Volume 2, Issue 10, October 2024 ISSN (E): 2938-3765

PHYSICOCHEMICAL REGULARITIES OF ANTIGEN-ANTIBODY INTERACTION

Umarova Tamila Abdufattoevna
Assistant of the Department of Clinical Laboratory
Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD

Kudratova Zebo Erkinovna
PhD, Associate Professor of the Department of Clinical Laboratory
Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD

Norxujayeva Adolat
Cadet (Laboratory Doctor) of the Department of Clinical Laboratory
Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD

Toshpulatova Madina
Cadet (Laboratory Doctor) of the Department of Clinical Laboratory
Diagnostics with a Course of Clinical and Laboratory Diagnostics PGD
Samarkand State Medical University Samarkand, Uzbekistan

Abstract
Antibodies formed in response to the introduction of antigens into the body interact specifically with these antigens. The primary interaction is based on the general principles of any bimolecular reaction. Since in this case the product of the reaction is an antigen-antibody complex, the immune reaction is reversible and is described by the same kinetic and thermodynamic parameters as any complexation process.

Keywords: antibodies, antigens, affinity, reaction, specific.

Introduction

The degree of correspondence between the antigenic determinant and the antigen-binding region of the active centre of the antibody (immunological specificity) is determined by chemical and spatial complementarity, which is due, on the one hand, to the interaction of the electron clouds of the reacting chemical groups and, on the other hand, to steric repulsion forces. On the quantitative side, the specificity of antigen-antibody interaction is characterised by the antibody affinity or the equilibrium constant of immunocomplex formation (Ka, dimension l/mol) or its decay (Kd = 1/Ka, dimension mol/l). Ka =k+1/ k-1 The usual range of antibody affinity (Ka) is 105 - 1011 M-1. The maximum values of binding constants are characteristic of antigens with pronounced hydrophobic properties or interacting with the active centre of the antibody by a sufficiently large region of the molecule. Since the antibody molecule has two or more antigen-binding centres, and in addition

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

Licensed under a Creative Commons Attribution 4.0 International License.

is able to interact with several antigenic determinants of the antigen molecule, the actual process of interaction of a polyvalent antibody with a polyvalent antigen is more complex and is characterised by functional affinity or avidity. From the quantitative point of view, bivalent interactions are almost three orders of magnitude stronger than monovalent ones. The difficulty in determining the affinity (or antibody binding constant) is due to the following reasons: the heterogeneity of antibodies in terms of physicochemical properties, including affinity for the antigen, the difficulty in determining the total amount of specific antibodies, and the possibility of complex formation in the case of polyvalent antigens [1,2].

However, for practical purposes, particularly for use in enzyme immunoassays, it is sufficient to know the effective values characterising the total properties of the antibodies used. For monoclonal antibodies, the determined values of the affinity constants are true values. All methods for the determination of free and bound antigen concentrations can be divided into two large groups. The former includes methods in which the separation of free and bound antigen is achieved by selective precipitation, affinity binding (immobilisation) or gel filtration. For low molecular weight antigens (haptens), equilibrium dialysis is used. The second group includes methods based on changes in the physicochemical properties of antigens (or antigen-bound tags) when complexed with antibodies: quenching or enhancing fluorescence, changing the degree of fluorescence, inhibiting enzymatic activity. For the quantitative method of calculating the complexation constants of antigen-antibody reactions, the most common methods are those based on measuring the equilibrium concentrations of the complex at a constant concentration of one of the reagents and varying the concentration of the second reagent [5,6,7]. In the Scatchard coordinates [AgAt]/[Ag] from [AgAt] (or B/F from B, B - bound, F -free) we obtain a straight line, the tangent of the slope of which is equal to the value of -Ka, and the segment cut off on the abscissa axis is the constant concentration of one of the reagents.The formation of antigen-antibody complex is a reversible process, i.e. the binding constant (affinity) of this complex is determined by the ratio of the association rate constant k1 to the dissociation rate constant k-1. The values of the association reaction rate constant for most antigens are large and approach the diffusion-controlled limit up to (108 M-1c-1). In the case of protein antigens, their values are approximately two orders of magnitude smaller and range from 105 to 5,106 M-1c-1. The observed differences in the affinity of antibodies are mainly due to differences in the values of the dissociation rate constant (10-3 - 10-7 c-1). For experimental determination of the association rate constant, one of the following approaches can be used: study of the initial reaction rates at known initial concentrations of each reagent, study of the dependence of the product (complex) formation rate at excess of one of the reagents and varying the concentration of the second reagent [7,8,9].

Determination of the dissociation rate constant of the complex is carried out by direct measurement of the dissociation rate of the complex under conditions of its irreversibility. For this purpose, one of the following approaches is used. 1. After the establishment of equilibrium in the system, dilution with a large excess of buffer is carried out. Under these conditions (Vdiss >>Vass), the dissociation of the complex will be described by an exponential curve, the straightening of which allows us to determine the numerical value of k-1. 2. Substances capable of rapidly and completely binding or removing the free ligand are introduced into the system. If the rate of removal of the free ligand is substantially greater than the rate of disassociation of the complex, the observed rate

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

Licensed under a Creative Commons Attribution 4.0 International License.

of decomposition of the complex is described by a first order reaction and is characterised by the constant k-1. After equilibrium is established in the antibody-labelled antigen system, an excess of free unlabelled antigen is added. Under these conditions, the process of changing the concentration of the labelled antigen-antibody complex is described by first-order kinetics, the rate constant of which corresponds to k-1 [10,11,12].

Reagents:

The immunoglobulins used in such test systems, the so-called conjugate can be derived from anti-species antibodies (e.g. rabbit antibodies against human immunoglobulin antibodies (e.g. rabbit antibodies against human immunoglobulins) or antibodies directed against human immunoglobulins antibodies directed against human immunoglobulins of a specific class (M, G, A). Depending on which antibodies are used, the test system will detect in the sample either specific antibodies, regardless of their class, or antibodies of only a certain class (e.g. only immunoglobulin G or only immunoglobulin M). Depending on which antigens are used, all immunoassay test systems for antibody detection are divided into: 1. Lysate - in which the native antigen (lysed or ultrasound-treated infectious agent obtained in culture) is used; 2. Recombinant - using genetically engineered protein analogues of certain protein antigens of the pathogen; 3. Peptide - using chemically synthesised protein fragments. The general direction of development of ELISA diagnostics is from lysate test systems, which are commonly referred to as first generation test systems, to recombinant and peptide test systems. As a solid phase in most commercial diagnostic preparations polystyrene 96-well plates or polystyrene spheres are used [firms 'DIA-plus', 'ROCHE','ABBOTT']. The main requirements for the solid phase in IFA include: - resistance to the solutions used in the reaction; -high specific capacity, i.e. the ability to sorb on its surface antibodies or antigens in the amounts required for the reaction in combination with the least possible non-specific sorption of proteins from the samples and conjugates under investigation [9,10,11,12].

The most common way of immobilisation of antibodies or antigens is ad sorption, when a part of molecules due to ionic and hydrophobic interactions, as well as the formation of hydrogen bonds is attached to the surface of the solid phase. Enzymes as tags in immunoassays. The fundamental possibility of using enzymes as tags in immunoassays is due to the extremely high sensitivity of enzyme detection in solution. Amplification systems are known to detect the presence of only a few hundred enzyme molecules in 1 ml of solution. The main requirements to enzyme molecules for their use as tags are the following: high specific catalytic activity, availability, possibility of obtaining the enzyme in a highly purified state, preservation of catalytic activity after chemical modification when obtaining enzyme-antibody (antigen) conjugates, stability, simplicity and sensitivity of the method for determining the concentration (activity) of the enzyme [2,3].

A variety of enzymes can be used for enzymatic labelling of antigens or antibodies: horseradish peroxidase, alkaline phosphotase, beta-galactosidase, etc. Peroxidase catalyses the reaction AH2 + H2O2 ------- A + 2H2O Different compounds can be used as AH2. Thus, the reduced colourless phenylenediamine in the peroxidase reaction is converted into an oxidised coloured form with an optical absorption maximum at 435 nm, recorded photometrically. Alkaline phosphatase catalyses the hydrolysis of phosphoric esters: 4-nitrophenylphosphate is converted into 4-nitrophenol, which

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

Licensed under a Creative Commons Attribution 4.0 International License.

is detected by optical density at 405 nm; 4 methylumbelliferylphosphate is converted into 4-methylumbelliferone, which fluoresces with high quantum yield at 450 nm (fluorescence is excited at 365 nm).β-Galactosidase catalyses the hydrolysis of lactose to form glucose and galactose. If 4-methylumbelliferyl-β-D-galactoside is used instead of the natural substrate, the hydrolysis results in the formation of galactose and 4-methylumbelliferone, which is registered fluorimetrically. All commercial test systems use horseradish peroxidase, the choice of which is determined by its high specific catalytic activity, availability, stability, and ease of detection. The most commonly used substrate reagent is ortho-phenylenediamine (OPD) with hydrogen peroxide, the oxidation product of which is recorded photometrically. To stop the enzymatic reaction, a 'stop reagent' is used, which is added in equal amounts to all test and control samples. The most common 'stop reagent' is sulphuric acid. The results are recorded spectrophotometrically at a wavelength of 490 nm [4,5].

References

1. Kudratova Z. E. et al. Current modern etiology of anemia //Open Access Repository. – 2023. – Т. 10. – №. 10. – С. 1-4.
2. Burxanova D. S., Umarova T. A., Kudratova Z. E. Acute myocarditis linked to the administration of the COVID 19 vaccine //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 11. – С. 23-26.
3. Кудратова З. Э. и др. Атипик микрофлора этиологияли ўткир обструктив бронхитларининг ў зига хос клиник кечиши //Research Focus. - 2022. - Т. 1. - №. 4. - С. 23-32.
4. Kudratova Z. E, Normurodov S. Etiological structure of acute obstructive bronchitis in children at the present stage - Thematics Journal of Microbiology, 2023. P.3-12.
5. Kudratova Z. E., Tuychiyeva S. K. Atipik mikroflora etiologiyali o'tkir obstruktiv bronxitlar etiopatogenezining zamonaviy jixatlari. Research Focus, 2023, B. 589-593.
6. Kudratova Z. E., Karimova L. A. Age-related features of the respiratory system. Research Focus,Tom 2, P. 586-588.
7. Исомадинова Л. К., Даминов Ф. А. Современная лабораторная диагностика хронического пиелонефрита у детей //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 112-116.
8. Isomadinova L. K., Daminov F. A. Glomerulonefrit kasalligida sitokinlar ahamiyati //Journal of new century innovations. – 2024. – Т. 49. – №. 2. – С. 117-120.
9. Isomadinova L. K., Qudratova Z. E., Shamsiddinova D. K. Samarqand viloyatida urotiliaz kasalligi klinik-kechishining o'ziga xos xususiyatlari //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10. – С. 51-53.
10. Isomadinova L. K., Qudratova Z. E., Sh B. F. Virusli gepatit b fonida Covid-19 ning klinik laborator kechish xususiyatlari //Journal of new century innovations. – 2023. – Т. 30. – №. 3. – С. 60-65.
11. Isomadinova L. K., Yulayeva I. A. Buyraklar kasalliklarning zamonaviy diagnostikasi //Центральноазиатский журнал образования и инноваций. – 2023. – Т. 2. – №. 10 Part 3. – С. 36-39
12. Kudratova Zebo Erkinovna, Tamila Abdufattoevna Umarova, & Sirojeddiova Sanobar. (2024). Modern types of immunoenzyme analysis methods old problems. Web of Discoveries: Journal of Analysis and Inventions, 2(6), 67–70.

Web of Medicine: Journal of Medicine, Practice and Nursing
webofjournals.com/index.php/5

Licensed under a Creative Commons Attribution 4.0 International License.