Technical Decision-Making with Higher Order Structure Data: Specific Binding of a Nonionic Detergent Perturbs Higher Order Structure of a Therapeutic Monoclonal Antibody

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ABSTRACT: Robust higher order structure (HOS) characterization capability and strategy are critical throughout biopharmaceutical development from initial candidate selection and formulation screening to process optimization and manufacturing. This case study describes the utility of several orthogonal HOS methods as investigational tools during purification process development. An atypically high level of residual detergent in a development drug substance batch of a therapeutic monoclonal antibody triggered a root cause investigation. Several orthogonal biophysical techniques were used to uncover and characterize a specific interaction between the detergent and the antibody. Isothermal titration calorimetry (ITC) was used to quantify the molar ratio and affinity of the binding event, and circular dichroism (CD) spectroscopy and differential scanning calorimetry (DSC) were used to evaluate corresponding impacts on secondary/tertiary structure and thermal stability, respectively. As detergents are used routinely in biopharmaceutical processing, this case study highlights the value and power of HOS data in informing technical investigations and underlines the importance of HOS characterization as a component of overall biopharmaceutical analytical control strategy. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1543–1547, 2015

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INTRODUCTION

Higher order structure (HOS) methods are an integral part of the overall characterization strategy for biotherapeutics. Perturbation of secondary and/or tertiary structure may negatively impact product quality through increased aggregation propensity and/or reduced potency.[1] Biophysical techniques such as electronic/vibrational spectroscopy, calorimetry, light scattering, and analytical ultracentrifugation are all widely used for HOS characterization.[2–7] This Note is part of a series of case studies intended to highlight how HOS methods are currently being used to inform technical decision-making in biopharmaceutical development.

Detergents (surfactants) are commonly used during drug substance manufacturing and included in final drug product. Although some data suggest the possible modulation of antigen–antibody interactions by nonionic detergents,[8] they are generally considered to be inert in biopharmaceutical process and formulation design. Triton® X-100 is a nonionic detergent composed of an aromatic hydrocarbon head group and a hydrophilic polyethylene oxide tail—$C_{14}H_{22}O(C_2H_4O)_n$, average n ~9.5. Triton® X-100 and similar detergents are routinely used for inactivation of enveloped viruses during biopharmaceutical purification. Triton® X-100 concentrations greater than 0.025% (w/v) (~0.39 mM) are typically required for virus inactivation.[9,10]

Analytical testing of a development drug substance batch of an IgG1 monoclonal antibody (mAbX) reported an atypical level of residual Triton® X-100, which posed a potential toxicology concern. This work describes the use of isothermal titration calorimetry (ITC), near- and far-UV circular dichroism (CD), and differential scanning calorimetry (DSC) to interrogate the nature of the interaction between Triton® X-100 and mAbX and inform technical decision-making related to the application of detergent viral inactivation in the mAbX purification process.

MATERIALS AND METHODS

Materials

Triton® X-100 was purchased from Sigma–Aldrich (St. Louis, Missouri). IgG1 monoclonal antibody mAbX was obtained from Bioprocess Research and Development, Eli Lilly & Company (Indianapolis, Indiana). All experiments were performed in Dulbecco's phosphate buffered saline (DPBS) (Thermo Fisher Scientific, Waltham, Massachusetts). Protein concentration was determined spectrophotometrically using a calculated extinction coefficient.[11] mAbX from the drug substance batch with the atypically high level of residual Triton® X-100 is referred to as "detergent-exposed" throughout.

CD Spectroscopy

Circular dichroism spectra were collected at ambient temperature using an Aviv 62NT instrument (Aviv Biomedical, Lakewood, New Jersey). The concentration of mAbX in all CD experiments was ≈1.9 mg/mL. Three protein sample scans were averaged, corrected by subtracting an average of three

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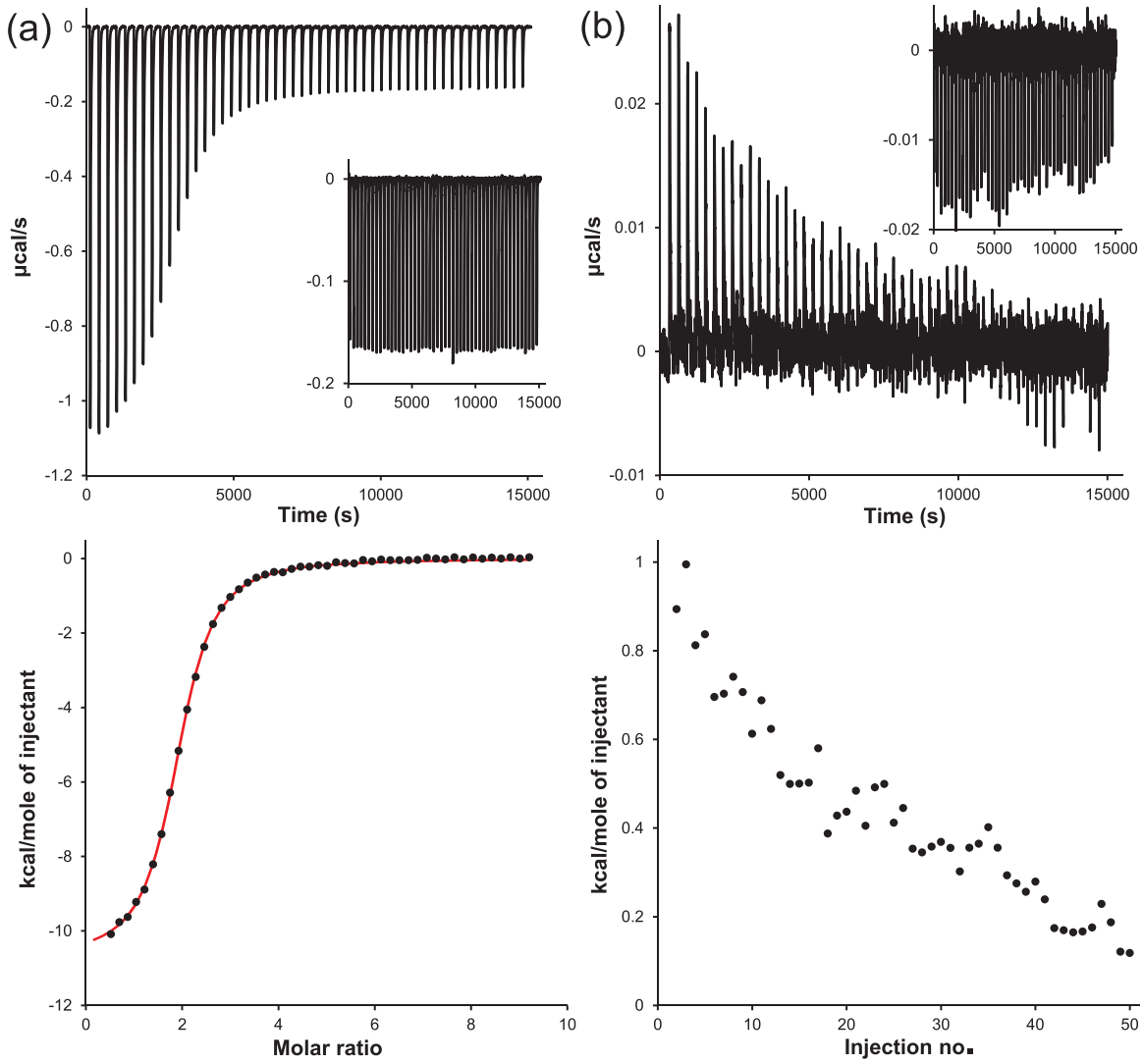

Figure 1. (a) Representative plot and binding isotherm (red line shows the best fit) for titration of 0.75 mM (0.048%, w/v) Triton® X-100 into 0.012 mM mAbX solution at 20°C. (b) Representative plot and dissociation isotherm for titration of 0.343 mM detergent-exposed mAbX into DPBS buffer at 20°C. Insets in the upper panels show control titrations [0.75 mM (0.048%, w/v) Triton® X-100 into DPBS and 0.3559 mM detergent-free mAbX into DPBS, respectively].

buffer blank scans, and then converted to mean residue molar ellipticity.

Isothermal Titration Calorimetry

Isothermal titration calorimetry binding and dilution experiments were conducted at 20°C using a VP-ITC instrument (MicroCal, Northampton, Massachusetts). The binding ITC experiment consisted of 50 injections of 0.75 mM (0.048%, w/v) Triton® X-100 stock into 0.012 mM mAbX solution at constant stirring (310 rpm). Each injection volume was 4 µL with a 9.6 s duration. The delay (wait) between injections was 5 min. In the dilution ITC experiment, a 0.343 mM solution of detergent-exposed mAbX was titrated into DPBS, with all other parameters as described above. All titration data were corrected for the heats of dilution and/or demicellization of Triton® X-100 from control experiments. ITC data were analyzed using Origin 7.0 software (MicroCal). The model describing a single set of independent identical binding sites (OneSites in Origin 7.0) was used for regression of the binding isotherm.

Differential Scanning Calorimetry

Differential scanning calorimetry was performed using VP-DSC instrument (MicroCal). Data were collected in the 20°C–90°C range at a scanning rate of 1°C/min, and mAbX concentration was ≈1.9 mg/mL. The resulting thermograms were corrected for the heat capacity of the solvent by subtraction of corresponding buffer scans. No thermal transitions were observed in control scans of these buffers versus DPBS (data not shown). The data were analyzed using Origin 7.0 software (MicroCal).

RESULTS AND DISCUSSION

Affinity, Molar Ratio, and Reversibility of Triton® X-100–mAbX Interaction

Binding of Triton® X-100 to mAbX was characterized using ITC. Titration of concentrated Triton® X-100 into a solution of mAbX produced exothermic peaks and a characteristic

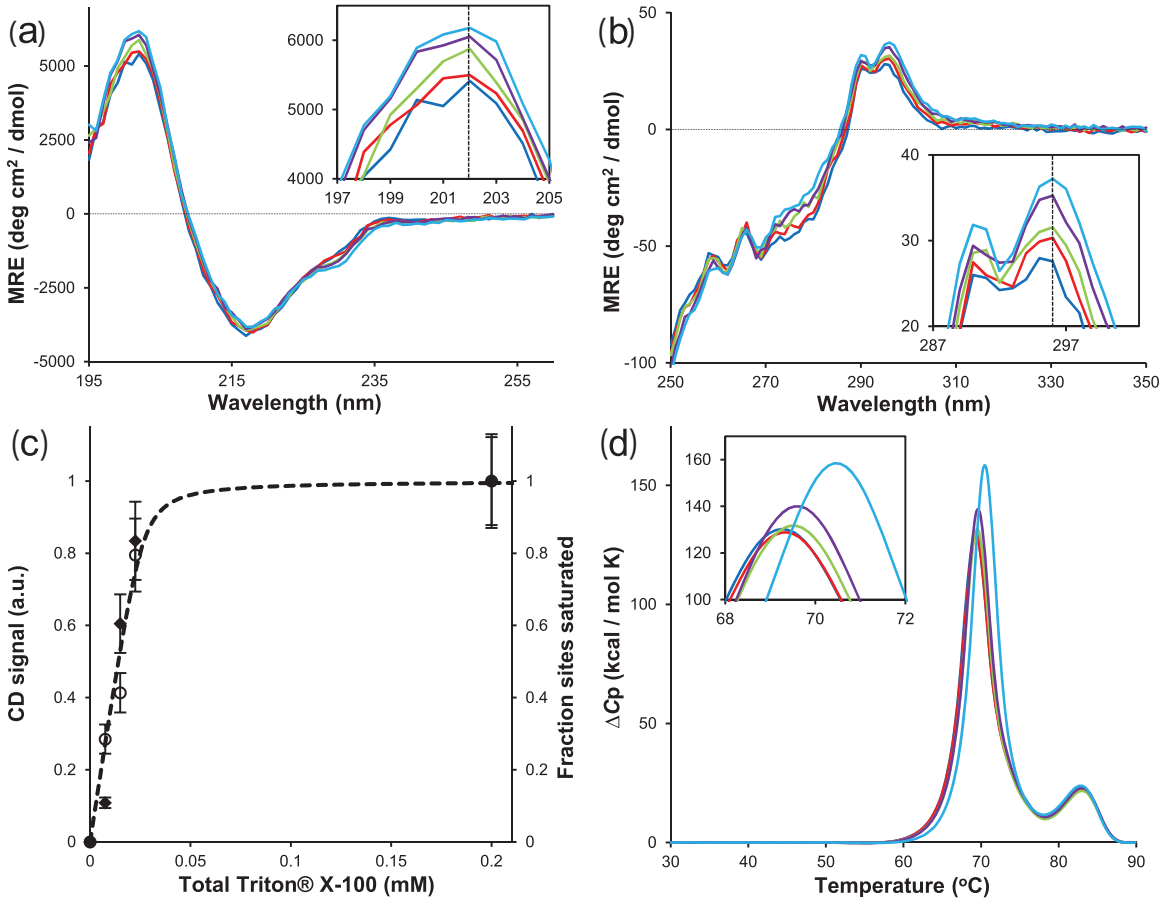

Figure 2. Circular dichroism spectra of mAbX in the (a) far-UV (195–260 nm) and (b) near-UV (250–350 nm) regions at increasing concentrations of Triton® X-100 [0 mM—blue trace, 0.0075 mM (0.00048%, w/v)—red trace, 0.015 mM (0.00096%, w/v)—green trace, 0.0225 mM (0.00143%, w/v)—purple trace, and 0.2 mM (0.01275%, w/v)—cyan trace]. Insets show ellipticity at 202 and 296 nm, respectively. (c) Dependence of normalized ellipticity signal change [$\Delta MRE_\lambda/MRE^0_\lambda$ normalized to unity at 0.2 mM (0.01275%, w/v) Triton® X-100] at 202 nm (filled diamonds) and 296 nm (open circles) on total Triton® X-100 concentration. Error bars represent SD. Dotted line is the predicted binding curve plotted as fraction sites saturated versus total ligand concentration assuming $K_D = 1\ \mu M$, N = 2, [mAbX] = 1.9 mg/mL. (d) DSC thermograms of mAbX at increasing concentrations of Triton® X-100 [colors are as in panels (a) and (b)]. Inset shows the position and magnitude of the major transition peak.

sigmoidal binding isotherm (Fig. 1a). The regression of the binding isotherm indicated an enthalpy-driven interaction[12] [$\Delta H = -(10.73 \pm 0.04)$ kcal/mol and calculated $-T\Delta S = (2.68 \pm 0.04)$ kcal/mol] with two moles of independent binding sites per mole of protein ($N = 1.900 \pm 0.005$) and 1 μM affinity [$K_A = (1.00 \pm 0.02) \times 10^6\ M^{-1}$]. Importantly, only the baseline heat of dilution was measured at concentrations above the Triton® X-100 critical micelle concentration and up to Triton® X-100–mAbX molar ratio of 86 (see Supporting Information, Fig. S1), indicating binding by detergent monomers only and not by micelles.

Reversibility of Triton® X-100 binding was tested by dilution ITC experiments. Although quantitatively fitting the ITC dilution data was intractable because of low signal–noise, the titration clearly yielded endothermic peaks and hyperbolic-like isotherm (Fig. 1b), both characteristic of dissociation of a complex.[13,14] Overall, ITC data show reversible, enthalpy-driven binding of Triton® X-100 monomers to mAbX at 2:1 molar ratio.

Triton® X-100 Binding Impacts HOS and Thermal Stability of mAbX

The impact of Triton® X-100 binding on the HOS of mAbX was evaluated using CD spectroscopy. Far- and near-UV CD spectra of mAbX were collected in the presence of increasing concentrations of Triton® X-100 (Figs. 2a and b). Although the overall shape of far-UV spectra as well as peak positions and their magnitudes remained consistent with IgG fold,[3] the ellipticity of the positive band at 201 nm increased as a function of Triton® X-100 concentration (Fig. 2a, inset). Similarly, ellipticity decreased in the 225–240 nm region. These changes in the far-UV region may be interpreted in terms of binding-induced increase in secondary structure content, a phenomenon reported for other systems including antibodies.[15,16] Near-UV CD spectra exhibited a Triton® X-100 concentration-dependent ellipticity change over a broad wavelength range (Fig. 2b). Significant signal change occurred between 290 and 300 nm, in the spectral region where bands from Trp side chains usually manifest (Fig. 2b, inset);[17] the changes around 280 nm were

masked by high intrinsic noise resulting from protein absorbance. The signal changes in the near-UV region can be potentially explained either by a change in the tertiary structure of mAbX or by direct binding of Triton® X-100 to aromatic side chains; the latter possibility cannot be ruled out given numerous examples of enthalpy-driven hydrophobic interactions.[18,19] The normalized signal change at both 202 nm (far-UV CD) and 296 nm (near-UV CD) agree well with the binding affinity and molar ratio determined by binding ITC (Fig. 2c).

The impact of Triton® X-100 binding on thermal stability of mAbX was tested using DSC. Both the apparent midpoint unfolding temperature (T_m) and enthalpy for the major transition peak (≈70°C), corresponding to unfolding of IgG1 CH2 and Fab domains,[20,21] increased with increasing Triton® X-100 concentrations, whereas the other transition (≈83°C), corresponding to the CH3 domain unfolding,[20,21] remained unaffected (Fig. 2d). The onset temperature of unfolding also increased with increasing Triton® X-100 concentration. Although the observed T_m shift qualitatively agreed with the proposed binding affinity (see Fig. 2d, inset and Supporting Information, Fig. S2), precise determination of K_D from calorimetric measurements was complicated by the irreversible thermal unfolding of mAbX and the possible increase of cooperativity of independent binding sites at T_m and above.[22] A detailed analysis of binding by DSC is beyond the scope of this report.

Taken together, far- and near-UV CD data indicate that Triton® X-100 binding perturbs the native secondary, and potentially tertiary, structure of mAbX. The results of detergent titration monitored by far- and near-UV CD agree well with 2:1 binding molar ratio determined by ITC. Furthermore, DSC data show a ligand-dependent increase of thermal stability of mAbX, which we speculate is due to Triton® X-100 binding to either the CH2 or Fab domain.

CONCLUSIONS

Several orthogonal HOS methods were used to uncover and characterize a specific interaction between a detergent commonly used for viral inactivation in biopharmaceutical processing and a therapeutic IgG1 mAb. Triton® X-100 was shown to bind specifically and reversibly to mAbX at a 2:1 molar ratio with 1 μM affinity. This binding event resulted in perturbations to secondary, and potentially tertiary, structure as well as changes to the thermal stability of mAbX. Although all of the biophysical data suggested a stabilizing impact of the detergent binding, this nevertheless presented potential risks with respect to comparability for future mAbX batches. For example, routine process definition, optimization, and scale-up conducted throughout the purification development process could result in variable levels of Triton® X-100 in the drug substance. This in turn could result in apparent inconsistencies in HOS between batches. Beyond the potential comparability risk, there was also the risk with respect to the toxicity of the Triton® X-100 itself. In this case, targeted biophysical characterization with orthogonal HOS methods demonstrated conclusively that the root cause of atypical levels of residual Triton® X-100 was the specific binding of the detergent to mAbX. This critical information helped enable informed technical decisions related to mAbX purification process design, including the use of alternative viral inactivation methods.

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