Prediction and Reduction of the Aggregation of Monoclonal Antibodies

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Abstract

Protein aggregation remains a major area of focus in the production of monoclonal antibodies. Improving the intrinsic properties of antibodies can improve manufacturability, attrition rates, safety, formulation, titers, immunogenicity, and solubility. Here, we explore the potential of predicting and reducing the aggregation propensity of monoclonal antibodies, based on the identification of aggregation-prone regions and their contribution to the thermodynamic stability of the protein. Although aggregation-prone regions are thought to occur in the antigen binding region to drive hydrophobic binding with antigen, we were able to rationally design variants that display a marked decrease in aggregation propensity while retaining antigen binding through the introduction of artificial aggregation gatekeeper residues. The reduction in aggregation propensity was accompanied by an increase in expression titer, showing that reducing protein aggregation is beneficial throughout the development process. The data presented show that this approach can significantly reduce liabilities in novel therapeutic antibodies and proteins, leading to a more efficient path to clinical studies.

Introduction

Protein particles are assemblies built up of native and/or denatured proteins [1] that generally have a negative impact on manufacturability, stability, safety, titers, immunogenicity, and solubility of biologics in general [2–6]. Here, we investigate the impact of β-aggregation-prone sequences on protein particle formation and assess our ability to predict and suppress antibody particle formation based on this structural mechanism alone. β-aggregation is the process of association of proteins, predominantly through the formation of intermolecular beta-sheet structures by short aggregation-prone regions (APRs) of the polypeptide sequence [7]. Despite the fact that APRs are mostly hydrophobic in nature, they require other key properties such as a high β-sheet propensity and a low net charge. Common methods of aggregation prediction are geared toward the identification of APRs in the primary sequence [2–5,8,9]. These prediction methods establish the theoretical aggregation potential of the protein in the unfolded state, called the "intrinsic aggregation propensity". To nucleate aggregation, an APR must be solvent exposed in order to form stable interactions with other like sequences. However, in most globular proteins,

these APRs are buried inside the hydrophobic core of the native structure, where they are prevented from triggering aggregation by the thermodynamic stability of the protein [7,10,11]. Noteworthy exceptions are APRs occurring at exposed sites of functional importance such as protein–protein interaction interfaces [12–15]: here, the functional requirements of the site appear to lead to the emergence of APRs that can be problematic when the protein is not engaged in functional interactions. The effective aggregation of a protein is thus dependent on the population of aggregation-compatible conformations in which the APRs are exposed. The interplay of physicochemical parameters such as protein and ion concentrations, pH, and temperature contributes to determine the concentration of aggregation-prone conformers in a protein solution. Therefore, the challenge for relatively large and thermodynamically stable proteins like antibodies is identifying sequences that will aggregate under native conditions. The solution to this challenge lies in the distinction between APRs that are thermodynamically protected by folding and those that occur in aggregation-competent conformations that can form without major unfolding transitions (Fig. 1a). The latter regions would be the critical APRs that determine aggregation propensity under native conditions. We previously demonstrated for protective antigen and alpha-galactosidase that mutations in these critical APRs dramatically reduce the overall aggregation rate of the protein and increase the amount of soluble produced protein in mammalian cells. These mutations introduced the so-called suppressing gatekeeper residues that oppress aggregation locally [16]. Here, we investigated if the approach is transferable to the engineering of monoclonal antibodies, which is in itself non-trivial given the difference in architecture and size between antibodies and the previously studied cases. Moreover, we also wanted to test if the method would allow the sorting of aggregation-prone antibodies from less aggregation-prone ones, which would be extremely valuable in prioritizing lead candidates for therapeutic applications early during development.

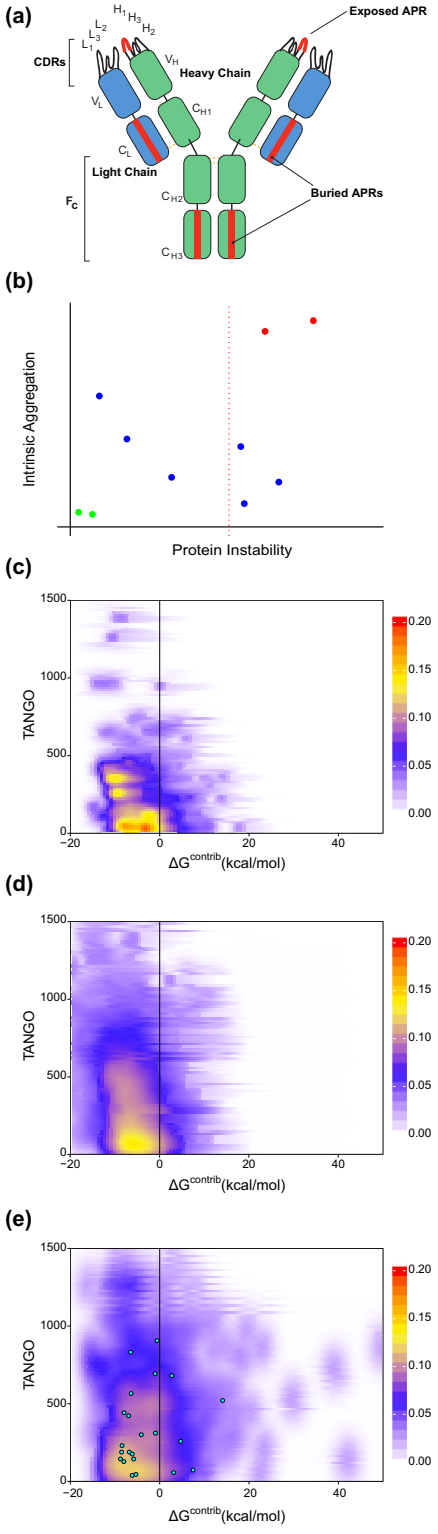

Fig. 1. *In silico* analysis of aggregation propensity in antibody crystal structures. (a) Schematic representation of possible locations of APRs in monoclonal antibodies. APRs in CDRs would be more problematic than APRs buried in the immunoglobulin fold. (b) Stretch-plots: representation of aggregation propensity and local stability of APRs. Problems increase toward the top right of the plot; ideally, APRs would be located in the bottom left. (c) Density plot of all APRs located in the FR of over 2000 antibody structures from the abYsis database [24]. (d) Density plot of aggregation propensity and local stability of APRs in globular protein structures. The analysis is based on a set of 2650 high quality structures (*R*-factor of <0.20 and resolution of <1.9, with 30% sequence identity) of globular proteins generated using the Whatif software suite [25]. (e) Density plot of all APRs overlapping with CDRs of all antibody structure from the abYsis database. Cyan dots: APRs overlapping with CDRs of the 11 model antibodies used in the study.