Linking the Solution Viscosity of an IgG2 Monoclonal Antibody to Its Structure as a Function of pH and Temperature

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ABSTRACT: Although the viscosity of concentrated antibody solutions has been the focus of many recent studies, less attention has been concentrated on how changes in protein structure impact viscosity. This study examines viscosity profiles of an immunoglobulin G (IgG) 2 monoclonal antibody at 150 mg/mL as a function of temperature and pH. Although the structure of the antibody at pH 4.0–7.0 was comparable at lower temperatures as measured by second derivative UV absorbance and Fourier transform infrared spectroscopy, differences in 8-anilino-1-naphthalene sulfonate (ANS) fluorescence intensity indicated small structural alterations as a function of pH. Below the structural transition onset temperature, the viscosity profiles were pH dependent and linearly correlated with fluorescence intensity, and followed semilogarithmic behavior as a function of temperature. The transitions of the viscosity profiles correlated well with the major structure transitions at a protein concentration of 150 mg/mL. The viscosity correlated particularly well with ANS fluorescence intensity at 0.2 mg/mL below and above the structural transition temperatures. These results suggest: (1) ANS can be an important measure of the overall structure and (2) hydrophobic interactions and charge–charge interactions are the two major physical factors that contribute collectively to the high viscosity of concentrated IgG solutions. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:4291–4304, 2013

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INTRODUCTION

Monoclonal antibodies (mAbs) have become increasingly important as therapeutic agents, especially as treatments for patients with cancer and autoimmune disorders.[1] The desired dosage form for patient convenience often requires these molecules to be self-administrated in prefilled syringes via subcutaneous injection. Because of the limitations in the maximum solution volume used for subcutaneous injection (~1 mL), high-concentration protein solutions (>100 mg/mL) are often required. Highly concentrated antibody solutions have raised significant stability and formulation delivery challenges, including aggregation and solution viscosity.[2]

Developing a better understanding of the causes and mechanisms of the elevated viscosity of concentrated antibody solutions is an area of active research.[3–6] Solution viscosity appears to have a nonlinear relationship to protein concentration and is often strongly affected by solution pH, ionic strength, presence of inorganic salts, as well as the intrinsic structural nature of the antibody itself.[3,5,7] Initial assumptions were that the high-solution viscosity at pH values close to the pI value was primarily because of reversible self-association mediated by electrostatic interactions.[2] Later studies suggested the higher solution viscosity of certain antibodies was because of the network-like associations of Fab regions rather than Fc associations.[4] The viscosity of protein solutions at high concentrations can be related to the second virial coefficient (B22)[6] and the interaction parameter (K_d) as represented by the slope of the mutual self-diffusion coefficient (D_m) as a function of protein concentration.[8] Experimental values for B22 and K_d can be obtained by several different approaches, including static[9] and dynamic light scattering,[10–12] ultracentrifugation,[6] and self-interaction chromatography.[13,14] Such measurements, however, require relatively low-concentration antibody solutions and extrapolation to more viscous solutions.[9,10,12]

Studies have suggested that increases in antibody solution viscosity may be related to charge–charge interactions, excluded volume effects, short-range van der Waals and apolar interactions, dipole–dipole interactions, as well as a variety of other interactions between mAb molecules.[5,7,8,15–17] Even in cases where experimental results have pointed to charge–charge interactions as the major source of elevated viscosity behavior, the nature and source of such interactions often remain to be more clearly defined. In addition, the significance and contribution of each of the possible noncovalent interactions, in combination with charge–charge interactions, to the overall viscosity of a high-concentration protein solution remain unclear. Two recent studies have shown that hydrophobic organic salts can significantly diminish the viscosity of high-concentration antibody solutions, suggesting these bulky organic salt molecules can disrupt the intermolecular transient networks between protein molecules because of their

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apolarity.[18,19] The results of He et al.[20] concerning the effect of sugars on the viscosity of antibody solutions also support a related conclusion because these sugars, lacking both significant apolarity and charge, were found to further increase the viscosity of antibody solutions.[20] In a more recent study, the viscosity of antibody mixtures was found to be reasonably described as the geometric sum of the viscosity of each solution,[21] suggesting that related protein–protein interactions are nonspecific in nature.

Although significant progress has been achieved, a further understanding of the factors affecting viscosity without careful consideration of their structural basis would be incomplete. In earlier studies concerning to structure and viscosity relationships, antibodies were assumed to have a similar structure,[3,5] although results[4,16] using circular dichroism to link antibody secondary structure to solution viscosity were preliminary and inconclusive. Liu et al.[22] recently showed that viscosity measurements can be used as a tool to monitor the thermal stability of IgG antibodies. As expected, the unfolding behavior could be inferred by examination of changes in viscosity.[22] Kamerzell et al.[23] revealed possible protein-specific secondary structure changes as a function of viscosity caused by increasing protein concentrations using two-dimensional vibrational correlation spectroscopy and argued that hydrogen bonding and electrostatics are primarily responsible for the intermolecular association that results in nonideal viscosity behavior.[23] However, so far there have not been any additional studies to examine protein solution viscosity profiles as a function of structural changes under different environmental conditions. Therefore, the purpose of this study is to characterize solution viscosity profiles and structural changes of a model IgG2 mAb at 150 mg/mL under different conditions of pH and temperature. A better understanding of the impact of antibody structure on the viscosity of high-concentration antibody solutions can lead to additional insights into how to better develop protein formulations with improved solution properties.

MATERIALS AND METHODS

Materials

The mAb was purified according to Amgen platform conditions. The antibody solution was dialyzed into a series of 20 mM citrate–phosphate (C–P) buffers at an ionic strength of 0.15 (adjusted with NaCl). The C–P buffers were prepared using a 0.4 M citric acid solution employing citric acid monohydrate (Fisher Scientific, Pittsburgh, Pennsylvania) and a 0.4 M sodium phosphate dibasic solution prepared from sodium phosphate dibasic anhydrous (Sigma, St. Louis, Missouri). All reagents were purchased from Sigma unless otherwise noted.

Protein dialyses were performed at 4°C using Slide-A-Lyzer® dialysis cassettes, 10,000 MWCO (molecular weight cut-off) (Pierce, Rockford, Illinois) against C–P buffers at pH 4.0–7.0. The protein was concentrated using CENTRICON® centrifugal filter devices (with a MWCO of 50k or less) centrifuged at 2000–3000 g at lower temperature. The concentration was then adjusted to approximately 150 mg/mL with the corresponding buffers. The protein concentration was measured with an Agilent 8453 UV–Visible spectrophotometer (Palo Alto, California) employing a theoretical extinction coefficient of 1.47 mL/(mg.cm) after dilution to approximately 0.2 mg/mL with the corresponding buffers.

Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectra were collected using a Bruker Tensor 27 spectrometer (Bruker Optics GmbH, Ettlington, Germany) equipped with an MCT detector and a Haake DC 30/K20 temperature controller (Thermo Electron, Newington, NH) . The FT-IR instrument employing a BioATR cell was purged with nitrogen throughout all experiments. Single-beam interferograms at 150 mg/mL were acquired using OPUS software (version 6.5) (Bruker Optics Inc., Billerica, MA) from 10°C to 90°C at 2.5°C intervals. The interferograms of a corresponding buffer blank were collected at the same temperatures. FT-IR spectra of the protein were obtained in the range of 4000−900 cm^{-1} at a resolution of 4 cm^{-1}. The resultant FT-IR spectra were processed with atmospheric (H_2O and CO_2) compensation and with a nine-point Savitsky–Golay smoothing function. The spectra in the amide I region (1710–1590 cm^{-1}) were used for further data processing. The resultant spectra were then normalized using a vector normalization function. The vector normalization first calculates the average absorbance value of each spectrum from 1710 to 1590 cm^{-1}. The average value calculated was then subtracted from the spectrum, which causes the spectrum to be centered around $y = 0$. This step is followed by calculating the sum of squares of all y values, and the respective spectrum is then divided by the square root of this sum. The spectrum at 10°C was then subtracted from the spectra at each temperature to obtain the differential spectra under each pH condition.

UV Absorption Spectroscopy

Protein samples at 150 mg/mL at pH 4.0–7.0 were loaded into a 10-μm path length cuvette and UV absorbance spectra were collected with the corresponding buffers used as blanks using an Agilent 8453 UV–Visible Spectrophotometer. The UV absorbance spectra of the protein at 0.5 unit intervals were collected at a temperature interval of 2.5°C from 10°C to 87.5°C. The equilibration and integration time at each temperature were 300 and 15 s, respectively. The total time for each run was approximately 4.5 h. The optical density (OD) reading at 350 nm was used to monitor the aggregation behavior of the antibody. Second-derivative spectra were calculated using a nine-point data filter and a third degree polynomial function and then smoothed by 99 interpolated points between each raw data point. The peak positions were obtained using the peak-picking function of Origin 7 software (OriginLab Corporation, Northampton, MA).

Extrinsic 8-Anilino-1-Naphthalene Sulfonate Fluorescence and Light Scattering Intensity

8-Anilino-1-naphthalene sulfonate (ANS) fluorescence was used to measure tertiary structural changes upon subjecting the protein to thermal stress. ANS was added to the protein solution at dye to protein molar ratio of 0.5, because higher molar ratios could result in protein precipitation as well as reducing the viscosity of high-concentration protein solutions.[19,24] ANS fluorescence spectra of the above samples at pH 4.0–7.0 were collected using a cuvette method in duplicates as reported earlier.[25] A spectrofluorometer (Photon Technology International, Lawrenceville, New Jersey) equipped with a turreted four-position Peltier-controlled cell holder and a xenon lamp was used in the study. The fluorescence of each sample (165 μL) in rectangular cuvettes (2×10 mm^2) was excited at 374 nm and

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