Journal of Pharmaceutical Sciences 105 (2016) 623–629

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

Suppression of Methionine Oxidation of a Pharmaceutical Antibody Stored in a Polymer-Based Syringe

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ARTICLE INFO

Article history:
Received 29 June 2015
Revised 1 September 2015
Accepted 15 September 2015
Available online 13 October 2015

Keywords:
mass spectrometry
biopharmaceuticals characterization
oxidation
stability
protein formulation
protein aggregation

ABSTRACT

Oxidation of methionine residues is one of the well-known deteriorations in monoclonal antibody (mAb) therapeutics. Because methionine oxidation may affect their efficacy and pharmacokinetic profile, oxidation levels should be strictly controlled during their storage period. In this study, we revealed that when a therapeutic antibody was filled into a cyclo olefin polymer-based syringe and stored in a blister pack with an oxygen absorber, the methionine oxidation production under thermal or light stress was suppressed because of the reduction in the concentration of dissolved oxygen. Also unexpectedly, fewer amounts of the high-molecular-weight species and the acidic variants of the antibody were generated under thermal or light stress. Although the high-molecular-weight species contains methionine oxidants at similar levels to those in a monomer species, they were likely to be constituted from a higher amount of the oxidative species of internal disulfide linkage, tyrosine, or histidine. Because the dissolved oxygen could be readily removed from the mAb solution in the polymer-based syringe owing to its high gas permeability, this study shows the advantages of the polymer-based syringe with an oxygen absorber over glass syringes in terms of the suppression of the methionine oxidation and oxidative high molecular species.

Introduction

Oxidation of methionine residues is one of the most frequently observed chemical deteriorations in therapeutic monoclonal antibodies (mAbs).[1-3] It has been reported that the methionine residues in mAbs are rapidly oxidized by hydroxyl radicals produced under thermal stress or by singlet oxygen produced under light stress.[4] The oxidation of methionine residues in mAbs may affect not only their biological efficacy, especially for the effector functions,[5] but also serum half-lives.[6,7] Therefore, suppression of methionine oxidation is imperative to maintain the quality of mAb pharmaceuticals. However, there is no practical way to completely prevent the methionine oxidation in mAb pharmaceuticals now. Even the best formulation can preserve the oxidation levels in the mAb pharmaceuticals stored under refrigerated conditions only up to some extent. Although freezing is the best way to almost completely prevent the methionine oxidation, it is not practical because the necessity of a deep freezer is an obstacle for the delivery of the products to the market.

In this study, we focused on a prefilled syringe with a container and closure system to investigate if the system can effectively prevent the methionine oxidation of an antibody in a solution. The level of methionine oxidation in a mAb was investigated by controlling the concentration of dissolved oxygen and the source of reactive oxygen species (ROS).[8] First, the mAb was filled into a cyclo olefin polymer-based or glass syringe, and with or without an oxygen absorber, which has the potential to remove dissolved oxygen, was exposed to thermal stress in the dark. Additionally, the mAb, with or without an oxygen absorber, filled into the polymer-based or glass syringe was exposed to light stress at 25°C.

The oxidation levels of the stressed mAb were quantified by mass spectrometry using the extracted ion chromatograms. In addition, the purity of the stressed mAb, with or without an oxygen absorber, was investigated with size-exclusion HPLC (SE-HPLC) and cation-exchange HPLC (CEX-HPLC). Formations of aggregates were

This article contains supplementary material available from the authors upon request or via the Internet at http://dx.doi.org/10.1002/jps.24675.

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http://dx.doi.org/10.1002/jps.24675
0022-3549/

discussed based on the oxidation levels of methionine residues in the high-molecular-weight species (HMWS) and the analytical results of the capillary gel electrophoresis (cGE).[9] From the results of the above, compatibility of the polymer-based and glass syringes with therapeutic mAbs was discussed in terms of their abilities to be degassed of the dissolved oxygen, the suppression of the methionine oxidation, and the level of the oxidative high molecular species.

Materials and Methods

Stress Testing

An IgG1 mAb was prepared at a concentration of 5 mg/mL in 20 mM phosphate, 150 mM sodium chloride, pH 7.0. A 500 μL aliquot of the sample was filled into a PLAJEX™ syringe, which is a newly developed polymer-based prefillable syringe with a 1 mL long staked needle (27 G) and was provided by TERUMO Corporation (Tokyo, Japan). A PLAJEX™ syringe is a silicone oil-free system and is made of cyclo olefin polymer with a butyl rubber plunger stopper coated with an i-coating™ technology (a proprietary coating).[10,11] Also, a 500 μL aliquot of the sample was filled into a silicone oil-free 1-mL glass syringe (Top Corporation, Tokyo, Japan) with a butyl rubber plunger stopper. Each syringe was placed into the polymetric multilayer blister pack as shown in Figure 1. Then, a small sachet of oxygen absorber (Ageless, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan), in which iron-based fine particles consume oxygen molecules, was placed beside the syringe. As control samples, syringes were placed into the blister packs without the oxygen absorber. Following the complete heat sealing of the blister packs, the packed syringe systems were stored at 5°C. For the thermal stress testing, the samples were stored at 40°C without light irradiation for 8 weeks to test thermal stability. Sampling points were 0, 2, 4, and 8 weeks. For the photostability testing, the samples were exposed to a light at 2000 lx at 25°C for 25 days, which corresponds to the ICH guideline Q1B. Sampling points were 0, 7, 14, and 25 days. Each sample was stored at −80°C before use, and all analyses were performed at the same time.

Evaluation of the Methionine Oxidation by Peptide Mapping

After denaturation of 100 μg of IgG1 with 8 M Gdn·HCl, the sample buffer was exchanged to the digestion buffer (100 mM Tris−HCl buffer, 0.02% polysorbate 80, pH 8.0) with MicroSpin G-25 columns (GE Healthcare, Little Chalfont, UK). The samples were digested with the addition of 3.2 μg of sequencing grade modified trypsin (Promega, Fitchburg, WI) followed by incubation at 37°C for 60 min. The digestion reaction was stopped with the addition of 10 μL of 25% trifluoroacetic acid (TFA). The digested peptides were separated with reversed-phase HPLC using an LC1200 system (Agilent Technologies, Palo Alto, CA) employing an AdvanceBio Peptide Map 2.1 x 150 mm^2, 2.7 μm column (Agilent Technologies). Mobile phase A contained a mixture of water and TFA (1000:1), and mobile phase B contained a mixture of water, acetonitrile, and TFA (400:3600:3). A linear gradient program of the mobile phase B from 0% to 45% was carried out over 60 min at the column temperature of 50°C and a flow rate of 0.2 mL/min. The eluted peptide fragments were detected with a LTQ/XL Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an electrospray ion source in the positive ion mode for the m/z of 300 to 2000. Oxidation of methionine residues were quantitated using each extracted ion chromatogram for the theoretical $m/z \pm 10$ ppm of the Met256 or Met432 containing peptide.

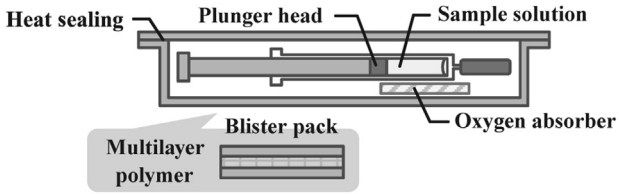

Figure 1. Schematic diagram of container and closure systems.

SE-HPLC

A 250 μg aliquot of the sample was loaded into two tandemly connected TSKgel SuperSW mAb HR columns (Tosoh Company, Tokyo, Japan) and separated by an isocratic flow of 100 mM phosphate, 400 mM sodium chloride, pH 7.0. The flow rate was 0.5 mL/min, the column temperature was operated at 25°C, and the elution was observed at UV 280 nm. The peaks eluted before the main peak were evaluated as HMWS and the peaks eluted after the main peak were evaluated as low-molecular-weight species (LMWS).

CEX-HPLC

A 25 μg aliquot of the sample was loaded into a ProPac WCX-10 column (Thermo Fisher Scientific) and separated by linear gradient flow of the mobile phase A (20 mM sodium phosphate, pH 6.8) and mobile phase B (500 mM sodium chloride in the mobile phase A). The ratio of the mobile phase B started from 9% and ended at 18%. The flow rate was 0.7 mL/min, the column temperature was operated at 40°C, and the elution was observed with UV at 280 nm. The peaks eluted before the main peak were evaluated as acidic peak groups (APGs) and the peaks eluted after the main peak were evaluated as basic peak groups.

Sodium Dodecyl Sulfate cGE

An 8 μL of the sample was added into 85 μL of sodium dodecyl sulfate (SDS) sample buffer (Beckman Coulter, Brea, CA). Followed by the addition of a 2 μL aliquot of 10 kDa internal standard (Beckman Coulter), 5 μL of iodoacetamide or 5 μL of dithiothreitol was added as the non-reduced or reduced sample, respectively. The samples were incubated at 70°C for 10 min and loaded into the bare fused-silica capillary (50 μm internal diameter). The mobilization was conducted by the application of an electric field at −15 kV with a UV detection at 220 nm. The quantitation is based on the relative corrected peak area percentages of the peaks.

Data Analysis

The levels of the variants, that is, oxidation, acidic variants, HMWS, and LMWS, were analyzed by linear regression to the first order reaction equation: $c = 100 - A_0 \cdot e^{-kt}$. Here, c is each level of the variant (%) after the degradation, A_0 is the initial purity of the product (%), k is a first order reaction constant (day^{-1}), and t is a storage period (day).

Results and Discussion

Oxidation Levels Under the Thermal Stress

Met256 residue in the CH_2 region and the Met432 residue in the CH_3 region were focused on as oxidation indicators because they are predominantly oxidized in IgG1 molecules by oxidative stress.[7]