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Thermodynamic stability contributes to immunoglobulin specificity

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Antigen-binding specificity of immunoglobulins is important for their function in immune defense. However, immune repertoires contain a considerable fraction of immunoglobulins with promiscuous binding behavior, the physicochemical basis of which is not well understood. Evolution of immunoglobulin specificity occurs through iterative processes of mutation and selection, referred to as affinity maturation. Recent studies reveal that some somatic mutations could compromise the thermodynamic stability of the variable regions of immunoglobulins. By integrating this observation with the wealth of data on the evolution of novel enzyme activities, we propose that antibody specificity is linked to the thermodynamic stability of the antigen-binding regions, which provides a quantitative distinction between highly specific and promiscuous antibodies.

Specificity of immune receptors

Molecular recognition has a basic role in the function of the immune system. The immune system has the potential to discriminate between distinct molecular species, which is essential to evoke reactions to pathogen-associated molecules and to avoid potentially detrimental reactions to self-constituents [1,2].

The repertoire of antigen-binding receptors expressed on B lymphocytes and their soluble counterparts, the immunoglobulins (antibodies), is characterized by enormous sequence diversity. This allows the immune system to recognize a myriad targets, encompassed in a theoretically infinite antigenic space. Although extensive, the sequence diversity available at a given time within the repertoire of antigen receptors is less than the potential diversity of antigens. This apparent paradox is reconciled by the observation that many antigen-binding receptors and antibodies are promiscuous (also referred to as polyreactive); that is, they are able to bind multiple structurally unrelated antigens [3–5].

Thus, normal immune repertoires comprise immunoglobulins that vary in their potential to discriminate between structurally unrelated antigens. It was proposed that antibodies that recognize different antigens with similar values of the binding affinity (which can also be expressed as similar changes in the free energy) are promiscuous [6]; by contrast, highly specific antibodies are characterized by a marked difference in values of binding affinity to cognate and to unrelated antigens. The two categories of immunoglobulin in a healthy immune repertoire are not sharply distinguished but rather exist as a continuum of species with different degrees of promiscuity [1,3].

Specific and promiscuous immunoglobulins have different but complementary roles. Thus, promiscuous molecules might provide the adaptability required for the surveillance of the immune system and contribute as a first line of defense against pathogens, whereas highly specific immunoglobulins are important for the elimination of pathogens and for building memory within the system [2,5,7].

The molecular features that determine the specificity of antibodies have been the subject of many studies (recently reviewed in [8]). However, these studies failed to provide a quantitative trait that explains why some antibodies bind antigen promiscuously, whereas others have more stringent antigen specificity. The antigen-binding promiscuity of antibodies has been often associated with the mutation status of the genes encoding the variable regions of the immunoglobulins. Recent work suggests that somatic mutations influence the physicochemical properties of antibody variable regions. Based on these findings and on the wealth of investigations on promiscuity of other proteins, we propose here a hypothesis that connects the basic physical phenomenon of thermodynamic stability of protein folds with the functional property of immune specificity. We concentrate our discussion on the antigen-binding specificity of B cell receptors (BCR) and their soluble counterparts, antibodies. However, the same principles may also be valid for other proteins.

Antibody specificity and the presence of somatic mutations

During adaptive immune responses, antigen-stimulated B cells undergo an iterative microevolutionary process of mutation and selection. This process (termed 'affinity maturation') results in a gradual accumulation of mutations in the genes encoding the antigen-binding sites of immunoglobulin molecules, and the selection of cells expressing

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BCR that bind with an increased affinity to the target antigen [9,10].

Antigen-binding promiscuity has been frequently associated with the maturation status of the genes encoding variable regions of antibodies. Early works implied that antigen-binding promiscuity is most often found among molecules that have not accumulated somatic mutations in the genes encoding their variable regions (i.e., they have a germ-line configuration), or have accumulated only a limited number of mutations [11–13]. This is especially valid for natural antibodies that are produced by a subset of B cells defined as B-1 cells, which do not undergo the affinity maturation process [14]. Moreover, it was demonstrated that, as a result of the affinity maturation process of antibodies, the spectrum of recognized antigens is narrowed [15–18].

However, other studies suggest that the relation between the maturation status of antibodies and their antigen binding promiscuity is not necessarily linear. By cloning and expressing antibodies from human conventional B cells at different developmental stages, it was demonstrated that a high percentage of the B cells at early developmental stages express polyreactive antibodies (approximately 50%) [19]. However, owing to negative selection processes where all B cells that express self-reactive antibodies are removed in the bone marrow, only 5–6% of the naïve B cell repertoire that reaches the circulation express promiscuous germ-line antibodies [19]. This implies that the antigen-binding promiscuity of germ-line antibodies that enter the circulation is not as widespread a phenomenon as initially thought. Further studies revealed that memory immunoglobulin M (IgM)+ B cells, which accumulate spontaneous somatic mutations outside of the affinity maturation process, experience a further loss of promiscuity and their repertoires contain only approximately 1% promiscuous immunoglobulins [20]. Interestingly, the antigen-binding promiscuity 'reappears' at later stages of conventional B cell ontogeny. Thus, a high percentage of memory immunoglobulin G (IgG)+ B cells that are generated following the affinity maturation process express promiscuous antibodies (20%) [21]. Importantly, it was proven that the promiscuity in IgG+ B cells is acquired following accumulation of somatic mutations [21].

Furthermore, many of the human antibodies that exhibit high neutralization potency towards a broad spectrum of HIV-1 strains, designated as 'broadly neutralizing antibodies' (bNAbs), display antigen-binding promiscuity [22–25]. Indeed, it was demonstrated that >65% of HIV-1 envelope-specific antibodies, isolated from patients with broadly neutralizing HIV-1 serum activity, are promiscuous [23,26]. This frequency is higher than the 20% of promiscuous antibodies expressed by affinity-matured B cells from healthy individuals [21]. Interestingly, most bNAbs have a high number of somatic mutations in the genes encoding their variable regions (40–100 mutations) that considerably surpasses the number of somatic mutations usually introduced in affinity-matured antibodies in the course of 'conventional' immune responses (15–20 mutations) [24–27].

Thus, it becomes clear that the promiscuous antigen-binding behavior of antibodies may emerge at any stage of the affinity maturation process. This raises questions about the nature of a common denominator of promiscuity that is shared by antibodies with varying numbers of somatic mutations.

Antibody specificity and protein stability

The notion of the existence of one protein sequence that is able to assume a variety of 3D conformational states (folds), although counterintuitive to the well-established dogma of 'one sequence–one structure–one function', seems a more widespread phenomenon in nature than was initially thought [28–31].

Details of the promiscuous behavior of antibodies have been obtained by structural and biophysical analyses that collectively underline the role of conformational dynamics for antigen-binding promiscuity [16,32–35]. Thus, antibodies that have a higher structural pliability of their polypeptide chains can sample and accommodate a large number of alternative conformational states, each able to recognize a distinct antigenic structure [16,18,33,34,36].

However, a phenomenological consideration of the conformational flexibility of an antigen-binding site might not be sufficient to explain fully the promiscuous behavior of antibodies. Indeed, numerous studies have demonstrated that the variable regions of immunoglobulins in germ-line configurations have a high level of structural dynamics [15,18,34,35,37,38]. Yet, as stated above, few B cells that express germ-line immunoglobulins with promiscuous antigen-binding potential reach the periphery [19]. Rather, many antibodies acquire binding promiscuity after the affinity maturation process [21]. To explain this apparent contradiction in the literature, one should examine the consequences of enhanced flexibility and take into account parameters defining the origin of molecular flexibility and binding promiscuity, such as the thermodynamic stability of the protein fold.

Each protein has an intrinsic thermodynamic stability that is defined by the difference in free energy (Gibbs energy, ΔG) between its folded and unfolded states. Protein stability is mainly determined by the opposing effects of favorable changes in enthalpy, due to the transfer of hydrophobic residues from the aqueous medium to the nonpolar medium of the protein interior, versus unfavorable changes in entropy, caused by restriction of configurational freedom of the polypeptide chain [39,40]. The net changes in the free energy that determine protein stability are only marginal: in the range of 20–80 kJ mol^{-1} [40]. Many factors, including temperature, pH, solvent composition, and point changes in the protein sequence (i.e., mutations), can perturb protein stability [41,42].

Converging evidence suggests that proteins with higher conformational dynamics of the polypeptide chain have a higher propensity for promiscuous activities [43–46]. A recent study revealed that the proteins of RNA viruses that have high mutation frequencies are characterized by loose packing, high flexibility, and low thermodynamic stability [45]. These proteins express high tolerance to mutations and have binding promiscuity. However, functional promiscuity and tolerance to mutation might also be typical for ancestral proteins that are characterized by high stabilities [47].