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Review

Microfluidic Approaches for the Characterization of Therapeutic Proteins

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ABSTRACT

In the last decades, the pharmaceutical market has experienced an increase in the number of therapeutic proteins. The high activity and selectivity of these macromolecules is often achieved at the expense of complex structures, which exhibit several biophysical properties that must be carefully controlled and optimized for the successful development of these drugs as well as for guaranteeing their quality and safety. This need has motivated the application of a variety of biophysical techniques to analyze properties of therapeutic proteins and protein solutions including interactions, aggregation, solubility, viscosity, and thermal stability. After briefly summarizing currently available experimental approaches, we highlight the emerging possibilities offered by advances in microfluidic technology for the analysis of therapeutic proteins during manufacturing and formulation.

Introduction

In the last decades, the pharmaceutical market has experienced the approval of an increasing number of protein-based products for the treatment of a broad range of severe disorders, including tumors, autoimmune diseases, and diabetes.[1,2] The success of therapeutic proteins is largely due to a series of attractive features which are difficult to obtain with small-molecule drugs, including high activity, high selectivity, and the reduction of side effects. Indeed, the flexible architecture of proteins allows one to engineer a variety of functionalities by modifying the primary sequence of the proteins or by conjugating the protein scaffold with polymers[3] or small molecules.[4]

A drawback of this engineering procedure lies in the complex macromolecular structure of the resulting product, which during the processing can be exposed to changes in buffer composition, interfaces, and shear stress. These conditions can harm the stability of the protein structure, which must be carefully controlled for the successful application of the drug.[5-7] The characterization and control of protein stability are therefore crucial aspects during each phase of the product life span, from early-stage development to production, storage, and delivery.

The task of preserving protein structure in solutions is particularly challenging because proteins may experience a broad range of chemical degradations (e.g., deamidation, oxidation upon light exposure) and physical instabilities.[8-10] The latter include phase transitions and the kinetic-controlled formation of soluble aggregates and subvisible particles, which are often triggered by chemical modifications or by the formation of nonnative structures.[11] In particular, protein aggregation has been associated with immunogenic responses leading to the inactivation of the drug or to inflammatory reactions.[7,12] Therefore, strict regulatory conditions are applied to the characterization of protein aggregation. In intravenous delivery, potential factors leading to aggregation include the interactions between proteins and several interfaces, extractables, and leachables as well as particulate materials originating from processing, and from the combined effect of air/water interface and mechanical forces.[13-15] In subcutaneous administration, crucial properties of the formulation are the solubility of the drug and the viscosity of the solution at the high protein concentrations required by the delivery (70-200 mg/mL).[16] Hydrophobic/hydrophilic interfaces generated for instance by the presence of silicone oil droplets in the syringes can also potentially induce aggregation.[17]

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In the context of preformulation studies, the evaluation of protein stability is a central step aiming at predicting the most suitable solution composition for a specific protein. The goal of such strategies is the reduction of the time and the extent of development activities needed during the following clinical stages.[18]

In addition to the formulation and the delivery of the drugs, protein stability plays a key role in assessing the developability of the product, including the level of expression from cells and the yield of recovery during downstream operations.[19]

Proteins characterized by particularly high instabilities toward chemical or physical degradations could still reach successful clinical development through the optimization of cell lines, upstream and downstream processes as well as formulation buffers.[19] However, this optimization is both labor and time consuming, and the success depends on the nature of the instabilities and their impact on safety and efficacy. Therefore, the selection of stable lead candidates as well as the identification of potential instabilities is an important aspect during the early stages of the process.[18] In this context, methods that generate useful information by consuming a limited amount of material are highly attractive.[20] Moreover, these methods are useful to optimize conditions for downstream processing on a large scale by prescreening a large number of conditions consuming a limited amount of material.

Getting a full picture of the stability of therapeutic proteins involves the characterization of a large number of properties of proteins and protein solutions, including solubility, self-association, aggregation, changes in secondary and tertiary structure, viscosity, thermal stability, stability at interfaces, and nonspecific binding.[19,21] The need of preserving protein structure in biopharmaceuticals has motivated the development and the application of a broad range of experimental and theoretical approaches to characterize and optimize these properties by engineering the protein primary sequence or modulating the buffer conditions. Since most of these properties arise at the molecular level from protein-protein and protein-surface interactions, particular attention has been given to the characterization of intermolecular forces.

In this review, after briefly summarizing the broad range of currently available methods, we discuss the emerging possibilities offered by recent advances in microfluidic technology for the experimental characterization of protein solutions. We focus on selected important properties of solutions of therapeutic proteins, namely protein interactions and formation of aggregates, nature and amount of impurities, viscosity, and thermal stability.

Biophysical Characterization of Therapeutic Proteins

The experimental characterization of protein stability exhibits a series of technical challenges that are largely related to the broad scales of time and sizes associated with the process. For instance, aggregation events can generate a variety of protein species with different morphologies, with size ranging from few nanometers to several hundreds of microns, in a time scale spanning hours, months, or even years.[22] As a consequence, a comprehensive biophysical analysis requires the application of many techniques in parallel. The formation of aggregates is characterized by a series of methods including—among many others—size exclusion chromatography coupled with in line multiangle light scattering for the analysis of soluble species as well as turbidity, flow imaging analysis, and centrifugation assays for the characterization of subvisible particles.

The detailed description of the state-of-the-art biophysical techniques currently available for the analysis of therapeutic proteins has been extensively treated in several reviews, to which the reader is referred.[13,23-25]

Most of these approaches are already routinely implemented with a high level of automation and throughput, although the amount of material required by the analysis can vary significantly depending on the technique and may not always be affordable, in particular during the early stages of the process. As a consequence, a driving need persists for the development of strategies aimed at reducing the amount of time and material required for the characterization of the product stability.

Among the various biophysical properties of therapeutic proteins, particular attention has been dedicated to the characterization and modeling of physical intermolecular interactions, which include a large number of weak noncovalent forces such as electrostatic, hydrophobic, solvation, and polar effects.[26-28] These forces are commonly described in a coarse-grained manner via the second virial coefficient (B_{22}), which integrates the contribution of different molecular forces over the distance between 2 proteins. A negative B_{22} is indicative of global attraction forces, whereas a positive B_{22} corresponds to a net repulsive interaction. Hard spheres characterized by net interactions equal to 0 exhibit a positive B_{22} due to excluded volume effect. The B_{22} is most often measured by osmometry,[29] light scattering techniques,[26,30] analytical centrifugation,[31] and self-interaction chromatography.[32] Weak intermolecular interactions can also be quantified by polyethylene-glycol induced liquid-liquid demixing[33] as well as by the evaluation of the protein-protein interaction parameter (k_D), which is typically measured by dynamic light scattering (DLS) and has been shown to correlate with B_{22}.[26,34] Recently, attractive methods based on affinity capture self-interaction nanoparticle spectroscopy have been developed to measure protein-protein interactions with higher throughput.[35-39] Other important high throughput approaches include self-interaction chromatography,[32,40] cross-interaction chromatography,[41] and biolayer interferometry.[42]

Weak intermolecular interactions, which become particularly important at high protein concentration,[43] have been shown to correlate with increased viscosities of protein solutions. In some cases, the increase in viscosity occurs because of the formation of transient clusters induced by weak protein-protein interactions.[44-49] Weak intermolecular forces are thus emerging as a good global parameter for prediction of product developability[36] and can be modulated for instance by changing the net charge of the variant regions of antibodies[50] or by introducing hydrophobic salts in the formulation.[51] Indeed, the net charge of the variant regions of antibodies has been shown to significantly affect intermolecular interactions and solution viscosity under several formulation conditions.[50]

Despite the key role played by intermolecular forces in dictating the properties of proteins and protein solutions, there is currently no individual parameter or property capable of comprehensively describing or predicting the protein behavior.

Microfluidic Technology for Therapeutic Proteins

Recent progress in microfluidic technologies is paving the way for novel approaches in the biophysical characterization of therapeutic proteins, which can complement the pool of available conventional methods. The manipulation of matter at the micron scale offers a series of attractive advantages, which can be broadly summarized in 2 aspects:

First, microfluidic approaches can reproduce current bulk techniques on a smaller scale. This miniaturization reduces the time and the amount of valuable material which are required by the analysis, therefore leading to an increased throughput.

Second, microfluidics allows to perform operations that are not achievable on the bulk scale. A clear example is the investigation of phase transitions under confinement in the volume scales from pL