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Effect of methionine oxidation of a recombinant monoclonal antibody on the binding affinity to protein A and protein G

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ABSTRACT

Oxidation of methionine (Met) residues is one of the most common protein degradation pathways. Two Met residues, Met256 and Met432, of a recombinant fully human monoclonal IgG1 antibody have been shown to be susceptible to oxidation. Met256 and Met432 are located in the antibody CH_2–CH_3 interface and in close proximity to protein A and protein G binding sites. The effect of oxidation of these susceptible Met residues on the binding to protein A and protein G was investigated in the current study. Incubation of the antibody with 5% *tert*-butyl hydroperoxide (tBHP) resulted in a nearly complete oxidation of Met256 and Met432, while incubation with 1% tBHP resulted in mixed populations of the antibody with different degrees of Met oxidation. Oxidation of Met256 and Met432 resulted in earlier elution of the antibody from protein A and protein G columns when eluted with a gradient of decreasing pH. Analysis by ELISA and surface plasmon resonance (SPR) revealed decreased binding affinity of the oxidized antibody to protein A and protein G. It is therefore concluded that oxidation of the Met256 and Met432 residues of the recombinant monoclonal antibody altered its interaction with protein A and protein G resulting in a decrease in binding affinity.

1. Introduction

Methionine (Met) is one of the most susceptible residues to oxidation. Oxidation of Met has been shown to affect the structure, stability and biological functions of a variety of proteins [1]. Oxidation of Met is a major instability factor of protein pharmaceuticals [2] including monoclonal antibodies [3].

Oxidation of Met residues has been reported for mouse, humanized and fully human monoclonal antibodies. Oxidation of one Met on the light chain and four Met residues on the heavy chain of a murine monoclonal antibody, OKT3 after storage at 2–8 °C for 14 months to 3 years has been reported [4,5]. Shen et al. [6] reported oxidation of Met255 and Met431 of a recombinant humanized monoclonal antibody, HER2, after incubation with tBHP at room temperature for 20 h. Lam et al. [7] reported oxidation of Met255 and Met431 after incubation of HER2 at 30 and 40 °C or exposure to intense light at 27 °C for 2 weeks. Chumsae et al. [8] reported that Met256 and Met432 were oxidized after storage at 25 °C for 12 months or incubation with tBHP. These two susceptible Met residues in humanized [6,7] and fully human [8] antibodies are at the same positions of CH_2-15.1 and CH_3-107 based on the IMGT unique numbering system [9]. They are located in the CH_2–CH_3 interface [10] in the AB-LOOP and FG-LOOP in the three-dimensional structure [9] (Fig. 1).

IgG antibodies can be divided into two functional units, the Fab region for antigen binding and the Fc region for effector functions as well as for binding to protein A and protein G. Oxidation of Met256 and Met432 in the Fc region is not expected to affect antigen binding because of the presence of the highly flexible hinge region. This assumption was confirmed by a study using the recombinant humanized monoclonal antibody, HER2 [7], where no antigen binding differences were detected after oxidation of these susceptible Met residues. On the other hand, oxidation of Met256 and Met432 may affect the binding of Fc to Fcγ receptors, the first component of complement (C1q), neonatal Fc receptor (FcRn), protein A and protein G. Fcγ receptors and C1q share overlapping as well as distinct binding sites in the hinge proximal region of the CH_2 domain [11–13], while the binding sites of FcRn [13,14], protein A [10] and protein G [15] are located around the CH_2–CH_3 interface.

Protein A and protein G are commonly used for affinity chromatography to purify antibodies. The binding sites of the antibody to protein A and protein G are in the same vicinity as the Met residues that are susceptible to oxidation. Therefore, the effect of Met oxidation on the binding affinity of a recombinant fully human monoclonal IgG1 antibody to protein A and protein G was investigated in this study. Met256 and Met432 of the recombinant

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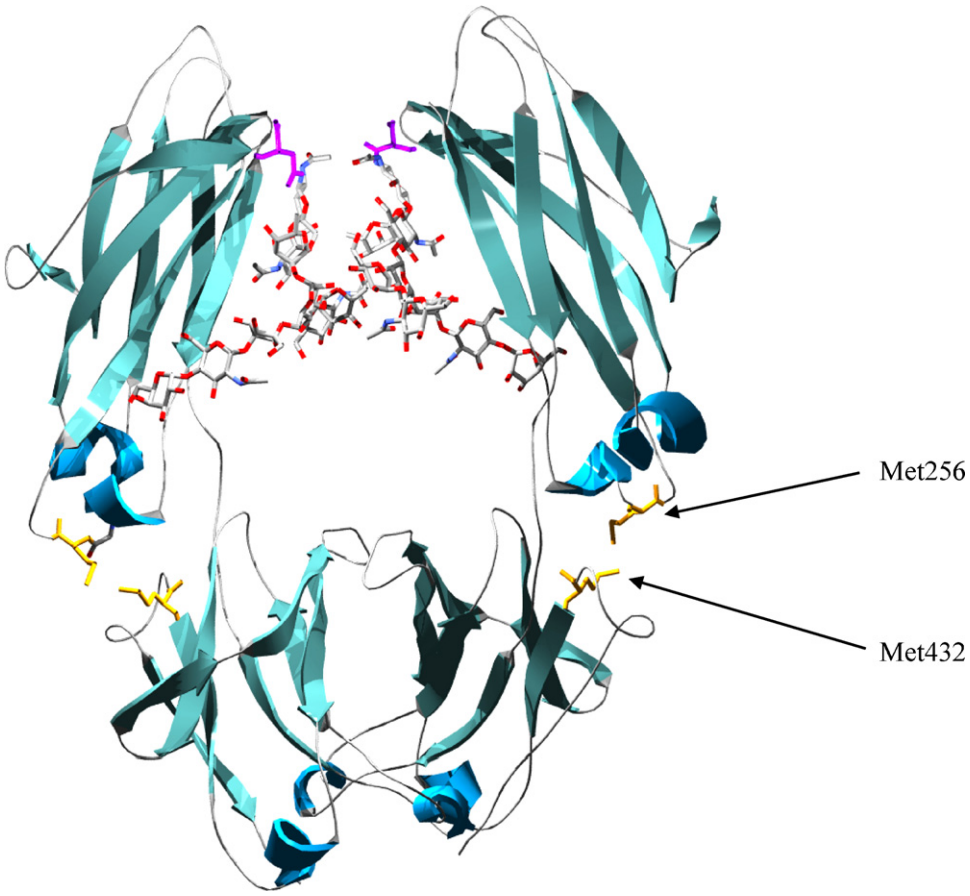

Fig. 1. Crystal structure of human IgG1–Fc. Met256 and Met432 are labeled in the structure. The structure was drawn using public information.

antibody were oxidized by incubation with different amounts of the oxidizing reagent, tBHP. Levels of oxidation were determined using liquid chromatography–mass spectrometry (LC–MS). Binding affinity of the antibody with different levels of oxidation was assessed using protein A and protein G chromatography, ELISA and surface plasmon resonance analysis.

2. Materials and methods

2.1. Materials

The recombinant fully human monoclonal antibody was produced by a transfected chinese hamster ovary (CHO) cell line and purified at Abbott Bioresearch Center (Worcester, MA). *Tert*-butyl hydroperoxide (tBHP) and dithiothreitol (DTT) were purchased from Sigma (St. Louis, MO). Formic acid (FA) was purchased from EMD (Madison, WI). Lys-C, phenylmethylsulfonyl fluoride (PMSF) and *N*-octylglucoside were purchased from Roche (Indianapolis, IN). Acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ).

2.2. Oxidation of methionine residues

The recombinant monoclonal antibody was diluted with phosphate buffered saline (PBS) to 10 mg/mL. Oxidizing reagent tBHP was included in the sample preparations at a final concentration of 1 or 5% and the samples were incubated at room temperature for 18 h. A sample diluted with PBS to 10 mg/mL without the addition of tBHP was also incubated at room temperature for 18 h and used as a control, referred to as the native antibody. The native and tBHP treated samples were dialyzed against 4 L of PBS at 4 °C overnight using a dialysis tubing with a molecular weight cut-off of 6000–8000 (Spectrum Laboratories, Inc. Rancho Dominguez, CA). Three changes of PBS were made during this period (6–8 h between each change). The concentrations of the recovered samples were determined by UV 280 nm using the calculated extinction coefficient based on the known sequence of this antibody.

2.3. Protein A and protein G chromatography

A Shimadzu HPLC and a Poros A (4.6 mm × 50 mm, Applied Biosystems, Framingham, MA) or Poros G column (4.6 mm × 50 mm, Applied Biosystems) were used for the analysis of the samples.

For protein A chromatography, PBS was used as mobile phase A and 0.1 M acetic acid and 0.15 M sodium chloride, pH 2.9, was used as mobile phase B. One hundred microliters of each sample at 10 mg/mL in PBS was loaded on the poros A column. After loading each sample, the column was washed with 100% mobile phase A for 10 min, then the sample was eluted from the column using a gradient of increasing mobile phase B to 60% at a rate of 2% mobile phase B/min. The column was washed for 10 min using 100% mobile phase B and then equilibrated using 100% mobile phase A for another 10 min before the next injection. The flow-rate was set at 2 mL/min and the elution of the antibody was monitored by UV at 214 and 280 nm.

For protein G chromatography, a much harsher elution condition was necessary. PBS was used as mobile phase A, while mobile phase B was 0.1 M acetic acid and 0.15 M sodium chloride at a pH of 2.5. One hundred microliters of each sample at 10 mg/mL in PBS was loaded on the poros G column at 20% mobile phase B. The col-

umn was washed using 20% mobile phase B for 10 min and then a gradient of increasing mobile phase B from 20 to 100% at a rate of 2%/min was used to elute the antibody. The column was washed with 100% mobile phase B for 10 min and then equilibrated with 20% mobile phase B for 10 min before the next injection. The flow rate was set at 2 mL/min and antibody elution was monitored using UV 214 nm and UV 280 nm.

For detailed analysis, fractions from protein A and protein G chromatography were collected. Approximately 5 mg of the antibody oxidized with 1% tBHP was loaded onto the columns and then eluted using the conditions as described previously. Fractions were collected every 2 min during the elution. The fractions were then concentrated using Amicon Ultra-4 centrifugal filter devices with a molecular weight cut-off of 30 kDa (Millipore, Billerica, MA). Protein concentrations were determined by UV 280 nm and the calculated extinction coefficient based on the known amino acid sequence of the antibody.

2.4. Lys-C digestion and LC–MS analysis

The levels of oxidation were determined by LC–MS. The oxidized samples and the collected protein A and protein G chromatography fractions were diluted to 0.5 mg/mL using PBS. Lys-C was added to 100 μL of each sample to a final 1:200 (w:w) Lys-C:antibody ratio. Digestion was allowed to proceed at 37 °C for 30 min. PMSF prepared in methanol was added to each sample at a final concentration of 2 mM to stop the Lys-C digestions. The samples were then reduced with 20 mM DTT at 37 °C for 5 min.

An Agilent HPLC (Santa Clara, CA) and a protein C4 column (Vydac, 150 mm × 1 mm i.d., 5 μm particle size, 300 A pore size) were used to desalt, separate and introduce samples into a Qstar mass spectrometer (Applied Biosystems). Ten microliters of each sample was loaded separately at 95% mobile phase C (0.08% FA in Milli-Q water) and 5% mobile phase D (0.08% FA in acetonitrile). After 5 min at 5% mobile phase D, proteins were eluted off the column by increasing mobile phase D to 65% within 35 min. The column was washed with 95% mobile phase D for 5 min and then equilibrated with 5% mobile phase D for 10 min before the next injection. The flow rate was set at 50 μL/min. The column oven temperature was set at 60 °C. The mass spectrometer was operated at positive mode with a scan range of m/z 800–2500. IonSpray voltage was set at 4500 V and the source temperature was set at 350 °C.

2.5. ELISA

The binding affinity of the antibody with and without Met oxidation to protein A and protein G was compared using a direct ELISA method. The native and the oxidized antibody were first diluted in 50 mM bicarbonate buffer, pH 9.4, to 20 μg/mL, then the samples were further diluted serially with a 2-fold dilution of each step. One hundred microliters of the serially diluted samples were immobilized onto three 96-well plates. The plates were incubated overnight at 4 °C with shaking. After removal of the unbound antibody, the plates were blocked with SuperBlock blocking buffer (Pierce, Rockford, IL) (300 μL/well) for 1 h at 37 °C with shaking. The plates were then washed five times with PBS with 0.1% Triton X-100 using a plate washer (Tecan, Zurich, Switzerland). The plates were incubated for 1 h at 37 °C with 100 μL/well with either protein A-horse radish peroxidase (HRP) conjugate (Millipore, Billerica, MA), protein G–HRP (Millipore) or goat anti-human IgG–HRP (Jackson ImmunoResearch, West Grove, PA) that was diluted 50,000 times in SuperBlock. After one wash with PBS, 100 μL/well of TMB substrate, K Blue (Neogen, Lansing, MI), was added then incubated at room temperature for 5 min. The reaction was stopped by the addition of 100 μL/well of 1N phosphoric acid. The plates were then read at 450 nm using a Spectramax Plus plate reader (Molecular Devices, Sunnyvale, CA). The data was fitted using SoftMax Pro Version 4.8 (Molecular Devices).

2.6. Analysis by surface plasmon resonance

Binding kinetics of the native and 5% tBHP treated sample to protein A and protein G was measured by surface plasmon resonance using a Biacore 2000 or Biacore 3000 instrument (Biacore AB, Uppsala, Sweden). Protein A (GE Healthcare, Chicago, IL) or protein G (GE Healthcare, Chicago, IL) was immobilized on a CM5 research grade biosensor chip using a standard amine coupling kit. Unreacted moieties on the biosensor surface were blocked with ethanolamine. The native and the 5% tBHP treated samples were diluted in HEPES buffered saline (HBS) with 0.015% surfactant P20 (Biacore AB, Uppsala, Sweden). A series of the native or tBHP treated samples in the concentration ranges of 0.078–100 nM were injected over the protein A or protein G coated chip at a flow rate of 75 μL/min. Refractive index was recorded and used to calculate the kinetic parameters. Surfaces were regenerated with two subsequent 30 μL injections of 10 mM glycine (pH 1.5) at a flow rate of 75 μL/min. The data was fitted with a 1:1 binding model using BiaEvaluation software (Biacore AB, Uppsala, Sweden).

3. Results

3.1. Oxidation of the antibody with 1 and 5% tBHP

The recombinant monoclonal antibody used in this study contains a total of 10 Met residues with one (Met4) on each light chain and four (Met34, Met83, Met256 and Met432) on each heavy chain. A previous study showed that only Met256 and Met432 in the Fc region were susceptible to oxidation [8]. This result was further confirmed by the analysis of the native and tBHP treated samples in the current study. No oxidation in the Fab region was observed either in the native or in the 5% tBHP treated sample (data not shown). A mass spectrum corresponding to the Fc region of this antibody from the native is shown in Fig. 2A. The observed molecular weight of 26,620 Da was in good agreement with the calculated molecular weight of 26,618 Da, which corresponded to reduced Fc with a core fucosylated biantennary complex oligosaccharide without terminal galactose residues and without the C-terminal lysine residue on the heavy chain. One minor peak and two major peaks were observed in the sample after treatment with 1% tBHP with molecular weights of 26,620, 26,636, and 26,651 Da (Fig. 2B), which corresponded to reduced Fc with zero, one or two oxidation sites, respectively. Based on this result, it was concluded that the intact antibody contained either zero, one, two, three or four sites of oxidation. Two peaks with molecular weights of 26,636 and 26,652 Da were observed for the Fc after treatment with 5% tBHP (Fig. 2C), which corresponded to reduced Fc with one or two sites of oxidation respectively and intact antibody with two, three or four sites of oxidation. No significant level of oxidation was detected in the native antibody, indicating that incubation of the antibody without the addition of tBHP did not cause oxidation to a detectable level.

3.2. Protein A and protein G chromatography

The native antibody and the samples after incubation with either 1% tBHP or 5% tBHP were analyzed by protein A chromatography. As shown in Fig. 3, native antibody eluted off the column in the time window of 23–27 min. The antibody after incubation with 5% tBHP eluted in the time window of 16–22 min. The antibody after incubation with 1% tBHP eluted in the time window of 18–27 min.

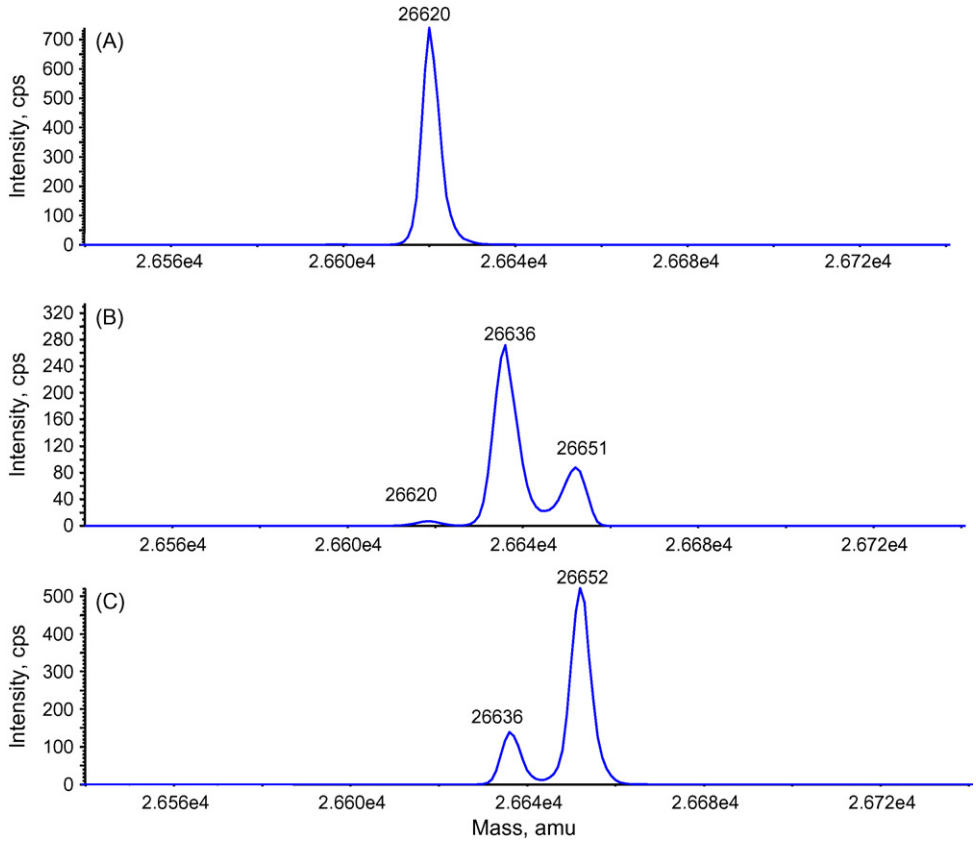

Fig. 2. Mass spectra of the reduced Fc of the recombinant monoclonal antibody. The data was acquired from the native (A), 1% tBHP (B) and 5% tBHP (C) treated samples. Only the spectra corresponding to the reduced Fc with Gal 0 and without C-terminal Lys are shown in the figure.

These results clearly demonstrated that oxidation decreased the retention time of the antibody. There was a shoulder in front of the main peak in the native antibody, which indicated that a low percentage of oxidation was likely present in this sample due to sample handling. A small peak that eluted after the main peak was observed in the sample that was treated with 5% tBHP and this was probably due to oxidation to a relatively lower level compared to the main peak. Several peaks were recognized in the sample treated with 1% tBHP, which indicated that different levels of antibody oxidation were differentiated by the protein A column.

A similar trend was observed when the antibody with or without treatment with either 1 or 5% tBHP was analyzed by protein G chromatography (Fig. 4). The sample treated with 5% tBHP eluted earlier than the sample treated with 1% tBHP. The native sample eluted last. Therefore, oxidation of Met256 and Met432 also decreased the retention times of the antibody from protein G chromatography. However, unlike protein A chromatography, no distinct peaks were observed in the samples after oxidation with 1 or 5% tBHP, which suggests that protein A offered a slightly better resolution.

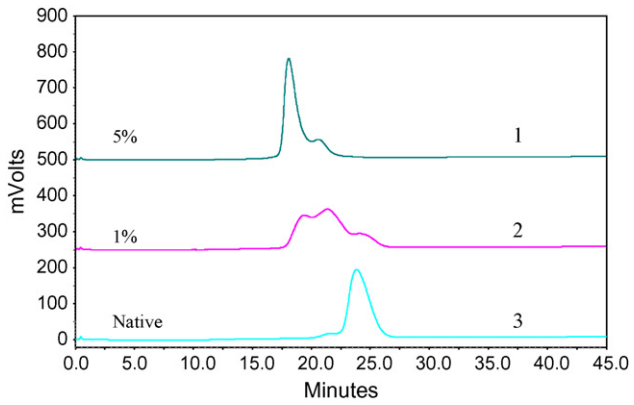

Fig. 3. Protein A chromatograms of the recombinant monoclonal antibody. The data was recorded from analyses of the 5% tBHP (1) and 1% tBHP (2) treated samples and the native antibody (3).

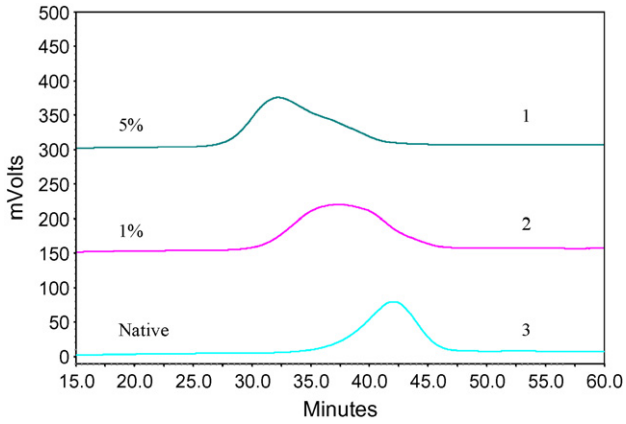

Fig. 4. Protein G chromatograms of the recombinant monoclonal antibody. The data was recorded from analyses of the 5% tBHP (1) and 1% tBHP (2) treated samples and the native antibody (3).

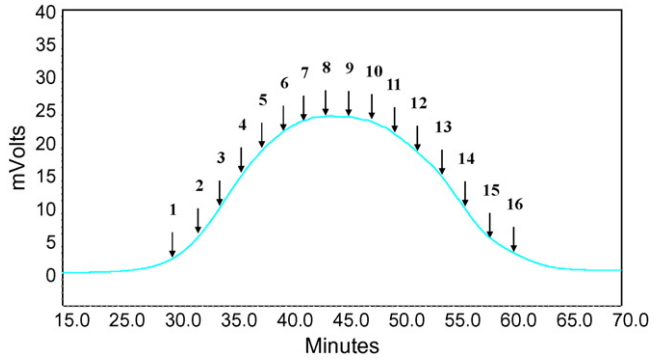

Fig. 5. A typical protein G chromatogram of the antibody after treatment with 5% tBHP. The numbers indicate the starting points of the different fractions that were collected.

3.3. Protein A and protein G chromatography fractions

To further confirm that oxidation of Met256 and Met432 decreased the binding affinity of the antibody to protein A and protein G columns, fractions from the 1% tBHP treated sample were collected for LC–MS analysis. A typical protein G chromatogram is shown in Fig. 5 to illustrate how the fractions were collected. Each fraction was digested with Lys-C to obtain Fab and Fc regions. After reduction, the samples were analyzed by LC–MS. As shown in Fig. 6, the reduced Fc from the earlier fractions from protein G chromatography contained higher levels of oxidation, while the reduced Fc from the later fractions contained lower levels or no Met oxidation sites. A similar trend was observed when the fractions from protein A chromatography using 1% tBHP treated sample was analyzed (data not shown). Antibody with four sites of Met oxidation

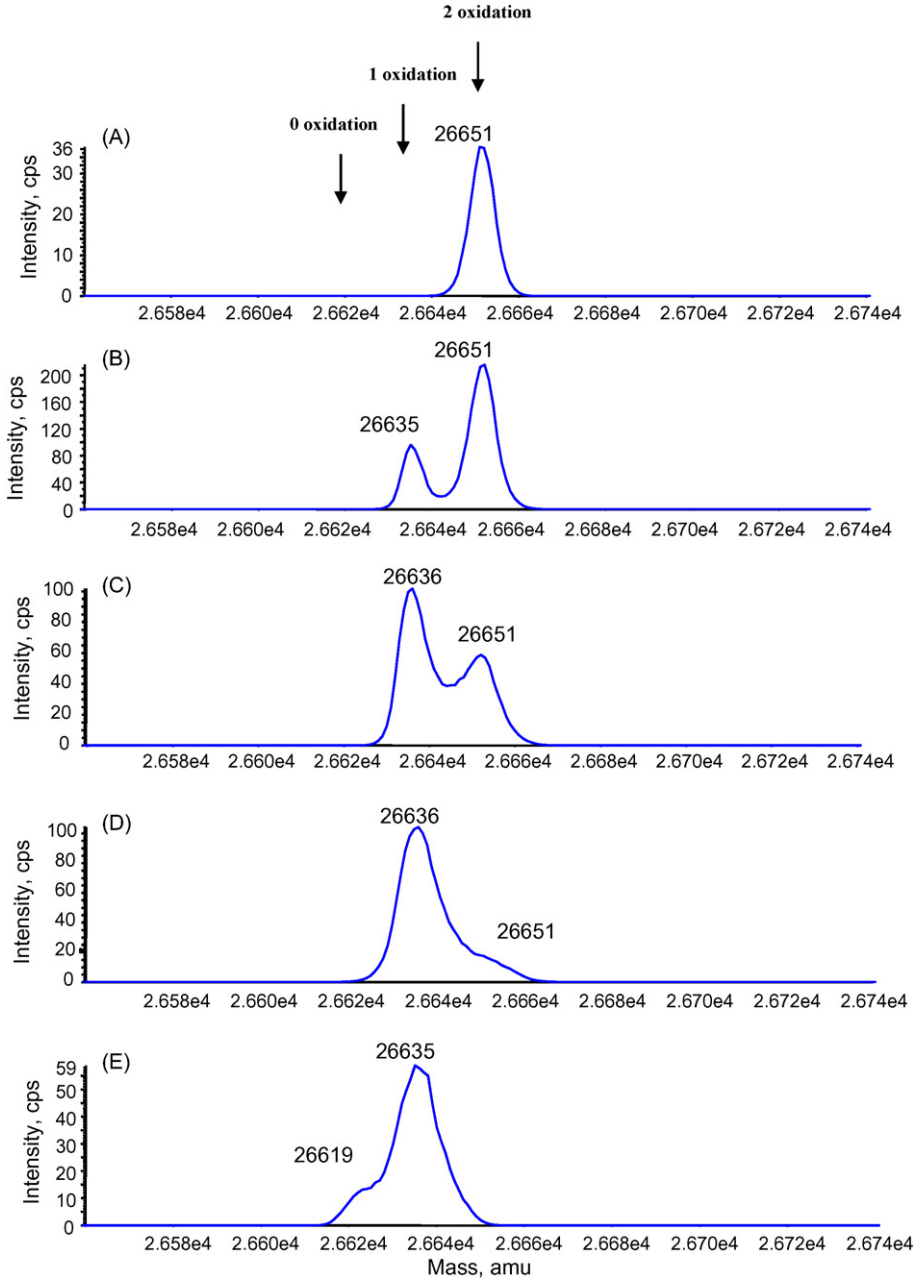

Fig. 6. Representative mass spectra of the protein G chromatography fractions. The spectra were acquired from fractions 4 (A), 6 (B), 8 (C), 10 (D) and 12 (E). The peaks corresponding to the different number of oxidation sites are labeled in panel A.

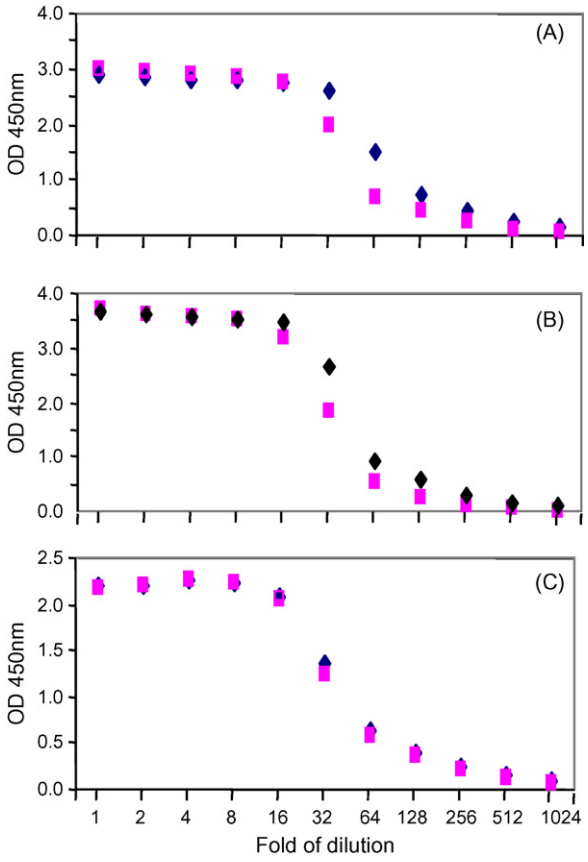

Fig. 7. Binding ELISA of the antibody to protein A and protein G. The native (diamond) and 5% tBHP treated antibody (square) were immobilized and then detected with either protein A–HRP (A), protein G–HRP (B) or anti-human IgG–HRP (C).

eluted from the protein A column first, antibody with two or three sites eluted next and antibody with zero or one site of Met oxidation eluted last. These results confirmed the earlier finding that oxidation of Met caused the antibody to elute earlier from the protein A and protein G columns. Furthermore, it demonstrated that there was a gradual decrease in the binding affinity to the antibody with the gradual increase in the levels of oxidation.

3.4. ELISA

The binding affinity of the native and 5% tBHP treated sample to protein A (Fig. 7A) and protein G (Fig. 7B) was measured by ELISA. The data was fitted using a four-parameter logistic curve fitting using the SoftMax software (Molecular Devices). The *C* values, which represent the concentration of the antibody at 50% maximum binding capacity, are shown in Table 1. The *C* values obtained with the oxidized antibody were higher. Therefore, binding of the oxidized antibody to protein A and protein G was weaker compared

Table 1
Antibody concentration at 50% of the maximum binding capacity

HRP-conjugate	Samples	*C* values (mg/mL)
Protein A	Native	2.99E−4
	5% tBHP	5.06E−4
Protein G	Native	4.64E−4
	5% tBHP	6.24E−4
Goat anti-human	Native	5.22E−4
	5% tBHP	5.76E−4

Table 2
Binding kinetics of the native and 5% tBHP treated samples

Samples	On-rate (k_a) ($M^{-1}\,s^{-1}$)	Off-rate (k_d) (s^{-1})	Equilibrium dissociation constant (K_D) (M)
Protein A			
Native	6.82E5	1.74E−4	2.59E−10
5% tBHP	15.6E5	17.6E−4	11.3E−10
Protein G			
Native	1.25E6	1.93E−4	1.54E−10
5% tBHP	1.22E6	4.02E−4	3.33E−10

to the native antibody. The binding difference was not due to the difference in the immobilization efficiency between the native and the oxidized antibody as both resulted in similar signals when probed with anti-human IgG antibody (Fig. 7C).

3.5. Analysis by surface plasmon resonance

Analysis by ELISA demonstrated that oxidation of Met256 and Met432 decreased binding affinity of the oxidized antibody to both protein A and protein G. However, this analysis did not identify if this change was due to changes in association or dissociation rates. To characterize this, the binding kinetics of native and 5% tBHP treated antibody to protein A and protein G, were compared using surface plasmon resonance. Inspection of the sensorgrams (Fig. 8) revealed faster dissociation of the oxidized antibody from both protein A and protein G. The data were fitted using a 1:1 binding model. The fitted parameters from these fits are summarized in Table 2. The binding affinity to both protein A and protein G was reduced for antibody treated with tBHP. Oxidation of Met256 and Met432 resulted in approximately a 2-fold increase in the on-rate and 10-fold increase in the off-rate of the antibody to protein A, which led to a 4.2-fold increase in equilibrium dissociation binding constant (K_D). The on-rate was similar for the native and the oxidized antibody to protein G, but the off-rate of the oxidized antibody increased 2.1-fold, which resulted in a 2.2-fold increase in the K_D.

In summary, oxidation of Met256 and Met432 of the antibody resulted in a greater effect of the antibody binding to protein A than to protein G as revealed by SPR (Table 2) as well as ELISA (Table 1). In addition, the decreased binding affinity of the oxidized antibody to protein A and protein G was mainly due to an increase in the off-rate.

4. Discussion

Two methionine residues in the CH_2–CH_3 domain interface are conserved in human IgG [16] and are susceptible to oxidation [6,7,8,17]. Oxidation of these residues decreased the stability of the CH_2 domain [18], while no effect on antigen binding affinity was observed. These Met residues are located in the regions of IgG molecules that are important for binding of the neonatal receptor (FcRn) [13], protein A [10] and protein G [15]. The effect of oxidation of these susceptible Met residues on the binding to protein A and protein G was investigated using a fully human monoclonal IgG1 antibody.

Only Met256 and Met432 of the fully human IgG1 antibody was oxidized when the antibody was incubated with tBHP, which is a mild oxidizing reagent that specifically oxidizes surface-exposed Met residues to form sulfoxide [18]. Oxidizing reagent tBHP has been used to probe the susceptibility of Met residues of recombinant monoclonal antibodies [6–8]. In agreement with previous studies using humanized [6,7] and fully human [8] recombinant monoclonal antibodies, only Met residues, Met256 and Met432,

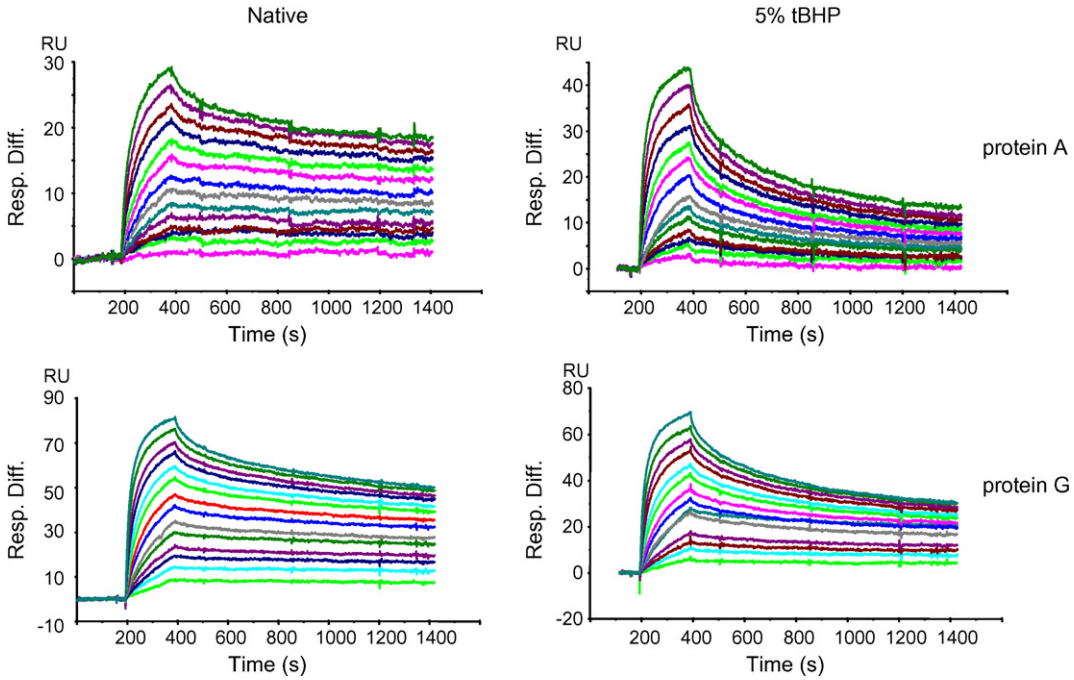

Fig. 8. SPR analysis of the binding of the antibody to protein A and protein G. The sensorgrams were acquired from the binding of the native (left) and oxidized (right) antibody to protein A (upper) and protein G (lower).

were oxidized when the antibody was incubated with either 1 or 5% tBHP. As demonstrated by mass spectrometry analysis, incubation of this antibody with 1% tBHP generated reduced Fc with zero, one or two Met oxidation sites, which suggested that the intact antibody contained either zero, one, two, three or four Met oxidation sites. Incubation with 5% tBHP generated reduced Fc with mainly two sites, which indicated that at the intact level, the antibody was mostly completely oxidized.

Oxidation of Met256 and Met432 decreased the binding affinity of the antibody to protein A and protein G as measured by chromatography, ELISA and surface plasmon resonance. The decreased affinity of the oxidized antibody to protein A and protein G could be due to a combination of three effects including a direct effect, effect by modulating local structures around residues that are involved in direct binding and effect by modulating the global structure of the antibody around the binding sites. Firstly, crystal structures of human IgG–Fc in complex with fragments of protein A [10] and protein G [15] showed direct contact of Met256 with protein A and protein G through hydrophobic interactions. Such a contact was also detected by NMR analysis of the mouse IgG–Fc in complex with fragments of protein A and protein G in solution [19,20]. Met432 also makes direct contact through a hydrophobic interaction with protein G [15]. Oxidation of Met to Met sulfoxide results in increased polarity, which is expected to decrease the binding to protein A and protein G. Secondly, oxidation of Met256 and Met432 could also change the binding affinity indirectly by modulating the local structure. In close proximity to Met256, residues L255 (L251 in human IgG), I257 (I253 in human IgG) and S258 (S254 in human IgG) are in direct contact with protein A and protein G [10,15]. In close proximity to Met432, residues such as N438 (N434 in human IgG), H439 (H435 in human IgG), and Y440 (Y436 in human IgG) are in direct contact with protein A [10], while H437 (H433 in human IgG), N438, Y440 and Q442 (Q438 in human IgG) are in direct contact with protein G [15]. A change in the chemical property of Met256 and Met432 could cause local structural changes around the residues that are directly involved in binding, which may lead to a decrease in the binding affinity of the neighboring amino acids to protein A or protein G. Thirdly, it has been demonstrated that oxidation of Met256 and Met432 can cause a significant decrease in the stability of the CH_2 domain [17], which suggests that a rather global conformational change occurs in the CH_2 domain. This conformational change can also cause decreased binding affinity to protein A and protein G.

There is another factor that needs to be considered when comparing the earlier elution of the oxidized antibody from both the protein A and protein G columns. The antibody was eluted off the column using a gradient of decreasing pH. It has been well documented that low pH can induce a significant conformational change in the CH_2–CH_3 interface [21–23]. If exposure to low pH during elution caused a more significant conformational change in the oxidized antibody than in the native antibody, it could lead to an earlier elution of the oxidized antibody. Therefore, the earlier elution of the oxidized antibody from the protein A and protein G column was likely due to both the decreased binding affinity to protein A and protein G as detected by ELISA and surface plasmon resonance analysis and instability caused by oxidation under low pH conditions.

It is worthwhile to mention that surface plasmon resonance revealed more complicated binding kinetics for antibody binding to protein A and protein G at higher analyte (antibody) concentrations. Although the simplest available model that provided adequate goodness-of-fit parameters was chosen, a more detailed study of the binding kinetics, which is beyond the scope of this paper, is necessary to adequately explain these observations. It is important to note that analysis of our data using more complex models did not change the effects being reported, and therefore do not affect our conclusions.

In summary, oxidation of Met residues in the CH_2–CH_3 domain interface decreased the binding affinity of a recombinant fully human monoclonal antibody to protein A and protein G. The decreased binding affinity was evidenced by an earlier elution of the oxidized antibody from protein A and protein G chromatography, by ELISA and surface plasmon resonance. Furthermore, other

proteins that bind to the CH_2–CH_3 region of the antibody may also be affected by oxidation of Met432 and Met256. For example, FcRn binds to this region of the antibody [13,14] and a change in binding affinity could have an impact on the half-life of the antibody in vivo. Protein A and protein G are the two most commonly used ligands for affinity purification of antibodies. The findings from this study provide useful information on the use of protein A and protein G affinity chromatography to possibly detect and separate antibodies modified by Met oxidation.

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