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Review

Stability engineering of the human antibody repertoire

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ABSTRACT

Human monoclonal antibodies often display limited thermodynamic and colloidal stabilities. This behavior hinders their production, and places limitations on the development of novel formulation conditions and therapeutic applications. Antibodies are highly diverse molecules, with much of the sequence variation observed within variable domain families and, in particular, their complementarity determining regions. This has complicated the development of comprehensive strategies for the stability engineering of the human antibody repertoire. Here we provide an overview of the field, and discuss recent advances in the development of robust and aggregation resistant antibody therapeutics.

1. Introduction

The number of human monoclonal antibody candidates has increased rapidly in recent years, and now represent the largest single class of molecules entering clinical studies [1]. Despite the rapid growth of the antibody therapeutics market over the past twenty to thirty years, hurdles remain that limit their manufacture. Key factors relate to the variable and often limited stability of human antibodies, which negatively impact on many production processes including expression [2,3], purification [4] and formulation [5].

Antibodies are complex multidomain proteins and mechanisms governing their thermodynamic [6] and, in particular, colloidal [7] stability are not fully understood. Human antibodies of the commonly used immunoglobulin G (IgG) type consist of a total of twelve domains, which can be further divided into two chains (heavy and light) and variable (V_H, V_L) and constant (C_H1, C_H2, C_H3, C_L) domains (Fig. 1). Only limited diversity is observed among constant domains, with a total of four isotype (IgG1–4) and two light chain ([κ] kappa and [λ] lambda) classes expressed in humans. Consequently, isotype differences can explain only a limited proportion of the observed stability variation in the human antibody repertoire [8]. A higher proportion of sequence diversity is observed among antibody variable domains, which are assembled through genetic recombination of variable, diverse (V_H only) and joining segments (VDJ-recombination). Further diversity is then introduced into framework, and in particular complementarity determining regions (CDR), through somatic hypermutation, followed by clonal selection of the repertoire. As a result of these processes, the overwhelming majority of sequence variation within the human antibody repertoire is observed within the CDR regions of a limited range of human variable domain families. In this review, we summarize the influence of the observed antibody diversity on colloidal and thermodynamic stabilities and discuss recent advances to improve these properties through engineering approaches.

2. Protein aggregation

Aggregation is a complex process by which proteins can form alternative colloidal states, which are different from the native state, but otherwise energetically favorable [9,10]. It is generally believed that such aggregate species are predominantly formed via unfolded or partially unfolded states [11]. Protein aggregation is increasingly recognized as a problem affecting the manufacturability of human therapeutic antibodies, shelf life and efficacy [12]. Importantly, the presence of aggregates has also been linked to increased immunogenicity, with effects ranging from mild skin irritation to anaphylaxis [13].

Although it can be assumed that stabilization of the native state over alternative aggregate states has occurred during evolution [14], it is important to note that the production of monoclonal antibodies exposes these molecules to a wide range of non-physiological

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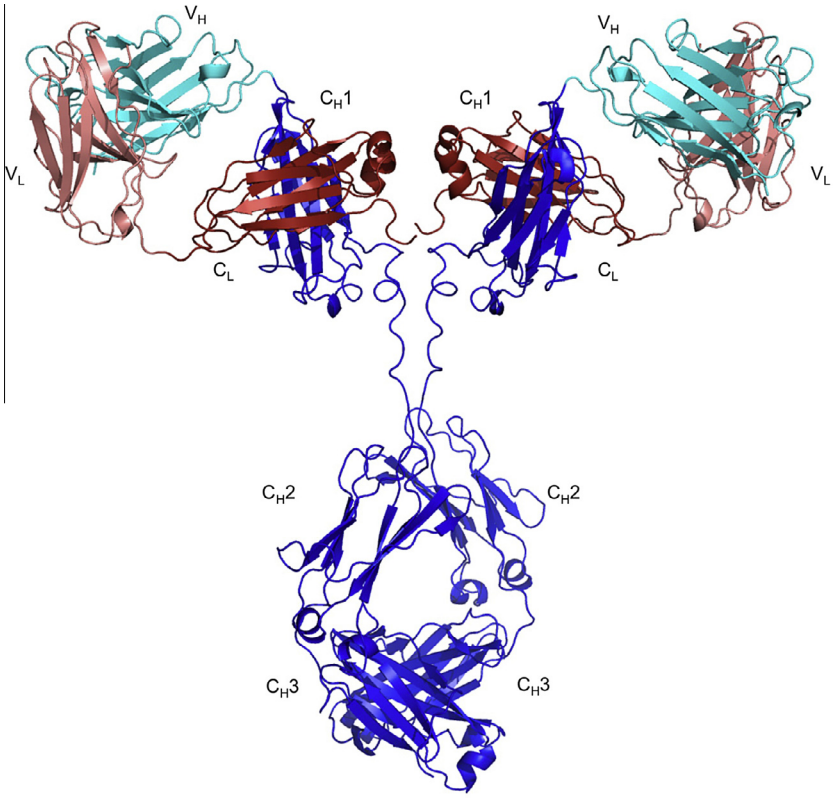

Fig. 1. Structure of the human IgG molecule. The molecule is formed by two heavy chains (consisting of V_H, C_H1, C_H2 and C_H3 domains) and two light chains (consisting of V_L and C_L). Figure was generated using PYMOL.

processes and conditions. These include recombinant expression, purification, concentration, viral inactivation, filtration, formulation, freeze/drying, transport and long-term storage. Throughout these steps the antibody molecule may encounter several stress factors that can dramatically increase its propensity to aggregate (including variations of temperature, pH, protein concentrations, ionic strength, exposure to air–water interfaces and mechanical stress). A further driver of protein aggregation has been a growing trend towards formulations that allow sub-cutaneous administration routes. This requires formulation at high protein concentrations (at around 100 mg/ml) in syringes for self-injection, which places increased demands on colloidal stability [15].

3. Stability of human antibody isotypes and constant domains

The four human IgG isotypes differ in their stabilities and biological functions, namely their potential to induce cellular killing through antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). While the human IgG1 isotype induces powerful ADCC and CDC responses, this is not the case for human IgG2, which is particularly suitable for applications where cellular killing is not required (such as neutralization of soluble ligands) [16]. Unlike the other human isotypes, IgG4 also has a naturally occurring potential to form a bispecific molecule [17] (it has been recently shown that this dual specificity can be grafted onto other isotypes [18]). Human IgG3 is not commonly used due to its longer hinge region, which renders it susceptible to proteolysis [19]. The vast majority of human antibody therapeutics currently in clinical practice and development are of the IgG1 isotype [16].

Conflicting evidence exists in respect to the colloidal stability of the various human IgG isotypes. A recent study of eleven different IgG1 and IgG2 antibodies concluded that, after high temperature storage, the IgG2 isotype is more prone to aggregation [20]. Similar findings were also obtained when subjecting these isotypes to high salt conditons [21] and from isotype switching studies [22]. The latter study confirmed that the human IgG1 isotype is less prone to aggregation when compared to IgG2 or IgG4. However, the authors also found that this isotype is more prone to fragmentation, especially at low pH, due to a non-enzymatic site in the upper hinge region. The hinge regions have also been implicated in the observed differences in aggregation propensity as the IgG2 hinge contains two additional cysteines in each heavy chain compared to IgG1, and is prone to the display of free cysteine residues [23,24]. Differences in the thermal stability of human constant domains have also been reported, with the C_H3 domains of human IgG1 and IgG2 displaying similar melting temperatures, much higher than what was observed for IgG4 [8]. In general, C_H3 domains exhibit more favorable biophysical properties than C_H2 domains [25]. This is reflected by melting temperatures of 8–10 °C higher than those observed for C_H2 domains [8,26].

Although human IgG1 therefore appears less aggregation prone than other isotypes, this is not universally the case. For example, a recent study found that the IgG1 isotype variant of an anti-LINGO-1 antibody exhibited a much higher propensity to aggregate than an IgG2 variant containing identical variable regions [27]. Studies such as these demonstrate that predicting the aggregation propensities of IgG molecules based on isotype can be difficult at the best of times. Moreover, evidence suggests that the aggregation propensity of human IgG molecules is greatly influenced by their variable domains [8,27,28]. This has the effect that antibodies with identical constant domains, but different variable domains, can vary widely in their stability profiles [5].