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RESEARCH PAPER

Quantitative Correlation between Viscosity of Concentrated MAb Solutions and Particle Size Parameters Obtained from Small-Angle X-ray Scattering

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ABSTRACT

Purpose To investigate the relationship between viscosity of concentrated MAb solutions and particle size parameters obtained from small-angle X-ray scattering (SAXS).

Methods The viscosity of three MAb solutions (MAb1, MAb2, and MAb3; 40–200 mg/mL) was measured by electromagnetically spinning viscometer. The protein interactions of MAb solutions (at 60 mg/mL) was evaluated by SAXS. The phase behavior of 60 mg/mL MAb solutions in a low-salt buffer was observed after 1 week storage at 25°C.

Results The MAb1 solutions exhibited the highest viscosity among the three MAbs in the buffer containing 50 mM NaCl. Viscosity of MAb1 solutions decreased with increasing temperature, increasing salt concentration, and addition of amino acids. Viscosity of MAb1 solutions was lowest in the buffer containing histidine, arginine, and aspartic acid. Particle size parameters obtained from SAXS measurements correlated very well with the viscosity of MAb solutions at 200 mg/mL. MAb1 exhibited liquid–liquid phase separation at a low salt concentration.

Conclusions Simultaneous addition of basic and acidic amino acids effectively suppressed intermolecular attractive interactions and decreased viscosity of MAb1 solutions. SAXS can be performed using a small volume of samples; therefore, the particle size parameters obtained from SAXS at intermediate protein concentration could be used to screen for low viscosity antibodies in the early development stage.

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KEY WORDS MAb · SAXS · Viscosity

ABBREVIATIONS

D_{max}^{app}	Apparent maximum dimension
EMS	Electromagnetically spinning
IFT	Indirect fourier transformation
$p(r)$	Pair–distance distribution function
q.s.	quantity sufficient
R_g^{app}	Apparent radius of gyration
SAXS	Small-angle X-ray scattering

INTRODUCTION

Monoclonal antibodies (MAbs) and MAb-based drugs have become a major class of biopharmaceuticals for treating numerous diseases such as cancer, inflammation, allergies, infectious diseases, and cardiovascular diseases, and are seeing rapid market growth. Owing to the low potency of MAb, many diseases targeted by MAb therapy require injections of several mg/kg. Although MAb therapeutics for oncology are often administered intravenously, for chronic diseases (*e.g.*, rheumatism) requiring frequent dosing it is preferable to provide a subcutaneous administration option that can be conducted by the patient at home and which thereby contributes to increasing patient compliance. However, an injection volume of more than approximately 1.5 mL is not suitable for subcutaneous administration. Therefore, MAb solutions for subcutaneous administration with a long dosage interval need to be concentrated to 100 to 200 mg/mL, which presents several challenges. As previously reported, the high viscosity of MAb solutions is a major issue affecting many aspects, including their stability and also their manufacturability and usability for subcutaneous injection (1). For example, high viscosity may result in a decrease in the performance of the tangential flow filtration used to change the buffer and concentrate the protein, and in a

decrease in the filling accuracy during the filling process. Furthermore, the injection time during subcutaneous administration is prolonged with high viscosity, resulting in increased risk of unsuccessful administration.

In recent years many researchers have been focusing on clarifying the mechanisms governing the viscosity of highly concentrated MAb solutions. By applying analytical ultracentrifugation techniques to a highly viscous MAb solution Liu *et al.* revealed that the reversible self-association caused by electrostatic attraction induces an increase in viscosity at high concentrations (2). In addition, through titration studies using the full-length MAb and the Fab fragments, Kanai *et al.* revealed that the self-association of the MAb solution can be attributed to the intermolecular Fab–Fab interaction (3). Furthermore, Yadav *et al.* suggested that the attractive interaction may be due to charge–charge and charge–dipole interactions at the surfaces of the Fab regions of the MAb molecule, giving a reasonable explanation for the pH dependency of the rheological profile observed (4). These hypotheses were subsequently supported by experimental results showing that replacing the charged residues in the CDR of the MAb molecule resulted in a drastic reduction in viscosity (5). These results suggest a scenario in which, for some types of antibodies, heterogeneous charge distribution on the antibody surface induces intermolecular electrostatic attraction and thereby self-association, resulting in high viscosity. The self-association that induces high viscosity is reversible, and the self-associated molecules spontaneously dissociate at low antibody concentrations; therefore, it cannot be detected by size-exclusion chromatography which is commonly used for evaluating aggregation of therapeutic MAbs (6). In order to detect self-association, partly concentrated MAb solutions must be analyzed directly. However, the preparation of concentrated MAb solutions is time-consuming and requires extensive laboratory resources and quantities of materials that are not usually available in the early development stage.

In this study, we demonstrated that the particle size parameters obtained from small-angle X-ray scattering (SAXS) measurements correlated very well with the viscosity of highly concentrated MAb solutions. SAXS measurements can be performed using a small volume of samples; therefore, the particle size parameters obtained from SAXS measurements could be used to screen for low viscosity antibodies in the early development stage. Additionally, we also suggest that the simultaneous addition of basic and acidic amino acids effectively suppresses the intermolecular attractive interactions and decreases the viscosity of some types of protein solutions.

MATERIALS AND METHODS

Materials

The humanized IgG monoclonal antibodies MAb1 (IgG4, 146 kDa), MAb2 (IgG2, 147 kDa), and MAb3 (IgG1, 145 kDa) were manufactured and purified by Chugai Pharmaceutical (Tokyo, Japan). The theoretical isoelectric points (pI) of MAb1, MAb2, and MAb3 are 6.6, 5.7, and 9.0, respectively. All MAbs recognize different antigens. 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 I. The protein concentrations of MAb solutions were determined by UV absorbance at 280 nm.

Viscosity Measurements

The viscosity of MAb solutions was measured by an electromagnetic spinning (EMS) method using an EMS viscometer (Kyoto Electronics Manufacturing, Kyoto, Japan) (7). 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 (40–200 mg/mL) 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.

Small-Angle X-ray Scattering (SAXS)

SAXS measurements were performed to examine the protein interactions of MAb solutions at concentrations of 1 or 60 mg/mL by using a SAXSess mc^2 system (Anton Paar, Graz, Austria) with line-collimated Cu K_α radiation ($\lambda = 0.1542$ nm). The scattering patterns of MAb solutions were recorded by a two-dimensional imaging-plate detector. The exposure time of X-ray was 30 min (1 mg/mL) or 10 min (60 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

Table I Buffer Conditions Used in This Study

Buffer name	Buffer conditions	Experiments		
		Viscosity	SAXS	Phase behavior
5 mM citrate/10 mM NaCl/NaOH buffer (low-salt buffer)	5 mM citrate, 10 mM NaCl, NaOH (q.s.), pH 6.0			√
5 mM citrate/50 mM NaCl/NaOH buffer	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	√	√	
5 mM citrate/150 mM NaCl/NaOH buffer	5 mM citrate, 150 mM NaCl, NaOH (q.s.), pH 6.0	√	√	
20 mM His/150 mM Arg/HCl buffer	20 mM His, 150 mM Arg, HCl (q.s.), pH 6.0	√	√	
20 mM His/150 mM Arg/Asp buffer	20 mM His, 150 mM Arg, Asp (q.s.), pH 6.0	√	√	

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

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

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 (8). The $p(r)$ was normalized by the area under the curve. The apparent radius of gyration [R_g^{app} (nm)] was also evaluated by Guinier approximation (using the q range satisfying the criteria $q^*R_g^{app} < 1.3$).

Phase Behavior

The three MAb solutions were formulated at a concentration of 60 mg/mL in 5 mM citrate/10 mM NaCl/NaOH buffer. The MAb solutions were incubated at 25°C for 1 week, and then the phase behavior was observed.

RESULTS

Viscosity Measurements

First, we compared the viscosity of the three MAb solutions in 5 mM citrate/50 mM NaCl/NaOH buffer at 25°C (Fig. 1a). As previously reported, the viscosity of each MAb solution exponentially increased with increasing MAb concentration from ~150 mg/mL (2). The MAb1 solutions exhibited the highest viscosity, followed in order by MAb2 and MAb3. The viscosity of MAb1, MAb2, and MAb3 solutions at a concentration of 200 mg/mL was 219.3, 30.5, and 12.7 mPa·s, respectively (Table II).

Second, we examined the temperature dependence of the viscosity of MAb1 solutions in 5 mM citrate/50 mM NaCl/NaOH buffer in the range from 15 to 35°C (Fig. 1b). The viscosity of MAb1 solutions decreased with the increase in temperature; however, the change was substantially reduced at about 35°C. The viscosity of 200 mg/mL MAb1 solutions at 15, 20, 25, 30, and 35°C was 635.3, 355.7, 219.3, 131.0, and 87.1 mPa·s, respectively (Table II).

Third, we examined the buffer dependence of the viscosity of MAb1 solutions at 25°C using four different types of buffer (Fig. 1c). The viscosity of MAb1 solutions drastically decreased with the increase in NaCl concentration from 50 to 150 mM. The viscosity of MAb1 solutions decreased further in 20 mM His/150 mM Arg/HCl buffer, and decreased most in 20 mM His/150 mM Arg/Asp buffer in which Asp is used as a counterion to His and Arg. The viscosity of 200 mg/mL MAb1

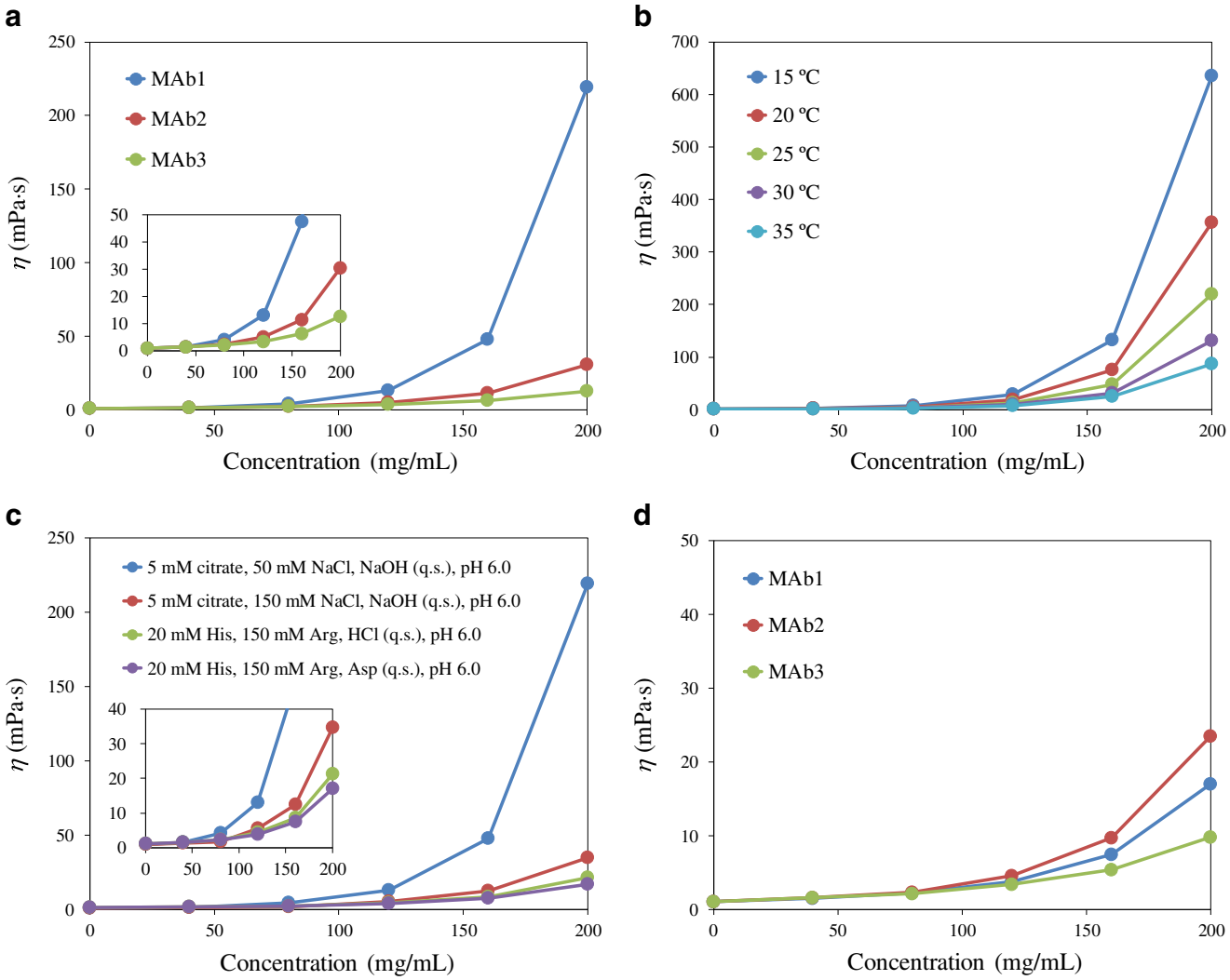

Fig. 1 Viscosity as a function of MAb concentration. (**a**) The viscosity of MAb1, MAb2, and MAb3 solutions in 5 mM citrate/50 mM NaCl/NaOH buffer at 25°C. Inset depicts the zoomed viscosity profiles from 0 to 50 mPa·s. (**b**) The viscosity of MAb1 solutions in 5 mM citrate/50 mM NaCl/NaOH buffer at 15, 20, 25, 30, and 35°C. (**c**) The viscosity of MAb1 solutions in 5 mM citrate/50 mM NaCl/NaOH, 5 mM citrate/150 mM NaCl/NaOH, 20 mM His/150 mM Arg/HCl, and 20 mM His/150 mM Arg/Asp buffers at 25°C. Inset depicts the zoomed viscosity profiles from 0 to 40 mPa·s. (**d**) The viscosity of MAb1, MAb2, and MAb3 solutions in 20 mM His/150 mM Arg/Asp buffer at 25°C.

solutions in 5 mM citrate/50 mM NaCl/NaOH, 5 mM citrate/150 mM NaCl/NaOH, 20 mM His/150 mM Arg/HCl, and 20 mM His/150 mM Arg/Asp buffers was 219.3, 34.6, 21.3, and 17.0 mPa·s, respectively (Table II).

Finally, we compared the viscosity of the three MAb solutions at 25°C in the 20 mM His/150 mM Arg/Asp buffer, which had most effectively reduced the viscosity of MAb1 solutions (Fig. 1d). The viscosity of MAb2 solutions was not substantially reduced by using 20 mM His/150 mM Arg/Asp buffer; therefore, MAb2 solutions exhibited the highest viscosity, followed in order by MAb1 and MAb3. The viscosity of MAb1, MAb2, and MAb3 solutions at a concentration of 200 mg/mL was 17.0, 23.6, and 9.8 mPa·s, respectively (Table II).

SAXS Measurements

In order to investigate the relationship between viscosity and protein interactions of MAb solutions, SAXS measurements were conducted under the same experimental conditions as used for viscosity measurements (*i.e.*, the same MAb species, buffer conditions, and temperature).

First, we compared the association state of the three MAb solutions in 5 mM citrate/50 mM NaCl/NaOH buffer at 25°C by SAXS measurements. The scattering intensity was normalized to the MAb concentration. The MAb solutions at 60 mg/mL were diluted to 1 mg/mL, and the scattering profiles were obtained. At 1 mg/mL, MAb1 solution exhibited the highest scattering intensity; however, there was no difference between MAb2 and MAb3 (Fig. 2a). On the other hand,

Table II Particle Size Parameters [R_g^{app} (nm) and D_{max}^{app} (nm)] Obtained by SAXS Measurements at a Concentration of 60 mg/mL, and Viscosity of MAb Solutions at a Concentration of 200 mg/mL

MAb type	Buffer conditions	Temperature (°C)	R_g^{app} (nm) [a] at 60 mg/mL	D_{max}^{app} (nm) [a]	Viscosity (mPa·s) [b] at 200 mg/mL
MAb1	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	15	14.6	56	635.3 ± 2.1
	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	20	12.6	49	355.7 ± 1.2
	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	25	10.4	42	219.3 ± 2.3
	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	30	10.1	39	131.0 ± 0.0
	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	35	9.0	37	87.1 ± 0.4
	5 mM citrate, 150 mM NaCl, NaOH (q.s.), pH 6.0	25	6.8	28	34.6 ± 0.6
	20 mM His, 150 mM Arg, HCl (q.s.), pH 6.0	25	5.5	21	21.3 ± 0.0
	20 mM His, 150 mM Arg, Asp (q.s.), pH 6.0	25	4.9	17	17.0 ± 0.0
MAb2	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	25	5.5	22	30.5 ± 0.2
	20 mM His, 150 mM Arg, Asp (q.s.), pH 6.0	25	5.2	20	23.6 ± 0.3
MAb3	5 mM citrate, 50 mM NaCl, NaOH (q.s.), pH 6.0	25	4.3	16	12.7 ± 0.1
	20 mM His, 150 mM Arg, Asp (q.s.), pH 6.0	25	3.8	14	9.8 ± 0.2

[a] The data represent a single experiment

[b] The data represent the mean ± standard deviation of three experiments

at 60 mg/mL, the differences in scattering intensity were clearly observed at low scattering angles (Fig. 2a). The normalized scattering intensity of MAb1 was increased by the increase in concentration, whereas that of MAb2 and MAb3 was decrease. Interestingly, MAb2 exhibited higher scattering intensity than MAb3 and this trend was different from that at 1 mg/mL. 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. From the IFT of the scattering data obtained at 60 mg/mL, the $p(r)$ of each MAb solution was obtained (Fig. 3a). The $p(r)$ of the MAb2 and MAb3 solutions exhibited two broad peaks in the range of 0–10 nm, which is characteristic of the $p(r)$ of monomeric IgG1, IgG2, and IgG4 (9–11). On the other hand, the $p(r)$ of the MAb1 solution exhibited a broad shape with an additional unclear shoulder at 20–30 nm, which was strikingly different from that of the MAb2 and MAb3 solutions. From the x-intercept of $p(r)$, the D_{max}^{app} of each MAb solution at 60 mg/mL was also determined. The D_{max}^{app} values for MAb1, MAb2, and MAb3 were 42, 22, and 16 nm, respectively (Table II).

Second, we examined the temperature dependence of the association state of MAb1 solutions (60 mg/mL) in 5 mM citrate/50 mM NaCl/NaOH buffer in the range from 15 to 35°C. The scattering intensity at low scattering angles was decreased by the increase in temperature (Fig. 2b). The broad shoulder of $p(r)$ at 20–30 nm detected at 25°C became clearer along with the decrease in temperature, whereas a new broad shoulder at 30–40 nm became apparent at 30°C and 35°C (Fig. 3b). Similarly to the scattering intensity, D_{max}^{app} decreased with the increase in temperature, and the change was substantially reduced at about 35°C. The D_{max}^{app} values for MAb1 at 15, 20, 25, 30, and 35°C were 56, 49, 42, 39, and 37 nm, respectively (Table II).

Third, we examined the buffer dependence of the association state of MAb1 solutions (60 mg/mL) at 25°C using four different buffers. The scattering intensity at low scattering angles drastically decreased with the increase of NaCl concentration from 50 to 150 mM and decreased further by using amino acids as buffer components (Fig. 2c). The broad shoulder of $p(r)$ at 20–30 nm that was present in the 5 mM citrate/50 mM NaCl/NaOH buffer disappeared in 5 mM citrate/150 mM NaCl/NaOH buffer, in which a new peak at around 20 nm became obvious (Fig. 3c). This peak also disappeared in 20 mM His/150 mM Arg/HCl buffer, in which a new tiny shoulder at around 15 nm further became apparent. Finally, this new peak also almost disappeared in 20 mM His/150 mM Arg/Asp buffer. It is noteworthy that there was a clear distinction in the $p(r)$ between 20 mM His/150 mM Arg/HCl and 20 mM His/150 mM Arg/Asp buffers, although the scattering intensity of MAb1 solutions was almost equal in these two buffers. The D_{max}^{app} values for MAb1 solutions in 5 mM citrate/50 mM NaCl/NaOH buffer, 5 mM citrate/150 mM NaCl/NaOH buffer, 20 mM His/150 mM Arg/HCl buffer, and 20 mM His/150 mM Arg/Asp buffer were 42, 28, 21, and 17 nm, respectively (Table II).

Finally, we compared the association state of the three MAb solutions (60 mg/mL) at 25°C in 20 mM His/150 mM Arg/Asp buffer. The scattering intensity of MAb1 and MAb3 solutions substantially decreased with the buffer change; on the

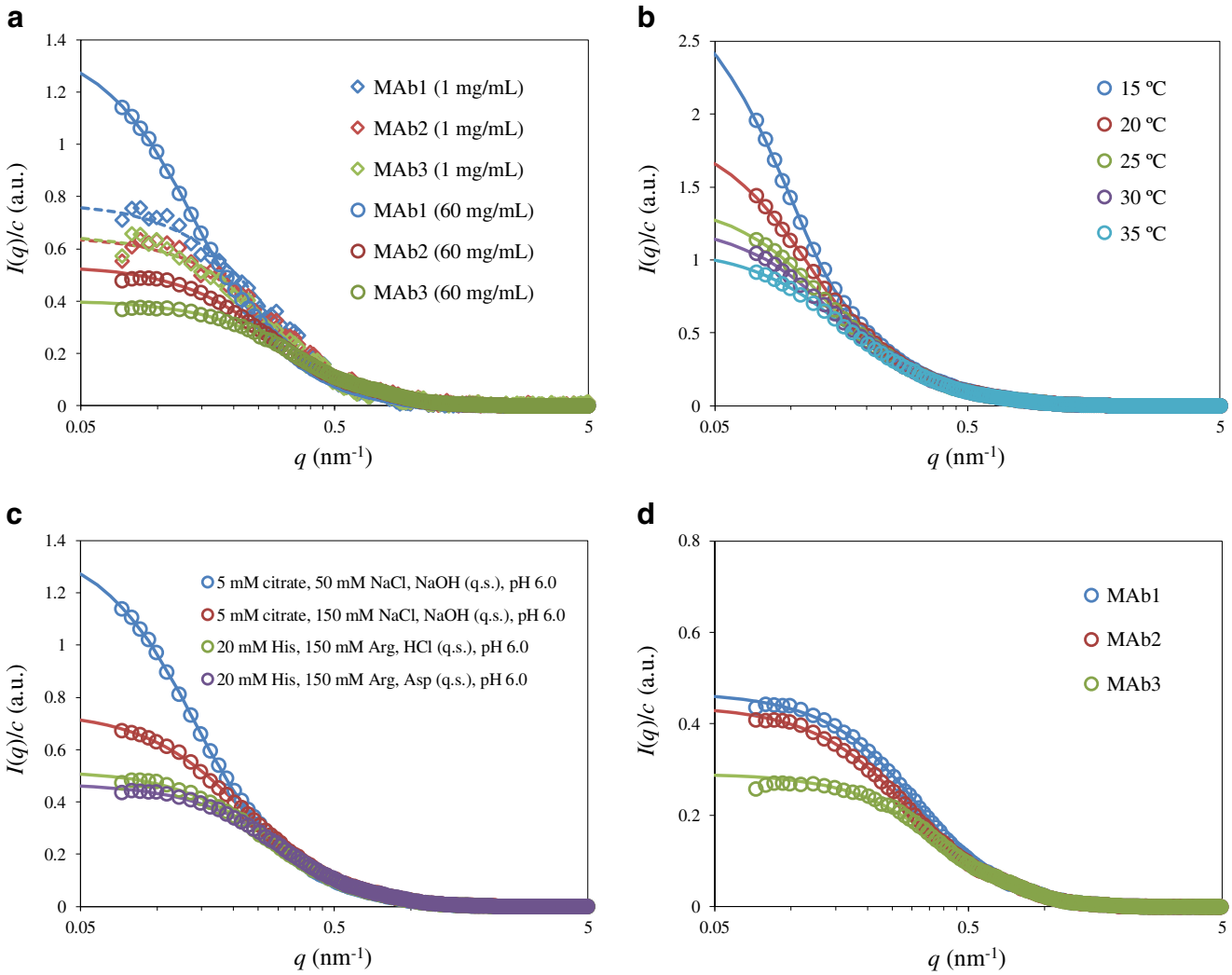

Fig. 2 SAXS profiles of MAb solutions. (**a**) SAXS profiles of MAb1, MAb2, and MAb3 solutions at 1 and 60 mg/mL in 5 mM citrate/50 mM NaCl/NaOH buffer at 25°C. (**b**) SAXS profiles of MAb1 solutions at 60 mg/mL in 5 mM citrate/50 mM NaCl/NaOH buffer at 15, 20, 25, 30, and 35°C. (**c**) SAXS profiles of MAb1 solutions at 60 mg/mL in 5 mM citrate/50 mM NaCl/NaOH, 5 mM citrate/150 mM NaCl/NaOH, 20 mM His/150 mM Arg/HCl, and 20 mM His/150 mM Arg/Asp buffers at 25°C. (**d**) SAXS profiles of MAb1, MAb2, and MAb3 solutions at 60 mg/mL in 20 mM His/150 mM Arg/Asp buffer at 25°C. Lines are the result of fitting by indirect Fourier transformation.

other hand, the contribution of this buffer was relatively small in the MAb2 solution (Fig. 2d). As a result, the scattering intensity of MAb1 and MAb2 solutions became almost equal. The $p(r)$ of MAb1 and MAb3 solutions was clearly sharpened; however, that of MAb2 solution was not obviously affected by the buffer change (Fig. 3d). The D_{max}^{app} values for MAb1, MAb2, and MAb3 were 17, 20, and 14 nm, respectively (Table II).

To relate the viscosity and the particle size parameters obtained from SAXS measurements at 60 mg/mL, the values of viscosity at a concentration of 200 mg/mL were plotted as a function of R_g^{app} (Fig. 4a) obtained by Guinier analysis and as a function of D_{max}^{app} (Fig. 4b) using the results of a series of experiments (Table II). The viscosity was strongly correlated with both R_g^{app} and D_{max}^{app}. We confirmed that three additional humanized IgG monoclonal antibodies also provides similar linear correlation to that in Fig. 4 (see Supplementary Material).

Phase Behavior

We observed the phase behavior of the three MAb solutions in a low-salt buffer (*i.e.*, 5 mM citrate/10 mM NaCl/NaOH buffer) at 25°C. The MAb3 solution maintained a clear liquid state, whereas the MAb1 solution exhibited liquid–liquid phase separation and the MAb2 solution became opalescent (Fig. 5).

DISCUSSION

As previously reported, 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) (2–5). In fact, Singh *et al.* have recently

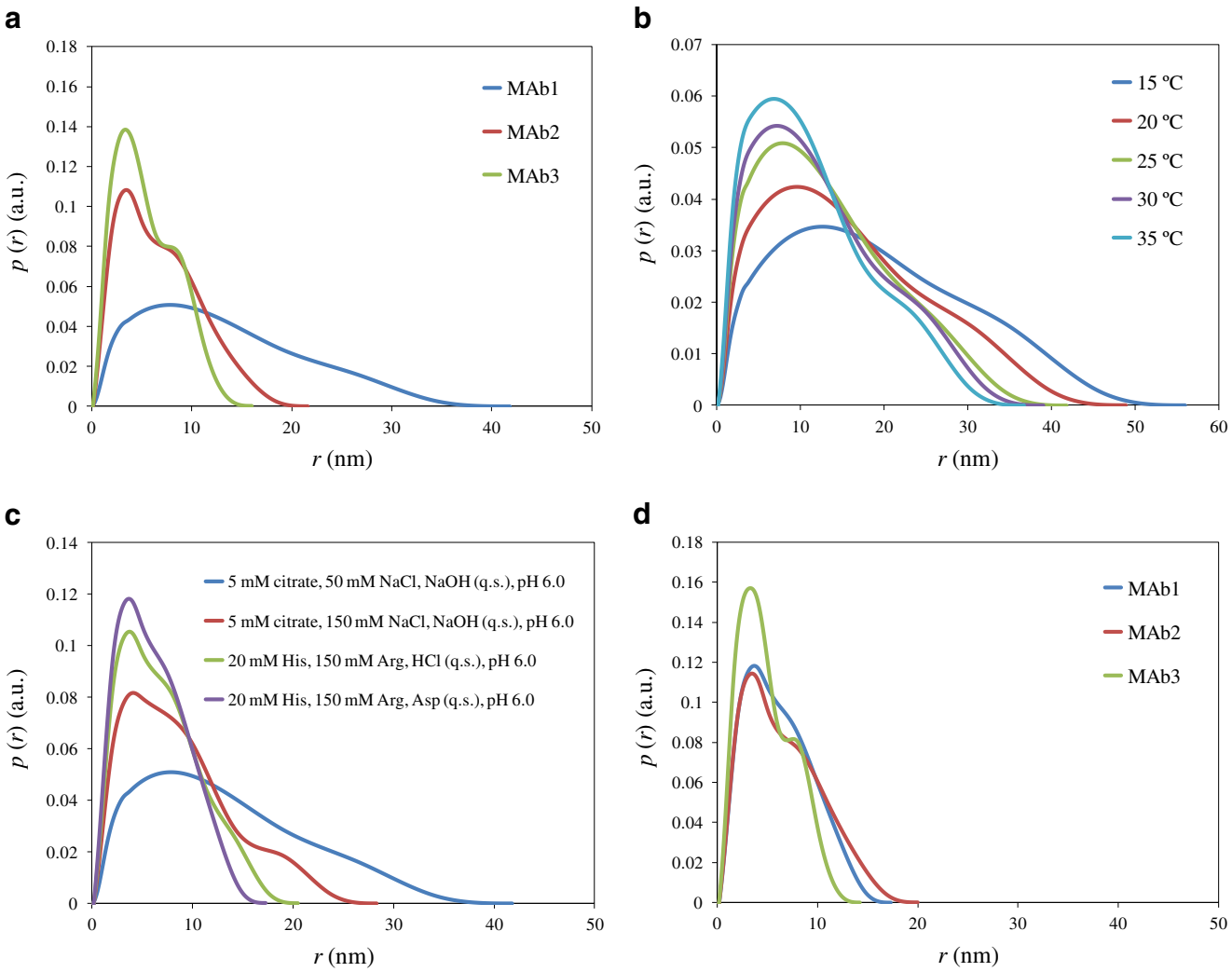

Fig. 3 Pair–distance distribution function [$p(r)$] of MAb solutions at 60 mg/mL. (**a**) The $p(r)$ of MAb1, MAb2, and MAb3 solutions in 5 mM citrate/50 mM NaCl/NaOH buffer at 25°C. (**b**) The $p(r)$ of MAb1 solutions in 5 mM citrate/50 mM NaCl/NaOH buffer at 15, 20, 25, 30, and 35°C. (**c**) The $p(r)$ of MAb1 solutions in 5 mM citrate/50 mM NaCl/NaOH, 5 mM citrate/150 mM NaCl/NaOH, 20 mM His/150 mM Arg/HCl, and 20 mM His/150 mM Arg/Asp buffers at 25°C. (**d**) The $p(r)$ of MAb1, MAb2, and MAb3 solutions in 20 mM His/150 mM Arg/Asp buffer at 25°C.

demonstrated by using dielectric relaxation spectroscopy that the pH dependence of viscosity of some types of antibody is synchronized with that of the dipole moment, suggesting that dipole–dipole interaction plays an important role in governing the viscosity behavior of the antibody at high concentration (12). In addition, some antibodies with high viscosity have been reported to exhibit liquid–liquid phase separation or opalescence at low salt concentrations in accordance with the fact that electrostatic attraction is enhanced by decreased salt concentrations (13, 14). In this study, the highly viscous properties of MAb1 solutions can be attributed to electrostatic attraction because MAb1 solution exhibited liquid–liquid phase separation at a low salt concentration and the viscosity was substantially reduced by increased NaCl concentration. In MAb2 solutions, too, electrostatic attraction may partially contribute to the viscosity behavior because MAb2 solutions exhibited opalescence at a low salt concentration.

With respect to development of subcutaneous injections of therapeutic MAbs, highly viscous MAb solutions, such as MAb1, pose many technical challenges regarding manufacturability and usability in addition to problems of stability (1); therefore, even a slight reduction in viscosity may play an important role in the development of such MAb therapeutics. It is well established that Arg reduces protein–protein and protein–surface interactions; therefore, Arg is commonly used as a formulation additive to suppress the aggregation of biopharmaceuticals (15). In this study, the simultaneous addition of basic and acidic amino acids effectively reduced the viscosity of MAb1 solutions, which we attributed to the suppression of the attractive interactions as detected by the change in particle size parameters. The simultaneous addition of basic and acidic amino acids can contribute to the improvement of colloidal stability of some types of proteins (16); Shukla *et al.* proposed that the crowding due to the presence

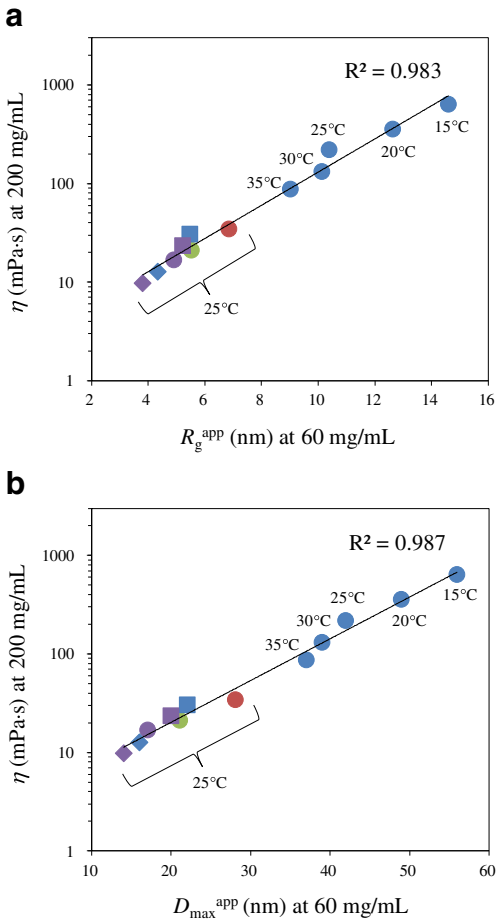

Fig. 4 Correlation between the viscosity at 200 mg/mL and (**a**) the apparent radius of gyration [R_g^{app} (nm)] and (**b**) the apparent maximum dimension [D_{max}^{app} (nm)] of MAb solutions at 60 mg/mL. MAb1 (*circle*), MAb2 (*square*), and MAb3 (*diamond*) in 5 mM citrate/50 mM NaCl/NaOH (*blue*), 5 mM citrate/150 mM NaCl/NaOH (*red*), 20 mM His/150 mM Arg/HCl (*green*), or 20 mM His/150 mM Arg/Asp (*purple*) buffers were used at 15, 20, 25, 30, or 35°C.

Fig. 5 Phase behavior of MAb solutions at a concentration of 60 mg/mL in 5 mM citrate/10 mM NaCl/NaOH buffer observed after 1 week storage at 25°C.

of an enhanced number of Arg and glutamic acid (Glu) molecules on the protein surface suppresses protein–protein association (17). On the other hand, we have recently reported that, in contrast to addition of Arg hydrochloride alone, addition of Arg–Glu and Arg–Asp mixtures increases the conformational stability of IgG1 (18). Taking these results together, it appears that the simultaneous addition of basic and acidic amino acids may confer excellent stabilization in terms of both colloidal and conformational stability for some types of proteins. Meanwhile, the combination of basic and acidic amino acids did not substantially reduce the viscosity of MAb2 solutions. One possible explanation for this result is that, in addition to electrostatic attraction, van der Waals attraction, which could not be shielded by the addition of amino acids, contributes to the attractive interactions of MAb2 solutions because the pI (5.7) of the MAb2 molecule was close to the pH (6.0) investigated in this study.

Our study revealed that the simultaneous addition of basic and acidic amino acids effectively reduces the viscosity of some types of proteins; however, this combination effect could not reduce the viscosity of the MAb1 solutions to the same degree as that of the MAb3 solutions. 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. Several research groups have reported that the osmotic second virial coefficient (19) and the diffusion interaction parameter (20, 21), which can be obtained using small quantities of samples, correlate with the viscosity that arises at high concentrations. These findings are very useful for developing high concentration antibody products because these parameters can be used as qualitative indicators to predict relative values of viscosity among sample solutions; however, the predictability is reported to decrease in the presence of attractive interactions and is not suitable for quantitative prediction. On the other hand, it is known that SAXS is one of the useful tools to study the intermolecular protein interactions and thereby self-association (clustering) of proteins (22, 23). In this study, we obtained the apparent particle sizes by intentionally assuming that there is no interaction between the particles and applying the IFT technique to the scattering data obtained at a middle protein concentration (*i.e.*, 60 mg/mL), where the particle–particle interaction becomes apparent (24). Justification for choosing the 60 mg/mL is described in Supplementary Material. We speculated that the apparent particle size parameters (*i.e.*, R_g^{app} and D_{max}^{app}) could be used as indicators of viscosity that arises at high concentrations because these parameters would be overestimated by the attractive interactions which induces high viscosity. In fact, the viscosity at 200 mg/mL was strongly correlated with both R_g^{app} and

D_{max}^{app}, indicating that the attractive interactions and thereby self-association increases the viscosity at high concentrations, as previously reported (23, 25). Interestingly, the viscosity and the particle size parameters obtained under different experimental setups achieved by changing MAb species, buffer conditions, and temperatures exhibited a single correlation line for each particle size parameter, demonstrating the versatility of the SAXS method for quantitative viscosity evaluation. The EMS viscometer used in this study needs more than 90 μL of sample solutions for viscosity measurements, and other viscometers using smaller sample volumes sometimes cannot measure the viscosity of adsorptive MAb solutions. On the other hand, SAXS measurements can be performed using a small volume of samples (~10 μL); therefore, the particle size parameters could be used to screen for low viscosity antibodies in the early stages of development. In addition, the viscosity at high concentrations can be predicted not qualitatively but quantitatively and thereby we can understand the accurate product profile in the early stages of development; therefore, SAXS technique also contributes to front-loading of manufacturing process development and primary container/device development for the subcutaneous injection and thereby potentially can shorten the total development period. These findings from this study could contribute to delivering new antibody drugs with high quality to patients more speedy. However, further detailed research using many types of antibody molecule is needed in order to clarify the limit of predictability and draw the general conclusion. The concentration range in which self-association starts to form also should be clarified to optimize the concentration for SAXS screening method.

CONCLUSION

SAXS analyses detected the differences in the protein interactions of MAb solutions depending on MAb species, buffer conditions, and temperature. Interestingly, 20 mM His/150 mM Arg/Asp buffer suppressed the attractive interactions and decreased the viscosity of highly viscous MAb1 solutions more strongly than did 20 mM His/150 mM Arg/HCl buffer. This result suggests that the simultaneous addition of basic and acidic amino acids effectively improves the colloidal stability of MAb1 solutions. The particle size parameters (D_{max}^{app} and R_g^{app}) obtained by SAXS measurements of MAb solutions at a concentration of 60 mg/mL correlated very well with the viscosity of MAb solutions at a concentration of 200 mg/mL. SAXS measurements can be performed using a small volume of samples; therefore, these parameters could be used to screen for low viscosity antibodies in the early development stage.

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