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Thermally induced degradation pathways of three different antibody-based drug development candidates

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ABSTRACT

Protein-based medicinal products are prone to undergo a variety of chemical and physical degradation pathways. One of the most important exogenous stress condition to consider during manufacturing, transport and storage processes is temperature, because antibody-based therapeutics are only stable in a limited temperature range.

In this study, three different formats of antibody-based molecules (IgG1, a bispecific scFv and a fab fragment) were exposed to thermal stress conditions occurring during transport and storage. For evaluation, an analytical platform was developed for the detection and characterization of relevant degradation pathways of different antibody-based therapeutics. The effect of thermal stress conditions on the stability of the three antibody-based formats was therefore investigated using visual inspection, different spectroscopic measurements, dynamic light scattering (DLS), differential scanning calorimetry (DSC), electrophoresis, asymmetric flow field-flow fractionation (AF4) and surface plasmon resonance technology (SPR).

In summary, thermal stress led to heterogeneous chemical and physical degradation pathways of all three antibody-based formats used. In addition, identical exogenous stress conditions resulted in different kinds and levels of aggregates and fragmentation products. This knowledge is fundamental for a systematic and successful stabilization of protein-based therapeutics by the use of formulation additives.

1. Introduction

Antibody-based medicinal products have been established in the pharmaceutical industry in last 2 decades (Wang et al., 2007). To expand the capabilities of this class of medicinal products, other formats have been developed in addition to the classical IgG molecules (Schrama et al., 2006; Stockwin and Holmes, 2003). One of the most challenging tasks during the development of these drugs is the prevention of degradation reactions during manufacture, transport and storage processes. To maintain safety and efficacy of such molecules, one has to deal with different kinds of degradation reactions, which are divided into chemical and physical degradation pathways (Daugherty and Mrsny, 2006; Krishnamurthy and Manning, 2002; Manning et al., 2010). Protein degradation products may have an increased immunogenicity or a different pharmacokinetic pathway which may influence the mode of action (Philo and Arakawa, 2009; Vazquez-Rey and Lang, 2011). Actually, the identification and characterization of these different and heterogeneous degradation pathways are a demanding challenge in the pharmaceutical industry, because they are not fully understood. Based on the broad variety of degradation pathways and structural complexity of the protein molecules, no single analytical technique is able to cover the vast spectrum of all possible instability reactions (Mahler et al., 2010; Ripple and Dimitrova, 2012). Therefore, a well selected combination of complementing analytical methods has to be selected for the characterization of protein degradation products.

Elevated temperature is one important exogenous stress condition to be considered during manufacturing, transport and storage processes. Like other proteins, antibody-based therapeutics are only stable in a limited temperature range. Temperature above the point of maximum thermodynamic stability will have several effects on antibody-based medicinal product. In general high temperature causes an unfolding of the secondary or tertiary structure and results in a denatured state of the protein. Partial unfolding of the protein structure often involves an exposition of hydrophobic

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motifs, which may cause diverse kinds of aggregation pathways. Beside physical degradation, high temperature can also induce chemical degradation like oxidation ore cross-linking of the protein molecules (Wang et al., 2007).

In the study presented here, we investigate and compare the degradation pathways of three different formats of antibody-based molecules (IgG1, a bispecific scFv and a fab fragment) induced by high temperature. To gain more insight into the physico-chemical properties of the degradation products, an analytical platform of orthogonal and complementary analytical techniques was developed. The results allow identifying key mechanisms of the interactions between differently structured antibody-based molecules and high transport- and storage temperatures. Finally, thermal stress leads to heterogeneous chemical and physical degradation pathways of all three antibody-based molecules used and caused different kinds and levels of aggregates and fragmentation products.

2. Materials and methods

2.1. Antibody-based molecules

A human monoclonal antibody of the IgG1 subclass, a bispecific scFv and a fab fragment were provided by Bayer Pharma AG (Fig. 1). The antibody was stored in a formulation at a pH of 7.5 at a concentration of 3.00 mg/mL. The bispecific scFv was formulated at a pH of 7.5 at a concentration of 0.72 mg/mL and the fab fragment at a pH of 5.5 at a concentration of 3.00 mg/mL.

2.2. Stress conditions of the antibody-based molecules

Previous studies had shown that a storage temperature of 60 °C for 72 h for the antibody, 40 °C for 28 d for the bispecific scFv and 60 °C for 72 h for the fab fragment were appropriate conditions to induce efficient and identifiable degradation.

2.3. Dynamic light scattering (DLS)

The hydrodynamic radius was determined with a DynaPro™ plate reader (Wyatt Technology Europe GmbH, Dernbach, Germany) combined with the software DYNAMICS (version 7.1.0.25, Wyatt). 50 µL of the undiluted and filtered (0.22 µm PVDF-Filter (Millex® Syringe-driven Filter Unit, Millipore, Billerica, USA)) protein solution was measured in a 384-well plate (384 round well plate, Polystyrol, Thermo Scientific, Langenselbold, Germany). Each sample was recorded for 5 s ten times ($n = 4$).

2.4. Differential scanning calorimetry (DSC)

The thermal stability of the antibody-based molecules was analyzed using a VP-Capillary DSC System (MicroCal, Freiburg, Germany) in combination with the software Origin™ (version 7.0, OriginLab). The protein samples were diluted to 0.5 mg/mL with formulation buffer and investigated over a temperature range from 15 °C to 105 °C and at a heating rate of 1 °C/min.

2.5. Far-UV circular dichroism (far-UV CD)

CD spectra were recorded using a J-815 spectrometer (JASCO Germany GmbH, Gross-Umstadt, Germany) in a quartz cell (path length 0.2 mm; Hellma® Analytics, Müllheim, Germany). For far-UV measurements the concentration of the antibody was 1.1 mg/mL and the fab fragment 0.3 mg/mL. The secondary structure of the bispecific scFv was determined using the FT-IR spectroscopy due to the high absorption of the formulation buffer. Each CD spectrum was collected from 240 nm to 190 nm; scanning speed 20 nm/min; data pitch of 0.1 nm; digital integration time 2 s; band width 1 nm; accumulation of nine measurements with background correction using the formulation buffer. The molar ellipticity was calculated. The secondary structure percentage determined using the Contin algorithm of the software CDPro Analysis (JASCO Germany GmbH, Gross-Umstadt, Germany).

2.6. Fourier-transformation infrared spectroscopy (FT-IR)

The FT-IR spectroscopy was used to analyze the secondary structure of the bispecific scFv and performed with the Tensor-27/Hyperion 2000 (Bruker Optik GmbH, Ettlingen, Germany) at 25 °C. The spectra were recorded from 3100 cm^{-1} to 1000 cm^{-1} with an AquaSpec transmission cell. With a resolution of 4 cm^{-1} 60 spectra were accumulated for every injection and background corrected. All recorded spectra were processed further by accumulating three injections, calculation of the second derivatives (smoothed by 17 points) and vector normalization. To determine the secondary structure the amide I region of the native and the stressed bispecific scFv were compared.

2.7. Intrinsic fluorescence

The intrinsic fluorescence emission spectrum of tryptophan was recorded between 300 nm and 450 nm with an excitation wavelength of 280 nm using a LS 55 Fluorescence Spectrometer (Perkin Elmer, Waltham, USA) with a quartz cell (path length of 10 mm); scanning speed of 100 nm/min; excitation and emission slits of 2.5 nm. The antibody concentration was 0.05 mg/mL, the bispecific scFv concentration 0.02 mg/mL and the fab fragment concentration 0.01 mg/mL. For the evaluation five measurements were accumulated and the value of the emission maximum was determined.

2.8. Detection of amyloid fibrils

The extrinsic fluorescence emission spectrum of Thioflavin T (Sigma-Aldrich, St. Louis, USA) was recorded using a LS 55 Fluorescence Spectrometer (Perkin Elmer, Waltham, USA) with a quartz

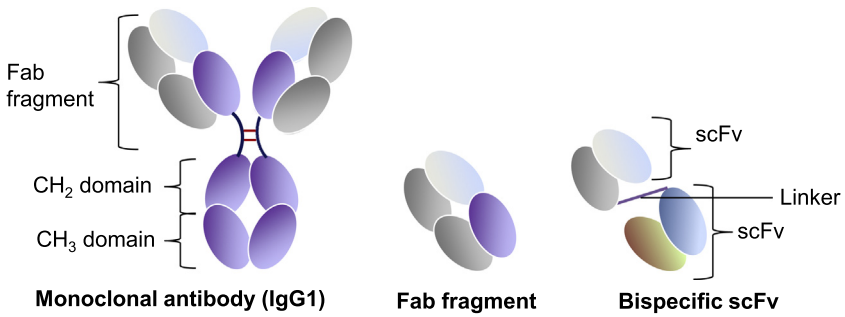

Fig. 1. Characteristic structure elements of three antibody-based therapeutics used.