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Review

Principles and engineering of antibody folding and assembly☆

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ABSTRACT

Antibodies are uniquely suited to serve essential roles in the human immune defense as they combine several specific functions in one hetero-oligomeric protein. Their constant regions activate effector functions and their variable domains provide a stable framework that allows incorporation of highly diverse loop sequences. The combination of non-germline DNA recombination and mutation together with heavy and light chain assembly allows developing variable regions that specifically recognize essentially any antigen they may encounter. However, it also requires tailor-made mechanisms to guarantee that folding and association of antibodies is carefully controlled before the protein is secreted from a plasma cell. Accordingly, the generic immunoglobulin fold β-barrel structure of antibody domains has been fine-tuned during evolution to fit the different requirements. Work over the past decades has identified important aspects of the folding and assembly of antibody domains and chains revealing domain specific variations of a general scheme. The most striking is the folding of an intrinsically disordered antibody domain in the context of its partner domain as the basis for antibody assembly and its control on the molecular level in the cell. These insights have not only allowed a better understanding of the antibody folding process but also provide a wealth of opportunities for rational optimization of antibody molecules.

In this review, we summarize current concepts of antibody folding and assembly and discuss how they can be utilized to engineer antibodies with improved performance for different applications. This article is part of a Special Issue entitled: Recent advances in molecular engineering of antibody.

1. Introduction

The human immune defense depends on reliable and efficient discrimination of self from non-self. In all higher vertebrates, antibodies play a major role in this task and protect the organism against threats ranging from toxins to microbial pathogens [1]. Antibodies are complex glycoproteins that are secreted in large quantities by plasma cells [2,3]. They are made up of heavy chains (HCs) and light chains (LCs) with the simplest human antibodies being hetero-tetramers that comprise two HCs and two LCs each (Fig. 1). Five antibody classes exist in humans (IgA, G, D, E and M) that differ in their HCs (αHC, γHC, δHC, εHC and μHC) comprising either four (αHC, γHC, δHC) or five (εHC, μHC) immunoglobulin (Ig) domains. Only two classes of light chains (κLC and λLC) are shared between the different human antibody classes. Even though the various antibody classes differ in their biological functions and structural details, they have two important features in common that enable them to defend the organism against most infectious or toxic challenges it may encounter: variable regions that bind to antigens and constant regions that link the antibody and its bound antigen to the cellular immune defense (Fig. 1).

The constant region of antibodies incorporates multiple biological functions. It binds to Fc receptors inducing antibody-dependent cell-mediated cytotoxicity (ADCC) and activates the complement system [4]. The constant region furthermore has an important impact on the biological half-life of antibodies due to its binding to the neonatal Fc receptor that induces antibody recycling after cellular uptake into endosomes [5,6].

Variable regions of one HC and one LC each form a composite antigen binding surface (paratope) (Fig. 1). Antigen binding sites of the variable regions are generated by non-germline genetic rearrangements, non-templated base pair additions and hypermutation in a micro-evolutionary process [7,8] that gives rise to proteins that can bind essentially any target structure with high affinity and specificity. An array of techniques has been developed, from ribosome, phage and yeast display to genetically engineered mice with a human antibody repertoire to obtain antibodies with the desired specificities [9–11]. Recently, this possible repertoire has even been further extended by generating bispecific antibodies [6,12–14].

Together, the highly specific binding of antibodies and their link to the cellular immune response have rendered them the most widespread

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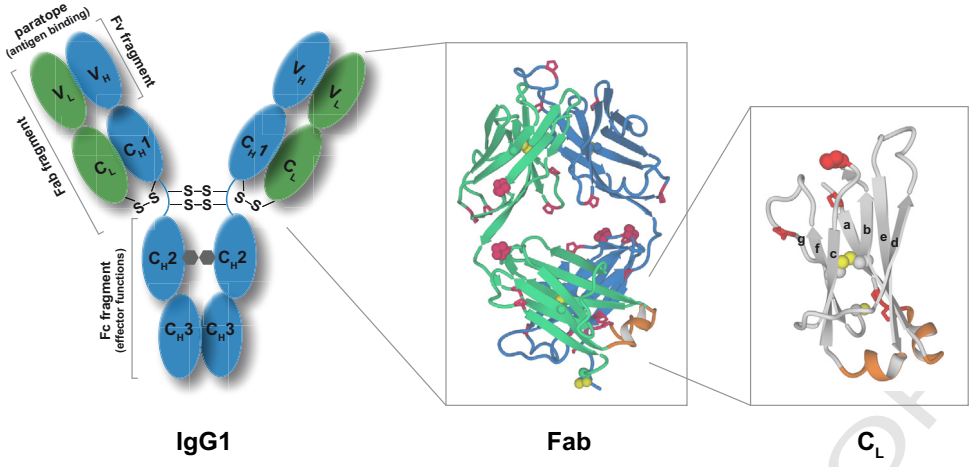

Fig. 1. Schematic and structural elements of the IgG1 antibody molecule and the Ig fold. Left panel: The IgG1 antibody consists of two heavy chains (blue) and two light chains (green) that together form two identical antigen binding sites (paratopes), which are part of the Fab fragment (fragment antigen binding). The smallest complete dimeric antigen binding entity is the Fv fragment (fragment variable). Effector functions are mediated via the Fc fragment (fragment crystallizable). The C_H2 domains are glycosylated (gray hexagons) and disulfide bridges (S – S) connect the two heavy chains as well as the heavy and light chains. Middle panel: The Fab fragment is connected by an interchain disulfide bond and each of the domains has one buried disulfide bond (yellow, CPK representation). Proline residues within the Fab fragment are highlighted in red. *Trans* prolines are shown in a stick representation, and *cis* prolines are shown in a CPK representation. The small helices in the C_L domain, which are found in all most antibody constant domains except for e.g. C_H1, are colored in orange. Right panel: As shown for the C_L domain, the constant domain Ig fold consists of seven strands (a–f), two of which (b and f) are connected by the buried disulfide bond. Prolines and small helices are depicted in the same manner as in the middle panel.

diagnostic and therapeutic biomolecules that can be used for a variety of applications from detecting and marking tumors in vivo, via treating autoimmune diseases to combating cancer cells [6]. Recent developments have given rise to antibodies that are able to pass the blood brain barrier, opening up a whole new field of diseases that could potentially be targeted with tailored antibodies [15,16]. Thus, a vital interest exists in optimizing antibody production, stability and half-life in the organism as well as in inhibiting side reactions like aggregation, which can cause serious side effects including immunogenicity [17–20]. As strategies to engineer specificity have been extensively reviewed elsewhere [9–11, 21,22], in the following, we will focus on recent insights into the determinants of in vitro and in vivo antibody folding and assembly pathways, with an emphasis on how these can be used to design better antibody therapeutics.

2. Engineering antibodies: stability and folding pathways

Each of the antibody domains shows the Ig fold, the most widespread protein topology in secretory pathway proteins, with variable domains belonging to the V-set and constant domains to the C-set [23,24]. Ig domains show a Greek key β-barrel topology generally composed of either seven (C-type) or nine (V-type) β-strands [23,24] (Fig. 1). A characteristic of this topology is that β-strands that are adjacent in space are not necessarily adjacent in sequence (Fig. 1). Most of the published studies on antibody optimization have focused on the antigen binding variable regions due to their immediate role in target recognition and the possibility to design smaller binding molecules without constant domains [20,25]. The opposite, however, applies when it comes to studies that have assessed Ig folding pathways in detail. These mostly focused on antibody constant domains or other members of the Ig superfamily [26–29].

Mutational approaches combined with kinetic analyses of a protein folding process allow the structural assessment of protein folding transition states (Phi value analysis) [30–33]. This approach has revealed that Ig domains initiate their folding by a nucleus composed of Ig strands b, c, e and f around which the Ig fold forms [34–36] (Fig. 1). However, antibody domains comprise several structural features that significantly influence this general mechanism with direct implications for protein engineering.

2.1. Disulfide bonds in antibody folding

A defining characteristic of most antibody domains is an intramolecular buried disulfide bridge perpendicular to the two sheets of the Ig fold [37] (Fig. 1). Early on, it has been recognized that this disulfide bond plays a crucial role in the stability of antibody domains in vitro [38–40] and complete antibodies in vivo [41]. Besides, the oligomeric structure of antibodies is stabilized by disulfide bonds connecting LCs and HCs as well as inter-HC disulfide bonds [42,43] (Fig. 1). In the case of the constant domain of antibody LCs (C_L domain, Fig. 1), the internal disulfide bridge accelerates folding and inhibits off-pathway misfolding reactions [44,45]. Of note, in antibody constant domains the internal disulfide bridge connects strands b and f (Fig. 1), which are part of the common Ig topology folding nucleus [34–36]. For the variable domains, the intramolecular disulfide bond, also connecting strands b and f, similarly reduces their tendency to aggregate [46]. Together this argues for an intimate link between folding of antibody domains and formation of their disulfide bond which was also found in vivo [47] where disulfide bonds in antibody domains and between antibody chains already form co-translationally [41,42]. Disulfide bond formation is aided by protein disulfide isomerases (PDIs) in vivo [48,49] and in vitro [50] (Fig. 2). The time window for the action of PDI can be extended in the presence of the Hsp70 chaperone BiP [51], a major ER-resident protein involved in the folding, assembly and quality control of antibodies in the cell [52,53] (Fig. 2), by inhibiting premature collapse of the polypeptide chains. Engineering additional disulfide bonds into the Ig fold or between Ig domains is one of the most widespread approaches to stabilize antibody domains. A significant stabilization can be achieved this way [54–58] as described in several recent reviews [59–62]. A particularly well studied example in this regard is Fv fragments, the most simple dimeric binding module derived from antibodies, which comprise only one V_L and one V_H domain (Fig. 1). Nowadays, Fv fragments are mainly used as single chain Fv fragments (scFv) where the C-terminus of the V_L and the N-terminus of the V_H domain are connected by a peptide linker [63,64]. Disulfide bonds have been shown to be particularly useful to stabilize scFv fragments either by engineering the antigen binding site [65], or by, more generally applicable, the framework regions to stabilize V_L–V_H heterodimers [66,67]. Indeed, dissociation of the variable domains is often an initial step preceding aggregation of scFv fragments under challenging conditions and thus disulfide bond engineering can