Acta virol. 37: 388-394, 1993

AN OPERATIONAL MODEL OF THE ANTIGEN-ANTIBODY INTERACTION

VOJTECH MUCHA

Institute of Virology, Slovak Academy of Sciences, Dúbravska cesta 9, 842 46 Bratislava, Slovak Republic

Received October 21, 1992

Summary. – A description of the antigen-antibody interaction based on the mass action law is applied to the complex system of a heterogeneous antigen and a mixture of antibodies. A model of this system is constructed and mathematically described. It provides a tool for the simulation of immunoassays.

Key words: antigen; antibody; interaction; operational model

Introduction

Various immunological methods have been developed to detect and utilize antigen-antibody interaction (Steward, 1978). Among these, methods which detect antigen-antibody complexes and allow to estimate the bound-to-free ratio for a given component, either an antigen or an antibody, are of great importance. Any of these methods can be used for the detection and quantification of specific antibodies, antigens, and their interactions (e. g. affinity of antibody). Equilibrium dialysis, equilibrium filtration, equilibrium sedimentation, radioimmunological methods, and immunoenzymatic methods are examples of such methods.

Theoretical approach to the immunoassay has been found useful for processing of experimental data, interpretation of experimental results, and design of the immunoassay itself (Feldman and Rodbard, 1971; Rodbard, 1971; Walker and Keane, 1977).

In this paper we describe a model of the antigen-antibody interaction, based on the mass action law, which provides a tool for the simulation of immunoassays. This model reflects the data about the structure of viral antigens and its antigenic properties, known structure and function of antibodies, and the data on the antigen-antibody interaction. Most of these data were obtained by the use of monoclonal antibodies (for review see Yewdell and Gerhard, 1981).

THE MODEL

Epitope, paratope, antigenic site

An antigen and an antibody bind to each other by their surface areas: antigenic determinant(s) and antibody combining site(s). These areas contribute to the binding energy of antigen-antibody interaction. A paratope is a population of identical and equivalent antibody combining sites of the same specificity and affinity. An epitope is a population of the antigenic determinants delineated by their interaction with a given paratope. As a consequence of such presumption, both epitope and paratope are rather populations of the elements of the same or similar properties than individual objects.

As stated above, each individual paratope which interacts with an antigen defines at least one epitope. Therefore several contiguous areas to which mixture of paratopes binds may be located on the surface of an antigen. Such areas are called antigenic sites. An individual antigenic site is built from overlapping antigenic determinants. A relatively simple homogeneous antigen may have generally several distinct antigenic sites on its surface which has been proved on the basis of the competitive binding tests with homogeneous antigen and a set of monoclonal antibodies (Wiley *et al.*, 1981).

Construction of the model

In spite of the fact that almost all surface of the antigen may be antigenic (Benjamin *et al.*, 1984), a paratope most usually react only with one antigenic site on the surface of antigen monomer. For example, so far there is no evidence of any monoclonal antibody binding to more than one antigenic site on the Influenza virus haemagglutinin monomer (Poumbourios *et al.*, 1990). It folows from the mass action law that for homogeneous and monovalent both antigen and antibody the epitope-paratope interaction at equilibrium is described by the equation

$$K = \frac{B}{F \times G} \tag{1}$$

where K is equilibrium constant of the reaction, B is concentration of the bound epitope-paratope pairs (complexes), F and G are concentrations of a free epitope and a free paratope, respectively. Formula (1) is also valid for an antibody with repeated combining sites under condition that they act independently each from the other (Fazekas de St. Groth, 1979).

Interaction of a simple homogeneous antigen with a mixture of antibodies

In our model we assume that a simple homogeneous antigen is monovalent in

reaction with a single paratope (monoclonal antibody). It follows from the definition of the antigenic site that the system composed of a simple homogeneous antigen and a polyclonal antibody (which we understand to be a mixture of monoclonal antibodies) consists generally of m nonoverlapping antigenic sites and of N distinct paratopes. Since an antigen is monovalent in reaction with each individual paratope, a single paratope is supposed to react only with one antigenic site. In our model we assume that on an antigen molecule only one antigenic determinant within an antigenic site can be occupied at the same time. Individual antigenic sites, however, act independently, i. e. binding of an antibody to one antigenic site has no influence upon binding properties of the other antigenic site. Generally, we assume that the equilibrium constant of an elementary interaction between an individual antigenic binding site and an individual antibody combining site (site-site interaction) is not affected by the occupancy of the other binding sites on the surface of an antigen and/or an antibody molecule: both antigenic sites and antibody combining sites are independent.

In experimental tests the accuracy of the antibody affinity estimation is restricted even for simple reactions. Therefore, in our model we consider finite number, n, of discrete distinguishable values of the equilibrium constant K, $C_1,..,C_n$, which represent rounded values of actual constants. Since the same spectrum of the equilibrium constants is considered for each of m antigenic sites, there are only $N = m \times n$ distinguishable paratopes in our model system, at most n for each antigenic site. The paratopes with $K = O$, i. e. exhibiting no binding to epitope, can be formally included.

At equilibrium, taking into account the assumptions stated above, our model system is described by $m \times n$ competing equations, each one written for an individual paratope:

$$K = \frac{B_i(K)}{F_i \times G_i(K)} \qquad (2)$$

$$i = 1,..,m, \quad K = C_1,..,C_n$$

Here a paratope is determined by the indices i, K, which denote that the paratope reacts with the i -th antigenic site with the equilibrium constant K. The following abbreviations are used in (2):

F_i concentration of the free i -th antigenic site

$G_i(K)$ concentration of the free paratope which interacts with i -th antigenic site with the equilibrium constant K.

$B_i(K)$ concentration of the bound i -th antigenic site occupied by the paratope which interacts with i -th antigenic site with the equilibrium constant K.

Due to the mass conservation

$$p_i = B_i + F_i, \qquad q_i(K) = Bi(K) + G_i(K)$$

$$B = \sum_K B_i(K), \quad \sum_{i=1}^{m} \sum_K q_i(K) = q \qquad (3)$$

$$i = 1,..,m$$

where summation through K is performed over all distinguished values of the equilibrium constant,
$C_1,..,C_n$, and the following abbreviations are used:

p_i total input concentration of the i -th antigenic site
$q_i(K)$ total input concentration of the paratope which interacts with i-th antigenic site with equilibrium constant K
B_i concentration of the bound i -th antigenic site
q overall total concentration of paratopes.

Taking into account the mass conservation (3), formula (2) can be transformed to the form

$$R_i = \sum_K \frac{K \times b_i(K)}{1 + Kp_i(1-X_i)} \times q \qquad (4)$$

$$i = 1,..,m$$

where summation through K is performed over all distinguished values of the equilibrium constant, $C_1,..,C_n$. $X_i = B_i/p_i$ and $R_i = X_i/(1-X_i)$ are bound-to-total and bound-to-free ratios of the i -th antigenic site respectively. For convenience we have introduced a proportion (relative concentration) of an individual paratope as $b_i(K) = q_i(K)/q$.

Interaction of a heterogeneous antigen with a mixture of antibodies

In the antigen-antibody binding assays, the antigen is usually not present in a homogeneous form (Walker and Keane, 1977). As a consequence of its isolation, purification, processing, or aging, the originally homogeneous antigen is splitted into several antigen subpopulations. The individual antigen subpopulations may differ in their immunoreactivity. Therefore we assume that there are several distinct homogeneous antigen subpopulations in the model system.

Let us consider a system consisting of a finite number, s, of antigen subpopulations and a mixture of paratopes. Let us suppose that we can

distinguish n discrete rounded values of the equilibrium constant, $C_1,..,C_n$, as in the case of the homogeneous antigen above. Let the system have the following properties:
(1) The antigen of each subpopulation has an equal number, m, of ordered and independent antigenic sites. An individual antigenic site is given by the pair of indices k,i , $k = 1,..,s$, $i = 1,..,m$, , which defines the i -th antigenic site of the k -th antigen subpopulation.
(2) An individual paratope is given by the index i and the ordered set of s indices K_k , $k = 1,..,s$, which means that a given paratope interacts with the i -th antigenic site of the k -th antigen subpopulation with the equilibrium constant K_k. Each of the indices K_k, $k = 1,..,s$, can attain values $C_1,..,C_n$.
For the sake of simplicity we shall denote an ordered set of indices $K_1,..,K_s$ by {K}, so a paratope will be determined by the indices i and {K}.

Now the model of the interaction of a heterogeneous antigen with antibodies can be mathematically described. At equilibrium, taking into account the same assumptions as in the case of the homogeneous antigen above, namely (i) monovalency of the antigen in the reaction with each individual paratope, (ii) competition of paratopes reacting with the same antigenic site, and (iii) independence of both the antigenic sites and the antibody combining sites, plus competition of the antigen subpopulations for the antibody, our model system is descsribed by $m \times n^s \times s$ competing equations, each one written for one elementary interaction between an individual paratope and an individual antigenic site, i. e. for a site-site interaction:

$$K(k) = \frac{B_{ki}(\{K\})}{F_{ki} \times G_i(\{K\})} \tag{5}$$

$$k = 1,..,s \quad , \quad i = 1,..,m \quad ,$$
$$\{K\} = K(1)..K(s) \; : \quad K(k) = C1,..,Cn$$

where $B_{ki}(\{K\})$ is a concentration of the bound i -th antigenic site of the k -th antigen subpopulation, occupied by the paratope given by its indices i and {K}, F_{ki} is a concentration of the free i -th antigenic site of the k -th antigen subpopulation, and $G_i(\{K\})$ is a concentration of the free paratope given by indices i and {K}.

Due to the mass conservation

$$p_{ki} = B_{ki} + F_{ki} \; , \; q_i(\{K\}) = B_i(\{K\}) + G_i(\{K\})$$
$$B_{ki} = \sum_{\{K\}} B_{ki}(\{K\}) \; , \; B_i(\{K\}) = \sum_{k=1}^{s} B_{ki}(\{K\}) \tag{6}$$

$$i = 1,..,m \; , \; k = 1,..,m$$

where summation through {K} is performed over all distinct ordered sets of values of equilibrium constants, and the following abbreviations are adopted:

p_{ki} total input concentration of the i -th antigenic site of the k -th antigen subpopulation

$q_i(\{K\})$ total input concentration of a paratope given by the indices i and {K}

B_{ki} concentration of the bound i -th antigenic site of the k -th antigen subpopulation

$B_i(\{K\})$ concentration of the bound paratope given by its indices i and {K}.

Taking into account the mass conservation (6), formula (5) can be transformed to the form:

$$R_{ki} = \sum_{\{K\}} \frac{K(k) \times b_i(\{K\})}{1 + \sum_{r=1}^{s} K(r) \times P_{ri} \times (1-X_{ri})} \times q \qquad (7)$$

$$\sum_{i=1}^{m} \sum_{\{K\}} b_i(\{K\}) = 1 \ , \ k = 1,..,s \ , \ i = 1,..,m$$

where $X_{ki} = B_{ki}/p_{ki}$ is a bound-to-total ratio of the i -th antigenic site of the k -th antigen subpopulation, $R_{ki} = X_{ki}/(1-X_{ki})$ is a bound-to-free ratio of the i -th antigenic site of the k -th antigen subpopulation,
$b_i(\{K\}) = q_i(\{K\})/q$ is a proportion of a paratope given by its indices i and {K}, q being a total input concentration of all paratopes.

In conclusion, our model is declared by a triplet of positive integers (m,n,s): a number of antigenic sites on each antigen subpopulation, a number of distinguishable values of equilibrium constant of the antigen-antibody interaction, and a number of antigen subpopulations taking part in the interaction. The model is described by the set of equations (7) by means of the parameters $C_1,..,C_n$, which represent rounded values of the equilibrium constants of the interaction distinguished by the given model, and $b_i(k)$, which represent distribution of the paratopes according to antigenic sites and equilibrium constants. Given these parameters and the concentrations of all antigenic sites, variables p_{ki}, and total input concentration of all paratopes q, the set of $m \times s$ equations (7) for unknown bound-to-total ratios of all antigenic sites, X_{ki}, can be solved numerically.

Discussion

This model evolved from the need in interpretation of results of radioimmu-

noanalytical experiments concerning the interaction of the influenza virus antigens and the corresponding antibodies. It provides a tool for the simulation of most immunoassays. An important feature of our model is its ability to describe binding of a heterogeneous antibody to an arbitrary number of antigen subpopulations. The basic mathematical formula (7) of our model can be derived from the formula (23) of Rodbard and Feldman (1975) under the conditions stated in this work.

To use our model for simulation of experiments, analogues of experimentally estimated quantities have to be constructed. Such an analogue can be represented by a function with bound-to-total ratios from the formula (7) as arguments. That function has to be specified according to particular experimental conditions.

The model can be applied to simulate immunoassays whenever a bound-to-total ratio (or equivalent data) for the binding sites or molecules for one or several components of the system can be estimated.

References

Benjamin, D. C., Berzofsky, J. A., East, I. J., Gurd, F. R. N., Hannum, C., Leach, S. J., Margoliash, E., Michael, J. G., Miller, A., Prager, E. M., Reichlin, M., Sercarz, E. E., Smith-Gill, S, J., Todd, P. E., and Wilson, A. C. (1984): The antigenic structure of proteins: A reappraisal. *Ann. Rev. Immunol.* **2**, 67–101.

Fazekas de St. Groth, S. (1979): The quality of antibodies and cellular receptors, pp. 1–42. In I. Lefkovits and S. Bernis (Eds): *Immunological Methods,* Academic Press, New York - San Francisco - London.

Feldman, H., and Rodbard, D. (1971): Mathematical theory of radioimmunoassay, pp. 158–203. In W. D. Odell and W. H. Doughaday (Eds): *Competitive binding assays,* Lippincott, Philadelphia.

Poumbourios, P., Brown, L. E., White, D. O., and Jackson, D. C. (1990): The stoichiometry of binding between monoclonal antibody molecules and the hemagglutinin of Influenza virus. *Virology* **179**, 768–776.

Rodbard, D. (1971): Statistical aspects of radioimmunoassay, pp. 204–259. In W. D. Odell and W. H. Doughaday (Eds): *Competitive Protein Binding Assays,* Lippincott, Philadelphia.

Rodbard, D., and Feldman, H. A. (1975): Theory of protein-ligand interaction, pp. 3–16. In B. W. O'Malley and J. G. Hardman (Eds): *Methods in Enzymolog*, vol. 36, Academic Press, New York - San Francisco - London.

Steward, M. W. (1978): Introduction to methods used to study antibody-antigen reactions. In D. M. Weir (Ed.): *Handbook of Experimental Immunology,* vol. 1, ch. 16, Blackwell, Oxford.

Walker, W. H. C., and Keane, P. M. (1977): Theoretical aspects of radioimmunoassay, pp. 87–130. In G. E. Abraham and E. M. Dekker (Eds): *Handbook of Radioimmunoassay,* vol. 5, Dekker, N. Y.

Wiley, D. C., Wilson, I. A., and Skehel, J. J. (1981): Structural identification of the antibody binding sites of the Hong Kong influenza hemagglutinin and their involvement in antigenic variation. *Nature* **289**, 373–378.

Yewdell, J. W., and Gerhard, W. (1981): Antigenic characterization of viruses by monoclonal antibodies. *Ann. Rev. Microbiol.* **35**, 185–206.