High Throughput Formulation Screening for Global Aggregation Behaviors of Three Monoclonal Antibodies

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ABSTRACT: Global aggregation behaviors of three distinct monoclonal antibodies were characterized by high throughput, multiassay analysis. First, extensive screening of formulations was performed using both incubation at elevated temperature and differential thermal scanning. In incubation studies, formulation conditions representing native favored, native favored but with particulate formation, unfolding with slow aggregation, and fast aggregation with or without phase separation were mapped across a wide range of pH and ionic strength. The sample types or aggregation kinetic scenarios were classified based on fluorescence spectroscopy, light scattering, and micron particle count. Furthermore, apparent melting point was determined for each formulation condition by differential thermal scanning. The global aggregation behaviors and their apparent melting points together highlight the common underlying aggregation pathways and kinetics for the three antibodies. Overall, incorporating multistage aggregation mechanisms in multivariate data analysis provides valuable insights to what and how high throughput techniques can be implemented. Understanding global aggregation behaviors is a key element toward development of rational screening approach. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2120–2135, 2011

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INTRODUCTION

High throughput screening is desirable at early stage formulation development due to material limitation and stringent timeline. The practical objective is to identify "aggregation hot spots" within a formulation space according to historical experiences and patent considerations. The screening results can then guide fine-tuning work at a later stage once the drug candidate enters clinical pipeline. Intensive development of a sustained, platform-based high throughput screening technology has occurred in recent years.[1,2] The effort is largely driven by advances in automation in many conventional techniques, including spectroscopy, light scattering, and imaging. For biopharmaceutical companies, the increased net cast for formulation candidates is an essential part in the product development. This is because, the current protein formulation development is still largely empirical, with little prediction capability on long-term storage stability.[3–5] Often very limited excipients and solution conditions are tested due to resource limitation at the preclinical stage. Without a broad screening or a strategic approach based on mechanistic understanding, final formulation might not be optimal and the product release can be jeopardized. For example, nonnative and irreversible aggregation even at a low level may compromise product purity, potency, and safety.[6–9] Most recently, presence of subvisible particulates in biological product has become an increasing concern with regulatory agencies.[10,11] The formation of unwanted and potentially immunogenic particles through the aggregation pathway is a common challenge. However, the time course prediction of soluble and insoluble aggregate formation is inherently challenging due to complex, multistage aggregation kinetics.[4,12]

Several high throughput analytical techniques were demonstrated in previous studies[1,13–18]:

Additional Supporting Information may be found in the online version of this article. Supporting Information

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optical density, extrinsic and intrinsic fluorescence, dynamic and static light scattering, particle count, and micro- or nano-size imaging. They are based on 96-well plate reader or equivalent analysis format. Theoretically, these assays relate directly or indirectly to aggregate nonnative structure and/or particle size. However, currently there is no systematic study to compare them, and there are no guidelines regarding their applicability in any particular protein formulation screening. On one hand, analytical techniques can only render accessible information of sample characteristics, and its output is limited by assay specifics. On the other hand, it is the experimental condition that determines what type of sample will be generated. Understanding such dynamics is especially important because recent studies[19,20] demonstrated that under a wide range of conditions, the same protein can undergo different aggregation pathways and kinetics to produce aggregates with various sizes, structures, morphologies, and possible phase transition. In practice, such sample is further complicated by possible mixture of monomers, oligomers, and high-order aggregates due to sampling at an arbitrary time point. Their convoluted mass, structural identity, size, and morphology are unlikely to be fully captured and resolved by any single technique. Therefore, multiple characterization techniques are often required to provide complementary information.

The choice of analytical techniques relies on the understanding of divergent characteristics in all possible sample characteristics. The experimentally observed sample characteristics are governed by underlying aggregation mechanisms. Currently it is not possible to have *a priori* knowledge as to what kind of sample will form under a specific experimental condition. Nevertheless, potential sample scenarios can be categorized based on how samples are formed using theoretical multistage aggregation model simulations.[12,21 22] The common pathways that are applicable to many experimentally observed aggregation scenarios are: (1) initial (partial) conformational change to form reactive monomeric conformers, (2) reversible oligomerization and irreversible nucleation, (3) aggregate growth either by monomer addition or by aggregate–aggregate association, and (4) maturation of aggregates in either soluble or insoluble state. The kinetic competition and interaction among different pathways determine the experimental sample properties,[19,23,24] for example, aggregates that primarily grow by monomer addition often exhibit chain polymers or ordered filaments morphology with narrow size distribution, whereas aggregates predominately formed by aggregate–aggregate association have much larger size with high polydispersity or in the form of insoluble precipitants. In contrast, one would expect to detect only minor amount of nonnative oligomers if conformation change is rapid but aggregate growth is considerably slower. It is a common fact that early stage drug candidates are less characterized and as such formulation screening can yield unexpected changes in reaction pathways and kinetics. Because there is little capability to predict the kinetic mechanisms without experimentation, it is more advantageous to include multiple detection methods to capture all possible sample types. Furthermore, samples can be formed from different types of accelerated stability experiment. Some experiments are based on differential thermal scanning, whereas others are based on extended incubation at elevated temperature.[1,18] Often it is presumed that thermal scanning for conformation stability is sufficient to predict aggregation propensity in its shelf life. However, in theory, the thermal unfolding can only predict the unfolding pathway, even though it is a prerequisite in most aggregation scenarios.[3,12,24–29]

To address many of the aforementioned challenges, this study explored accelerated stability study using both differential thermal scanning and constant temperature incubation, and based on a 96-well plate format (see *Materials and Methods* section). Extensive formulation conditions were screened for three distinct monoclonal antibodies. Background information regarding the antibodies is listed in Table 1. Overall, they are sufficiently different with respect to their native sequences and folded structures: the sequence-based aggregation propensity as per complementarily determining regions (CDR) model[30] varies, and the total surface charge (isoelectric point) is effectively apart. Therefore, their intrinsic stability and aggregation behaviors are expected to vary. The screened formulation conditions are compiled in Table 2. They provide a sufficiently large formulation space (pH, ionic strength, and additives) to explore possible aggregation behaviors. The significant variations in both antibodies and formulations served the purpose to test the general applicability

Table 1. Information for Antibodies Used in this Study

	mAb1	mAb2	mAb3
IgG type	IgG2m4	IgG2m4	IgG1
Aggregation propensity[a]	Medium	High	Low
Weight-average molecular weight (kDa)[b]	147 (1.01)	152 (1.01)	153 (1.00)
pI[c]	7.3	8.4	9.1

[a] Rank is based on sequence complementarily determining regions (CDR) analysis.[30] The rank is according to calculated aggregation index (normalized by residue number), which is based on contributions from hydrophobicity, beta sheet propensity, and charge for each CDR.

[b] On the basis of SEC-Viscotek; polydispersity (ratio of weight–average molecular weight over number–average molecular weight) is indicated in parenthesis.

[c] On the basis of capillary isoelectric focusing.

pI, isoelectric point.