Antigen–Antibody Binding

Introductory article

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Antibodies are a family of glycoproteins that bind specifically to foreign molecules (antigens). The binding between antibodies and antigens has high specificity and affinity resulting from various structural and energetic aspects.

Article Contents

Introduction

Antibodies (immunoglobulins) are produced by the immune system of vertebrates and are essential for the prevention and resolution of infection by foreign invaders such as viruses. Antibodies are a family of variable glycoproteins that bind specifically to foreign molecules (antigens). The most striking feature of antigen–antibody interactions is their high specificity and a nity. In this article, the structural and energetic aspects of antigen–antibody binding are described, focusing in particular on how an antibody specifically recognizes its cognate antigen and binds to it tightly.

A binding strength between an antigenic determinant in an antigen (epitope) and an antigen-binding site in an antibody (paratope) is termed a nity. Each antibody unit has at least two antigen-binding sites, and is therefore bivalent, or multivalent, to its antigen. The functional combining strength of an antibody with its antigen, which is related to both the a nity of the reaction and the valencies of the antibody, is termed avidity. The significance of avidity to antigen–antibody binding is also described.

Antigen–Antibody Binding

Antibody structure

The basic structure of an antibody (immunoglobulin) molecule comprises two identical light chains and two identical heavy chains linked together by disulfide bonds. There are five classes of immunoglobulins (IgG, IgA, IgM, IgD and IgE), which di er in amino acid sequence and number of domains in the constant regions of the heavy chains. There are two di erent isotypes of light chains (λ and κ). Immunoglobulin G (IgG) is the major type of immunoglobulin in normal serum and the most extensively investigated (**Figure 1**). The remarkable feature of the antibody molecule is revealed by comparison of amino acid sequences from various immunoglobulin molecules. This shows that immunoglobulins are composed of various copies of a folding unit of about 100 amino acids, each of which forms an independent similar structure called the immunoglobulin fold (**Figure 2**). The N-terminal domain of each polypeptide (heavy and light chains) is highly variable, while the remaining domains have constant sequences. The former domain is called the variable region (V region), while the latter is the constant region (C region). In addition, a comparison of V region sequences shows that variability is not uniformly distributed but concentrated into three areas called the hypervariable regions.

Investigations into the structures of various antigen–antibody complexes have demonstrated that the domain structure of the antibody molecule is a β barrel consisting of nine antiparallel β strands (V regions) and seven C regions, and that the hypervariable regions are clustered at the end of the variable domain arms (**Figure 3**). The antigen-combining site of antibodies is formed almost entirely by six polypeptide segments, three from light variable domains and three from heavy variable domains. These segments show variability in sequence as well as in number of residues, and it is this variability that provides the basis for the diversity found in the binding characteristics of the di erent antibodies. These six hypervariable segments are often referred to as the complementarity-determining regions or CDRs.

It was realized that a more constant sequence of residues outside of the CDRs was required to maintain the essential immunoglobulin fold that results in the CDRs being brought into three-dimensional proximity. These residues are referred to as the framework regions. The framework residues do not usually form bonds with the antigen. However, they are essential for producing the folding of the V domains and maintaining the integrity of the binding site. Thus, the antibody-binding sites are formed by six segments of variable structure (CDRs) supported by a sca olding of essentially invariant architecture (framework regions). This characteristic structure has led to several approaches to the artificial design of novel antibodies by grafting new CDRs onto existing antibodies (**Figure 3**).

Canonical structure of CDRs

The antigen-binding specificity of an antibody is defined by the physical and chemical properties of its CDR surface. These in turn are determined by the conformation of the

individual CDRs, by the relative disposition of the CDRs, and by the nature and disposition of the side-chains of the amino acids in the CDRs. The structure of antibodies shows that antibodies can recognize infinitely variable antigens by varying the amino acid sequences of the CDR loops (i.e. the surface structures of antigen-recognizing regions). While the structure of the CDR loops might vary randomly, there are certain preferred conformations. Such preferred conformations can be deduced from the lengths and sequences of these regions.

CDR loop conformations in the antigen–antibody complexes whose three-dimensional structures have been solved by X-ray crystallographic study have been extensively analysed. Examination of the sequences of variable domains of unknown structure has shown that many have CDR loops that are similar in size to those of one of the known structures and contain identical residues at the sites responsible for the observed conformation. For five of the six hypervariable regions of most immunoglobulins (the only exception is CDR of heavy chain 3), there seems to be only a small repertoire of main-chain conformations, most of which are known from the set of immunoglobulin structures so far determined. These observations have interesting implications for the molecular mechanism involved in the generation of antibody diversity, since the combination of limited numbers of CDR loop structure creates infinite specificity toward foreign molecules. Sequence variations within the hypervariable regions modulate the surface that these canonical structures present to the antigens, altering the specificity and a nity of the antibodies. Sequence variations within both the

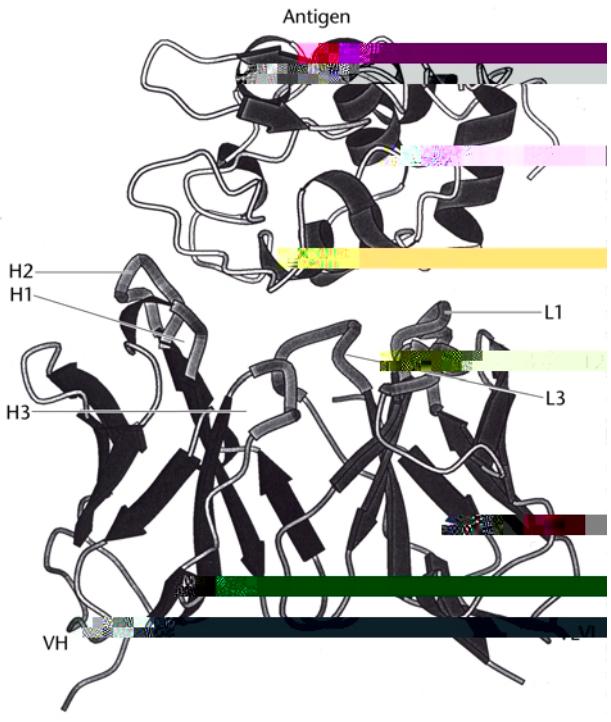

Figure 3 Structure of complementarity-determining regions (CDRs). Variable domains of a murine immunoglobulin G (composed of heavy chain and light chain) are shown (from HyHEL10 structure, Tsumoto and Kumagai, unpublished results). The hypervariable regions, CDRs comprising the antigen-binding site are shown by thick lines. These are located at the one edge of the β-barrel structure. Drawn using MOLSCRIPT.

framework and the hypervariable regions shift the canonical structures relative to each other by small but significant amounts.

Contrary to other biomolecular interactions, antibodies can recognize various foreign antigens (e.g. small molecules, DNA, soluble proteins, surface proteins on viruses) by varying the hypervariable regions. The six CDR loops from the two chains at the rim of the eight-strand barrel provide an ideal arrangement for generating antigen-binding sites of di erent shapes depending on the size and sequence of the loops. Di erent CDR structures form according to the antigen structure. These CDR structures can create flat, extended binding surfaces for protein antigens, a specific groove for a peptide, DNA and carbohydrate, or specific deep binding cavities for small molecules called haptens. A hapten is a simple chemical molecule that has the ability to bind antibody and can induce specific antibody production when it is attached to a carrier molecule such as albumin or Ficoll.

It has been pointed out that the distribution of amino acids in variable domains seems to be biased, and certain residues (Tyr, Trp and Asn) seem to have a propensity for being in the CDRs, and for participating in antigen recognition. It seems that the aromatic side-chains (Tyr, Trp) are more exposed to the solvent than in usual water-soluble proteins, and they are frequently found to be involved in the interaction with the ligand. This is explained by their large size (hydrophobic e ect), large polarization (van der Waals interactions), ability to form hydrogen bonds, and rigidity (less loss of conformational entropy upon complexation). Thus, the concentration of aromatic rings would give a certain 'stickiness' to the CDRs and give diverse specificity to antibodies. Specificity for a particular antigen would arise from the complementarity of the shapes of the interacting surfaces created by the proper positioning of the aromatic rings and the correct location of polar and/or charged groups.

Role of structural changes upon complex formation in antigen–antibody binding

On comparison of an antigen structure and the antibody with its complex, local conformational changes (often called induced fitting) of both molecules have often been observed, creating high specificity and a nity. It can be supposed that induced fitting of an antibody to its antigen is critical for high specificity and a nity. Induced fitting can be achieved (1) by small movements of side-chains, (2) by structural modifications such as deformation of CDR loops, or (3) by a change in the relative orientation of variable domains. All of these also seem to play a critical role in antigen–antibody interaction. In some antigen–antibody complexes, the significance of (3) has been suggested. As for (1) and (2), although the flexibility of the binding site leads to entropic loss upon complex formation, a greater interaction due to a more precise fitting must result in an overall increase in binding energy. Favourable energy change is in part or completely compensated for by unfavourable entropic loss (see below). Therefore, the binding characteristics must be analysed structurally by both antigen-free and complex form.

It is known that diversity in the germline antibody population is generated by the association of V, D and J gene segments with additional diversity occurring at the joining regions (**Figure 4**). In addition, somatic hypermutation, which alters the specific amino acid by specific mutations of the gene encoding V regions, provides further diversity and leads to increased a nity and specificity as the immune response proceeds, generating a nity-matured antibody. A comparison of the structures of germline antibodies and a nity-matured antibodies shows that the former display significant conformational changes upon complex formation, whereas the antigens bind to the mature antibodies by a lock-and-key mechanism. Thus, it can be speculated that germline antibodies may adopt

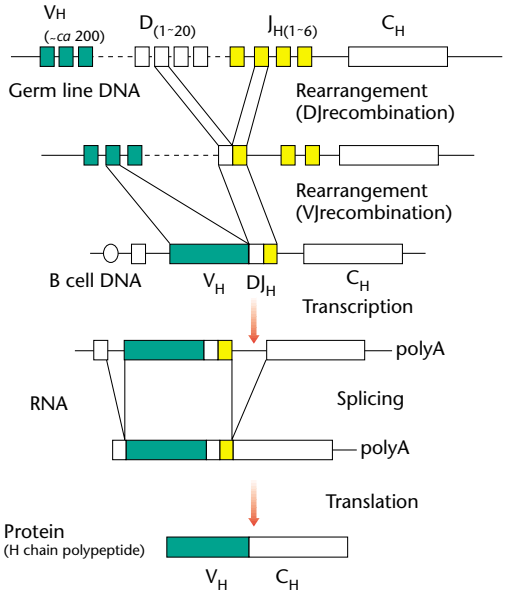

Figure 4 Molecular basis for antibody diversity. Only the case of heavy chain has been shown. In the human genome, one of the about 80 VH genes (in mouse, about 100) recombines with one of 30 D segments (in mouse, about ten), and one of six J segments (in mouse, four) producing a functional V-D-J gene in the B cell. The recombined DNA is transcribed, spliced and translated into a polypeptide chain. Half of the VH genes in human B cell seem to be pseudogenes.

multiple configurations upon antigen binding, and combined with somatic hypermutation, this could stabilize the configuration with optimal complementary to antigen. The structural plasticity of CDRs, especially in the case of germline antibodies, may adopt many di erent conformations, enabling them to accommodate many di erent antigenic structures, which might lead to polyreactivity (i.e. reactivity to various antigens). Thus, adequate stickiness and the plasticity of CDRs may create high specificity and a nity of antibodies toward target antigens.

Antigenic Epitope

Each antibody binds to a particular part of the antigen called the antigenic determinant (or epitope). The term epitope was first proposed by Jerne, to include surface configurations, haptenic groups, specific areas and so on. For protein antigens, it was later proposed that epitopes might be subdivided into sequential epitopes (involving a single continuous length of the polypeptide chain) and conformational epitopes, in which several discrete amino acid sequences, widely separated in the primary structure, come together on the surface when the polypeptide chain folds to form the native protein.

Various researchers have suggested that any macromolecule can be antigenic, and that all accessible areas of a protein can potentially be bound by antibodies. 'Discontinuous' seems to be a more accurate description of nonlinear epitopes since they are assembled from residues from di erent portions of the polypeptide chain. However, recent research has suggested that not all areas seem to contribute equally to the binding. Small sets of surface residues on antigens make a significant contribution to the interaction (called energetic epitope), while the rest of the antibody-binding regions seem to make additional contributions to the binding energy, although with some exceptions (antibody–idiotype antibody binding).

Limitation of antibody specificity – cross-reactivity

As mentioned above, the most striking feature of the antigen–antibody interaction is its high specificity and a nity. However, in some cases, antibodies can recognize other antigens. This is called cross-reactivity, i.e. the ability of a binding site to accommodate antigens other than the original immunogen. The structural basis of this cross-reactivity has been investigated in several antigen–antibody-binding systems. For example, antisteroid (progesterone)–antibody (DB3) cross-reacts with several steroids, and is seen, from X-ray crystallography, to bind to alternative binding subsites, i.e. di erent binding orientations of the steroids can be formed in these binding sites. Anti-hen egg lysozyme antibody (D11.15) cross-reacts with several avian lysozymes, in some cases with a higher a nity than that for the original immunogen, hen lysozyme (called heteroclitic binding). In this case residues di erent from the original immunogen were found to be located around the edge of the epitope. Thus, it was concluded that a stereochemically permissive environment for the variant antigen residues at the antibody–antigen interface was required for cross-reactivity.

Forces Involved in Antigen-binding

The binding of an antigen to an antibody takes place by the formation of multiple noncovalent bonds between the antigen and the amino acids of the binding site. The strength of a single antigen–antibody bond is the antibody a nity. It is produced by summation of the attractive and repulsive forces (van der Waals interactions, hydrogen bonds, salt bridges and hydrophobic force). The interaction of the antibody-combining site with antigen can be investigated thermodynamically and kinetically by using monovalent antibody fragments (fragments of variable regions or Fab).

In principle, the increase in van der Waals contacts and/or varied surfaces upon complexation correlates well with the a nity (the strength of a single antigen–antibody bond) for the antigen in the case of hapten–antibody binding. However, hydrogen bond formation and/or a saltbridge link (also called ion pairing, a noncovalent bond formed when a charged residue (e.g. aspartate) attracts its oppositely charged group (e.g. lysine)) seem to be required for specific recognition. In protein antigen–antibody interactions, the buried surface is almost the same (~ 750 Å^3), and creating shape complementarity between proteins is probably needed. Hydrogen bond formation is more frequently observed in comparison with other protein–protein interactions, and considered to be a critical specificity-determining factor. Saltbridge formation (e.g. aspartate–lysine) is not always seen, and seems not to be necessary. Although no gross conformational change upon binding has been observed, local induced fitting (see above) has been observed.

If a monovalent antibody fragment is used for analysis, the equilibrium of antigen–antibody binding is defined as:

$$+ \quad \overset{K}{\Longleftrightarrow} \quad [\ ]$$

where

$$K_a = [\text{Complex}] / [\text{Antibody}][\text{Antigen}].$$

Association and dissociation rate constants are defined as follows:

$$V_{ass} = k_{ass}\,[\text{Antibody}][\text{Antigen}] V_{diss} = k_{diss}\,[\text{Complex}] \quad [2]$$

where V_{ass} and V_{diss} represent the rates of association and dissociation, respectively, and k_{ass} and k_{diss} represent the rate constants of association and dissociation, respectively. At equilibrium V_{ass} is equal to V_{diss} and from eqns [1] and [2], the following equation is obtained:

$$K_a = k_{ass}/k_{diss} \quad [3]$$

The Gibbs' energy of formation (ΔG_0) of an antigen–antibody complex is given by:

$$\Delta G_0 = -RT \ln K_a \quad [4]$$

where R is the gas constant and T is temperature.

The free energy of complex formation represents a balance between enthalpic (ΔH_0) and entropic ($\Delta\ _0$) forces as defined by the equation:

$$\Delta G_0 = \Delta H_0 - T\Delta\ _0 \quad [5]$$

In general, antigens and antibodies in solution have to overcome large entropic barriers before they can form a tight binding. There is a loss of the entropy of free rotation and translation of the separate molecules as well as a loss of conformational entropy of mobile segments and of sidechains upon binding. On the other hand, entropy is gained when water molecules are displaced from the surfaces that become the new interface. This latter e ect is quite significant and, in the structures observed by X-ray analysis, it appears that water molecules are almost totally excluded from the interface by the close contact between antibodies and antigens. Enthalpic contributions arise from van der Waals interactions and hydrogen bond formation.

It is believed that the driving force in antigen–antibody binding originates from an increase in the entropy of solvent molecules displaced from the interface upon complexation (i.e. it is entropy-driven). On the other hand, hydrogen bond formation and van der Waals interactions make only a little contribution to the overall binding energy and act mainly to determine the specificity to the interaction. However, thermodynamic analyses have suggested that a considerable number of antigen–antibody interactions are enthalpy-driven, i.e. they make favourable enthalpy changes with some opposition from the negative entropy contribution to association.

As mentioned above, it has been suggested from crystal structures of antigen–antibody complexes that shape complementarity of binding surfaces (in the case of protein antigens) or close contact with small antigens (hapten, peptide and others) are important. In particular, almost all solvent molecules have been observed to be excluded from the interfaces, and therefore hydrophobic interactions are supposed to make a significant contribution to the interaction. However, a recent high-resolution crystallographic study shows that several water molecules remain in the interface and make hydrogen bonds with both antigen and antibody. The water molecule complements the imperfect structural complementarity between antigen and antibody and makes a significant contribution to the binding (about 1–2 kcal mol^{-1}). In addition to the direct antigen–antibody hydrogen bonds, solvent-mediated hydrogen bond formation should drive the interaction.

The structural basis of antigen–antibody binding is fundamentally important for clarifying the binding mechanism. However, for further discussion, a structural study using X-ray crystallographic study or nuclear magnetic resonance (NMR) should be combined with an energetic study using thermodynamics and kinetics. Recent advances in genetic engineering have enabled antibody fragments to be obtained more easily, and the mutants can be constructed more conveniently. Some antigen–antibody binding systems have been investigated using mutants, and the role of contact residues in the binding has been discussed. Thus, biological specificity and a nity often depend on very subtle structural parameters, and extensive research is in progress.

Kinetic analyses on several antigen–antibody bonds have been performed to investigate the mechanism of creating high specificity. Although K_a is extremely high ($\sim 10^{15}$ L mol^{-1}), in some protein–ligand interactions (avidin–i'3q–jN(qAHqdepejHWqij = (qrjHjHflV'qnjHWqejRoeiW

[3], the ceiling originates from the limits for association and dissociation rate constants. The maximum association rate constant for the binding of a monomeric protein antigen by its antibody is approximately 10^5–10^6, a value controlled by the di usion coe cients of the reactant molecules and verified experimentally. In contrast, no limitation for dissociation rate constant seems to exist, and a nity changes appear mostly as variations of the dissociation rate constant. Nevertheless, the k_{diss} of the naturally prepared antibody molecule is fixed at 10^{-3}–10^{-4}, and it is considered that dissociation rates which are too slow would not be selected in the immune system. Thus, the a nity ceiling of an antibody for any antigen is around 10^{10} L mol^{-1}.

Affinity and Avidity

The strength of a single antigen–antibody bond is termed the antibody a nity. It is produced by summation of the attractive and repulsive forces mentioned above. However, since each monoclonal antibody produced by hybridoma technology has two antigen-binding sites and antibodies obtained from serum contain polyclonal antibodies which can bind multiple antigenic determinants, antibodies are potentially multivalent in their reaction with antigen. When an antigen carrying multiple copies of the antigenic determinant (macromolecules or microorganisms) combines with a multivalent antibody, the binding strength is greatly increased because all of the antigen–antibody bonds must be broken simultaneously before the antigen and antibody can dissociate. Thus, total binding energy between a multivalent antigen and more than one of the antigen-binding sites of an antibody is greater than the summation of the a nity of each binding site for an antigen.

The strength with which a multivalent antibody binds a multivalent antigen is termed avidity, to di erentiate it from the a nity of the bond between a single antigenic determinant and an individual combining site. The avidity of an antibody for its antigen is determined by the sum of all of the individual interactions taking place between individual antigen-binding sites of antibodies and determinants on the antigens. The avidity of an antibody for its antigen strongly depends on the a nities of the individual combining sites for the determinants on the antigens. It is greater than the summation of these a nities if both antigen-binding sites of an antibody can combine with the antigen. The e ective range of antibody valence is from 2 (IgG) up to 10 (IgM), and the advantage of multivalence to the functional a nity (as opposed to the a nity of monovalent interactions, termed intrinsic a nity) is estimated to be 10^{3-7}. Thus, even when each antigen-binding site has only a low a nity (e.g. IgM produced early in immune responses), antibodies can function e ectively in the immune system.

Biological Significance of Antibody Affinity and Multivalency

An antibody with high a nity for its antigen can function most e ectively in the immune system (e.g. in biological reactions such as haemagglutination, virus neutralization, enzyme inactivation, haemolysis, immune selimnation,NSFSqbfjP = aith digh a oor vtarRHFentNHF‘Vfl’antigen ados notNHF3FX FWV = (9qihejNFWSVflqsowantrinsicjNflFWVSqannityjNHFWVF mf hbctiercaNWW’V’qarjNWSHVHqiirusje. nhus, hhe aost oe cin eo ainceacssNWWSVflqthejNSWWHainding an

Padlan EA (1990) On the nature of antibody combining sites: unusual structural features that may confer on these sites an enhanced capacity for binding ligands. *P oteins: t uctu e, Function, and Genetics* **7**: 112–124.

Padlan EA (1994) Anatomy of the antibody molecule. *Molecula Immunolo y* **31**: 169–217.

Roitt I, Brosto J and Male D (1996) *Immunolo y*, 3rd edn. London: Mosby Year Book.

Webster DM, Henry AH and Rees AR (1994) Antibody–antigen interactions. *Cu ent Opinion in t uctu al Biolo y* **4**: 123–129.

Wedmayer GJ, Patten PA, Wang LH, Schultz PG and Stevens RC (1997) Structural insights into the evolution of an antibody combining site. *cience* **27** : 1665–1669.