REVIEW OF ANTIGEN AND ANTIBODY INTERACTION

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Abstract

The synthesis of an antibody in response to the administration of an antigen is an adaptive phenomenon capable of exacting yet wide-ranging specificity. Under appropriate conditions it is responsive to, and specific for, an extraordinary variety of substances: proteins, carbohydrates, lipids, simple organic molecules and even, on the basis of recent observations, nucleic acids. Antigen-antibody interaction, or antigen-antibody reaction, is a specific chemical interaction between antibodies produced by B cells of the white blood cells and antigens during immune reaction. It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. The immune complex is then transported to cellular systems where it can be destroyed or deactivated.

Keywords: Fragment, Antigen-Binding, Hydrophobic Interactions

Introduction

There are several types of antibodies and antigens, and each antibody is capable of binding only to a specific antigen. The specificity of the binding is due to specific chemical constitution of each antibody. The antigenic determinant or epitope is recognized by the paratope of the antibody, situated at the variable region of the polypeptide chain. The variable region in turn has hyper-variable regions which are unique amino acid sequences in each antibody. Antigens are bound to antibodies through weak and non-covalent interactions such as electrostatic interactions, hydrogen bonds, Van der Waals forces, and hydrophobic interactions. Immunity developed as an individual is exposed to antigens is called adaptive or acquired immunity, in contrast to immunity developed at birth, which is innate immunity. Acquired immunity depends upon the interaction between antigens and a group of proteins called antibodies produced by B cells of the blood. There are many antibodies and each is specific for a particular type of antigen. Thus immune response in acquired immunity is due to the precise binding of antigens to antibody. Only very small area of the antigens and antibody molecules actually interact through complimentary binding sites, called epitopes in antigens and paratopes in antibody.

Literature review

Thole et al. suggested that antigen 85 complex, comprising the 85A, 85B, and 85C proteins, represents a dominant group of secreted 30-kDa proteins from M. tuberculosis, M. bovis, and other mycobacteria, and the 30-kDa protein cognates of M. bovis (P32) and M. tuberculosis are highly homologous. Wiker et al. demonstrated that the three components of the Ag 85 complex constitute a major portion of the secreted proteins in mycobacterial culture filtrates and exhibit mycolyltransferase activity. Drowart et al. detected distinct components probably corresponding to antigens 85A, 85B, and 85C in five of eight other mycobacterial species tested. Pal et al. stated that when the same bacteria are heat killed and injected alone or with adjuvant, only marginal protection or no protective response against a virulent challenge is detected. A role for actively secreted antigens in protective immunity has been proposed on this basis, and some articles have reported a limited degree of protection after immunization with secreted antigens. Harboe et al. stated that 38-kDa protein is a major constituent of M. tuberculosis culture fluid. It is also present in M. bovis BCG culture fluid, but in far lower concentrations. The M. tuberculosis H37Rv culture medium filtrate was purified biochemically by Romain et al. (1993). Heron et al. (1993) showed that among M. tuberculosis antigens studied, the 30/32 KDa antigen 85 (Ag85) complex has been the focus of intense research over the past several years and comprises three closely related proteins, 85A (32 KDa), 85B (30 KDa), and 85C (32.5 KDa) that possess enzymatic mycolyl-transferase activity.

Studies of memory immunity to tuberculosis conducted by Andersen et al. (1993) have demonstrated the specificity of immune, gamma interferon (IFN-g)-producing T lymphocytes in mice to be directed against two fractions of secreted proteins ranging in molecular mass from 3 to 9 kDa and from 25 to 31 kDa. Leao et al. described the immunological and functional characterization of proteins of the Mycobacterium tuberculosis antigen 85 complex using synthetic peptides.

Antibody structure

In an antibody, the Fab (fragment, antigen-binding) region is formed from the amino-terminal end of both the light and heavy chains of the immunoglobulin polypeptide. This region, called the variable (V) domain, is composed of amino acid sequences that define each type of antibody and their binding affinity to an antigen.

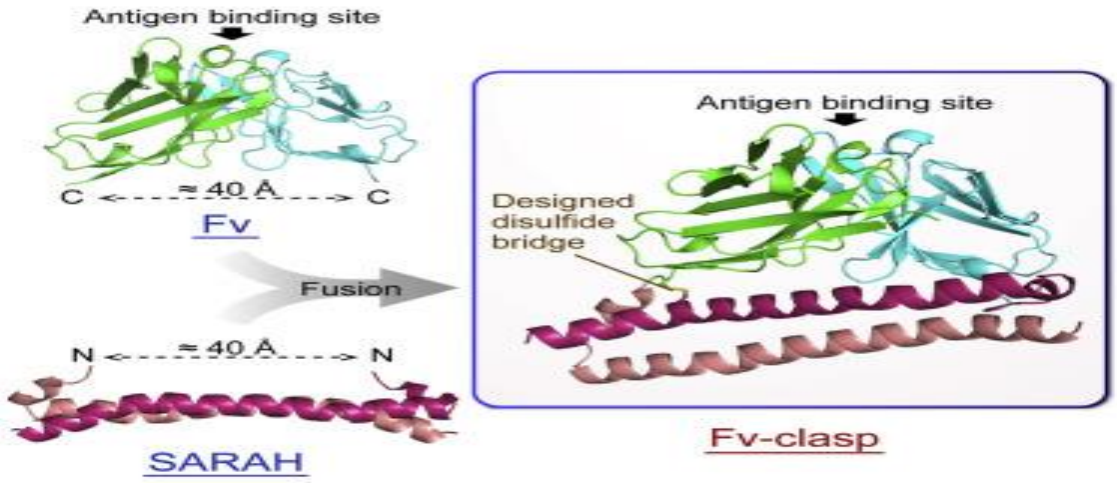

Figure 1. Structure of Antibody

The combined sequence of variable light chain (V_L) and variable heavy chain (V_H) creates three hypervariable regions (HV1, HV2, and HV3). In V_L these are roughly from residues 28 to 35, from 49 to 59, and from 92 to 103, respectively. HV3 is the most variable part. Thus these regions are the paratope, the binding site of an antigen. The rest of the V region between the hyper variable regions are called framework regions. Each V domain has four framework domains, namely FR1, FR2, FR3, and FR4.

Interactions between antigen and antibody

Interaction between antigen and antibody is a bimolecular association and it does not lead to an irreversible chemical alteration in either the antibody or the antigen. The association between an antigen and antibody involves various non-covalent interactions between the antigenic determinant (epitope) of the antigen and the variable-region (V_H/V_L) domain of the antibody molecule. The specific association of antigens and antibodies is dependent on hydrogen bonds, hydrophobic interactions, electrostatic forces, and van der Walls interactions, which are all weak and non-covalent in nature. So a large number of such weak interactions are required to form a strong antigen-antibody (Ag-Ab) interaction. These interactions can only take place if the antigen and antibody molecules are close enough for some of the individual atoms to fit into complementary recesses. The complementary regions of an antibody are its two antigen binding sites. Like antibodies, antigens can be multivalent, either through multiple copies of the same epitope, or through the presence of multiple epitopes that are recognized by multiple antibodies. Interactions involving multivalency can produce more stabilized complexes.

Properties of antigen-antibody

A strong antigen-antibody interaction depends on a very close fit between antigen and antibody. The combined strength of the non-covalent interactions between a single antigen-binding site on an antibody and a single epitope is the affinity of the antibody for that epitope. Affinity is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody. There exists a weak association between low-affinity antibodies and antigen, which dissociates easily whereas high-affinity antibodies bind antigen more tightly and remain bound longer.

The binding of an antibody (Ab) to its antigen (Ag) is reversible, so the binding reaction can be expressed as:

$$Ag + Ab \leftrightarrow Ag\text{-}Ab$$

Where k_1 is the forward rate constant and k_{-1} is the reverse rate constant. The ratio k_1/k_{-1} is the association constant K_a, a measure of affinity. K_a is the strength of the interaction and is expressed as

$$K_a = [Ag\text{-}Ab]/ [Ab] [Ag]$$

In this equation, [Ag-Ab] is the molar concentration of the antibody-antigen complex, and [Ab] and [Ag] are the molar concentrations of the antibody and antigen, respectively. Affinity constants can vary widely between different antibodies and antigens, and are affected by pH, temperature, and solvent.

The dissociation of the antigen-antibody complex can be expressed as:

$$Ag\text{-}Ab \leftrightarrow Ab + Ag$$

$$K_d = [Ab] [Ag]/ [Ab\text{-}Ag]$$

The affinity constants described above apply to single site interactions. However, all naturally occurring antibodies are multivalent and their functional affinity is dependent not only on their intrinsic affinity for antigen but also on the number of binding sites (2 for IgD,G and E and 10 for IgM). The association constant K_a, for binding of a univalent ligand to a multivalent antibody may be expressed as:

$$K_a = [Ab\text{-}Ag]/ [Ab] [Ag] = r/c(n-r)$$

where, at equilibrium, c is the concentration of free ligand, r represents the ratio of the concentration of bound ligand to total antibody concentration and n is the maximum number of binding sites per antibody molecule (the antibody valence). This expression can be rearranged to give the Scatchard equation:

$$r/c = K_a n - K_a r$$

A set of values of r and c can be obtained from a series of experiments in which the concentration of antibody is kept constant with a varying concentration of ligand and from these a plot (Scatchard plot) can be constructed in which r/c is plotted against r. From a Scatchard plot. Both the equilibrium constant (K_a) and

the number of binding sites per antibody molecule (n) or its valency can be obtained. If all antibodies have the same affinity, then a Scatchard plot gives a straight line with a slope of $-K_a$ and if the antibody mixture has a range of affinities (polyclonal antibodies), a scatchard plot gives a curved line whose slope is constantly changing.

Conclusion

Although the antibody/antigen interaction is of great practical and theoretical relevance, its direct study by force measurement is rendered more difficult by several factors. Both antibodies and antigens usually have complicated tertiary structures and need firm attachment to the force measuring device.

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