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Structural effect of deglycosylation and methionine oxidation on a recombinant monoclonal antibody

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Abstract

Methionine (Met) is one of the most susceptible amino acids to oxidation. Met256 (CH2-Met15.1) and Met432 (CH3-Met107) of a recombinant humanized monoclonal IgG1 antibody are located in the CH2 and CH3 domains, respectively. In three-dimensional structure, these two Met residues are close to the CH2-CH3 interface. In close proximity, oligosaccharides on the conserved asparagine (Asn) residues are enclosed in the CH2 domains. The relationship of Met oxidation with oligosaccharides and their effect on the structure of the antibody was investigated. Removal of oligosaccharides did not alter the oxidation rates of Met256 and Met432, however it caused significant structural changes as evidenced by the susceptibility of the deglycosylated antibody to trypsin and chymotrypsin. Oxidation of Met256 and Met432 did not cause significant conformational changes of the antibody with oligosaccharides, however oxidation of these Met residues accelerated degradation of the deglycosylated antibody. Analysis by mass spectrometry indicated that most of the protease cleavage sites were in the CH2 domains, which suggested that conformational changes induced by the removal of oligosaccharides and further by Met oxidation were local to the CH2 domains.

Keywords: Recombinant monoclonal antibody; Glycosylation; Oxidation; Mass spectrometry

1. Introduction

Glycosylation of the conserved asparagine (Asn) residue in the CH2 domain is the most common enzymatic modification of antibodies. Removal of oligosaccharides can induce significant structural changes. For example, it has been demonstrated that deglycosylated IgG-Fc cannot be crystallized under the conditions used for glycosylated Fc (Krapp et al., 2003). Structural changes have also been demonstrated by the increased susceptibility of deglycosylated and non-glycosylated antibodies to proteases (Dwek et al., 1995; Tao and Morrison, 1989) and decreased thermal stability of antibodies and their Fc fragments (Ghirlando et al., 1999; Liu et al., 2006; Mimura et al., 2000, 2001). The most dramatic differences between antibodies with and without oligosaccharides are Fc effector functions. Near complete loss of binding affinity of antibodies to the first component of complement (C1q) and Fc receptors have been reported in various antibodies without oligosaccharides (Boyd et al., 1995; Mimura et al., 2000, 2001; Nose and Wigzell, 1983; Tao and Morrison, 1989). The loss of Fc functionality is most likely a reflection of conformational changes as there are only minimal direct interaction between Fc oligosaccharides and the effector molecules including C1q and Fc receptors (Jefferis et al., 1998; Kato et al., 2000; Sondermann et al., 2000).

Oxidation of Met residues is a common non-enzymatic modification, which has been demonstrated to change protein structure, function and stability (Brot and Weissbach, 1983; Carp et al., 1982; Chugha et al., 2006; Gao et al., 1998; Kim et al., 2001; Kornfelt et al., 1999; Lu et al., 1999; Teh et al., 1987). There are several reports on the oxidation of Met residues in monoclonal antibodies. Although Met residues of different regions of antibodies have been oxidized under various conditions (Chumsae et al., 2007; Kroon et al., 1992; Lam et al., 1997; Matamoros Fernandez et al., 2001; Roberts et al., 1995; Shen et al., 1996), two Met residues in the Fc region of recombinant humanized monoclonal antibodies are most susceptible to oxidation (Chumsae et al., 2007; Lam et al., 1997; Shen et al., 1996). The two susceptible Met residues are located in the CH2 and CH3 domains in the primary structure, respectively, however in the three-dimensional structure, they locate close to the CH2-CH3 interface and the oligosaccharides, which are

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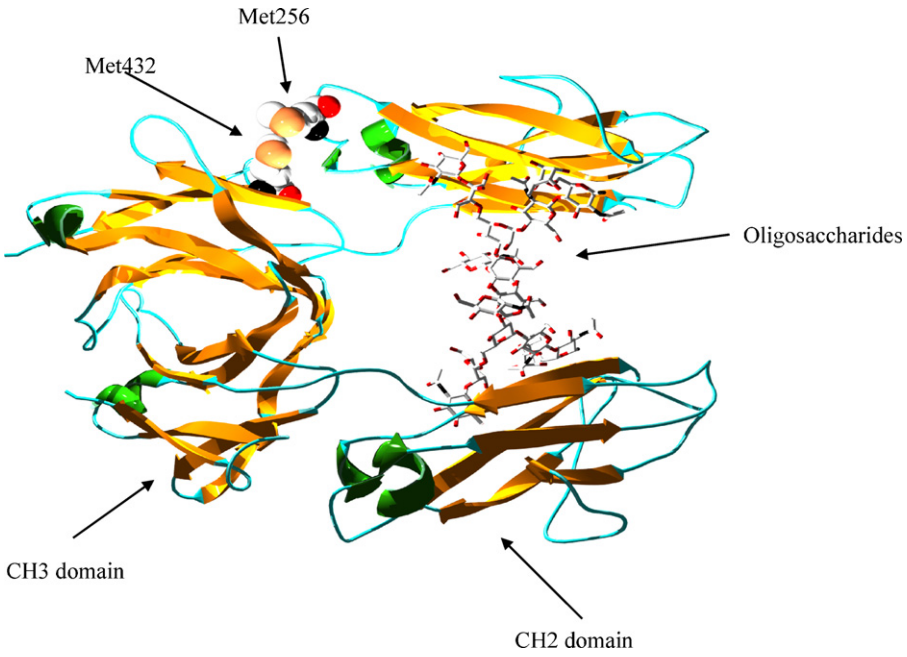

Fig. 1. Crystal structure of human IgG1-Fc drawn from published coordinates. Met256 (IMGT CH2-15.1) and Met432 (IMGT CH3-107) are labeled. The glycosylation site is Asn301 (IMGT CH2-84.4).

enclosed within the two CH2 domains (Fig. 1). Therefore, it is intriguing to study the relationship of Met oxidation and the presence or absence of oligosaccharides and their effect on the structure and stability of the antibody.

In this study, a recombinant humanized monoclonal antibody (IGHG1*01 with a kappa light chain) was used. Incomplete processing of the C-terminal lysine (Lys) residues results in the antibody with zero, one or two C-terminal Lys. The five major forms of oligosaccharides include three biantennary complex structure with core fucose with either zero (Gal 0), one (Gal 1) or two (Gal2) terminal galactose and two oligomannose with either five or six mannose residues. Each light chain has one Met (IMGT (Lefranc et al., 2003), FR1-Met4) residue. Each heavy chain has four Met residues with two in the Fab region (IMGT (Lefranc et al., 2003), CDR1-Met35 and FR3-Met91) and two in the Fc region (IMGT (Lefranc et al., 2005), CH2-Met15.1 and CH3-Met107). The antibody with and without oligosaccharides was oxidized with different amounts of *tert*-butyl hydroperoxide (tBHP). Oxidation sites and percentage were determined by liquid chromatography-mass spectrometry analysis (LC-MS). Conformational changes caused by the removal of oligosaccharides and Met oxidation were probed by limited trypsin and chymotrypsin digestion.

2. Materials and methods

2.1. Materials

The recombinant humanized monoclonal antibody was produced by transfected Chinese hamster (CHO) ovary cell lines and purified at Abbott Bioresearch Center (Worcester, MA). *tert*-Butyl hydroperoxide (tBHP), dithiothreitol (DTT), and iodoacetic acid were purchased from Sigma (St. Louis, MO). Formic acid (FA) was purchased from EMD (Madison, WI). PNGaseF was purchased from Prozyme (San Leandro, CA). Lys-C, chymotrypsin, phenylmethylsulfonyl fluoride (PMSF) and *N*-octylglucoside were purchased from Roche (Indianapolis, IN). Acetonitrile, trifluroacetic acid (TFA), guanidine hydrochloride and 1N hydrochloride acid (HCl) were purchased from J.T. Baker (Phillipsburg, NJ). Trypsin was purchased from Worthington (Lakewood, NJ).

2.2. Deglycosylation of the recombinant monoclonal antibody

The recombinant monoclonal antibody at approximately 70 mg/mL in formulation buffer (5.57 mM sodium phosphate monobasic, 8.69 mM sodium phosphate dibasic, 106.69 mM sodium chloride, 1.07 mM sodium citrate, 6.45 mM citric acid, 66.68 mM mannitol and 0.1% Tween) at pH 5.2, was diluted to 10 mg/mL using 10 mM sodium phosphate, pH 7.5. PNGaseF (2.5 mU/μL) was added to the diluted sample based on the ratio of 1 μL enzyme:500 μg antibody. *N*-Octylglucoside was included in the sample preparation to a final concentration of 1% to facilitate the removal of N-linked oligosaccharides. Digestion was allowed to proceed at 37 °C for 18 h. The same antibody diluted to 10 mg/mL using 10 mM sodium phosphate, pH 7.5, with 1% *N*-octylglucoside but without PNGaseF was also incubated at 37 °C for 18 h and used as a control.

2.3. Oxidation of methionine residues

The deglycosylated and control antibody samples were buffer exchanged to 10 mM sodium phosphate, pH 7.5 using Amicon Ultra-4 centrifugal filter device (Millipore, Billerica, MA) with a molecular weight cut-off of 10 kDa and then diluted to 10 mg/mL