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MODERN BASICS OF IMMUNOCHEMICAL REACTIONS

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Abstract
Immunochemical methods of analysis, based on specific binding of the determined compound by appropriate antibodies, have widely entered into analytical practice and are used in various fields of medicine, agriculture, microbiological and food industries, and for environmental protection purposes. The indication of the formed antigen-antibody complex can be realised by introducing a label into one of the initial components of the reaction system, which can be easily detected by an appropriate highly sensitive physico-chemical method.

Keywords: Isotopic, enzyme, fluorescent, paramagnetic, fluorescent.

Introduction

Isotopic, enzyme, fluorescent, paramagnetic, fluorescent, etc. tags have turned out to be very convenient for this purpose. Their use made it possible to increase the sensitivity of classical immunochemical methods of analysis by millions of times and to reduce the analysis time to a few minutes. Genetically foreign substances, entering the organism of higher animals and humans, are able to cause in them a number of specific processes aimed at their removal from the body. The body system performing this function is called the immune system, and the processes themselves are called immunological [4,5,7,8].

The formation of specific blood proteins - antibodies (immunoglobulins) - is one of the most important of these processes. Substances capable of causing specific immunological reactions in

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the body are called antigens. The ability of antigens to elicit an immune response is called immunogenicity, and the ability to form complexes with antibodies is called antigenicity. Antigens include proteins, polysaccharides, nucleic acids both in purified form and as components of various biological structures (cells, tissues, viruses, etc.). On the surface of a complex antigen molecule, functional groups or residues that determine antigenic specificity, called antigenic determinants or epitopes, can be identified. The number of epitopes on the surface of a complex molecule determines the valence of the antigen. The concept of antigenic determinant includes the sequence of chemical functional groups forming it and their spatial arrangement. In protein molecules, the antigenic determinant is formed by a set of amino acid residues. Antigenic determinants of proteins are of two types - sequential, i.e. representing the sequence of amino acid residues in the polypeptide chain, and conformational, formed by amino acid residues from different parts of the protein globule. In many cases, a single amino acid change in the structure of an antigenic determinant or a change in the conformation of a protein globule is sufficient to alter the antigenic specificity of a macromolecule. If two antigens have only partially identical antigenic determinants, they are called cross-reacting antigens. Low molecular weight substances that are not capable of inducing antibody formation but acquire immunogenic properties after conjugation with high molecular weight carriers, e.g. bovine serum albumin, are called haptens. Haptens include a wide range of natural compounds: peptide and steroid hormones, various drugs, antibiotics, vitamins, oligosaccharides, etc. The biological function of antibodies is to protect the organism from the penetration of foreign substances through the formation of strong specific immune complexes with the corresponding antigens and their subsequent removal from the organism. The ability of antibodies to form highly specific strong immune complexes with various substances and the possibility of obtaining antibodies in necessary quantities are the basis of immunochemical methods of analysis [1,2,3].

In the body, antibodies are produced by specific blood cells - B lymphocytes, each of which has up to 100,000 receptors of equal specificity on its surface, capable of recognizing any foreign antigen. An antigen, meeting in the bloodstream with a receptor complementary to it, leads to the selection of the corresponding B-lymphocyte, which then, transforming into a plasma cell and dividing repeatedly, forms a clone of cells. Each clone of plasma cells secretes antibodies that are homogeneous in structure. However, since the antigen activates a large number of B-lymphocyte types in the blood at once, which contain receptors with different degrees of specificity to the original antigen, this immune response and antibodies are called polyclonal. The serum of an animal containing antibodies specific to a given antigen is called antiserum, and it is usually indicated against which antigen and by which animal it is produced (e.g. rabbit antiserum against human erythrocytes). It is fundamentally important that polyclonal antibodies even against a single antigenic determinant are heterogeneous both in the structure of the active centre and in their physicochemical properties. If the antigen is polyvalent, for example, a protein, antibodies directed against each individual antigenic determinant are formed in the serum, which further complicates the composition of antibodies.

In the mid-70s, a fundamentally new way of antibody production was developed based on the fusion (hybridization) of lymphocytes of an immunized animal with myeloma cells to form new cells - hybrids. The peculiarity of such cells is their ability to multiply and produce antibodies

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under artificial conditions outside the body. With the help of special cloning methods it is possible to isolate one hybrid cell, which, multiplying, will secrete in unlimited quantities antibodies of only one kind - monoclonal antibodies, which are homogeneous both in specificity and in physicochemical properties. Immunoglobulins by their chemical structure belong to a large class of native compounds - glycoproteins, i.e. proteins containing oligosaccharides in their structure. Despite the great diversity of antibodies and their heterogeneity, all of them have some common structural elements that ensure the fulfilment of their main functions. According to their antigenic, effector properties and structural features, immunoglobulins are divided into five main classes: IgA, IgD, IgE, IgG and IgM. The common structural unit of all immunoglobulins is a complex of four polypeptide chains - two identical light chains with a molecular mass of 23 kD each and heavy chains with a molecular mass of 53000 each [5,6].

Each of the light chains is firmly connected to the NH2-terminal portions of the heavy chains due to the presence of interchain disulfide bonds and many weak hydrophobic, electrostatic and other inter-atomic interactions. Similar bonds exist between the free parts of the heavy chains. In general, the structure of such a complex resembles the Latin letter Y (or T) and is characteristic of immunoglobulins of classes IgG, IgD, and IgE. Upon the action of the proteolytic enzyme papain, the IgG molecule breaks down into three fragments, two of which are identical and retain the ability to bind antigens (the so-called Fab-fragments) and the third, capable of crystallisation (Fc-fragment), which is responsible for the effector function of antibodies. Another proteolytic enzyme, pepsin, breaks the peptide bond closer to the COOH end of the chain from the S-S bond between the H chains in the Fc fragment. This results in the formation of the so-called pFc'-fragment, representing the heavy chain residues and two Fab fragments connected by disulfide bonds, denoted as the F(ab')2-fragment. The antigen-binding centre is located in the NH2-terminal parts of the H- and L-chains [3,4].

Thus, each IgG molecule as well as F(ab')2-fragments contain two identical anti-gene-binding centres, while the Fab-fragment contains one. Antibody molecules have a large number of S-S bonds, which can be divided into 3 categories - interchain, intrachain and bonds between H-chains of separate four-chain complexes, which cause the formation of polymeric molecules - IgM and IgA. The structure of immunoglobulins of different classes is determined by the number and arrangement of S-S bonds in the molecules and the number of four-chain elements. IgM is present in serum as a pentamer of four-chain complexes linked by S-S bonds between the H-chains. Some serum IgA is also present as dimeric and tetrameric forms. Immunoglobulin light chains are of only two types, λ or χ, and are common to all five classes, while heavy chains have structural, immunological and chemical features specific to each immunoglobulin class [1,2].

When the amino acid sequence was examined, it was found that all light and heavy chains share one fundamental structural feature: they consist of two parts, a variable (V) and a constant (C). The constant or constant part of the light chains (CL) comprises 107 amino acid residues of the COOH-terminus, the constant part of the heavy chain is approximately three times (or four times in the case of IgM and IgA) longer than the variable part. The remaining sequences of amino acid residues in the NH2-terminal half of the light and heavy chains form the so-called variable regions (VC and VH). In each of the light chains of antibody molecules there are two intrachain disulfide bonds, the number of such bonds in the heavy chains is different. Each of the intrachain disulfide

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bridges forms a loop of 55-70 amino acid residues. According to X-ray structure analysis, peptide chain sections near the loop form a globular structure with about 110 amino acid residues. Such globules in the structure of antibody molecules are called domains [9,11].

The NH2-terminal domain of the heavy chain is denoted as VH, and the three subsequent domains in the constant region of the heavy chain as CH1, CH2 and CH3, and for the light chain as VL and CL, respectively. Antigen binding occurs in the solvent-accessible gap of the active centre formed by variable domains in the NH2-terminal part of the light and heavy chains. Fab and F(ab')2-fragments of immunoglobulins have the ability to bind antigens with the same efficiency as native antibody molecules. The basic principle of organisation of antigen-binding centres of immunoglobulins is the polycentric structure. Small antigenic determinants bind in a limited area of the active centre complementary to the given determinant. Large determinants can occupy almost the entire binding region [10,12].

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