Journal of Pharmaceutical Sciences 106 (2017) 3507-3514

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

Assessing the Impact of Charge Variants on Stability and Viscosity of a High Concentration Antibody Formulation

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ARTICLE INFO

Article history:
Received 24 May 2017
Revised 10 August 2017
Accepted 17 August 2017
Available online 30 August 2017

Keywords:
protein aggregation
protein formulation
biotechnology
stabilization
viscosity
charge isoforms
protein characterization
high concentration
monoclonal antibodies
physical stability

ABSTRACT

Characterizing molecular charge variants or isoforms is essential for understanding safety, potency, and bioavailability of antibody therapeutics. However, there is little information on how they influence stability and viscosity—properties governing immunogenicity and delivery. To bridge this gap, we studied antibody stability as a function of charge variant content generated via bioreactor process. We were able to systematically vary acidic variant levels as a function of bioreactor harvest time. Importantly, we do not observe any impact on aggregation behavior of a formulated antibody at high protein concentration as a function of acidic variant level. Furthermore, we confirm that acidic variants enriched using fractionation do not influence viscosity, colloidal or conformational stability. Interestingly, variants with the most acidic isoelectric points contribute disproportionately to formulation color. We discuss our findings in context of antibody manufacturing processes that may yield increased charge variant content.

Introduction

Monoclonal antibodies are the most common biopharmaceuticals targeting a variety of disease indications ranging from various cancers, rheumatoid arthritis, multiple sclerosis, cardiovascular and autoimmune diseases with annual sales revenues reaching $75 billion in 2013.[1,2] Apart from the wide range of treatable indications, the success of antibody therapy is also attributed to well-established methods for discovery and development.[3-6] Antibody manufacturing has advanced substantially from improvements in cell culture titers up to 5-10 g/L as well as downstream purification processes maximizing yield.[7-9] Additionally, better understanding of patient convenience and supply chain optimization has revealed the attractiveness of high concentration (≥150 mg/mL protein) liquid formulations.[10-12] In particular, such formulations enable lower-volume, less-frequent, self-administered therapeutics for subcutaneous delivery. Manufacturing concentrated antibody formulations using high-yield processes is therefore a popular initiative across the biopharmaceutical industry.[11]

However, development of safe and efficacious high-concentration antibody formulations is often met with challenges like protein aggregation, particle formation, chemical degradation, high viscosity, and phase separation.[13-16] Such problems arise from attractive intermolecular interactions leading to self-association, sequence hotspots prone to chemical modification, and interfacial instability that generates particle-forming nuclei.[17-20] Among intrinsic characteristics that impact stability and viscosity, the protein molecular surface charge or charge distribution has been identified as a key attribute.[21,22] Oppositely charged surface patches may engage in long-range attractive electrostatic self-interactions,[23-25] governed by even single amino acid residues in

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2017.08.016.

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http://dx.doi.org/10.1016/j.xphs.2017.08.016
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some cases.[26] To minimize such interactions, ionic strength—providing additives like sodium chloride and arginine hydrochloride are favored.[27-29] However, the asymmetry in charge distribution across the length of the antibody framework also mediates short-range attractive dipole interactions that prevail despite the presence of such excipients.[18,30] Interactions mediated by solvent-exposed hydrophobic patches also become prominent in the absence of electrostatic repulsion. Importantly, at concentrations exceeding 150 mg/mL, intermolecular separation distances are comparable to effective molecular size magnifying such interactions. Therefore, selecting mutants with minimal charge-related self-association is a focus of developability assessment before clinical development.[31]

Yet, any biologically synthesized and purified antibody formulation also contains an ensemble of heterogeneous molecular charge variants or isoforms.[32-35] These isoforms result from post-translational modifications (e.g., glycosylation variants in content of sialic acid, mannose, fucose, and glycosylation positions, nonclassical disulfide linkages, and spontaneous mutations),[34,36-39] chemical modifications during bioreactor process (e.g., glycation, oxidation, deamidation, aspartate isomerization, and N-terminal pyroglutamate formation),[40-43] degradation resulting in antibody fragments,[34,43] as well as soluble aggregates formed during downstream purification, membrane processing, and long-term on-shelf storage of drug product.[34] Such modifications can directly impact the complementarity-determining regions leading to a reduction in antigen-binding affinity, whereas others can impact the Fc region resulting in loss of binding to receptors regulating effector cell response or serum half-life. Indeed, several studies have assessed impact of charge isoforms in antibody bioavailability and *in vivo* biology.[34,44-49] Isoforms are especially relevant when scaling up upstream processes to produce higher antibody titers—selecting cell lines with higher productivity, modifying bioreactor feed strategy, extending bioreactor batch times, etc.[7,50] Processing larger titers with minimum product loss also requires modifying purification process parameters commonly resulting in extended run times, modified buffer constituents and pH, different affinity or ion-exchange resins, additional polishing steps and membrane processing.[9,51] For this reason, performing molecular comparability assessment across process changes is a key aspect of clinical and commercial drug development.[52]

Despite the importance of charge isoforms in antibody safety and efficacy, their impact on stability and viscosity—phenomena closely related to molecular charge—is not yet fully understood. Current knowledge on charge variant–mediated aggregation is restricted to chemical modifications over long-term or accelerated product storage, for example, metal-catalyzed or free radical oxidation, glycation, and deamidation.[20] Limited studies have examined variants generated via cell culture modifications including recent work on biophysical attributes of antibody glycoforms,[53] and the impact of disulfide reduction on drug substance quality.[54] However, controlling glycosylation and disulfide reduction are already well-recognized strategies to produce antibody therapeutics with molecular comparability. Thus, there is an unmet need to characterize isoforms generated during optimal cell culture and downstream purification. Additionally, there are no studies examining viscosity behavior as a function of charge isoforms. To bridge these gaps, we studied the impact of charge variants generated via modulating bioreactor batch time on aggregation of a human IgG1 monoclonal antibody (mAb1) and assessed viscosity behavior of fractionated isoform species. Here, we summarize our findings in context of heterogeneous antibody formulations that may arise in high-titer manufacturing.

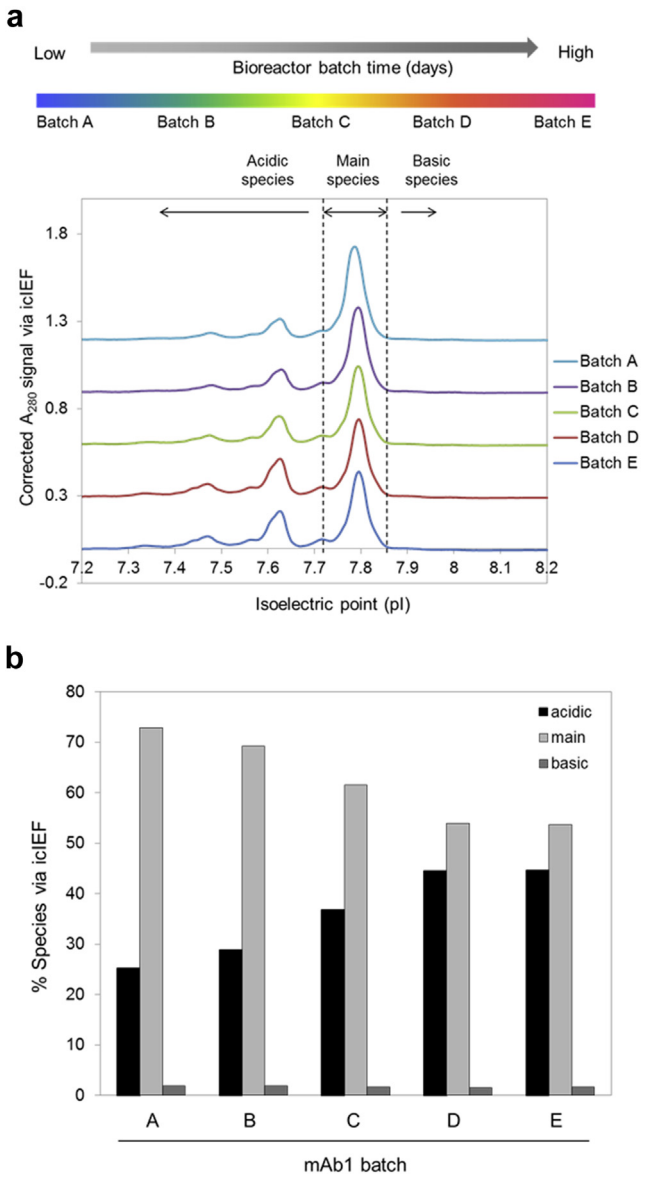

Figure 1. Modulating mAb1 charge variants via bioreactor batch time. (a) Charge heterogeneity profiles of each mAb1 batch via icIEF showing acidic, basic, and main molecular species. (b) Acidic, main, and basic species content in each mAb1 batch.

Results

Toward our goal, we performed a small-scale bioreactor run using an optimum feed strategy while harvesting product over a wide range of batch times ranging from the shortest batch (A) to longest batch (E) (see Methods). We posited that the variation in bioreactor batch time would yield mAb1 product with a wide range of charge variant content due to increased exposure of the molecule to reactive ingredients, post-translational modifications, and elevated temperature. Specifically, we expected an increase in charge isoform content starting from relatively pure main species in the shortest batch to a highly heterogeneous formulation in the longest batch. The selection of harvest times was based on a conventional operating range used in cell culture process development, spread over multiple days (data not shown).[7] After purifying the bioreactor product, we indeed observe that the acidic species (low pI isoforms) content increases systematically from 25.2% in batch A to 46.3% in batch E (Figs. 1a and 1b). On the other hand, the