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Effect of polyol sugars on the stabilization of monoclonal antibodies

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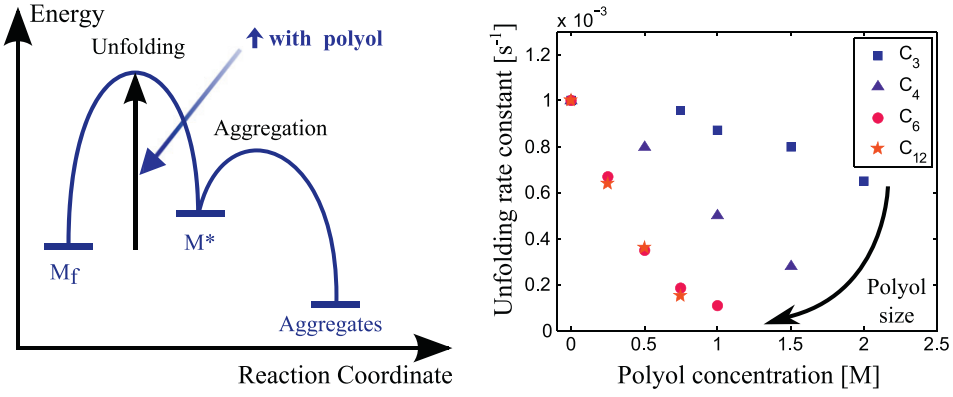
HIGHLIGHTS

- 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



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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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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