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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Received 17 July 2014; revised 12 October 2014; accepted 6 November 2014

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24293

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

Keywords: calorimetry (ITC); calorimetry (DSC); circular dichroism; proteins; protein structure; surfactants; thermodynamics

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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This article contains supplementary material available from the authors upon request or via the Internet at http://onlinelibrary.wiley.com

Journal of Pharmaceutical Sciences

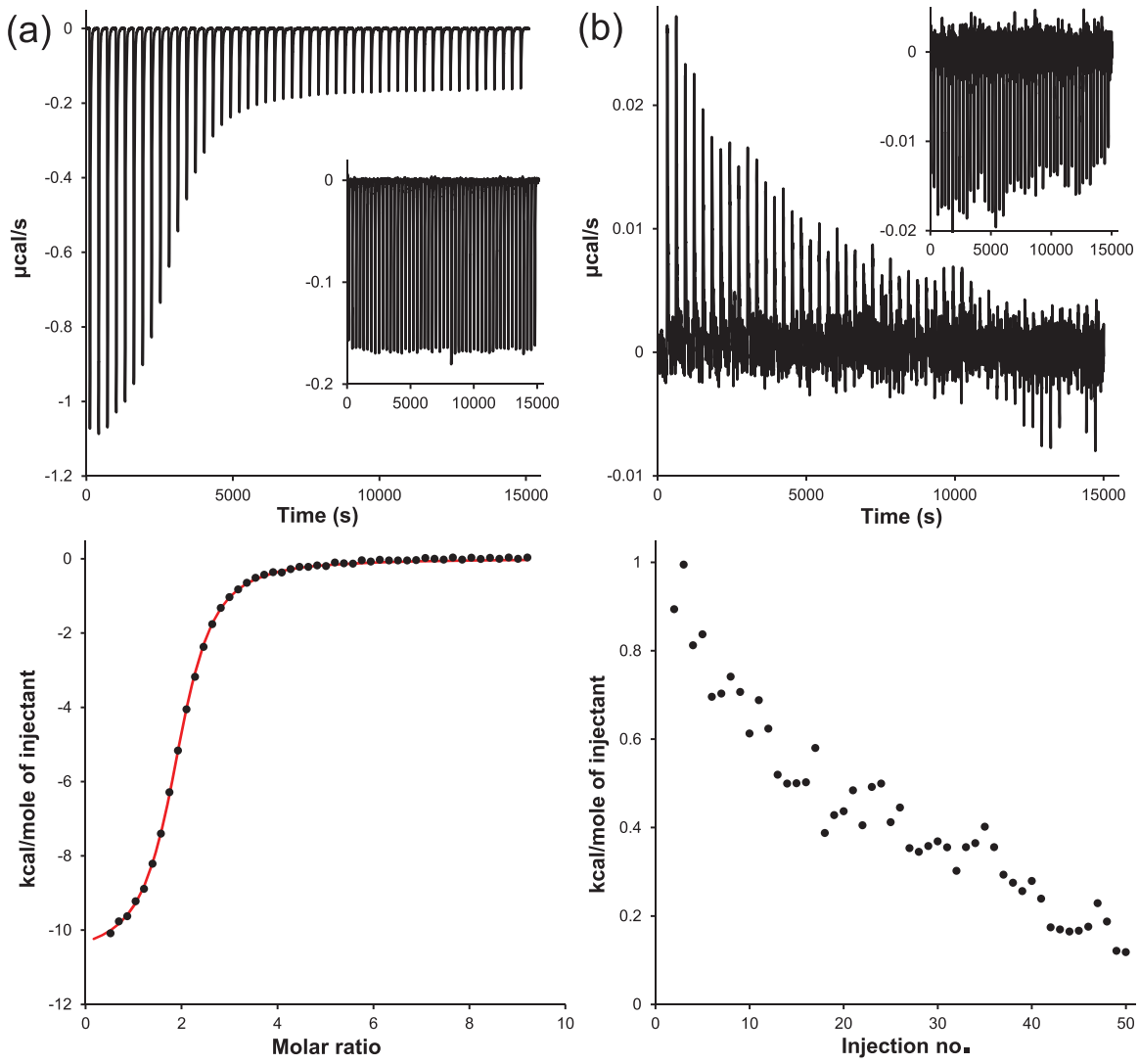

Figure 1. (a) Representative plot and binding isotherm (red line shows the best fit) for titration of 0.75 mM (0.048%, w/v) Triton® X-100 into 0.012 mM mAbX solution at 20°C. (b) Representative plot and dissociation isotherm for titration of 0.343 mM detergent-exposed mAbX into DPBS buffer at 20°C. Insets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

 DOI 10.1002/jps.24293