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A Systematic Multitechnique Approach for Detection and Characterization of Reversible Self-Association during Formulation Development of Therapeutic Antibodies

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ABSTRACT: In addition to controlling typical instabilities such as physical and chemical degradations, understanding monoclonal antibodies' (mAbs) solution behavior is a key step in designing and developing process and formulation controls during their development. Reversible self-association (RSA), a unique solution property in which native, reversible oligomeric species are formed as a result of the noncovalent intermolecular interactions has been recognized as a developability risk with the potential to negatively impact manufacturing, storage stability, and delivery of mAbs. Therefore, its identification, characterization, and mitigation are key requirements during formulation development. Considering the large number of available analytical methods, choice of the employed technique is an important contributing factor for successful investigation of RSA. Herein, a multitechnique (dynamic light scattering, multiangle static light scattering, and analytical ultracentrifugation) approach is employed to comprehensively characterize the self-association of a model immunoglobulin G1 molecule. Studies herein discuss an effective approach for detection and characterization of RSA during biopharmaceutical development based on the capabilities of each technique, their complementarity, and more importantly their suitability for the stage of development in which RSA is investigated. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3089–3099, 2013

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INTRODUCTION

With high specificity and avidity toward a variety of disease targets,[1] monoclonal antibodies (mAbs) represent the fastest growing class of therapeutics in the pharmaceutical marketplace.[2] Advances in protein expression and production technologies as well as improved *in vitro* libraries and selection strategies are some of the key contributing factors to their increasing popularity and success.[1,3]

Because of mAbs' high diversity and complexity, their development as safe and efficacious therapeutics can be challenging.[1,4] mAbs are known to undergo a variety of physical and chemical degradations during manufacturing, fill-finish operation, storage, transportation, and delivery. In addition, mAb optimization with a functional focus requires fine tuning of properties such as affinity, potency, specificity, pharmacokinetics, and effector function,[5–7] which in some cases can inadvertently impact stability. A comprehensive list of mAbs' degradation pathways and their corresponding mechanisms of action are detailed elsewhere.[8–11]

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Small proteins such as enzymes and hormones are often utilized in catalytic quantities and are dosed in small amounts. mAbs, however, are often dosed at stoichiometric quantities and hence require several hundred milligrams of the active product per dose. In addition, although the conventional intravenous administration can serve as an option to achieve the necessary high doses, the goal of providing greater patient convenience by lowering dosing frequency and self-administration strategies, presents subcutaneous (s.c.) injection as the preferred route of administration. The volumes of injection associated with s.c. administrations are typically limited to 1–1.5 mL because of tissue backpressure and injection pain. This significant reduction in injection volumes requires increase in formulation concentration to achieve the desired doses.[12]

Higher concentrations can introduce significant burdens on the formulation and the device design space and often limit the types of approaches that can be used to provide an acceptable stability and delivery profile. At higher concentrations, enhanced attractive intermolecular protein–protein interactions (e.g., charge–charge and hydrophobic interactions) often increase the propensity of a molecule to undergo reversible self-association (RSA), a phenomenon in which native, noncovalent, and reversible oligomeric species are formed.[13] Such a concentration-dependent RSA can, in some cases, lead to other undesired solution properties such as high viscosity,[14–16] opalescence,[17] and liquid–liquid phase separation.[18] For example, self-association-induced high viscosities can introduce significant manufacturability challenges, specifically for filtration-based operations such as Nano-filtration units in which reduced flux and clogging of the lines may occur.[19] Patient's confidence can be compromised because of issues with the drug appearance such as opalescence and phase separation, both of which could occur because of the formation of associated species in solution.[20,21] Depending on the association–dissociation kinetics, RSA can also negatively impact the drug's clearance rates and consequently the pharmacokinetic profiles.[22] In some cases, covalent linkages among the reversible oligomeric species can form over the extended 18–24 month storage shelf-life and lead to formation of irreversible, nonnative aggregates.[23]

Although more prevalent at higher concentration, recent published studies have demonstrated that RSA is not an exclusive property of higher concentration formulations and it can in fact occur in the low protein concentration range (<5–10 mg/mL), spreading the risks associated with self-association over a wide formulation space.[24] Therefore, early detection and characterization efforts, focused on mitigation of RSA, become very important in establishing effective formulation and process controls required for successful development of biopharmaceuticals.[19,25] Because the oligomeric and monomeric species exist in equilibrium with each other, formulation efforts such as optimization of protein concentration, pH, and ionic strength can shift the equilibrium toward the monomeric species.[26,27]

A variety of analytical techniques are available to detect and characterize self-association. These techniques include but are not limited to proton magnetic relaxation dispersion,[28,29] surface plasmon resonance,[30,31] isothermal titration calorimetry,[32] nuclear magnetic resonance,[33] fluorescence energy transfer,[34] mass spectrometry,[35] self-interaction nano-particle spectroscopy,[36] analytical ultracentrifugation (AUC),[20,37] dynamic light scattering (DLS), and composition-gradient multiangle static light scattering (CG-MALS).[38–41] The choice of the employed analytical techniques, not only based on their capabilities and complementarities but more importantly their suitability for a given stage of development, is an important factor for successful evaluation of RSA. Herein, we have employed a multitechnique (AUC, CG-MALS, and DLS) approach to comprehensively characterize the self-association propensity of a model immunoglobulin G1 (IgG1) molecule (referred to as "mAbA" herein). The three selected techniques are the most widely utilized methods by formulation scientists in both academic and industrial settings and were therefore utilized for investigations herein. The capabilities of each technique to detect and characterize protein intermolecular interactions, specifically self-association, are demonstrated and recommendations on an effective RSA detection/characterization approach are provided based on the pros and cons associated with each technique and the stage of formulation development studies.

MATERIALS AND METHODS

Materials

The model IgG1, mAbA, was obtained from bulk drug lots that were manufactured at MedImmune (Gaithersburg, Maryland). The sample purity (>99%) was analyzed and confirmed by size exclusion chromatography and gel electrophoresis. The mAbA samples for all experiments were prepared by dialyzing the stock at 20 mg/mL against 1× phosphate-buffered saline (PBS) buffer (GIBCO, Grand Island, New York) exhaustively over 24 h at refrigerated temperatures. Thermo scientific (Waltham, Massachusetts) Slide-A-Lyzer dialysis cassettes with molecular-weight cutoff of 3.5 kDa were used for dialysis. The concentration of mAbA after dialysis was determined by ultraviolet absorption spectroscopy ($A_{280\,nm}$) using an experimentally determined $E_{1\,cm}$ 0.1% of 1.54 mL mg^{-1} cm^{-1}. All experiments were performed over a concentration