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RESEARCH PAPER

The Photolysis of Disulfide Bonds in IgG1 and IgG2 Leads to Selective Intramolecular Hydrogen Transfer Reactions of Cysteine Thiyl Radicals, Probed by Covalent H/D Exchange and RPLC-MS/MS analysis

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ABSTRACT

Purpose The evaluation of photo-instability of biotherapeutic products is mandated by regulatory agencies. Photo-irradiation can induce oxidative modifications in proteins, which may lead to undesired biological and therapeutic consequences. Among the modifications, epimerization of amino acid residues can occur upon photo-irradiation of IgGs.

Methods We show here, that UV irradiation ($\lambda = 253.7$ nm) of IgG1 and IgG2 leads to the formation of intermediary carbon-centered radicals, validated by covalent incorporation of deuterium into the protein primary sequence.

Results By MS/MS analysis we identified the sites of deuterium incorporation, such as the sequence QD [303:304, HC], present in the peptide of WSVLTVWHQDWLNGK [294:309, HC] in both IgG1 and IgG2, and V [111, LC] and K [116, LC], present in the peptide VTVLGQPK [109:116, LC] in IgG2. Both peptides are in the proximity of intrachain disulfide bonds.

Conclusions The exposure of IgG1 and IgG2 to UV-light ($\lambda = 253.7$ nm) generates specific carbon-centered radicals. The latter were evidenced by a covalent H-D exchange reaction that likely occurred through a hydrogen atom transfer reaction between cysteine thiyl radical and C-H bond.

KEY WORDS carbon-centered radical · covalent hydrogen/deuterium (H/D) exchange · cysteine thiyl radical (CysS•) · intramolecular hydrogen transfer · photo-degradation

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INTRODUCTION

Immunoglobulin G (IgG) monoclonal antibody (mAb) biotherapeutics have gained significant attention and become a fast growing biotherapeutic class in the pharmaceutical industry (1–3). These protein therapeutics are subject to ultraviolet (UV) and/or visible light exposure during development, manufacturing, inspection, transportation, storage and administration to the patients. Antibodies contain multiple disulfide bonds, and, similar to many other proteins, are sensitive to light-induced degradation (4). Light testing is defined as an integral part of stress testing for submission in registration application for new molecular entities and associated drug products by the Food and Drug Administration (FDA) under The International Conference on Harmonization (ICH) Tripartite guideline (5). The guideline clearly notes that the intrinsic photostability characteristics of new drug substances and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change. A systematic approach to photostability testing is recommended covering, as appropriate, studies of i) tests on the drug substances, ii) tests on the exposed drug product outside of the immediate packing, and, if necessary, iii) tests on the drug product in the immediate packing, and, if necessary, iv) tests on the drug product in the marketing packing. The resulting instability information is an important element in the quality assurance for drug therapy, e.g., in determining the shelf life, storage instructions, and use life (i.e. stability in an opened container). For photo-labile drug substances and drug products, the formal labeling requirements are established by national/regional requirements.

IgG monoclonal antibodies contain multiple Cys residues, forming inter- and intrachain disulfide bonds. These inter- and intrachain disulfide bonds play an important role for protein stability. On the other hand, disulfide bonds are

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subject to light-induced degradation (4,6,7). Photoirradiation at $\lambda = 253.7$ nm results in the homolytic dissociation of disulfide bonds, leading to the formation of a cysteine thiyl radical (CysS•) pair (8–12). This CysS• radical pair engages in disproportionation (to thiol and thioaldehyde) as well as reversible hydrogen atom transfer reactions with C-H bonds of surrounding amino acids (13–17). Intramolecular hydrogen transfer reactions were confirmed by the covalent incorporation of deuterium into specific amino acid residues when peptides and protein thiyl radicals were generated in D_2O (16,17). These intramolecular hydrogen transfer reactions generate carbon-centered (C-centered) radicals, which ultimately may result in the formation of protein hydroperoxide, protein aggregates and/or protein fragmentation (14). In specific model peptides and proteins, such as insulin, we have demonstrated that reversible hydrogen transfer reaction between CysS• radicals and C-H bonds led to covalent hydrogen/deuterium (H/D) exchange (17), as outlined in Scheme 1 (16). Such reversible hydrogen transfer can also result in L- to D-amino acid conversion, as shown for L-Ala in a model peptide (15). UV irradiation at 254 nm is of potential concern to IgG stability because of its potential use for viral decontamination of biotechnology products (18).

IgG1 and IgG2 represent two important subclasses of IgG. They exhibit differences in the number and connectivity of interchain disulfide bonds. In IgG1, the interchain disulfide bonds link the light chain (LC) and the heavy chain (HC) at the hinge region, whereas in IgG2, the interchain disulfide bonds linking the LC and the HC are located to the junction between the variable and the constant region (19). Based on these different locations of the disulfide bonds, CysS• radicals generated through disulfide bond photolysis may react selectively with different amino acid residues in different domains of IgG1 and IgG2, generating intermediary carbon-centered protein radicals at different locations. Through covalent H/D exchange, we have probed in this paper the reactivity of disulfide bonds, and specifically CysS• radicals in IgG1 and IgG2 toward reversible intramolecular hydrogen transfer. Based on the underlying radical chemistry, sites of covalent H/D exchange indicate potential cleavage sites, i.e. may guide the analytical chemist in the search for fragmentation sites and mechanisms.

MATERIALS AND METHODS

Materials

IgG1 (37 mg/mL) and IgG2 (30 mg/mL) mAb stored as frozen solutions (−80°C) were supplied by Amgen Inc. (Seattle, WA, USA). Dithiothreitol (DTT, >99%) and diethylmaleate (DEM, 97%) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). N-ethylmaleimide (NEM, >99%) and ammonium bicarbonate (NH_4HCO_3) were obtained from Fluka (Saint Louis, MO, USA). Sequencing-grade trypsin and Glu-C were obtained from Promega Corp. (Madison, WI, USA). Deuterium oxide (D_2O, 99.9%) was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

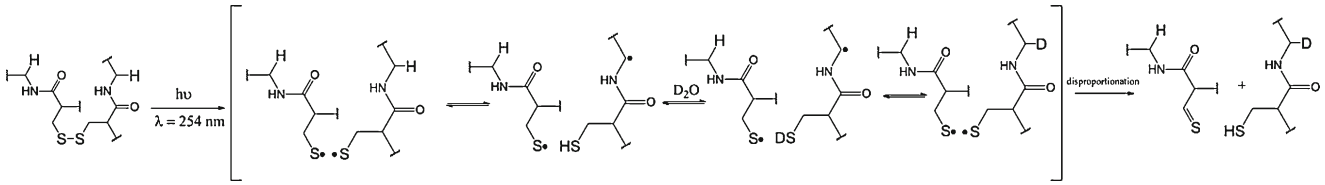
Reactions

UV Irradiation

The IgG1 and IgG2 stock solutions were exchanged into MilliQ H_2O or D_2O at room temperature in a nitrogen (N_2) box using an Amicon® ultra-0.5 centrifugal filter device equipped with a 10 kDa filter membrane (Millipore Inc., Bedford, MA, USA). The samples were incubated in D_2O for no longer than 1 h. The samples were centrifuged by means of a mini-centrifuge (Southwest Science, Roebling, NJ, USA) for 15 mins. An aliquot (200 µL) of each protein stock solution in H_2O or D_2O was transferred to a quartz tube and sealed with a rubber stopper. The sealed quartz tubes containing protein solutions were transferred from the N_2 box and irradiated at room temperature for 1 h with UV light at $\lambda = 253.7$ nm by means of four UV lamps (RMA-500, Southern New England, Branford, CT, USA) in a Rayonet Photo-chemical reactor (Southern New England, Branford, CT, USA).

Thiol Derivatization Reactions and Digestion

The protein solutions in D_2O were exchanged into H_2O by means of an Amicon® ultra-0.5 centrifugal filter device as described above. An aliquot of 100 µL of each protein solution in H_2O was added to 0.9 mL of NH_4HCO_3 buffer (50 mM, pH 7.8). The proteins were denatured by ramping



Scheme 1 proposed reaction mechanism to explain the formation of carbon-centered (C-centered) radical. The mechanism proceeds through an intramolecular hydrogen atom transfer reaction between $^{\alpha}C$ and CysS•.

the temperature from 35°C to 80°C using a water bath. Heat denaturing of the proteins was terminated when the solution turned cloudy. Thiol groups produced during photo- irradiation were derivatized with NEM (0.2 mM) for 1 h at 37°C. The remaining, intact disulfide bonds were reduced by DTT (2 mM) for another 30 mins at 37°C. Then DEM was added at a final concentration of 20 mM to the samples to derivatize the thiol residues resulting from the reduction of disulfide bonds by DTT. That is, the thiol residues produced by photo-irradiation were labeled with NEM whereas DEM was used to alkylate the thiols generated from the disulfide bonds through reduction by DTT. The solutions containing the derivatized proteins were subsequently transferred into 15-mL Falcon™ tubes (BD, Franklin Lakes, NJ, USA) containing 9 mL ethanol and stored at −20°C overnight. The solutions were then centrifuged at 14,000 x *g* to precipitate the proteins. After removal of the supernatant, the protein pellet was re-dissolved in 1 mL NH_4HCO_3 buffer (50 mM, pH 7.8). An aliquot of 100 µL of the derivatized protein solutions was digested first with sequencing-grade trypsin (15 µg) (two doses with an interval of 2 h). Then, the digestion was continued by the addition of sequencing-grade Glu-C (8 µg) by incubating the sample at 37°C overnight.

UPLC-MS and Nano-electrospray Ionization Time-of-Flight (ESI-TOF) MS Analysis

The samples were injected onto a Vydac MS C18 column (25 cm×1 mm, 3.5 µm) from Grace (Deerfield, IL, USA). The mobile phases A and B were composed of water, acetonitrile and formic acid at 99:1:0.08 (v:v:v, %) for A and 0:99:0.06 (v:v:v) for B. The samples were eluted on an Acquity ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, USA) by gradient elution at a flow rate of 12 µL. The gradient elution was composed of three segments defined by increasing contents of solvent B: 1% B for 1 min, 10 to 30% B increased within 8 mins, followed by an increase of B to 70% within 3 mins.

ESI mass spectra were acquired on a SYNAPT G2 high definition mass spectrometer (Waters Corp. Milford, MA, USA). Electrospray ionization mass spectra of the proteins were acquired by operating the SYNAPT G2 for maximum resolution with all lenses optimized on the $[M+2H]^{2+}$ ion from the $[Glu]^1$-fibrinopeptide B and Ar was admitted to the collision cell. The capillary voltage was set at 2.9 kV. The cone voltage was set at 45 V and then ramped to 85 V. The voltage of the extraction cone was maintained at 6 V. Source and desolvation temperatures were set at 95°C and 200°C, respectively. The gas flow at the cone and for desolvation was set at 40 L/h and 250 L/h, respectively. The spectra were acquired within a mass range of 100–2000 amu (amu: atomic mass unit). The data were accumulated for 0.5 s per cycle and processed using the software MassLynx (Waters Corp. Milford, MA, USA).

MS/MS Analysis

Collision-induced dissociation (CID) spectra were acquired by setting the MS^1 quadrupole to transmit a precursor mass window of ±0.2 amu centered on the most abundant isotopomer. MS/MS spectra were acquired with collision energies between 20 eV and 50 eV depending on the mass-to-charge ratio (m/z) of the parent ions.

Identification of Covalent H/D Exchange

The covalent incorporation of deuterium into the proteolytic peptides and their fragments was determined by the variation of base peak intensity (BPI) of the isotopic distribution peaks between the covalently deuterated peptide and the corresponding fully protonated peptide. The isotopic distribution peaks were normalized to the most abundant isotopic peak. The measurements of %BPI were repeated four times. A %BPI intermediate value is provided in the text as well as the average error. The %BPI peaks with a value equal to 100% represent the peaks used for normalization. Therefore, no error is reported for such measurement.

RESULTS

After photo-irradiation, LC-MS analysis of the proteolytic peptides from IgG2 in H_2O and D_2O was performed in order to identify peptides which have covalently incorporated at least one deuterium. Peptide sequence mapping was achieved by searching against a custom-built database of IgG2. A similar analysis was performed for IgG1. The sequence coverage was 87% and 82%, respectively for the HC and LC of IgG2, whereas the sequence coverage was 90% and 92%, respectively, for the HC and LC of IgG1. Two peptides displaying covalent incorporation of deuterium were identified for IgG2, peptide A, VVSVLTVVHQDWLNGK [294:309], located in the constant region (Fc) of the heavy chain (HC), and peptide B, VTVLGQPK [109:116], located in the light chain (LC). For IgG1, only peptide A was identified, which exhibited the same isotopic distribution profile as peptide A originating from IgG2. Therefore, the detailed discussion below will focus on the two peptides A and B originating from IgG2. For simplicity, any peptide analyzed by LC-MS and MS/MS originating from the non-irradiated or the photo-irradiated solutions which were prepared in H_2O, will be referred to as proteo-samples, and the ones prepared in D_2O as deutero-samples.

Peptide A: VVSVLTVVHQDWLNGK [294:309, HC] (m/z = 598.75, z = 3)

The comparison of the isotopic distributions of peptide A after photo-irradiation of IgG2 at $\lambda = 253.7$ nm in H_2O or D_2O reveals a mass shift towards higher masses for IgG2 photo-irradiated in D_2O compared to H_2O (Fig. 1a). In contrast, a comparison of the isotopic distributions of peptide A originating from non-irradiated IgG2 in H_2O and D_2O shows a perfect overlap (Fig. 1b). The deconvoluted isotopic distributions of peptide A generated in H_2O and D_2O give the final monoisotopic masses (and intensities) 1795.28 Da (4.9×10^5) and 1795.29 Da (4.3×10^5), respectively. We therefore conclude that approximately 1% (± 0.3% over the four repeated experiments) of the immunoglobulin had incorporated one deuteron atom in the peptide sequence A during the photolysis in D_2O.

MS/MS experiments were performed on peptide A originating from the proteo and deutero samples. MS/MS spectra of peptide A generated the following ions: b3-b4 (Fig. 2), and y1-y3, y5, y7-y9 and y11-y14 (Fig. 3). The isotopic distributions of these ions, originating from the UV-irradiated proteo and deutero samples were analyzed. The isotopic distributions of the b3 and b4 fragments overlap (Fig. 2). We, therefore, exclude that the amino acid residues VVSV [294:297, HC] incorporate any deuteron (Fig. 2). A comparison of the isotopic distributions of the y1, y2, y3, and y5 fragment ions of the proteo and deutero samples also shows no difference (Fig. 3). Thus, none of the amino acid residues WLNGK [305:309, HC] incorporates a deuteron. Further analysis of the fully protonated fragment ion y7 (QDWLNGK [295:309, HC]) originating from the proteo sample shows that its isotopic distribution displays ions with m/z 860.5, m/z 861.5 and m/z 862.5 with a relative abundance of 82 ± 4%, 100%, and 21 ± 4%, respectively. After photo-irradiation of IgG2 in D_2O, the y7 fragment ion of peptide A shows an isotopic distribution composed of the same ions with a relative abundance of 12 ± 6%, 100% and 36 ± 6%, respectively. Hence, the relative abundance of the ion with m/z 860.5 for the y7 fragment originating from peptide A of the photo-irradiated IgG2 in D_2O is *ca.* 15 ± 1% of that in H_2O, and the relative abundance increased significantly, *ca.* 70 ± 6%, for the isotopic peak with m/z 862.5. This difference in isotopic distributions between the fully protonated peptide A and peptide A originating from photo-irradiated IgG2 in D_2O indicates that deuterium is covalently incorporated into the amino acids composing fragment ion y7. The analysis of the isotopic distributions of the y8, y9, and y11-y14 fragment ions do not reveal any additional significant shifts toward higher masses (Fig. 3). Hence, we conclude that deuterium incorporation occurred largely in the amino acids Q [303, HC] and/or D [304, HC].

Peptide B: VTVLGQPK [109:116, LC] (m/z = 841.64, z = 1)

Like for peptide A, a comparison of the isotopic distributions of peptide B after photo-irradiation of IgG2 in H_2O and D_2O at $\lambda = 253.7$ nm exhibites a mass shift towards higher masses for photo-irradiated IgG2 in D_2O (Fig. 4a). The comparison of the isotopic distributions of peptide B originating from non-irradiated IgG2 in H_2O and D_2O shows no mass shift (Fig. 4b). The isotopic distribution of peptide B in H_2O is composed of ions with m/z 421.3, m/z 421.8 and m/z 422.3 with a relative abundance of 100%, 46 ± 2% and 13 ± 2%, whereas the same peptide after irradiation in D_2O is composed of the same ions with a relative abundance of 80 ± 3%, 100% and 40 ± 3%, respectively. The most intense peak in D_2O shifts to the ions with m/z 421.8, compared to the most intense ion with m/z 421.3 in

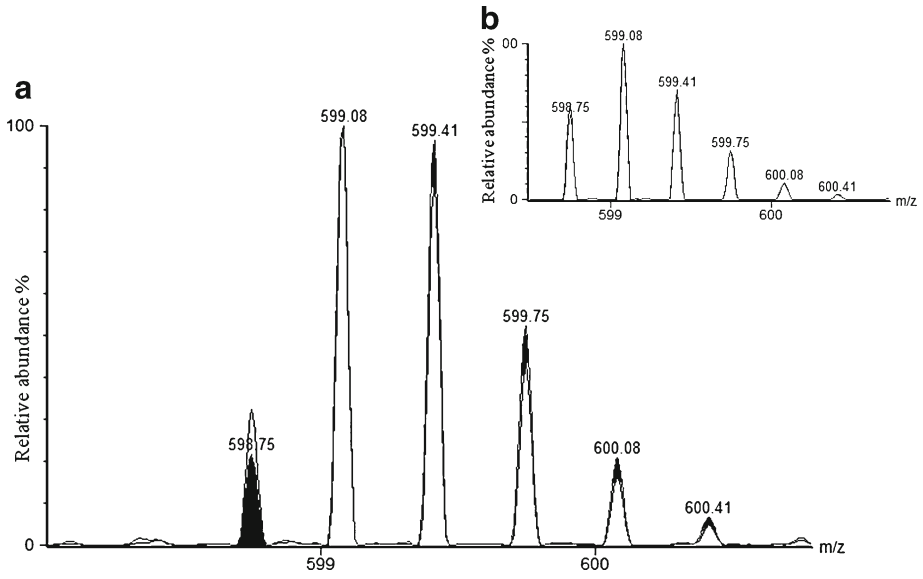


Fig. 1 MS spectra of peptide A, VVSVLTVVHQDWLNGK [294:309, HC], originating from IgG2 after proteolytic digestion: (**a**) photo-irradiated IgG2, (**b**) non photo-irradiated IgG2; IgG2 in H_2O (*empty peaks*) or in D_2O (*filled peaks*).

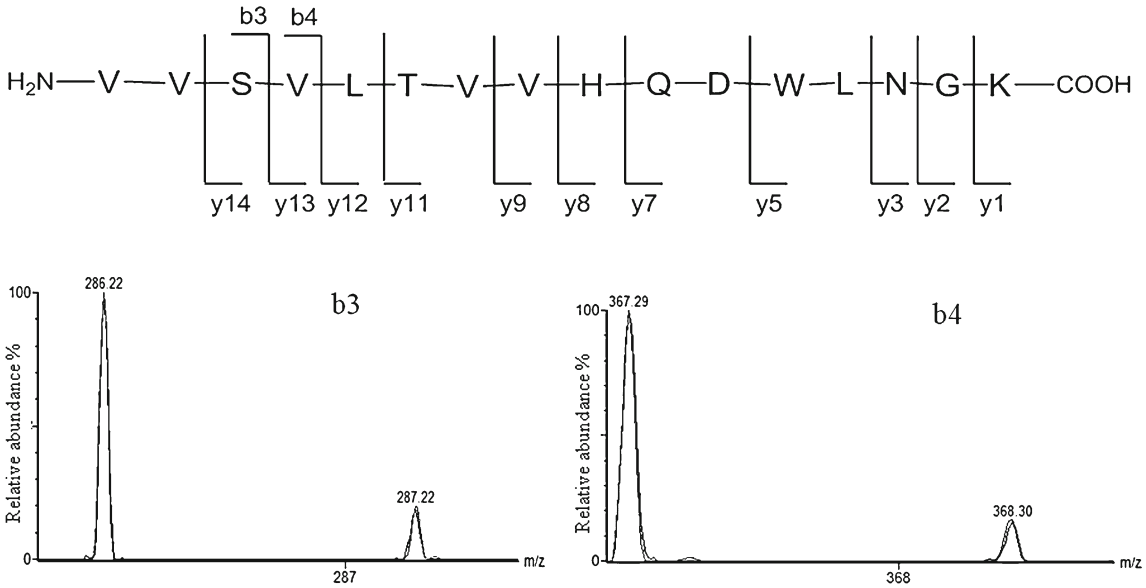


Fig. 2 MS/MS analysis and comparison of the isotopic distributions of the b fragment ions originating from peptide A, VVSVLTVVHQDWLNGK [294:309, HC], to localize the amino acid residues with covalently incorporated deuterium.

H_2O. The relative abundances of the isotopic peaks for the peptide in D_2O are *ca.* 110±3% higher for *m/z* 421.8, and *ca.* 200±5%higher for *m/z* 422.3 compared to the ones in H_2O. These results indicate that during the photo-irradiation of IgG2 in D_2O, peptide B covalently incorporated deuterium.

To specify the location of covalent deuterium incorporation in peptide B, MS/MS analysis was performed (Fig. 5). The isotopic distribution of the fully protonated y1 fragment is composed of ions with *m/z* 147.1 and *m/z* 148.1 with a relative abundance of 100% and *ca.* 10±1%. After photo-irradiation of IgG2 in D_2O, the corresponding fragment shows an isotopic distribution with a relative abundance of *ca.* 18±0.3% and 100%. The fact that the most intense peak shifted from *m/z* 147.1 to *m/z* 148.1 Da for a singly charged fragment ion indicates that nearly 100% of peptide B has covalently incorporated one deuteron into the y1 fragment. Therefore, K [116, LC] represents a site for deuterium incorporation during the photo-irradiation of IgG2 in D_2O. Further analysis of the fully protonated fragment of y7 (TVLGQPK [110:116]) originating from the proteo sample shows an isotopic distribution for ions with *m/z* 742.5, *m/z* 743.5 and *m/z* 744.5 with a relative abundance of 100%, 42±7% and 10±7%, respectively. After photo-irradiation of IgG2 in D_2O, the fragment ion y7 shows an isotopic distribution for ions with *m/z* 743.5, *m/z* 744.5 and *m/z* 745.5, with the same relative abundance of 100%, 42±3% and 10±3%, respectively, however 1 Da higher in mass than their corresponding ions in H_2O. This result indicates that one deuterium is incorporated into the sequence TVLGQPK [110:116]. Meanwhile, no significant change is observed for the isotopic distributions of the fragment ion b2 between the proteo and deutero samples. However, the b3-H_2O fragment ion shows an isotopic distribution for the ions with *m/z* 282.2 and *m/z* 283.2 with a relative abundance of 100% and 16±1%, whereas the fragment b3-H_2O originating from photo-irradiation in D_2O shows a relative abundance of 100% and 60±1% for the same ions, respectively. Variation of isotopic distributions for the fragment b3-H_2O towards the higher masses is observed for the deutero sample compared to the proteo one. We can, therefore, hypothesize that the residue V [111, LC] has incorporated deuterium during photo-irradiation of IgG2 in D_2O. In conclusion, the MS/MS analyses of the fragment ions of peptide B generated after photo-irradiation of the IgG2 in H_2O and D_2O, allow us to determine that V [111, LC] and K [116, LC] are two amino acid residues which covalently incorporates a deuteron during photo-irradiation of IgG2 in D_2O.

DISCUSSION

It was recently demonstrated that UV-irradiation (λ = 253.7 nm) of an IgG1 monoclonal antibody could lead to the formation of dithiohemiacetal and thioether products, originating from the photolytic cleavage of selected disulfide bonds (14). Specifically, the formation of the dithiohemiacetal requires disproportionation of a pair of CysS• radicals. An alternative reaction of CysS• radicals is a hydrogen transfer reaction, as illustrated in Scheme 1 (16), which can generate carbon-centered radicals on surrounding amino acid residues. The intermediary formation of carbon-centered radicals can be monitored by covalent H/D exchange when

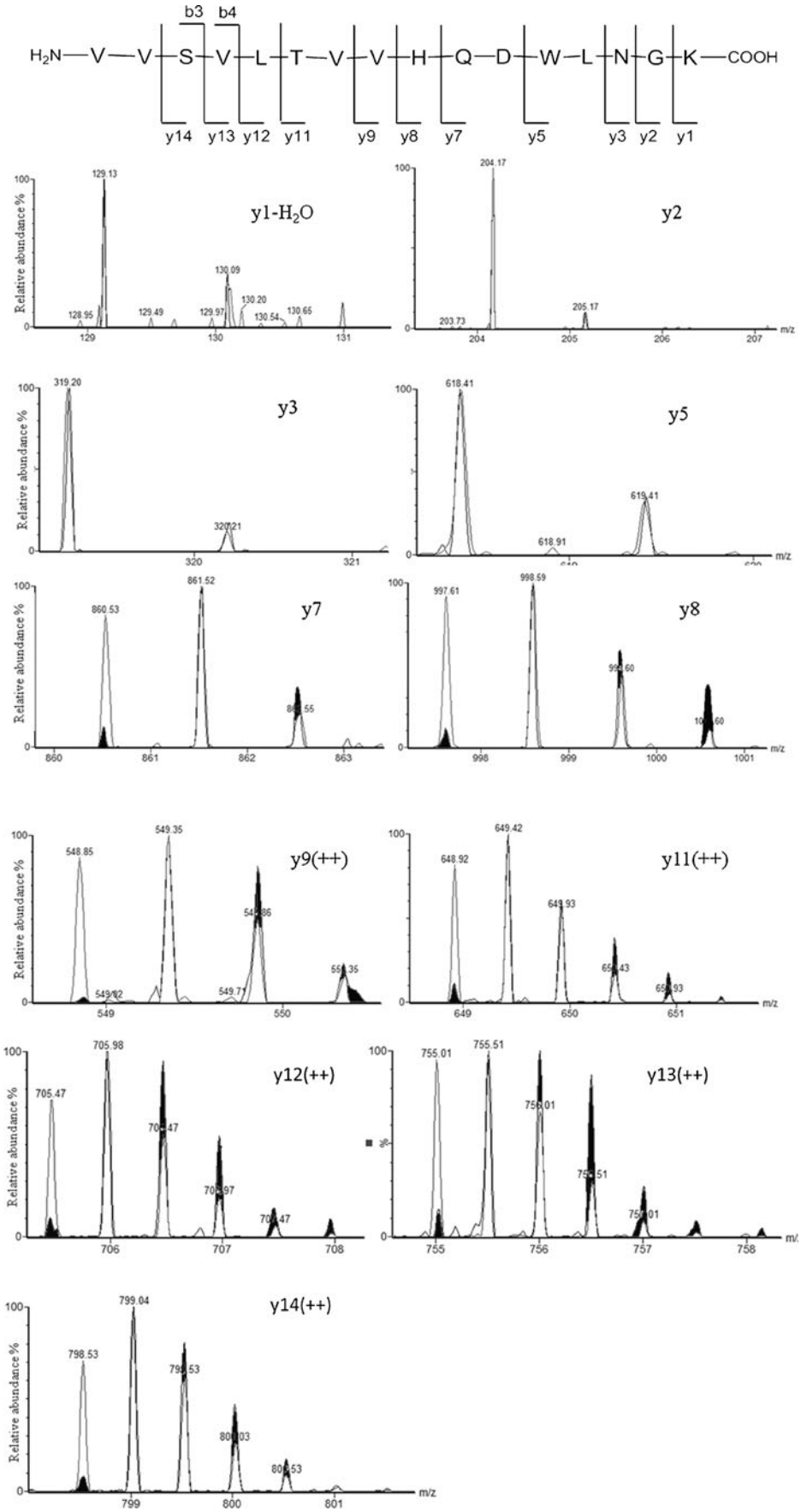


Fig. 3 MS/MS analysis of the y fragment ions originating from peptide A, VVSVLTVVHQDWLNGK [294:309, HC], to localize the amino acid residues with covalently incorporated deuterium.

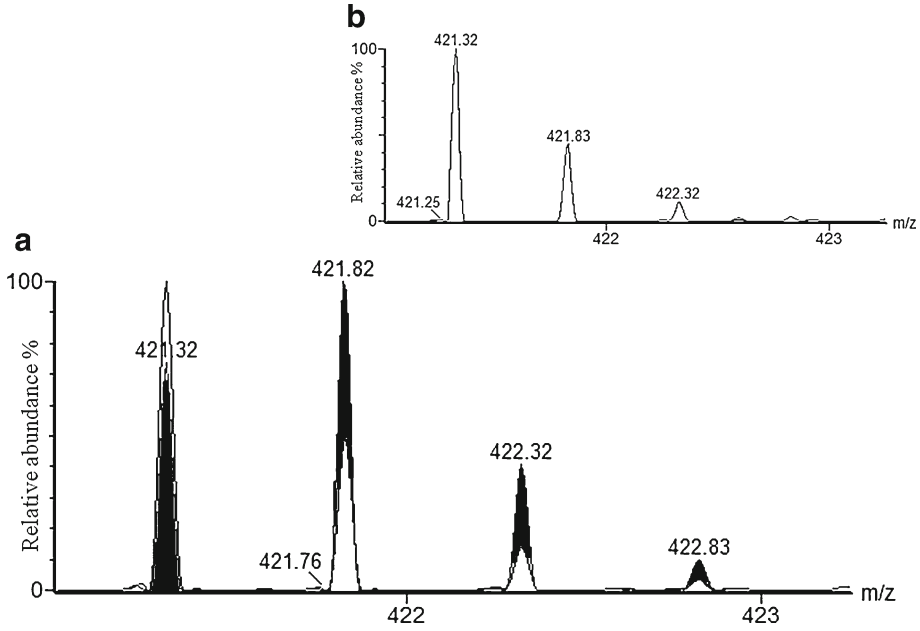


Fig. 4 MS spectra of peptide B, VTVLGQPK [109:116, LC] originating from IgG2 after proteolytic digestion: (**a**) photo-irradiated IgG2, (**b**) non photo-irradiated IgG2; IgG2 in H_2O (*empty peaks*) or in D_2O (*filled peaks*).

photolysis reactions are carried out in D_2O. Here, we have identified the selective incorporation of deuterium into two peptides in IgG2 and one peptide in IgG1, indicating that protein CysS$^{\bullet}$ radicals generated from IgG1 and IgG2 disulfide bonds abstract hydrogen atoms from specific IgG sequences. The two deuterated sequences are adjacent to disulfide bonds. The difference in deuterium incorporation in IgG1 and IgG2 suggests an influence of IgG structure and sequence on CysS• radical formation and reactivity. Four amino acids in IgG1 and IgG2 were identified as likely targets for covalent H/D exchange, a very small number compared to the size of IgG1 and IgG2, indicating a very high selectivity for the formation of carbon-centered radicals. Protein structure can influence the formation of carbon-centered radicals in various ways: first, protein structure and dynamics define the accessibility of an amino acid C-H bond to the protein CysS$^{\bullet}$ radicals generated during photo-irradiation. Second, secondary structure defines the homolytic bond dissociation energies (BDE) of $^{\alpha}$C-H bonds, were the BDE for an amino acid increases in the following order depending on the type of secondary structure the amino acid is embedded in: random coil < β-sheet < α-helix (20,21). Consistent

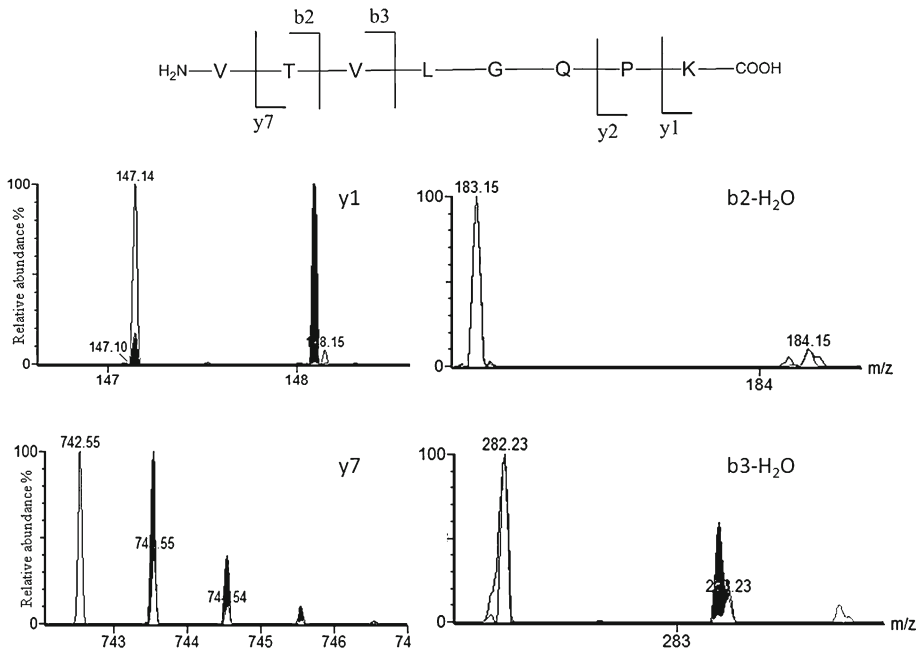


Fig. 5 MS/MS analysis of the b and y fragment ions originating from peptide B, VTVLGQPK [109:116, LC], to localize the amino acid residues with covalently incorporated deuterium.

with such an increase in BDE values, photochemically generated CysS$^{\bullet}$ radicals did not induce significant H/D exchange in α-helical sequences of human insulin (17). Nevertheless, covalent H/D exchange was observed in six different amino acids of human insulin, indicating a significantly higher propensity of protein CysS$^{\bullet}$ radicals to generate carbon-centered radicals in insulin as compared to IgG1 and IgG2. The structural basis for the high selectivity in IgG1 and IgG2 is subject to further investigation.

Importantly, the high sequence coverage of our HPLC-MS/MS analysis, especially for IgG2, ensures that the observed selectivity is not the result of missed peptides but is an inherent feature of IgG conformation and radical reactivity. More importantly, preliminary data on photolytic fragmentation of IgG1 (unpublished results) indicate, that peptide B (VTVLGQPK) is located in a domain of IgG1, which is sensitive to fragmentation. The actual mechanisms of photolytic fragmentation of IgG1 are currently under investigation. However, radical pathways, such as initiated through radical-dependent formation of carbon-centered radicals on specific amino acids of IgG1 may well be at the origin of fragmentation reaction.

Carbon-centered radicals such as formed through reaction with protein CysS$^{\bullet}$ radicals may be at the origin of IgG1 aggregation and fragmentation induced by light exposure. Our own experiments have indicated a pH-dependent sensitivity of IgG1 to both aggregation and fragmentation, with a higher propensity to light-induced aggregation at slightly alkaline pH (8.0) and a higher tendency for fragmentation at acidic pH (3.5) (22). A significant fraction of these aggregates was not through intermolecular disulfide formation, i.e. the aggregates could not be dissociated under conditions of reducing SDS-PAGE. Besides known pathways of aggregation through dityrosine formation (23), other carbon-carbon bonds or carbon-sulfur bonds generated through the recombination of radicals may contribute to the formation of these non-reducible aggregates. Importantly, such pathways have been characterized for the photo-irradiation of disulfide-containing model peptides (13–17).

The intermediary formation of carbon-centered radicals after homolytic disulfide cleavage is critical since it may permit the epimerization of amino acids and, consequently, conformational changes. Moreover, carbon-centered radicals can serve as the origins for the formation of covalent crosslinks and fragmentation of proteins.

CONCLUSION

The formation of intermediary carbon-centered radicals in IgG1 and IgG2 after UV-irradiation ($\lambda = 253.7$ nm) has been documented through the covalent incorporation of deuterium. This observed covalent deuterium incorporation into IgG1 and IgG2 irradiated in D_2O can be rationalized by intramolecular hydrogen transfer upon the formation of CysS• radicals. The formation of intermediary carbon-centered radicals in IgG monoclonal antibodies may lead to fragmentation, epimerization, and formation of covalent aggregates, major concerns of biotherapeutic product development. Further understanding of degradation pathways induced by photo-exposure would provide valuable insight for regulatory agency and industry in evaluating protein photo-instability.

ACKNOWLEDGMENTS AND DISCLOSURES

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