Concluding Summary: Proceedings of The AAPS Biotec Open Forum on “Aggregation of Protein Therapeutics.”

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With the advent of recombinant DNA technology and improvements in large-scale bioprocessing, it has been possible to produce large quantities of proteins to be used as therapeutic agents. One of the key challenges is that proteins can degrade both by chemical and physical degradation pathways. One of the most important pathways for protein physical degradation is the generation of protein aggregates. The ability of proteins to aggregate has been recognized from the early beginnings in protein biochemistry. A recent article in the AAPS Newsmagazine (June 2006) discusses the general impact of protein aggregation on biopharmaceutical development as well as the creation of the AAPS Protein Aggregation and Immunogenicity Focus group, which is affiliated with the Biotec Section. In June 2005, preceding the AAPS National Biotechnology Conference (NBC), the AAPS Biotec Section organized an Open Forum Meeting on “Aggregation of Protein Therapeutics.” This issue of *The AAPS Journal* presents selected papers that resulted from the presentations at this meeting. This Open Forum also included time for panel discussions with the presenters and the questions and responses were transcribed and are included in these proceedings.

The morning session of the Open Forum dealt with the impact of protein aggregation on bioprocessing, formulation, and quality of protein therapeutics. The keynote address by Amy Rosenberg, from the Division of Therapeutic Proteins, The Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), addresses the impact of protein aggregates on the potential immunogenicity of protein drugs. The contributed paper very nicely gives some keen insight from an immunologist’s perspective. Dr Rosenberg clearly reviews animal models as well as human clinical evidence for the role of protein aggregates in induction of an immune response.¹ An important concept that she discusses in some detail relates to not just the size or amount of aggregate present but the physical characteristics of the aggregate. The hypothesis is that multimers consisting of natively formed proteins may be efficient at eliciting a humoral antibody response that is independent of T cells. In addition, these antibodies may then be efficient in interacting with properly folded protein, which can result in neutralization of potency of the administered therapeutic, alteration of pharmacokinetics, or worse, in some cases,

neutralize the activity of existing endogenous protein. The remaining talks in this session focused on different aspects of how protein aggregates affect protein pharmaceutical quality and impact formulation and bioprocessing. Dr Edwin Moore gave an overview on how protein aggregation affects protein quality (article not included in this volume). In his presentation he reviewed how protein aggregates could result in important clinical consequences such as alteration of pharmacokinetics, loss of potency, and elicitation of immune responses. He also discussed mechanisms in the context of the well-known Lumry-Eyring model. This discussion included a brief summary of “environmental factors” encountered during bioprocessing that can influence the rate of protein aggregation. In particular, he stressed the importance of understanding surfaces encountered during bioprocessing and how these surfaces can lead to undesirable interaction with proteins resulting in altered conformations and ultimately in protein aggregates. Professor Ted Randolph (University of Colorado, Boulder) elaborated further on this issue by presenting previously published work on heterogeneous nucleation-controlled particulate formation in proteins.² The salient features of this work demonstrate that the driving force for particulate generation can arise from favorable interactions between proteins and solid surfaces, and is illustrated using spiking experiments with nuclei generated from protein absorbed onto silica particles. An understanding of such phenomena allows for better control of particulate formation by either decreasing the extent of adsorption to surfaces through the addition of surface-active agents such as Pluronics or the affinity of the surface adsorption by altering the electrostatics via changes in solution pH. The final presentation of the morning session by Mary Cromwell (Genentech, Inc) continued to explore the occurrence of protein aggregation during bioprocessing using several case study examples.³ This article goes through several examples that highlight how protein aggregation can occur at different stages in bioprocessing, such as during cell culture process, recovery/purification final formulation, and final fill/finishing. Particular attention is paid to the use of ultrafiltration/diafiltration technology and the impact of pumping of protein solutions resulting in mechanical stress that can generate protein aggregates. Overall this paper nicely lays out the many “lookouts” that one needs to

be aware of encountering during bioprocessing and suggestions for how to detect and avoid these problems.

The afternoon session of the Open Forum was dedicated to "Methods Used to Assess Protein Aggregation." The lead-off keynote presentation by Dr Michelle Frazier-Jessen (CDER, FDA) addressed FDA experience in measuring and minimizing protein aggregates (article not included in this volume). This presentation indicated that acceptable levels of aggregates in products would be dependent on manufacturing capabilities and experience, the product stability profile, the type of aggregates (reversible, irreversible, and so forth), impact on potency, and of course experience in preclinical in vivo and in vitro studies. Dr Frazier-Jessen also focused on a summary of methods used to characterize and quantify aggregates in protein solutions. It was also stressed that since each method has its own set of limitations it is important to use more than one method to verify the characterization. Dr John Philo (Protein Alliance Labs) elaborated further on this point by first discussing the wide spectrum of aggregate sizes and types and citing specific examples from his work to demonstrate how different biophysical techniques can be applied to study protein aggregates.⁴ Dr Stephen Berkowitz (Biogen) concentrates on the use of one technique, analytical ultracentrifugation (AUC), to show how this method can be used to assess performance of size exclusion chromatography (SEC), and, in fact, improve the SEC protocol.⁵ He also does a very nice job in outlining how the very popular SedFit analysis program created by Peter Schuck⁶ works by fitting data to the Lamm equation. The final presentation of the afternoon session, by Dr Jun Liu (Genentech, Inc), discusses the use of AUC and Field Flow Fractionation techniques.⁷ This article also describes particular steps that can be taken when designing the sedimentation velocity experiments to help increase the precision of the determinations when using the SedFit analysis. The procedures described have allowed the authors to reproducibly quantify aggregate levels at the 0.2% to 0.3% range with a well-characterized monoclonal antibody.

Overall, this Open Forum, which was well attended (~150 attendees), gave some nice in-depth talks on the concerns of how aggregates impact bioprocessing and protein quality, as well as what analytical techniques are being used to char-

acterize and quantify protein aggregates. The panel discussions⁸ also included in this issue addressed several points, and it was clear that there was high enthusiasm and desire for a follow-up Open Forum that included more discussion of analytical methods. This high level of interest resulted in a second Open Forum held prior to the AAPS NBC 2006 in Boston entitled "Analysis of Protein Aggregation and Self Association," which again drew more than 100 attendees. As a follow-up, the AAPS Protein Aggregation and Immunogenicity Focus Group in collaboration with the University of Colorado Center of Pharmaceutical Biotechnology and the FDA organized a workshop on Protein Aggregation held at Breckenridge, Colorado, September 26 to 27, 2006. This workshop included breakout sessions for further interactive discussion after each session and was attended by approximately 200 registrants. Hopefully these proceedings will whet the appetite and encourage more protein pharmaceutical biotechnologists to attend future workshops, and also to contribute to future programming and endeavors in this important area.

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