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Technical note

Small-scale screening method for low-viscosity antibody solutions using small-angle X-ray scattering

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ABSTRACT

In this study, we investigated the concentration range in which self-association starts to form in humanized IgG monoclonal antibody (mAb) solutions. Furthermore, on the basis of the results, we developed a practical method of screening for low-viscosity antibody solutions by using small-angle X-ray scattering (SAXS) measurements utilizing small quantities of samples. With lower-viscosity mAb3, self-association was not detected in the range of 1–80 mg/mL. With higher-viscosity mAb1, on the other hand, self-association was detected in the range of 10–20 mg/mL and was clearly enhanced by a decrease in temperature. The viscosities of mAb solutions at 160, 180, and 200 mg/mL at 25 °C quantitatively correlated very well with the particle size parameters obtained by SAXS measurements of mAb solutions at 15 mg/mL at 5 °C. The quantity of mAb sample required for the SAXS measurements was only 0.15 mg, which is about one-hundredth of that required for actual viscosity measurements at a high concentration, and such quantities could be available even at an early stage of development. In conclusion, the SAXS analysis method proposed in this study is a valuable tool for the development of concentrated mAb therapeutics with high manufacturability and high usability for subcutaneous injection.

1. Introduction

The viscosity of therapeutic monoclonal antibody (mAb) solutions is a major issue affecting many aspects including their stability [1] and also their manufacturability and usability for subcutaneous injection [2] in addition to their handling performance in laboratories [3]. As previously reported, small cluster (i.e., reversibly self-associated aggregate) is speculated to be responsible for viscosity behavior of high-concentration mAb solutions [4–6]. Furthermore, the high viscosity of some types of antibodies can be attributed to reversible self-association induced by heterogeneous charge distribution on the protein surface and the accompanying electrostatic attraction (e.g., dipole–dipole interaction) [7–13]. In order to avoid the risk of high viscosity with any certainty, antibody molecules with low viscosity must be selected during discovery and lead optimization. However, measurements of viscosity at high concentrations often require extensive laboratory resources and large quantities of samples, which are not usually available at such an early stage of development. We have recently reported that the particle size parameters obtained by small-angle X-ray scattering (SAXS) measurements of mAb solutions at a concentration of 60 mg/mL quantitatively correlated very well with the viscosity of mAb solutions at a concentration of 200 mg/mL [14]. SAXS measurements can be performed using a small volume of samples; therefore, these parameters could be used as quantitative indicators of viscosity at high concentrations, even in the early development stage. In this study, we investigated the mAb concentration range in which self-association starts to form. Furthermore, on the basis of the results, we developed a practical method of screening for low-viscosity antibody solutions by SAXS measurements using smaller quantities of samples.

Abbreviations: D_{max}^{app}, apparent maximum dimension; R_g^{app}, apparent radius of gyration; EMS, electromagnetically spinning; IFT, indirect Fourier transformation; $p(r)$, pair–distance distribution function; q.s., quantity sufficient; SAXS, small-angle X-ray scattering.

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2. Materials and methods

2.1. Materials

The humanized IgG monoclonal antibodies mAb1 (IgG4, 146 kDa), mAb2 (IgG2, 147 kDa), mAb3 (IgG1, 145 kDa), mAb4 (IgG1, 148 kDa), mAb5 (IgG2, 147 kDa), and mAb6 (IgG1, 145 kDa) were manufactured and purified by Chugai Pharmaceutical (Tokyo, Japan). The theoretical isoelectric points (pI) of mAb1, mAb2, mAb3, mAb4, mAb5, and mAb6 are 6.6, 5.7, 9.0, 9.3, 5.5, and 9.1, respectively. Histidine (His), arginine (Arg), and aspartic acid (Asp) were purchased from Ajinomoto Healthy Supply (Tokyo, Japan). All other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan). Buffer conditions chosen for investigation in this study are summarized in Table 1. In addition to NaCl [15] and Arg [16], we also used Asp as a stabilizer because of the combination effect of Arg and Asp [14,17,18]. The protein concentrations of mAb solutions were determined by UV absorbance at 280 nm.

2.2. Small-angle X-ray scattering (SAXS)

SAXS measurements were performed to examine the self-association behavior of mAb solutions (1–80 mg/mL, 10 μL) at 5 °C or 25 °C by using a SAXSess mc^2 system (Anton Paar, Graz, Austria) with line-collimated Cu Kα radiation (λ = 0.1542 nm) [14]. The scattering patterns of mAb solutions were recorded by a two-dimensional imaging-plate detector. The exposure time of X-ray was 60 min (1 mg/mL), 30 min (5 mg/mL), 10 min (10–20 mg/mL), or 5 min (40–80 mg/mL). The two-dimensional scattering intensities were integrated into one-dimensional scattering intensities [$I(q)$] as a function of the magnitude of the scattering vector $q = (4\pi/\lambda)\sin(\theta/2)$ by using SAXSQuant software (Anton Paar), where θ is the total scattering angle. For all experiments, the attenuated primary beam at $q = 0$ was monitored by using a semitransparent beam stop. SAXS profiles were calibrated for transmission by normalizing the zero-q primary intensity to unity. Background subtraction (capillary and corresponding buffer solution) and collimation correction (desmearing) were performed. $I(q)$ was normalized to the mAb concentration (c), which is referred to here as $I(q)/c$. We confirmed that the SAXS profiles were not changed by additional exposure of X-ray, indicating that there was no radiation damage (data not shown).

Assuming that there is no interaction between particles in the system (i.e., the structure factor $S(q) = 1$), then $I(q)$ is given by Fourier transformation of the pair–distance distribution function of the particle, $p(r)$, as

$$I(q) = 4\pi \int_0^\infty p(r) \frac{\sin qr}{qr} dr \quad (1)$$

where r is the distance between two scattering centers chosen inside the particle. We used the indirect Fourier transformation (IFT) technique to calculate $p(r)$ and determine the values of the apparent maximum dimension [D_{max}^{app} (nm)] of the particles [19]. The $p(r)$ was normalized by the area under the curve. The apparent radius of gyration [R_g^{app} (nm)] and the extrapolated scattering intensity [$I(q \to 0)/c$ (a.u.)] were also obtained by Guinier approximation (using the q range satisfying the criteria $q*R_g^{app} < 1.3$).

We also experimentally obtained structure factor [$S(q)$] by dividing $I(q)/c$ by the experimental form factor [$P(q)$] determined at very low concentration (i.e., 1 mg/mL). The extrapolated structure factors [$S(q \to 0)$] were obtained as a function of mAb concentration. The theoretical $S(q \to 0)$ of hard sphere with identical volume fraction was calculated by the following equation [20–22]

$$S(q \to 0) = (1-\phi)^4/(1+4\phi+4\phi^2-4\phi^3+\phi^4) \quad (2)$$

where ϕ is the volume fraction of the mAb molecule. The ϕ can be calculated by ϕ = concentration (mg/mL) * 0.74 (cm^3/g)/1000.

2.3. Viscosity measurements

The viscosity of mAb solutions was measured at 25 °C by an electromagnetic spinning (EMS) method using an EMS viscometer (Kyoto Electronics Manufacturing, Kyoto, Japan) [14,23]. The EMS viscometer consists of a rotor to which a pair of permanent magnets is attached, a brushless direct current motor, a flash lamp, a CCD video camera, and a thermoregulator. In the EMS method, a liquid sample and an aluminum ball are put in a glass tube, and then the aluminum ball is rotated by utilizing the moment caused by the Lorentz force. The viscosity of the liquid sample can be calculated from the rotational speed of the aluminum ball measured by using the flash lamp and the CCD video camera. For each viscosity experiment we put the mAb solution (160, 180, 200 mg/mL, 90 μL) and an aluminum ball (2 mm diameter) into a glass tube (6.3 mm inside diameter). The aluminum balls and the glass tubes were siliconized before use to prevent adsorption of sample components. The viscosity of each sample solution was constant at rotation speeds from 250 to 1000 rpm; however, the standard deviation of viscosity increased as the rotation speed decreased (data not shown). Therefore, we obtained all viscosity data at 1000 rpm. We also confirmed that the viscosity measured by EMS viscometer using siliconized aluminum balls is approximately equal to that measured by rheometer with cone-and-plate system and calculated by using the approximation equation for Newtonian fluid (data not shown).

Table 1
Buffers used in this study.

Buffer name	Buffer conditions
Buffer A	5 mM citrate, 100 mM NaCl, NaOH (q.s.), pH 6.0
Buffer B	5 mM citrate, 150 mM NaCl, NaOH (q.s.), pH 6.0
Buffer C	20 mM His, 150 mM Arg, HCl (q.s.), pH 6.0
Buffer D	20 mM His, 150 mM Arg, Asp (q.s.), pH 6.0

3. Results

The self-association behavior of mAb solutions was evaluated by SAXS measurements. Fig. 1 shows SAXS profiles of mAb1 and mAb3 solutions (1–80 mg/mL) in buffer D at 5 °C or 25 °C. Scattering intensity was normalized to the mAb concentration. The increase in scattering intensity in the small angle region indicates the formation of self-association and/or intermolecular attractive interactions, while the decrease in scattering intensity in the small angle region indicates the excluded volume effects and/or intermolecular repulsive interactions. Fig. 2(A) and (C) shows $I(q \to 0)/c$ and R_g^{app} as a function of mAb concentration. In the mAb3 solutions, $I(q \to 0)/c$ and R_g^{app} decreased with increasing mAb3 concentration at 25 °C. In order to investigate the detailed intermolecular interactions, $S(q \to 0)$ of mAb3, which reflect the net repulsive forces between the mAb molecules, was obtained (Fig. 2(B)). The $S(q \to 0)$ was lower than that predicted for hard sphere with an identical volume fraction. This result indicates that $I(q \to 0)/c$ and R_g^{app} of mAb1 were underestimated at high mAb concentrations owing to repulsive electrostatic interactions in addition to excluded volume effects [24]. In the case of mAb3, $I(q \to 0)/c$, $S(q \to 0)$, and R_g^{app} did not prominently change with the decrease in temperature from 25 °C to 5 °C. With mAb1 solutions at 25 °C, $I(q \to 0)/c$ decreased from 1 to 10 mg/mL, similarly to mAb3 solutions; however, it exhibited a broad peak in