Biophysical Chemistry 197 (2015) 40–46

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Effect of polyol sugars on the stabilization of monoclonal antibodies

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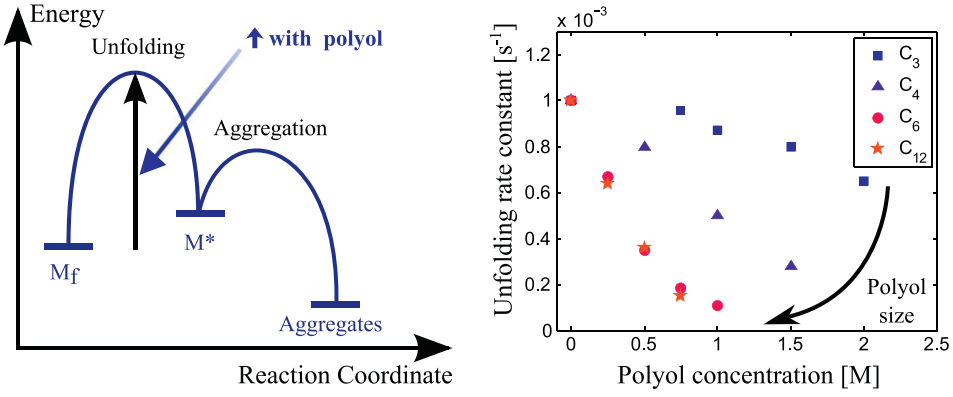
HIGHLIGHTS

- Sugars and polyols reduce mAb aggregation propensity by increasing the mAb conformational stability.
- The stabilization is interpreted from a kinetic perspective as an increase in the energy barrier of protein unfolding.
- An alternative thermodynamic view involving a shift of the native state ensemble towards a compact structure is proposed.
- The stabilization effect increases as a function of polyol size until a plateau is reached at large polyol sizes.
- The mAb stabilization depends both on the volume fraction filled by the polyol molecules and on the polyol chemistry.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 November 2014
Received in revised form 17 December 2014
Accepted 19 December 2014
Available online 27 December 2014

Keywords:
Therapeutic protein
Formulation
Excipient
Kinetic model
Aggregation mechanism
Protein unfolding

ABSTRACT

We investigate the impact of sugars and polyols on the heat-induced aggregation of a model monoclonal antibody whose monomer depletion is rate-limited by protein unfolding. We follow the kinetics of monomer consumption by size exclusion chromatography, and we interpret the results in the frame of two mechanistic schemes describing the enhanced protein stability in the presence of polyols. It is found that the stabilization effect increases with increasing polyol concentration with a comparable trend for all of the tested polyols. However, the stabilization effect at a given polyol concentration is polyol specific. In particular, the stabilization effect increases as a function of polyol size until a plateau is reached above a critical polyol size corresponding to six carbon atoms. Our results show that the stabilization by polyols does not depend solely on the volume fraction filled by the polyol molecules, but is also affected by the polyol chemistry.

1. Introduction

Improving the mechanistic understanding of the stabilization of proteins by cosolutes is of great interest in biological and biotechnological sciences. A relevant example is represented by the use of excipients in the formulation of protein-based drugs. Indeed, aggregation is one of the major degradation routes of protein therapeutics, often hindering rapid commercialization of potential protein drug candidates. The

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http://dx.doi.org/10.1016/j.bpc.2014.12.003
0301-4622/

optimization of the buffer formulation composition is one of the most common strategies to enhance protein stability and guarantee suitable product shelf-life [1,2]. Yet, predicting protein aggregation rates under given operating conditions is extremely challenging [3,4], and the buffer composition is currently largely optimized empirically by screening of the formulation conditions, which is both time and material consuming. Model-based approaches represent promising tools to gain fundamental knowledge on the mechanism of action of cosolutes on protein aggregation and can support the rational design of drug formulation stability studies.

Sugars and polyol sugars such as sorbitol are known to stabilize protein solutions and are widely used as excipients in drug formulation in order to hinder protein aggregation [5–9]. The impact of such cosolutes on the thermodynamic properties of proteins in aqueous solutions has been extensively investigated. Pioneering work by Timasheff and Arakawa indicated the preferential exclusion of sugars at the protein surface as a major mechanism of action. They provided evidence that unfavorable protein–solvent interactions favor native compact protein conformations with respect to more open unfolded structures [10–15]. However, despite several works examined the effect of sugars on the thermodynamics of protein unfolding (i.e. on the difference in the free energy between the native and unfolded states in the absence and presence of sugars), only a few studies were dedicated to the investigation of the impact of sugars and sugar alcohols on the kinetics of protein aggregation.

In this work, we combine experimental characterization with theoretical modeling to investigate the impact of sucrose and polyols (glycerol, threitol, sorbitol, maltitol) on the heat-induced aggregation of a model monoclonal antibody (mAb) whose monomer depletion kinetics is limited by the rate of protein unfolding. Although these conditions do not represent strictly the situation typically observed during the formulation step, this type of mechanistic study can provide fundamental insights on the effect of stabilizers on protein aggregation stability, which could be relevant also at lower temperatures. We follow the kinetics of monomer consumption by size exclusion chromatography and we interpret the experimental data within two mechanistic schemes. In the first scheme, the stabilization by sugars is described from a kinetic point of view by a decrease of the unfolding rate constant with increasing sugar concentration. In the second scheme, the stabilization by sugars is attributed to a change in the conformational equilibrium constant, shifting the native-state ensemble towards a more compact conformation, in line with results proposed in the literature [16–19]. We first apply these two schemes to the case of sorbitol, and then we investigate the effect of polyol size on the mAb stabilization.

2. Materials and methods

2.1. Materials

The monoclonal antibody used for this study was a glycosylated IgG1 provided by UCB Pharma, and will be denoted as mAb-1 in the following.

The antibody solution was dialyzed at a protein concentration of 20 g/L against a 20 mM histidine buffer at pH 6.5 using Slide-A-Lyzer cassettes from Thermo Fisher Scientific, with a cut-off molecular weight of 7 kDa. The volume of the dialysis buffer was five hundred-fold larger than the volume of the sample to be dialyzed. The buffer was renewed a first time after 2 h, and a second time after 4 h of dialysis. The dialysis was performed at 4 °C under gentle stirring for at least 18 h. The protein concentration of the stock solution after dialysis was checked by UV absorption at 280 nm.

All the samples for this study were prepared by diluting the stock solution with selected buffer solutions to reach the targeted protein and cosolute concentrations in 20 mM histidine buffer at pH 6.5.

All the chemicals were purchased from Sigma, with the highest purity available. The buffers were filtered through a 0.1 μm cut-off membrane filter (Millipore).

2.2. Isothermal aggregation kinetics

Isothermal aggregation kinetics were performed by incubating antibody samples at the reference protein concentration of 1 g/L at elevated temperatures in hermetically sealed HPLC vials containing 250 μL inserts (Agilent Technologies, part numbers 5182-0716, 5181-1270 and 5182-0721 for vials, inserts and caps, respectively). The vials were placed in a block-heater (Rotilabo H 250, Roth, Karlsruhe) for predetermined times. To improve heat transfer, 1 mL of aggregation buffer was added in the space delimited by the vial and the insert. Temperature was controlled by an oil bath with less than ± 0.1 °C variability, as verified with a thermocouple. All the experiments were carried out at the elevated temperature of 70 °C, except those aiming at determining the activation energy of protein unfolding, where the temperature was varied between 65 °C and 70 °C by step of 1 °C. Aggregated samples were quenched in ice for at least 3 min and analyzed immediately after by size exclusion chromatography.

It is worth mentioning that notable changes in the protein structure occur at the temperature of 70 °C used in this work, which is approximately the melting temperature of the mAb, as revealed by circular dichroism experiments carried out in a previous study [20]. Therefore, the experimental conditions used in this work are promoting protein unfolding.

2.3. Size exclusion chromatography (SEC)

Monomer conversion was monitored by size exclusion chromatography (SEC) with a Superdex 200 10/300 GL, 10 mm × 300 mm size-exclusion column (GE Healthcare, Uppsala, Sweden) assembled on an Agilent series HPLC unit (Santa Clara, CA, USA). The samples were eluted for 45 min at a constant flow rate of 0.5 mL/min using as mobile phase a 100 mM phosphate buffer containing 200 mM Arginine at pH 7.0, which has been shown previously to improve sample recovery [21]. The eluting species were detected by UV absorbance at 280 nm. The chromatograms were deconvoluted using OriginPro 8.5 (Academic) in order to determine the monomer content. The data reported in the figures correspond to the average and standard deviation (error bars) of at least two independent measurements.

3. Results and discussion

The mechanism of the heat-induced aggregation of the antibody considered in this work has been identified in the absence of cosolute in a previous study [20]. The aggregation mechanism is summarized in the energy diagram shown in Fig. 1(a), while the detailed description of the model can be found in the original paper [20]. In Fig. 1(a), the global aggregation pathway is schematically represented as a unimolecular unfolding event forming an aggregation-competent conformation followed by bimolecular collisions leading to the formation of aggregates. This kinetic scheme is further illustrated in Model 1 of Fig. 1(b), where a native folded conformation of the monomer M_f unfolds with a rate constant k_U to form an aggregation prone monomer M^*, which is then depleted by aggregation. In addition, it has been proven that protein unfolding is the rate-limiting step for monomer depletion [20], which implies that the concentration of M^* is small compared to that of the native monomer M_f.

In a subsequent study, the impact of sorbitol on the single elementary reactions of the multistep aggregation kinetics of mAb-1 has been investigated [22]. In Fig. 2(a), we show the time evolution of the monomer concentration of mAb-1 followed by size exclusion chromatography at various sorbitol concentrations. It can be observed that the presence of sorbitol significantly delays the kinetics of monomer depletion in a concentration dependent manner. It has been shown previously that the presence of sorbitol delays the kinetics of mAb-1 aggregation by specifically inhibiting the unfolding step, without impacting the aggregation events [22], as depicted in the energy diagram of

Fig. 1(a). This finding is consistent with other literature data showing that sugars and other polyols reduce mAb aggregation propensity by improving their conformational stability [23]. According to the mechanism proposed by Timasheff and Arakawa, the stabilizing effect of these cosolutes can be attributed to unfavorable protein–solvent interactions which favor compact native protein conformations with respect to more open unfolded structures [10–14]. It is worth mentioning here that the kinetics of monomer depletion remains limited by the rate of protein unfolding also in the presence of sorbitol. Indeed, aggregation experiments performed at various protein concentrations in the presence of sorbitol revealed that the kinetics of monomer depletion follows a first order process with a reaction rate constant independent of the protein concentration (data shown in the Supporting information). Moreover, kinetic analysis indicates that sorbitol increases the characteristic time of unfolding without impacting the characteristic time of aggregation, thus confirming that mAb aggregation remains unfolding rate limited in the presence of sorbitol [22].

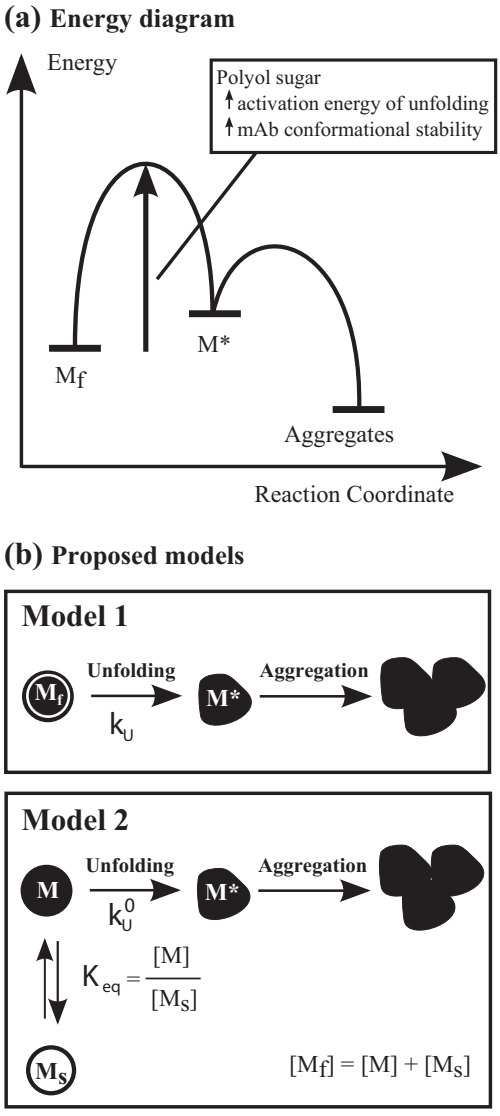

Fig. 1. (a) The aggregation pathway of mAb-1 can be described by a two-step mechanism involving protein unfolding (i.e. a conformational change leading to an aggregate-competent species) followed by aggregate growth. The presence of a polyol sugar inhibits mAb aggregation by delaying specifically the protein unfolding step. (b) Two kinetic models are proposed to describe the stabilization of mAb-1 by polyol sugars. In the first model, the unfolding rate constant of the native monomer (M_f) is reduced upon polyol sugar addition. In the second model, two conformations of the native monomer (M_f) are considered: one compact conformation which is protected from unfolding (M_s), and a second conformation which undergoes unfolding (M) and then leads to aggregate formation. The presence of the polyol sugar displaces the equilibrium between M and M_s towards M_s, but does not impact the unfolding rate constant of M.

3.1. Model proposal

3.1.1. Model 1

In the model developed in our previous work [22], and which is here summarized as Model 1 in Fig. 1(b), the stabilization effect of sorbitol is captured by modifying the value of the unfolding rate constant k_U at different sorbitol concentrations, with k_U decreasing upon an increase in sorbitol concentration. The deceleration of protein unfolding in the presence of sorbitol is attributed to the increase in the activation energy of unfolding due to the greater exclusion of the sugar molecules from the expanded transition state with respect to the native protein state [7]. Indeed, the increased surface area of the expanded transition state leads to higher unfavorable sugar–protein interactions as compared to the native state.

The impact of the presence of sugar on the activation energy of unfolding can be quantified within the transition state theory by using the following expressions:

$$\begin{cases} k_U = A\exp\left(\dfrac{-\Delta H_U^{\#}}{RT}\right) \\ A = \kappa\dfrac{k_B T}{h}\exp\left(\dfrac{\Delta S_U^{\#}}{R}\right) \end{cases} \quad (1)$$

where $\Delta H_U^{\#}$ and $\Delta S_U^{\#}$ are the enthalpy and entropy contribution to the free energy of activation, respectively, T is the temperature, R is the gas constant, k_B is the Boltzmann constant, h is the Planck constant and κ is the transmission coefficient.

Over a narrow range of temperatures, $\Delta H_U^{\#}$ and A can be assumed independent of the temperature and their values can be determined from the slope and y-intercept, respectively, of an Arrhenius plot. The free energy of activation can then be computed as:

$$\Delta G_U^{\#}(T) = \Delta H_U^{\#} - T\Delta S_U^{\#}. \quad (2)$$

3.1.2. Model 2

In this work, we also describe the stabilization by sugars in the frame of a second scheme, denoted as Model 2 in Fig. 1(b), where two native monomeric conformations are considered: M, which is subject to unfolding, and M_s, which is a more compact conformation of the native-state ensemble and which is characterized by a negligible unfolding rate constant. The presence of sorbitol shifts the conformational equilibrium towards the more compact state [16–18], which is poorly populated in the absence of cosolute. This structural conversion reduces the effective concentration of native monomer which can undergo unfolding, therefore delaying the kinetics of unfolding, and consequently the kinetics of aggregation.

It is worth highlighting that within Model 2, the unfolding rate constant k_U^0 is not affected by the presence of sorbitol and is equal to the value estimated in the absence of cosolute.

In the following, we connect mathematically the two proposed models. The formation of the aggregation-prone monomer M^* in Model 1 and in Model 2 can be expressed as follows:

$$\left.\frac{d[M^*]}{dt}\right)_{1,+} = k_U\left[M_f\right] \quad (3)$$

$$\left.\frac{d[M^*]}{dt}\right)_{2,+} = k_U^0[M] \quad (4)$$

where the concentration of folded monomer $[M_f]$ in Model 1 is equal to the sum of the concentrations of non-protected $[M]$ and protected $[M_s]$

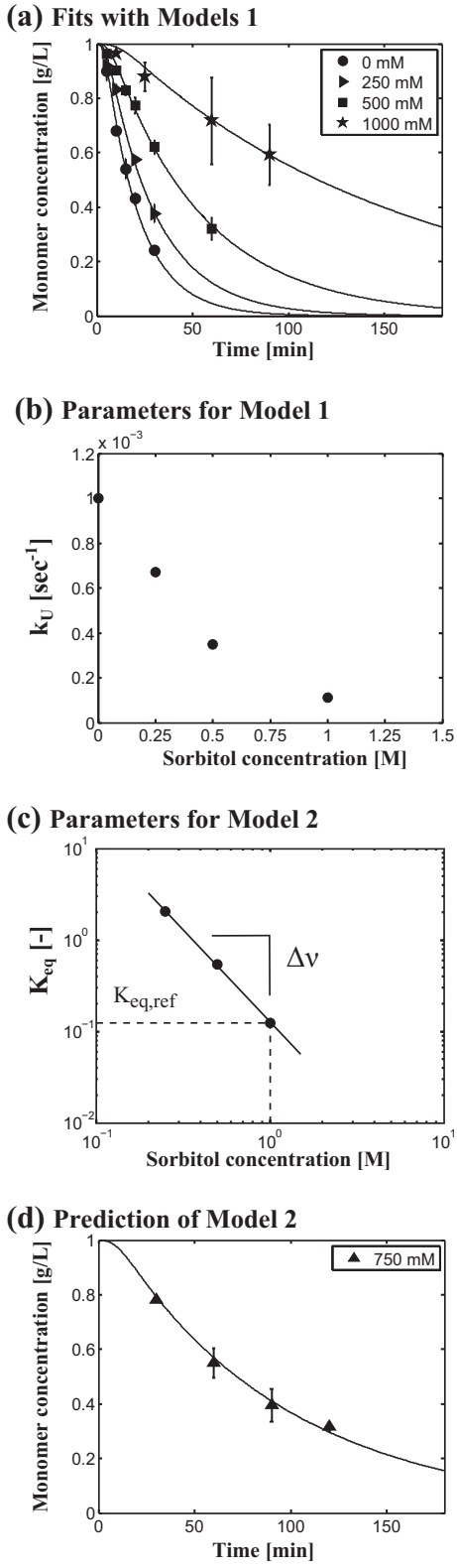

Fig. 2. (a) Time evolution of the monomer depletion ($[M_f]+[M^*]$) at mAb concentration of 1 g/L and at various sorbitol concentrations measured by SEC (symbols) and simulated according to Model 1 (lines). (b) Unfolding rate constant k_U evaluated in the frame of Model 1 as a function of sorbitol concentration. It appears that at zero sorbitol concentration $k_U^0 = 10^{-3}s^{-1}$. (c) Conformational equilibrium constant evaluated in the frame of Model 2, which provides the values of $\Delta\nu = -2$ and $K_{eq,ref} = 0.13$ according to Eq. (12). (d) Prediction of the monomer depletion kinetics at 750 mM sorbitol according to Model 2 and considering the parameters determined in (b) and (c).

folded monomer in Model 2:

$$\left[M_f\right] = [M] + [M_s]. \tag{5}$$

Since the formation of M^* must be the same in the two models, we can write:

$$k_U\left[M_f\right] = k_U^0[M]. \tag{6}$$

The conformational equilibrium constant in Model 2 is defined as:

$$K_{eq} = \frac{[M]}{[M_s]}. \tag{7}$$

Then, Eq. (5) reads:

$$\left[M_f\right] = [M] \times \left(1 + 1/K_{eq}\right). \tag{8}$$

Finally, Eqs. (6) and (8) can be combined to provide a relation between the unfolding rate constant of Model 1 and the equilibrium constant of Model 2, as follows:

$$k_U = \frac{k_U^0}{1 + 1/K_{eq}}. \tag{9}$$

Where k_U^0 corresponds to the value of k_U in the absence of sorbitol.

This relation shows that, from a mathematical point of view, the stabilization by sorbitol can be described equivalently either by a decrease in the unfolding rate constant of Model 1, or by a decrease in the conformational equilibrium constant of Model 2.

The Wyman linkage equation states that the equilibrium constant varies with the presence of sugar according to [12,24]:

$$\frac{\partial \log\left(K_{eq}\right)}{\partial \log(a_S)} = \Delta\nu \tag{10}$$

where a_S is the activity of the sugar and $\Delta\nu$ is the preferential binding parameter, i.e. the difference in ligand binding between the two native states involved in the equilibrium.

The preferential binding is a thermodynamic quantity which can be either positive or negative depending on the type of protein–cosolute interactions [11,12]. In the case of unfavorable protein–cosolute interactions (as for example with sugars or polyols), $\Delta\nu$ is negative, reflecting the preferential exclusion of the cosolute from the protein surface, or in other words the preferential hydration of the protein. In contrast, positive $\Delta\nu$ values correspond to favorable protein–cosolute interactions, which are typically encountered with denaturants such as urea or guanidine hydrochloride.

The activity coefficient of several sugar solutions, in a concentration range comparable to the values investigated here, was reported to be comprised between 1 and 1.1 [25,26]. In this study, we approximated the sugar activity coefficient to 1. The sugar activity a_S is therefore considered equivalent to its concentration $[S]$.

The integration of Eq. (10) then provides the following relation:

$$K_{eq} = K_{eq,ref}[S]^{\Delta\nu} \tag{11}$$

where the equilibrium constant $K_{eq,ref}$ is defined at the reference sugar concentration of 1 mol/L.

The parameters $\Delta\nu$ and $K_{eq,ref}$ can be easily estimated from the K_{eq} values obtained at several sugar concentrations by using the linearized form:

$$\log\left(K_{eq}\right) = \Delta\nu \times \log([S]) + \log\left(K_{eq,ref}\right). \quad (12)$$

It is worth noticing that the parameters k_U^0, $\Delta\nu$ and $K_{eq,ref}$ are independent on the sugar concentration. Therefore, once these three parameters are determined, the monomer depletion kinetics can be predicted at any sugar concentration lying in the range of parameter validity.

3.2. Stabilization by sorbitol

In the following, we apply the two models described previously to quantify the stabilization induced by the presence of sorbitol.

In this frame, we determine first the values of the unfolding rate constant k_U by fitting numerical simulations of Model 1 to experimental data acquired at several concentrations of sorbitol (0, 250, 500 and 1000 mM). The mass balances which were implemented to simulate the aggregation kinetics can be found in the Supporting information. The model simulations together with the experimental data are shown in Fig. 2(a), and the obtained k_U values are shown in Fig. 2(b), where it appears that at zero sorbitol concentration $k_U^0 = 10^{-3}\ s^{-1}$.

The impact of sorbitol on the activation energy of unfolding was then estimated within the transition state theory. For this purpose, the monomer depletion kinetics was monitored by SEC at several temperatures ranging between 65 and 70 °C (data shown in the Supporting information). In Fig. 3(a), we show the Arrhenius plot of the unfolding rate constant both in the absence and in the presence of sorbitol. The energies of activation of unfolding were then determined at the reference temperature $T_m = 70$ °C by using Eqs. (1)–(2) and assuming $\kappa = 1$. It can be seen in Fig. 3(b) that the presence of sorbitol increases both $\Delta H_U^{\#}$ and $\Delta S_U^{\#}$, but has only a little impact on $\Delta G_U^{\#}$. This observation suggests an enthalpy–entropy compensation mechanism, which is characteristic of many reactions in solutions [27–29]. In the presence of sugar molecules, the dehydration of the protein surface in the transition state is accompanied by an unfavorable increase in enthalpy related to the lower affinity of the sugar molecules for the protein surface with respect to water molecules; this unfavorable increase in enthalpy is only partially compensated by the favorable increase in entropy associated with the release of water molecules absorbed on the protein surface. Overall, the enthalpy increase dominates, leading to an increase in the free activation energy.

In a second step, we quantified the impact of sorbitol in the frame of Model 2 by computing the equilibrium constant K_{eq} with Eq. (9) at each sorbitol concentration, as shown in Fig. 2(c). The linearization provides $\Delta\nu = -2.0$ and $K_{eq,ref} = 0.13$ by using Eq. (12).

Finally, we used the parameters k_U^0, $\Delta\nu$ and $K_{eq,ref}$ previously determined to predict the monomer depletion at an additional sorbitol concentration (750 mM) by using Model 2. In Fig. 2(d), it can be seen that the prediction of the monomer depletion kinetics at 750 mM sorbitol is in very good agreement with the experimental data.

3.3. Impact of polyol size on mAb stabilization

We apply this modeling framework to other polyols (glycerol, threitol, sucrose, maltitol) with the aim to understand the effect of cosolute size on mAb stabilization. The cosolutes used in this study are listed in Table 1, together with some of their most meaningful physical properties.

The experimental data from size exclusion chromatography and the model simulations are presented in the Supporting information. In Fig. 4(a), we show the unfolding rate constant k_U evaluated in the frame of Model 1 as a function of cosolute concentration for all the cosolutes mentioned above. It is seen that, at a given cosolute molarity, the unfolding rate constant decreases with an increasing cosolute size.

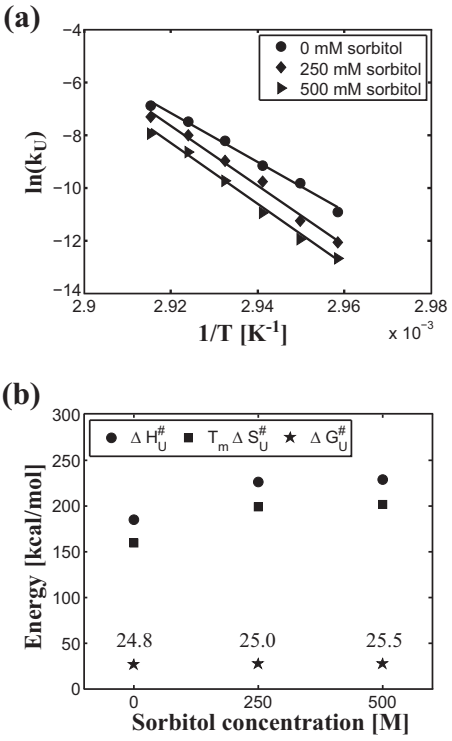

Fig. 3. (a) Arrhenius plot of the unfolding rate constant, in the absence and presence of 250 mM and 500 mM sorbitol. (b) Enthalpic and entropic contributions to the free energy of activation of unfolding as a function of sorbitol concentration evaluated from the Arrhenius plots of (a) at the reference temperature $T_m = 70$ °C.

This result indicates that large molecules have a stronger stabilization effect as compared to small molecules.

The values of conformational equilibrium constant K_{eq} as a function of cosolute concentration are presented in Fig. 4(b). The parameters $\Delta\nu$ and $K_{eq,ref}$ of Model 2 were determined by using Eq. (12) and are shown in Fig. 4(c) and Fig. 4(d), respectively.

Interestingly, the value of $\Delta\nu$ is close to −2 for all cosolutes under investigation, whereas $K_{eq,ref}$ is largely affected by the cosolute size. This means that the impact of polyol concentration on the mAb stability is similar for all the polyols investigated in this study, while the stabilization at a reference polyol concentration is polyol specific.

In particular, the stabilization effect is found to be enhanced with polyol size until a critical cosolute size corresponding to sorbitol (Fig. 4(d)) is reached. In contrast, the stabilization effect becomes almost independent on the polyol size for cosolute molecules larger than sorbitol. For example, sucrose (342 g/mol) and sorbitol (182 g/mol) are found to have similar $K_{eq,ref}$ values, close to 0.1.

Moreover, it is worth noticing that even though sucrose and maltitol have similar molar volumes, the stabilization by maltitol is somewhat more effective than that by sucrose (Fig. 4). This is possibly correlated to the number of –OH groups (and thus to the possible number of hydrogen bonds formed) of the two molecules. Indeed, maltitol carries nine –OH groups, while sucrose carries only eight –OH groups, due to its

Table 1
List of the cosolutes considered in this study together with some selected meaningful physical parameters.

Name	Chemical formula	Molecular weight [g/mol]	Molar volume [mL/mol]	# of –OH groups [−]
Glycerol	$C_3H_8O_3$	92.1	73.5 [30]	3
Threitol	$C_4H_{10}O_4$	122.1	86.7 [30]	4
Sorbitol	$C_6H_{14}O_6$	182.2	118.2 [30]	6
Sucrose	$C_{12}H_{22}O_{11}$	342.3	210.5 [31]	8
Maltitol	$C_{12}H_{24}O_{11}$	344.3	215.9 [32]	9

bi-cyclic structure. This observation suggests that the stabilization by polyol is not related exclusively to the volume occupied by the polyol molecules but also to the cosolute chemistry.

To confirm this hypothesis, we plot the unfolding rate constant as a function of the volume fraction occupied by the polyols in Fig. 5(a). It can be clearly seen that the results obtained with the various polyols do not overlap on one single curve. In particular, sorbitol appears to be the most stabilizing cosolute among the molecules investigated in this study, while glycerol is found to have a very weak stabilization effect. Similar conclusions are reached when plotting the conformational equilibrium constant as a function of the occupied volume fraction (figure not shown) since the values of K_{eq} of Model 2 are directly correlated to the values of k_U computed within Model 1 (Eq. (9)). These results show that the stabilization by polyol sugars does not depend only on the volume fraction occupied by the polyol molecules, thus suggesting that cosolute chemistry plays a central role in the mAb stabilization.

The effect of cosolute chemistry on mAb stability is also evidenced in Fig. 5(b), which shows the kinetics of monomer depletion in the absence and presence of 250 mM polyethyleneglycol (PEG). PEG200 and PEG300 exhibit comparable sizes with respect to the polyols used for this study, but they exert an opposite effect on the kinetics of monomer depletion: while polyols stabilize the antibody, PEG has a destabilizing effect, as commonly observed for several protein systems [33].

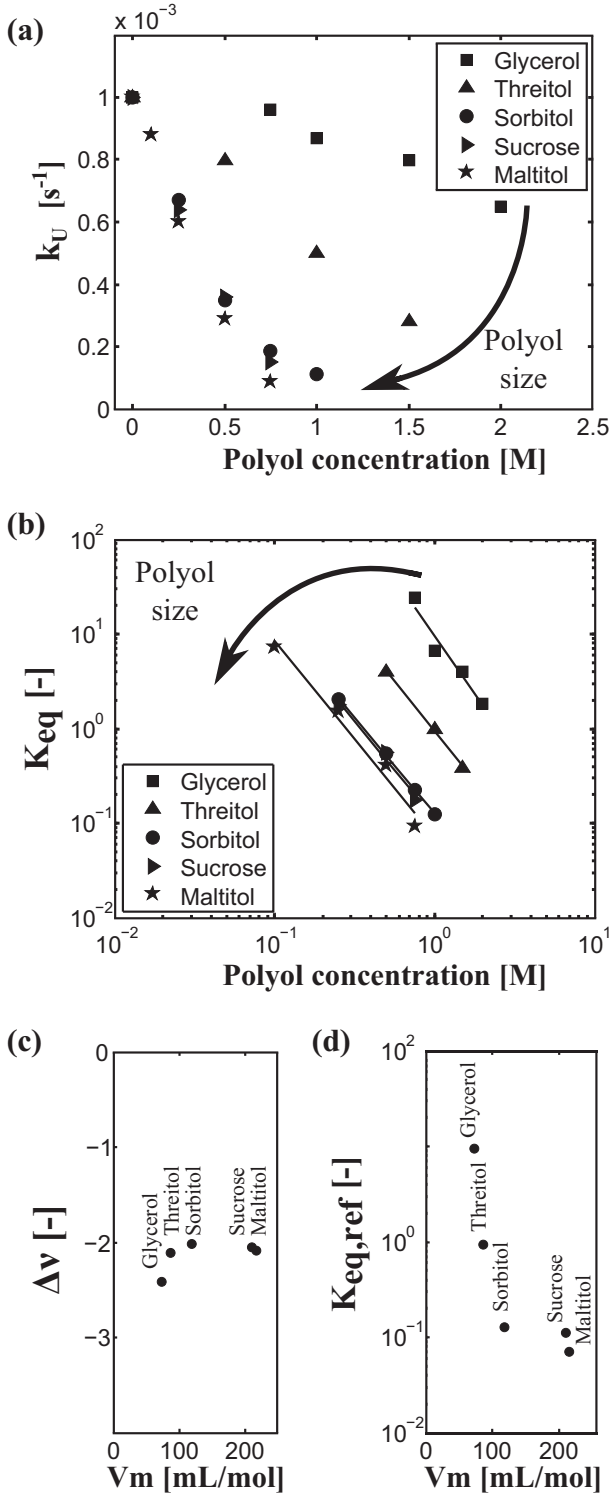

Fig. 4. (a) Unfolding rate constant k_U evaluated in the frame of Model 1 as a function of polyol concentration for polyols of an increasing size. (b) Conformational equilibrium constant evaluated in the frame of Model 2, which provides values of $\Delta\nu$ and $K_{eq,ref}$ by using Eq. (12). (c) $\Delta\nu$ as a function of the molar volume of the polyols. (d) $K_{eq,ref}$ as a function of the molar volume of the polyols.

4. Conclusions

We coupled experimental characterization with theoretical kinetic modeling to investigate the stabilization effect of polyols on the heat-induced aggregation of a monoclonal antibody whose monomer depletion kinetics is rate limited by protein unfolding. We interpreted the results within the frame of two mechanistic schemes. In the first scheme, the stabilization was described from a kinetic point of view, i.e. by an increase in the energy barrier preventing the protein from unfolding. In the second scheme, the inhibition effect observed in the presence of the sugar was interpreted from a thermodynamic point of view, i.e. by a shift of the native-state ensemble towards a more compact configuration.

We applied this framework to investigate the impact of polyol size on antibody stability. We found that the relative increase in the protein stability (expressed in terms of unfolding rate constant in model 1, or equilibrium constant in model 2) with increasing polyol concentration is similar for the different polyol molecules. However, at constant polyol concentration, the stabilization effect is highly polyol specific. In particular, we found that the stabilization effect is enhanced with increasing cosolute size for cosolute molecules smaller than a critical size, corresponding to sorbitol. In contrast, for cosolute molecules larger than sorbitol, the aggregation inhibition becomes almost independent of the polyol size. Our results show that the mAb stabilization does not depend only on the volume fraction occupied by the polyol, but is also affected by the polyol chemistry.

Acknowledgments

Financial support from the Fondation Claude et Giuliana and from the Swiss National Foundation (grant no. 200020_147137/1) is gratefully acknowledged. We also thank UCB Pharma (Braine l'Alleud, Belgium) for supplying the materials and for the financial support, Margaux Sozo for her help with the experiments and David Pfister forfruitful discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bpc.2014.12.003.

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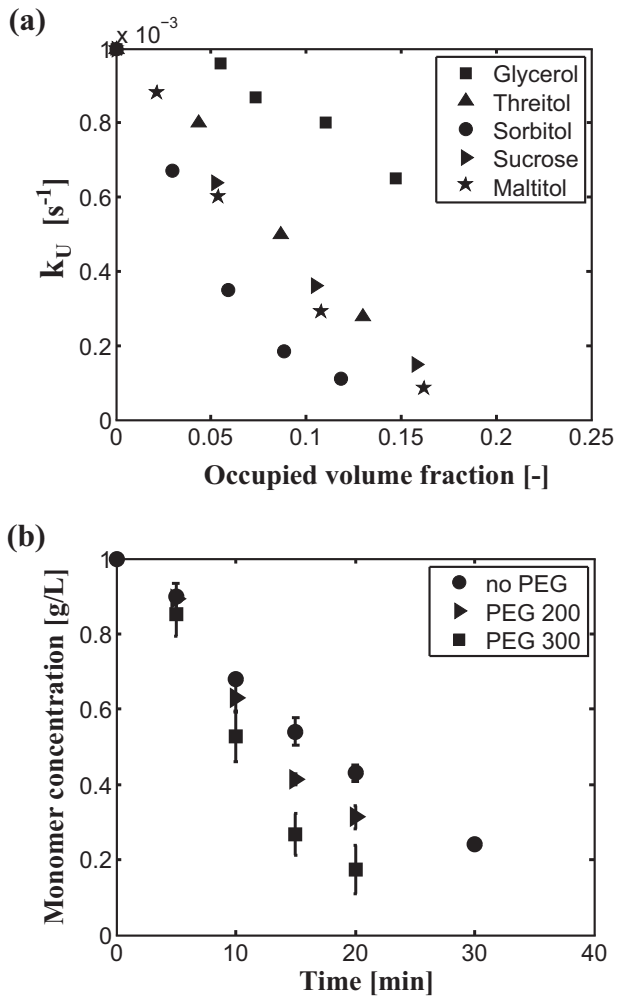

Fig. 5. (a) Unfolding rate constant as a function of the volume fraction occupied by the polyols for several polyol sugars. (b) Kinetics of monomer depletion of 1 g/L mAb solution in 20 mM histidine at pH 6.5 without PEG, with 250 mM PEG200 and with 250 mM PEG300.

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