Pharm Res (2011) 28:1552–1560
DOI 10.1007/s11095-011-0388-7

RESEARCH PAPER

Effect of Sugar Molecules on the Viscosity of High Concentration Monoclonal Antibody Solutions

Feng He • Christopher E. Woods • Jennifer R. Litowski • Lauren A. Roschen • Himanshu S. Gadgil • Vladimir I. Razinkov • Bruce A. Kerwin

Received: 13 November 2010 / Accepted: 31 January 2011 / Published online: 15 May 2011

ABSTRACT

Purpose To assess the effect of sugar molecules on solution viscosity at high protein concentrations.

Methods A high throughput dynamic light scattering method was used to measure the viscosity of monoclonal antibody solutions. The effects of protein concentration, type of sugar molecule (trehalose, sucrose, sorbitol, glucose, fructose, xylose and galactose), temperature and ionic strength were evaluated. Differential scanning fluorimetry was used to reveal the effect of the same sugars on protein stability and to provide insight into the mechanism by which sugars increase viscosity.

Results The addition of all seven types of sugar molecules studied result in a significant increase in viscosity of high concentration monoclonal antibody solutions. Similar effects of sugars were observed in the two mAbs examined; viscosity could be reduced by increasing the ionic strength or temperature. The effect by sugars was enhanced at higher protein concentrations.

Conclusions Disaccharides have a greater effect on the solution viscosity at high protein concentrations compared to monosaccharides. The effect may be explained by commonly accepted mechanisms of interactions between sugar and protein molecules in solution.

KEY WORDS dynamic light scattering · high throughput · monoclonal antibody · preferential exclusion · preferential hydration · sugar molecule · viscosity

ABBREVIATIONS

cP	centipoise
DLS	dynamic light scattering
DSF	differential scanning fluorimetry
Igg	Immunoglobulin G
mAb	monoclonal antibody

INTRODUCTION

Monoclonal antibodies are one of the most common classes of biotherapeutic molecules, with more than 150 products on the market and in development (1). To improve patient convenience, there has been a movement towards the use of high concentration protein solutions (i.e. > 100 mg/mL) in pre-filled syringes. At these concentrations, many protein solutions become highly viscous, posing considerable challenges for both processing and delivery (2). In ultrafiltration/diafiltration systems, high viscosity leads to high membrane backpressures and reduction in the flow rate. In pre-filled syringes, increases in viscosity may lead to difficulties during injection. Consequently, controlling solution viscosity is a high priority when developing high concentration protein formulations.

Protein-protein interactions are a primary cause of high viscosity and have received considerable attention from researchers. Both reversible self-interactions and electrostatic repulsions have been implicated as causes for the viscosity of protein solutions (3–5). When these interactions

F. He · C. E. Woods · V. I. Razinkov
Formulation and Analytical Resources, Amgen, Inc.
Seattle, Washington, USA

J. R. Litowski · L. A. Roschen · H. S. Gadgil · B. A. Kerwin
Analytical and Formulation Sciences, Amgen, Inc.
Seattle, Washington, USA

F. He (✉) · B. A. Kerwin (✉)
AW2/D3152
1201 Amgen Court West
Seattle, Washington 98119, USA
e-mail: fhe@amgen.com
e-mail: bkerwin@amgen.com

are electrostatically based, they can frequently be controlled by screening charges on the protein surface through the addition of salt. However, in some cases, salts can have detrimental effects on the stability of mAbs (6), and the observed destabilizing effects have been linked to increased levels of aggregation, particulation (6) and opalescence (7). Sugars are frequently used for formulation of mAbs (8). As a major component of many formulations, they may form up to 10% (w/v) of the formulation components. They are included for the dual roles of increasing protein stability and maintaining tonicity (8,9). Their ability to stabilize proteins is explained by the preferential exclusion and hydration model introduced by Timasheff and colleagues (10–12). The sugars are excluded from the region immediately surrounding the protein, leading to preferential hydration of the protein shell and increased chemical potential of the solution. This, in turn, shifts the equilibrium towards the more compact, natively folded state. The model was developed using low concentration solutions of proteins with relatively few interactions among the protein molecules. In high concentration protein solutions, protein-protein interactions lead to more complex behaviors, and the solution exhibits non-ideality. Molecular crowding forces protein molecules and other solutes into limited spaces, which increases the chance of protein-protein and protein-solvent interactions (13). These interactions include charge-charge attraction and repulsion, hydrogen bonding, hydrophobic interaction, etc. (14). Preferential exclusion of osmolytes, such as sugars, is enhanced at high protein concentrations (11). These interactions among protein and other solutes can lead to changes in physical properties of the solution, including light scattering patterns (15) and protein diffusion parameters (14). Recently, it has been demonstrated that increased viscosity at high protein concentrations may also be attributed to enhanced protein-protein interactions (3).

Although sugars have been used as microviscogen agents to mediate enzymatic reaction rates by increasing solution viscosity (16,17), to the best of our knowledge, the effect of sugars on solution viscosity at high protein concentrations has not been fully characterized. These types of studies are complicated by the fact that the traditional rheological techniques, such as the cone-and-plate method, require significant amounts of material and time (3,18–20). Recently, a high throughput analytical method utilizing dynamic light scattering (DLS) was developed and applied to protein viscosity measurements (21). The method was derived from a technique used to measure polymer solution viscosity (22). In the current study, the DLS method was employed to evaluate the viscosity of sugar-protein solution complexes. While the method cannot reveal shear effects, it is an excellent tool to compare relative viscosities among a large number of samples with minimal material requirements. In this report, we present a thorough viscosity characterization of solutions containing high protein concentrations and a variety of sugar molecules. To examine the possible role of preferential hydration and exclusion, we employed viscosity measurements at higher ionic strength and various temperatures, both of which strongly altered the solution viscosity of the sugar-protein system. These results are consistent with the effect of preferential hydration and exclusion reported in the literature. Furthermore, evaluations of protein thermal stability demonstrated that the effect of sugar molecules was enhanced at higher protein concentrations, where a significant increase in viscosity was also observed. This provides additional evidence that the effect of sugars on protein solution viscosity may be explained by preferential hydration and exclusion.

MATERIALS AND METHODS

Preparation of mAb and Sugar Solutions

MAb1 (an IgG1) and mAb2 (an IgG2) were purified by Amgen Inc. as described elsewhere (23). The purified mAbs were dialyzed (1:100,000 volume ratio) against a solution containing 10 mM sodium acetate, pH 5 at 4°C using dialysis cassettes with a 30,000 MWCO (Thermo Scientific, Rockford, IL). The dialyzed protein solutions were then concentrated by centrifugation using 10,000 MWCO Amicon Ultra-15® centrifugal filters (Millipore Corporation, Billerica, MA).

Sugars were purchased from Sigma-Aldrich Inc. (St. Louis, MO) at the highest chemical grades commercially available. Dry sugar powders were dissolved into distilled water, and 100 mM sodium acetate (pH 5) buffer was spiked into the sugar stock solutions to achieve a final buffer concentration of 10 mM sodium acetate. Sugar stocks contained the following sugar concentrations: trehalose and sucrose, 1.5 M; sorbitol, glucose, fructose, xylose, and galactose, 3 M. Concentrated mAb solutions were directly mixed with sugar stocks to achieve the desired protein and sugar concentrations, while 10 mM sodium acetate (pH 5) was used to adjust sample volume. For measurements of mAb solution viscosity in the presence of salt, 10 mM sodium acetate (pH 5) with 4.5 M sodium chloride was spiked into samples accordingly. All the final sugar concentrations were based on dilutions from the stock solutions.

Viscosity Measurements by Dynamic Light Scattering (DLS)

The solution viscosity was measured using a DLS method developed previously (21). In this method, the viscosity of the solution is determined based on a difference in the movement of beads in solution compared to the expected light scattering behavior due to Brownian motion alone.

Viscosity samples were prepared by mixing 49.5 μL of sample solution with 0.5 μL of polystyrene beads with a 101.5 nm nominal radius at 1.05 g/cm3 density (Thermo Scientific Inc., Fremont, CA). The DLS measurement was performed using a DynaPro™ Plate reader dynamic light scattering system (Wyatt Technology, Santa Barbara, CA) and a 384-well clear-bottom plate containing 25 μL of liquid sample in each well. The plate was centrifuged at 2,000×g for 3 min to eliminate air bubbles. Experimental temperature was reached by a block-heating system with flushing using nitrogen gas. Samples were allowed to equilibrate for 30 min prior to the first measurement. Each measurement contained 10 acquisitions and 30 s of data collection per acquisition. Assuming water viscosities as input, the hydrodynamic radii of the polystyrene beads were determined using the "Legacy" method available in the Dynamics™ software (Wyatt Technology, Santa Barbara, CA). The micro-viscosity was calculated by applying the Stokes-Einstein equation.

Protein Melting Detected by Differential Scanning Fluorimetry (DSF)

The high throughput DSF technique was employed to monitor mAb unfolding during temperature melting according to the previously described method (24). Briefly, a 96-well microplate was used during DSF with each well containing 19.5 μL of protein sample and 0.5 μL of SYPRO® Orange (Invitrogen Inc., Carlsbad, CA) that was diluted from the purchased stock 1:125 in water. The final dye concentration was 1/5000 of the initial product and equivalent to 1× working concentration used for protein gel staining. A CFX96 Real-Time PCR instrument (Bio-Rad Laboratories, Inc., Hercules, CA) was used, and the "FRET" channel setting was applied to record fluorescence changes during the DSF measurement. Samples were incubated at 20°C for 3 min prior to melting, during which the temperature was increased from 20 to 95°C at 0.2°C increments and an equilibration time of 12 s at each temperature. The hydrophobic exposure temperature, T_h, was reported as an indication of the transition mid-point of protein unfolding. The first-order derivative curves and the T_h values were determined using the CFX Manager™ software (Bio-Rad Laboratories, Inc., Hercules, CA).

RESULTS

Viscosity Assessment of mAb Solutions Containing Sugars

The sugar molecules included in this study are listed in Fig. 1. Among these sugars, trehalose and sucrose are disaccharides, while the other five are monosaccharides. These sugar molecules were selected to test the effect of differences in sugar structure and their hydrogen bonding propensity. While sugar solutions alone can be highly viscous, i.e. 60% (w/v) sucrose possesses a viscosity of 60 cP at room temperature (25), the sugar concentrations used in this study did not contribute significantly to the solution viscosity in the absence of protein. The viscosity values of buffer controls containing these sugars are summarized in Table I and as shown resulted in less than 2 cP of viscosity at 25°C.

The viscosity of protein solutions increases nonlinearly as the protein concentration increases (2,3,5,21,26). In this study, we evaluated two mAb molecules in combination with seven sugars using a high throughput DLS method described previously (21). In addition, viscosity measurements using a cone-and-plate instrument on selected samples at high protein and sugar concentrations agreed with the data obtained by DLS (not shown). Solution viscosity was measured as a function of protein concentration, and the results are shown in Fig. 2. The viscosity increase at lower protein concentrations is minimal, while the slope of viscosity change increases significantly at higher protein concentrations. The addition of sugars increased the solution viscosity at all protein concentrations studied with a greater increase observed as the protein concentration increased. The sugar concentrations (300 mM for disaccharides and 600 mM for monosaccharides) were selected to ensure that there was the same number of monosaccharide units in all the samples. For both mAb molecules, trehalose displayed the highest viscosity, followed by sucrose and sorbitol, while xylose manifested the smallest viscosity increase. The viscosity of sugar-protein solutions are significantly higher than that observed in protein-free solutions at the same sugar concentrations or sugar-free protein solutions.

Solution viscosity is also highly dependent upon sugar concentration. Both mAb1 and mAb2 were subjected to viscosity measurements as sugars were titrated into the solution. In general, the increase in viscosity appeared linear over the sugar concentrations examined (shown in Fig. 3). The increased viscosity showed a strong dependence on the number of monosaccharide units in both IgG types. To further evaluate the effect by sugars, mAb2 was used as a model protein in the following studies.

The Effect of Sugar on Viscosity is Altered by Salt and Temperature

Ions can significantly change the protein solution viscosity (3,4,21). Charged ions are thought to interact with charged amino acid side chains on the protein surface, altering protein-protein interactions and, as a result, decreasing the

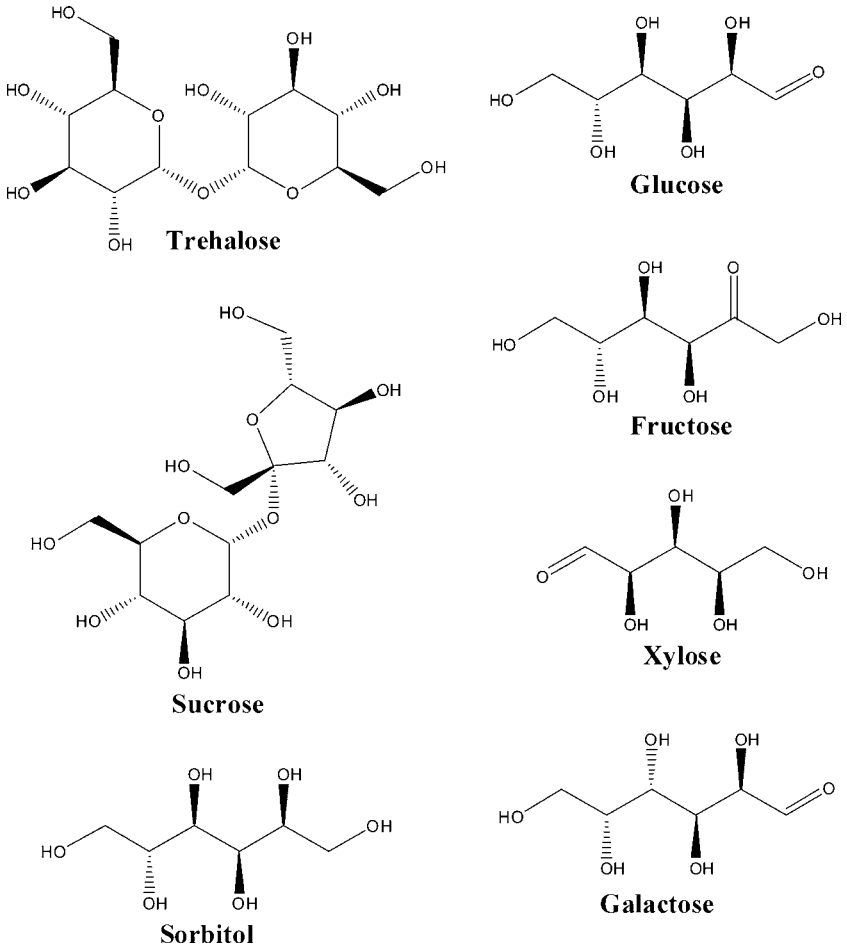

Fig. 1 Molecular structure of the sugars studied.

solution viscosity (3,4). To study the combined effects of ions and sugar molecules, mAb2 was evaluated at 177 mg/mL in the absence and presence of sodium chloride. The concentration of salt, 150 mM, was derived from a report published elsewhere (3) in order to achieve 50% of the viscosity mitigation. The results of the salt effect are illustrated in Fig. 4. The addition of salt reduced both the absolute solution viscosity, and the increase in viscosity induced by sugars. The monosaccharides generally showed lower viscosities than disaccharides, with the exception of sorbitol, which has viscosities similar to sucrose in both the absence and presence of salt. While NaCl certainly alters the general viscosity properties of the whole solutions, the data with NaCl suggest that the effect of sugars on protein viscosity can also be mitigated by ions in solution. Polyethylene glycol (PEG) is known to mediate solution osmolality and is widely used in pharmaceutical development. In this study, we used PEG-300 since it is the smallest PEG commercially available and the molecular mass is close to that of a disaccharide. PEG-300 displayed similar behavior to sugars in mAb solutions, increasing the viscosity as well, in spite of having a different structure (Fig. 4).

Table I Viscosity of Buffers Containing Sugar Molecules

Solution	% Sugar (w/v)[a]	Viscosity (cP)[b]
10 mM sodium acetate, pH 5	NA	0.91 ± 0.06
10 mM sodium acetate, pH 5, 300 mM Trehalose	10.27	1.53 ± 0.01
10 mM sodium acetate, pH 5, 300 mM Sucrose	10.27	1.26 ± 0.01
10 mM sodium acetate, pH 5, 600 mM Sorbitol	10.93	1.73 ± 0.06
10 mM sodium acetate, pH 5, 600 mM Glucose	10.81	1.19 ± 0.01
10 mM sodium acetate, pH 5, 600 mM Fructose	10.81	1.22 ± 0.03
10 mM sodium acetate, pH 5, 600 mM Xylose	9.01	1.18 ± 0.01
10 mM sodium acetate, pH 5, 600 mM Galactose	10.81	1.23 ± 0.03

[a] Percentage of sugar was calculated based on the anhydride form

[b] Solution viscosities were measured using the DLS method at 25°C as described in Materials and Methods. The results are presented as *average value* ± *standard deviation*, calculated from duplicate experiments

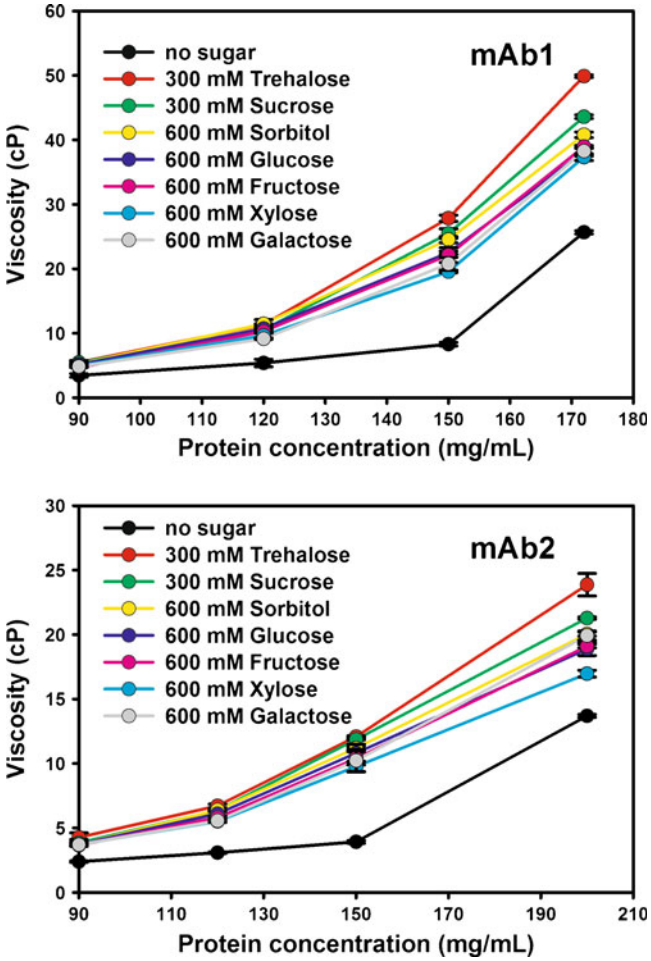

Fig. 2 Solution viscosity as a function of mAb concentration. All samples contained 10 mM sodium acetate, pH 5. Samples were measured by DLS, and the data points reflect average values and standard deviations calculated from duplicate experiments at 25°C. Lines are for guiding purposes only.

Another factor that contributes significantly to solution viscosity is temperature. In general, as temperature increases, the solution viscosity decreases (27). The impact of temperature was fully assessed in this study over a range of 5–35°C. The viscosity values followed a decreasing trend as temperature increased in all samples (Fig. 5a). In addition, the relative impact of added sugar on viscosity increase was also significantly affected by temperature (shown in Fig. 5b). Overall, the increase of viscosity due to sugars decreased as the temperature increased from 5 to 35°C. This observation confirms that the mechanism by which sugars increase protein solution viscosity is strongly mediated by temperature.

The Effect of Sugars on mAb Thermal Melting

Sugar molecules, such as trehalose and sucrose, have been shown to offer protection of a protein's structural integrity under environmental stresses (9,28–31). Traditionally, this protective effect has been assessed by differential scanning calorimetry (DSC) via temperature melting of the protein, where the stabilization is reflected by a shift of the transition to higher temperatures in the presence of sugars (30). Liquid-based DSC, however, is limited to relatively low protein concentrations, making it impossible to apply to high protein concentration solutions directly. Differential scanning fluorimetry (DSF) employing SYPRO Orange, on the other hand, is a tool that can be used to evaluate protein thermal stability over a wide range of protein concentrations during melting (24). Representative melting curves of mAb2 at two different concentrations with and without sucrose are shown in Fig. 6a. The effect by sucrose is minimal at 1 mg/mL protein, as shown by the nearly overlapping fluorescence traces. In contrast, a significant delay of thermal transition is observed in samples contain-

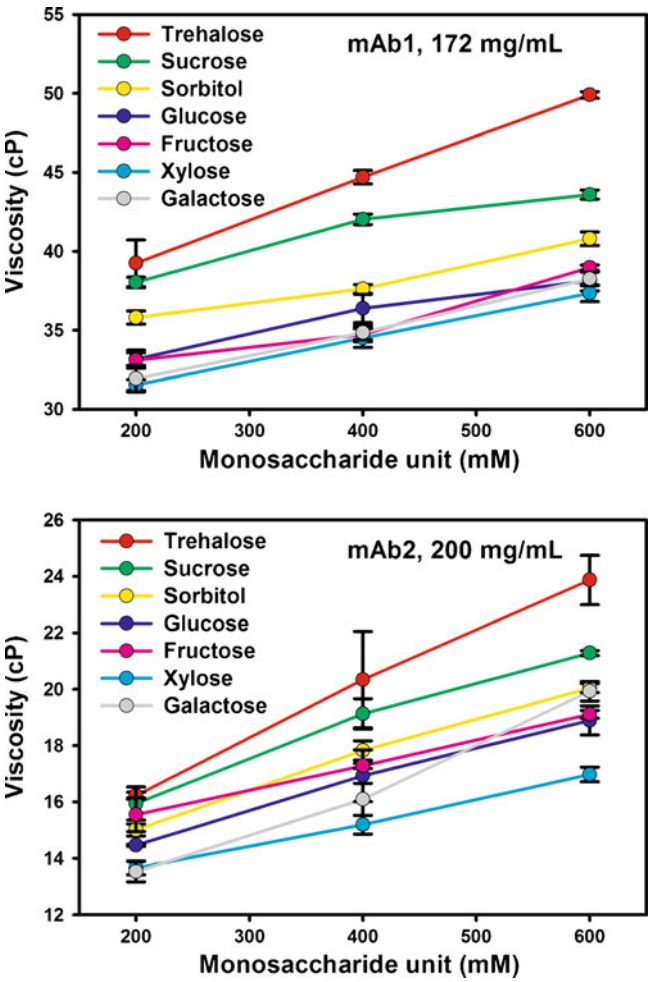

Fig. 3 Solution viscosity as a function of sugar concentration. All samples contained 10 mM sodium acetate, pH 5. Protein concentrations were constant: mAb1 at 172 mg/mL and mAb2 at 200 mg/mL. Sugar concentration was plotted as monosaccharide unit concentration. Average viscosities and standard deviations were determined from duplicate DLS measurements employed at 25°C. Lines are for guiding purposes only.

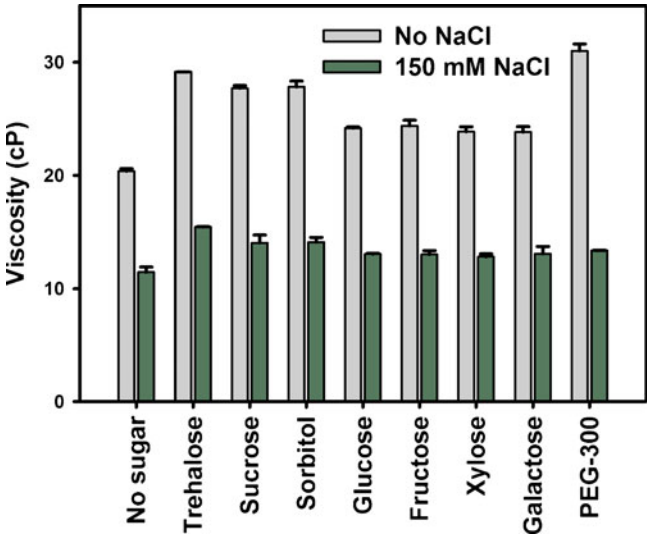

Fig. 4 The effect of NaCl on solution viscosity in the presence of sugars. All samples consisted of 177 mg/mL mAb2 in 10 mM sodium acetate, pH 5. Samples were measured at 25°C using DLS and results obtained from duplicate experiments. Polyethylene glycol at 300 Da (PEG-300) was included as a control.

ing 177 mg/mL mAb2. The hydrophobic exposure temperature, T_h, has been shown to correlate well with T_m as detected by DSC at low protein concentrations (24). The T_h values of mAb2 at both low and high concentrations are presented in Table II. At 1 mg/mL protein, the transition temperature of mAb2 is only mildly altered by all of the sugars, while the addition of sugar shifted it to higher temperature at 177 mg/mL protein. Figures 6b and c display the T_h values as a function of mAb2 concentration in the presence and absence of sucrose. The transition temperature of protein alone decreased with increasing concentration, presumably as a result of enhanced protein-protein interactions (24). The addition of 300 mM sucrose stabilized mAb2, as indicated by the rise in T_h (Fig. 6b). Furthermore, ΔT_h caused by sucrose increased with protein concentration from 80 to 180 mg/ml (Fig. 2). The T_h value increases shown in Fig. 6c as a function of sucrose concentration are also consistent with the viscosity results presented in Fig. 3.

DISCUSSION

Preferential interactions of protein with water and co-solvents and preferential exclusion of sugar molecules have been well characterized and reported (9–12,28–35). The surrounding hydration layer is known to modulate protein dynamics, which are reflected in changes of physical and chemical properties (11). When sugars are present in protein solutions, the preferential exclusion effect drives sugar molecules away from proteins and causes the local sugar concentration in the bulk solution to rise. This effect can be estimated by theoretical calculations of the solution volume occupied by proteins. For a mAb solution at 150 mg/mL with a hydrodynamic radius of 5 nm, the volume available for sugar molecules is 30% less than the same sugar concentration in a protein-free solution. If the protein radius increases to 6 nm, the volume available for sugars decreases by 55%. Although it is nearly impossible to accurately measure the exact hydrodynamic parameters of IgG molecules at high concentrations (15), it is reasonable to assume that the hydration layer can increase the effective

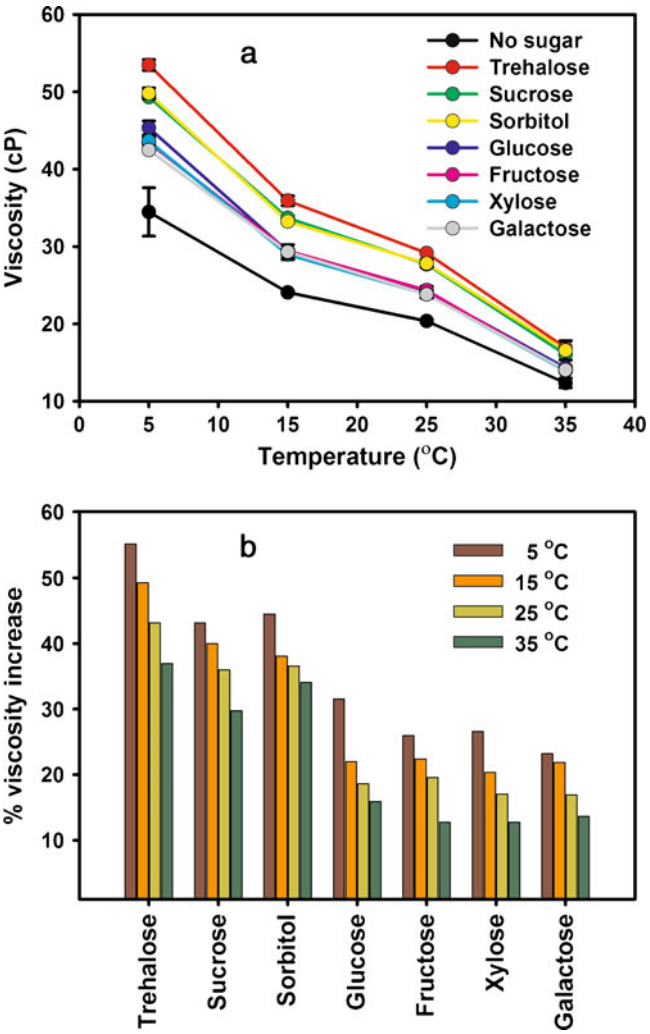

Fig. 5 The effects of temperature on the mAb2 solution viscosity in the presence of the studied sugars. Samples contained 177 mg/mL mAb2 in 10 mM sodium acetate, pH 5. (**a**) Solution viscosity as a function of temperature. Lines are for guiding purposes only. (**b**) Percent of viscosity increase by sugars. The Y-axis in (**b**) represents the percent of viscosity increase that was calculated as % viscosity increase $= 100\times(\text{viscosity}_{\text{with sugar}} - \text{viscosity}_{\text{without sugar}})/\text{viscosity}_{\text{without sugar}}$. The data presented in (**b**) were calculated using the average viscosities obtained from duplicate DLS measurements for each sample. Sugar concentrations trehalose and sucrose at 300 mM; sorbitol, glucose, fructose, xylose and galactose at 600 mM.

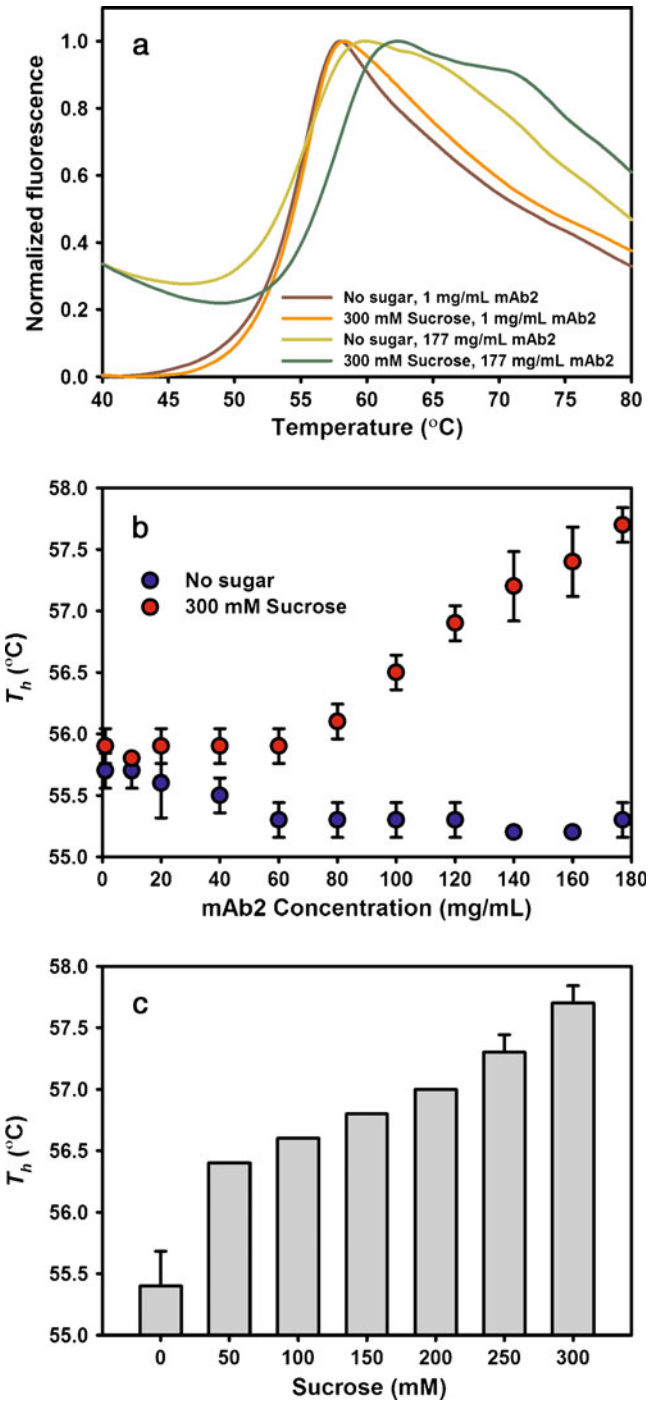

Fig. 6 Thermal melting results for mAb2. (**a**) Representative curves of normalized fluorescence intensity of SYPRO Orange are shown as a function of temperature. Samples contained 10 mM sodium acetate, pH 5. Protein unfolding was monitored by differential scanning fluorimetry. (**b**) Thermal melting results for mAb2 in the absence and presence of 300 mM sucrose. T_h was determined by differential scanning fluorimetry as described in Materials and Methods. All samples contained 10 mM sodium acetate, pH 5. (**c**) T_h as a function of sucrose concentration in 10 mM sodium acetate, pH 5, and 177 mg/mL mAb2. Both figures represent results obtained from duplicate measurements.

volume of a protein molecule, leaving less volume and water molecules in which to dissolve sugars. As a result, the effective sugar concentrations increase significantly, and this alone might lead to higher solution viscosities. In this study, we demonstrate that the mAb solution viscosity is increased by the seven different sugars examined. This effect is dependent upon both protein and sugar concentration (Figs. 1 and 2), consistent with the preferential hydration and exclusion mechanism. However, additional mathematical and experimental analyses are required to fully understand the role of sugar concentration in mediating protein solution viscosity. It is also not clear whether sugar and protein molecules form network interactions that may potentially lead to increased viscosity. The sugars selected for this study possess differences in their structure and molecular weight, but did not reveal any specific viscosity patterns that would indicate the involvement of any particular functional group(s). The results indicate that the effect of sugar molecules on protein solution viscosity is similar in both mAbs studied. A global mechanism that could explain these results is preferential hydration and exclusion (11,30,33,36). It has been shown that the degree of preferential exclusion from proteins is different among commonly used osmolytes (10). For example, trehalose has a smaller exchange constant, K_{ex}, than sorbitol, leading to a greater degree of exclusion from the protein surface (10). This could explain why the viscosity is higher among disaccharides such as trehalose than it is with monosaccharides such as sorbitol at similar monosaccharide concentrations. This would also be consistent with trehalose producing a higher viscosity than sucrose, as the trehalose has been shown to have a higher degree of

Table II Stabilization of Sugar Molecules Measured by Differential Scanning Fluorimetry (DSF)

Sugar[a]	ΔT_h[b]	
	1 mg/mL mAb2	177 mg/mL mAb2
Trehalose	1.0 ± 0.0	2.5 ± 0.1
Sucrose	0.4 ± 0.0	2.4 ± 0.0
Sorbitol	0.4 ± 0.0	2.3 ± 0.1
Glucose	0.3 ± 0.1	2.3 ± 0.1
Fructose	0.5 ± 0.1	2.1 ± 0.1
Xylose	0.2 ± 0.3	1.7 ± 0.1
Galactose	0.3 ± 0.1	2.0 ± 0.0

[a] All solutions contained 10 mM sodium acetate, pH 5. Sugar concentrations: trehalose and sucrose, 300 mM; sorbitol, glucose, fructose, xylose, galactose, 600 mM

[b] ΔT_h values were calculated as T_h (with sugar) - T_h (without sugar). DSF measurements were employed according to Materials and Methods. All ΔT_h values were determined by duplicate experiments and presented in the format of *average value* ± *standard deviation*

exclusion from proteins (10). In addition, disaccharides are usually excluded more from protein molecules compared to monosaccharides (10). This is consistent with the observation in this study that disaccharides result in higher viscosity than monosaccharides.

In a solution environment, co-solutes such as charged ions can occupy protein surfaces via preferential interactions, altering the dynamic features and compactness of proteins (10,11). In some cases, ions can also effectively decrease viscosity by modifying the protein surface charges and influencing protein-protein interactions (3,4,6,37,38). While cations often have similar effects on viscosity, anions display significant differences (3). This is probably due to the fact that many amino acid side chains are positively charged in the slightly acidic buffers typical of mAb formulations, and anions possess different binding affinities for these side chains. In this study, we evaluated the effect of ionic strength on solution viscosity in the presence of sugars (Fig. 4). Salt reduced both the overall viscosity and the degree of increase observed upon the addition of sugar. This may be explained by the rearrangement of the protein surface hydration layer induced by ions, reducing the exclusion of sugars from protein molecules, and thus decreasing the impact of sugars on the viscosity. Polyethylene glycols are also thought to be excluded from the surface of proteins (32), but to a lesser degree (10). Our results, on the other hand, show that the addition of PEG-300 results in slightly higher viscosity compared to sugars. This may be due to the interactions between the protein and PEG that lead to additional networking among molecules in addition to preferential exclusions (39).

It has been shown that the degree of preferential exclusion of sugar molecules from proteins is significantly decreased when temperature increases (32,35). As the temperature rises, protein molecules have higher mobility in solution, and sugar solubility increases, altering dispersion patterns among molecules. The temperature effect on solution viscosity is shown in Fig. 5. Although it is not surprising that protein viscosity decreases as temperature increases, the increased viscosity in the presence of the sugars also attenuates.

Sugars, such as sucrose, have been shown to provide structural protection to proteins and to delay unfolding transitions during thermal melting (30). The protective effect is presumably achieved through the ability of sugars to restrict the overall mobility of the protein molecules and shift the protein dynamics to favor the folded, compact structure. Sugars can also increase the chemical potential of the protein. All of these effects mentioned above can be attributed to the preferential exclusion of the sugar molecules from the protein in solution (33). The stabilization of the native conformation of a protein can be detected by DSC and other biophysical measurements at low protein and high sugar concentrations (29,30). The same measurements at high protein concentrations, however, are challenging due to the limitations of these techniques. DSF possesses distinct advantages in characterizing highly concentrated protein samples, and the results indicate that sugar molecules significantly delay the mAb melting transitions at high protein concentrations (Fig. 6). Although other protein behaviors at high concentrations, such as reversible aggregation and precipitation, might contribute to the fluorescence detection, our results clearly indicate that sugar molecules can delay the transitions at higher protein concentrations. At these conditions, it is safe to assume that sugar molecules are excluded more from the protein, and, simultaneously, the solution exclusion volume decreases significantly due to protein occupancy. As a result, the restriction of the native conformation and mobility of a protein by sugars is enhanced. DSF does not reveal significant differences among the sugars studied (Table II), indicating the sugar effect is governed by global, rather than specific mechanisms.

CONCLUSIONS

High viscosity is a major hurdle during the manufacturing, storage and delivery of high concentration protein therapeutics. Characterizations of the effects of sugars as excipients at different temperatures will help guide the process and product development of these pharmaceutical entities. The results presented here demonstrate that the sugar molecules increase protein solution viscosity, while disaccharides have a greater effect than monosaccharides. This may be explained by the preferential hydration/exclusion mechanism that mediates the interactions between sugar and protein molecules. The preferential interactions among sugar, solvent and protein molecules can be used to explain the mitigation of viscosity increase by sugars in the presence of salt and at higher temperatures. The observation of enhanced sugar effect on protein melting at higher protein concentrations can also be consistent with the preferential hydration and exclusion principle.

ACKNOWLEDGMENTS

The authors are grateful towards Fan Zhang and Dr. Jeremy Guo for supplying research materials. The authors would also like to thank Drs. R. Matthew Fesinmeyer, Gerald Becker, Linda Narhi, David Brems, and Michael Treuheit for critical reviews and fruitful discussions.

REFERENCES

1. Reichert JM, Rosensweig CJ, Faden LB, Dewitz MC. Monoclonal antibody successes in the clinic. Nat Biotechnol. 2005;23:1073–8.
2. Shire SJ. Formulation and manufacturability of biologics. Curr Opin Biotechnol. 2009;20:708–14.
3. Kanai S, Liu J, Patapoff TW, Shire SJ. Reversible self-association of a concentrated monoclonal antibody solution mediated by Fab-Fab interaction that impacts solution viscosity. J Pharm Sci. 2008;97:4219–27.
4. Liu J, Nguyen MD, Andya JD, Shire SJ. Reversible self-association increases the viscosity of a concentrated monoclonal antibody in aqueous solution. J Pharm Sci. 2005;94:1928–40.
5. Saluja A, Kalonia DS. Nature and consequences of protein-protein interactions in high protein concentration solutions. Int J Pharm. 2008;358:1–15.
6. Fesinmeyer RM, Hogan S, Saluja A, Brych SR, Kras E, Narhi LO, *et al.* Effect of ions on agitation- and temperature-induced aggregation reactions of antibodies. Pharm Res. 2009;26:903–13.
7. Salinas BA, Sathish HA, Bishop SM, Harn N, Carpenter JF, Randolph TW. Understanding and modulating opalescence and viscosity in a monoclonal antibody formulation. J Pharm Sci. 2010;99:82–93.
8. Banks DD, Hambly DM, Scavezze JL, Siska CC, Stackhouse NL, Gadgil HS. The effect of sucrose hydrolysis on the stability of protein therapeutics during accelerated formulation studies. J Pharm Sci. 2009;98:4501–10.
9. Arakawa T, Timasheff SN. Stabilization of protein structure by sugars. Biochemistry. 1982;21:6536–44.
10. Timasheff SN. Protein hydration, thermodynamic binding, and preferential hydration. Biochemistry. 2002;41:13473–82.
11. Timasheff SN. Protein-solvent preferential interactions, protein hydration, and the modulation of biochemical reactions by solvent components. Proc Natl Acad Sci USA. 2002;99:9721–6.
12. Timasheff SN, Xie G. Preferential interactions of urea with lysozyme and their linkage to protein denaturation. Biophys Chem. 2003;105:421–48.
13. Fernandez C, Minton AP. Static light scattering from concentrated protein solutions II: experimental test of theory for protein mixtures and weakly self-associating proteins. Biophys J. 2009;96:1992–8.
14. Saluja A, Badkar AV, Zeng DL, Nema S, Kalonia DS. Ultrasonic storage modulus as a novel parameter for analyzing protein-protein interactions in high protein concentration solutions: correlation with static and dynamic light scattering measurements. Biophys J. 2007;92:234–44.
15. Minton AP. Static light scattering from concentrated protein solutions. I: general theory for protein mixtures and application to self-associating proteins. Biophys J. 2007;93:1321–8.
16. Bulychev A, Mobashery S. Class C beta-lactamases operate at the diffusion limit for turnover of their preferred cephalosporin substrates. Antimicrob Agents Chemother. 1999;43:1743–6.
17. Sengerova B, Tomlinson C, Atack JM, Williams R, Sayers JR, Williams NH, *et al.* Bronsted analysis and rate-limiting steps for the T5 flap endonuclease catalyzed hydrolysis of exonucleolytic substrates. Biochemistry. 2010;49:8085–93.
18. Patapoff TW, Esue O. Polysorbate 20 prevents the precipitation of a monoclonal antibody during shear. Pharm Dev Technol. 2009;14:659–64.
19. Esnaashari S, Javadzadeh Y, Batchelor HK, Conway BR. The use of microviscometry to study polymer dissolution from solid dispersion drug delivery systems. Int J Pharm. 2005;292:227–30.
20. Redford SJ, Dickinson E, Golding M. Stability and rheology of emulsions containing sodium caseinate: combined effects of ionic calcium and alcohol. J Colloid Interface Sci. 2003;274:673–86.
21. He F, Becker GW, Litowski JR, Narhi LO, Brems DN, Razinkov VI. High-throughput dynamic light scattering method for measuring viscosity of concentrated protein solutions. Anal Biochem. 2010;399:141–3.
22. de Smidt JH, Crommelin DJA. Viscosity measurement in aqueous polymer solutions by dynamic light scattering. Int J Pharm. 1991;77:261–4.
23. Shukla AA, Hubbard B, Tressel T, Guhan S, Low D. Downstream processing of monoclonal antibodies–application of platform approaches. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;848:28–39.
24. He F, Hogan S, Latypov RF, Narhi LO, Razinkov VI. High throughput thermostability screening of monoclonal antibody formulations. J Pharm Sci. 2010;99:1707–20.
25. Chenlo F, Moreira R, Pereira G, Ampudia A. Viscosities of aqueous solutions of sucrose and sodium chloride of interest in osmotic dehydration processes. J Food Eng. 2002;54:347–52.
26. Monkos K, Turczynski B. A comparative study on viscosity of human, bovine and pig IgG immunoglobulins in aqueous solutions. Int J Biol Macromol. 1999;26:155–9.
27. Parmar AS, Muschol M. Lysozyme as diffusion tracer for measuring aqueous solution viscosity. J Colloid Interface Sci. 2009;339:243–8.
28. Arakawa T, Timasheff SN. The stabilization of proteins by osmolytes. Biophys J. 1985;47:411–4.
29. Khan RH, Shabnum MS. Effect of sugars on rabbit serum albumin stability and induction of secondary structure. Biochemistry (Mosc). 2001;66:1042–6.
30. Kim YS, Jones LS, Dong A, Kendrick BS, Chang BS, Manning MC, *et al.* Effects of sucrose on conformational equilibria and fluctuations within the native-state ensemble of proteins. Protein Sci. 2003;12:1252–61.
31. Xie G, Timasheff SN. Mechanism of the stabilization of ribonuclease A by sorbitol: preferential hydration is greater for the denatured then for the native protein. Protein Sci. 1997;6:211–21.
32. Gekko K, Morikawa T. Preferential hydration of bovine serum albumin in polyhydric alcohol-water mixtures. J Biochem. 1981;90:39–50.
33. Kendrick BS, Chang BS, Arakawa T, Peterson B, Randolph TW, Manning MC, *et al.* Preferential exclusion of sucrose from recombinant interleukin-1 receptor antagonist: role in restricted conformational mobility and compaction of native state. Proc Natl Acad Sci USA. 1997;94:11917–22.
34. Xie G, Timasheff SN. The thermodynamic mechanism of protein stabilization by trehalose. Biophys Chem. 1997;64:25–43.
35. Xie G, Timasheff SN. Temperature dependence of the preferential interactions of ribonuclease A in aqueous co-solvent systems: thermodynamic analysis. Protein Sci. 1997;6:222–32.
36. Shimizu S, Smith DJ. Preferential hydration and the exclusion of cosolvents from protein surfaces. J Chem Phys. 2004;121:1148–54.
37. Gokarn YR, Fesinmeyer RM, Saluja A, Cao S, Dankberg J, Goetze A, *et al.* Ion-specific modulation of protein interactions: anion-induced, reversible oligomerization of a fusion protein. Protein Sci. 2009;18:169–79.
38. Yadav S, Liu J, Shire SJ, Kalonia DS. Specific interactions in high concentration antibody solutions resulting in high viscosity. J Pharm Sci. 2010;99:1152–68.
39. Wang Y, Annunziata O. Comparison between protein-polyethylene glycol (PEG) interactions and the effect of PEG on protein-protein interactions using the liquid-liquid phase transition. J Phys Chem B. 2007;111:1222–30.