQC spectral data obtained by amino acid-selective 13C/15N-labeling. Chemical shift assignments were made for protein backbone resonances: Cα (99 %), Cβ (84 %), CO (80 %), CN (99 %), and N (99 %) (except for N of prolines). The spectral assignments at lower temperatures could be extrapolated by observing progressive spectral changes, depending on temperature, as exemplified by the spectrum at 42 °C (Supplemental Fig.1). The present spectral assignments indicate that a cluster of amino acid residues in the vicinity of the N-glycans, i.e. Gln295-Thr299 exhibit...
significant chemical shift differences in comparison with the previously reported assignments of human Fc produced in Escherichia coli (Liu et al. 2007).

The assignments for the $^1$H, $^{13}$C, and $^{15}$N backbone resonances of human IgG1-Fc have been deposited in the BioMagResBank database (http://www.bmrbr.wisc.edu) under the accession number 25224.

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