COMPUTER AIDED ANALYSIS OF ANTIGEN-ANTIBODY REACTIONS

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ABSTRACT

Antibodies are large molecules which are formed in the body to render antigens (causes of diseases) harmless. Bivalent antibodies and multivalent antigens may form a rich variety of different antigen-antibody complexes. The outcome of a reaction between an antigen and its antibodies can be analyzed experimentally by use of a gradient centrifugation technique.

We have used computer models to study the extent to which the outcome of an antigen-antibody reaction is predictable from simple assumptions.

First, a mathematical model for simulation of this particular centrifugal technique was constructed, allowing calculation of where in the centrifuge rotor a given complex would be located following centrifugation under precisely defined circumstances. Second, a program to predict the outcome of reactions between antigens and antibodies was written, based on the assumption that the kinds and the amounts of antigen-antibody complexes are determined by the concentrations of antigens and antibodies, respectively, together with a parameter describing their mutual affinity. Third, these programs were combined so as to calculate the expected outcome of antigen-antibody reactions as they appear after this type of centrifugal analysis. Finally, calculated results were compared to experimentally obtained results, and a fairly good agreement was found.

It is concluded that the main characteristical features of such reactions are predictable from the above mentioned assumptions by use of these computer models.

INTRODUCTION

When antigens and antibodies interact complexes containing different numbers of antigen and of antibody molecules will be formed. Some of these complexes can be regarded as beneficial because they (as intended in the immune system) trigger the mechanisms which eventually will result in the removal of the antigen in question. However, in some unfortunate cases the complexes, themselves, may cause or be indicative of serious secondary pathological processes.

In order to be able to study the conditions under which the beneficial and the harmful complexes are formed, computer models for simulation of the complex-forming reactions between antigens and antibodies have been formulated (1,2). These models are based on the assumption that the kinds and the amounts of antigen-antibody complexes which are formed under defined conditions are determined by the total concentrations of both components, their valences, and a parameter describing their mutual affinity.

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The outcome of an interaction between an antigen and its corresponding antibody can be studied experimentally by use of a gradient centrifugation technique called rate-zonal centrifugation (3). This method rests on relatively simple physical principles, and the sedimentation pattern can be predicted by use of numerical simulation models for this purpose (4,5).

In this communication we will describe a numerical method for simulation of zonal centrifugation of antigen-antibody complexes. Since this method, due to its physical foundation, in principle is correct, it can be used to study the extent to which the outcome of antigen-antibody interactions is predictable from the above mentioned assumptions.

EXPERIMENTAL METHODS

Complete description of the experimental technique is given in (3). Radioactive iodinated human serum albumin (HSA) was used as antigen, and rabbit anti-HSA IgG as antibody. Antigen and antibody were mixed at 37°C, and left overnight in a refrigerator before being centrifuged. Centrifugation was performed with a B-XIV zonal rotor for five hours at 46,000 rpm and at a temperature of 8°C. A 3-20% isokinetic sucrose gradient was used.

THE ANTIGEN-ANTIBODY MODEL SYSTEM

A macromolecular antigen has a certain number (f) of antigenic determinants, all of which are able to bind to one of the combining regions of an antibody molecule. The antibody molecule (an IgG) has two combining regions. Depending on the concentration of antigen and antibody molecules, and the affinity between the determinants and the combining regions, different types of complexes can be formed. In the presence of an overweight of antibody molecules the most prominent complexes will be some containing one antigen molecule surrounded by several (but not more than f) antibody molecules. The typical complex formed in antigen excess will consist of one antibody molecule and two antigen molecules. In the concentration range between these two extremes a rich variety of compositionally different complexes are formed.

An overall picture of the complex formation is given in Fig. 1 in the form of a reaction scheme. The reaction scheme also forms the basis for numerical methods for simulation of such reactions on a computer, using a multicompartmental method of analysis. The total amounts of antigen and antibody in the system are known values. Furthermore, an association constant, stating the ratio between the velocity constants for association and dissociation of individual complexes is known also. At the start of a simulation, all antigen and all antibody molecules are in their free form. In the first iterative cycle they will form some of the simplest complex AgAb. In subsequent cycles of calculations more of the simplest complex will be formed, and larger complexes will be formed gradually according to the actual conditions. After several cycles a state of equilibrium is reached where the concentration of individual complexes no longer changes. Hence, the calculations are terminated, and the complex distribution is provided as output.

SIMULATION OF ZONAL CENTRIFUGATION

Rate-zonal centrifugation is a centrifugal technique in which the sample particles in a gravitational field sediment through a density and viscosity gradient formed by a steadily increasing concentration of the gradient material, being sucrose in the present study.

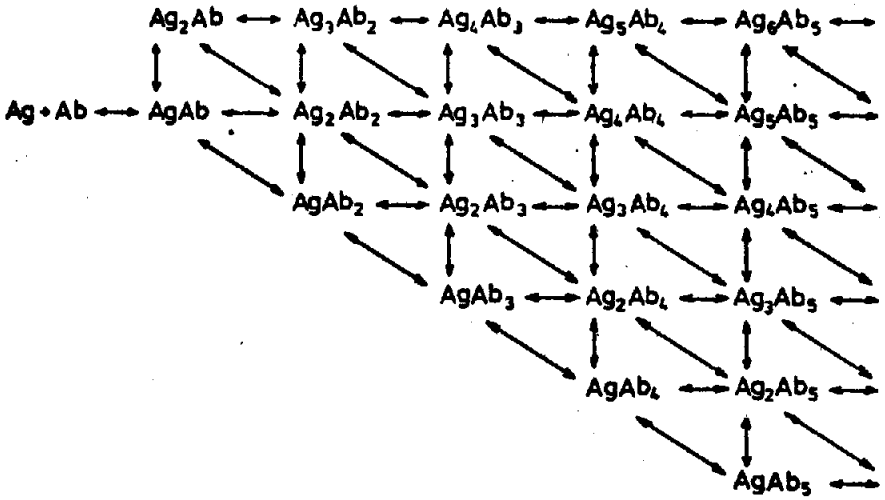

Figure 1. Reaction scheme for the interaction between a macromolecular antigen and a corresponding IgG. The antigen is denoted by Ag, the antibody by Ab. Vertical arrows reflect growth of a complex by addition of one antigen molecule, diagonal arrows addition of one antibody molecule, and horizontal arrows addition of the simplest complex AgAb.

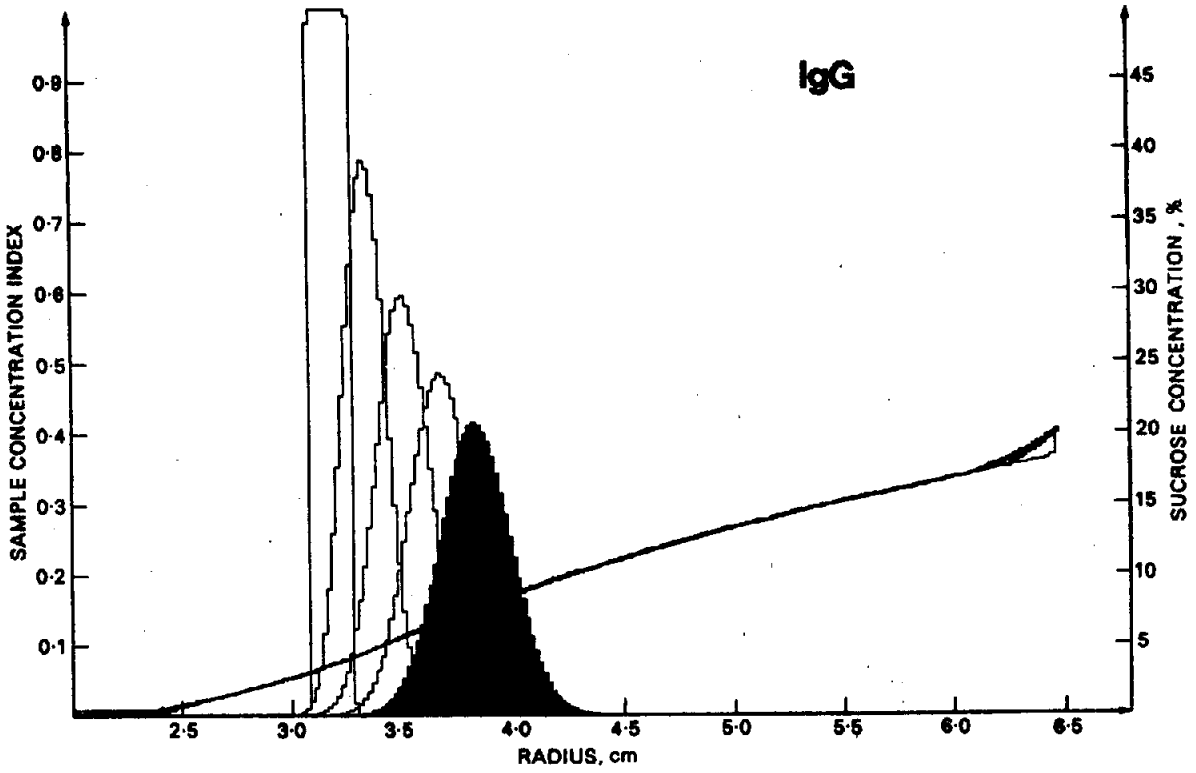

Figure 2. Computer plot of the result of a simulated run. IgG was used as sample material, and was centrifuged in an isokinetic gradient. The block zone to the left is the initial sample zone, while the dark hatched Gaussian zone is the final sample zone. The other Gaussian curves show three intermediate zones.

The flow across a unit area of an imaginary plane perpendicular to the centrifugal field, is given by

$$J = sc\omega^2 r - D(dc/dr)$$

where J is the amount of material passing through the plane, s is the sedimentation coefficient, c is the concentration of material, ω is the angular velocity of the rotor, D is the diffusion coefficient, and r is the distance from the plane to the axis of rotation.

A model zonal rotor is assumed to contain 206 imaginary planes, separating the internal part of the rotor in 207 narrow segments. The sample is started in the required number of segments, and the shape of the gradient is defined by the concentration of sucrose in each segment. In a series of iterative calculations the flow across each plane is calculated for sample particles as well as gradient materials. After each cycle the new concentrations in each segment are calculated from values of fluxes of material into the segments and the fluxes out of segments. Also new values of s and D are calculated in each cycle for new concentrations of gradient material before calculation of the flow of particles and gradient material. An example of output is given in Fig. 2.

The original program, being written for other purposes, was designed for use with very few different particles in each simulation. However, to simulate centrifugation of a mixture of different antigen-antibody complexes at least 23 different complexes (all in their own concentrations) plus the gradient material must be accounted for.

Inspection of Fig. 2 will reveal that a zone in the spinning rotor assumes a Gaussian shape. Hence, it is possible to characterize the final sedimentation pattern of a particle with known sedimentation and diffusion coefficients by the parameters of the corresponding Gaussian curve. Parameters for all complexes in the reaction scheme were calculated by single simulations of centrifugations for all theoretically possible complexes. The parameters obtained in this way are listed in Table 1. Following that, the total sedimentation pattern of a mixture of different complexes can be obtained by summation of the Gaussian curves for all involved complexes.

RESULTS AND DISCUSSION

Fig. 3 shows the experimental results obtained by rate-zonal centrifugation of a mixture of human serum albumin, its corresponding rabbit IgG, and the antigen-antibody complexes formed by the interaction. The first and largest peak contains the free unreacted antigen, while the second and wider peak contains the soluble complexes formed in this reaction. The range of sedimentation coefficients for these complexes are about 8 to 20, indicating that these complexes are relatively small. Fig. 4 shows the theoretical results obtained by a computer simulation of the antigen-antibody interaction as well as a simulation of the zonal centrifugation. Comparison between Fig. 3 and Fig. 4 shows that the experimental and the simulated results have several features in common. The peak of free antigen and the peak containing the soluble complexes are located in the same positions. The relative size of the two peaks is about correct, and even the trailing ends of the complex peak have finer structures in common. Hence, it is concluded: 1) that this approach can be used to study the extent to which the antigen-antibody simulation program makes correct predictions, and 2) that the tested model system actually makes predictions which are in good agreement with experimental predictions.

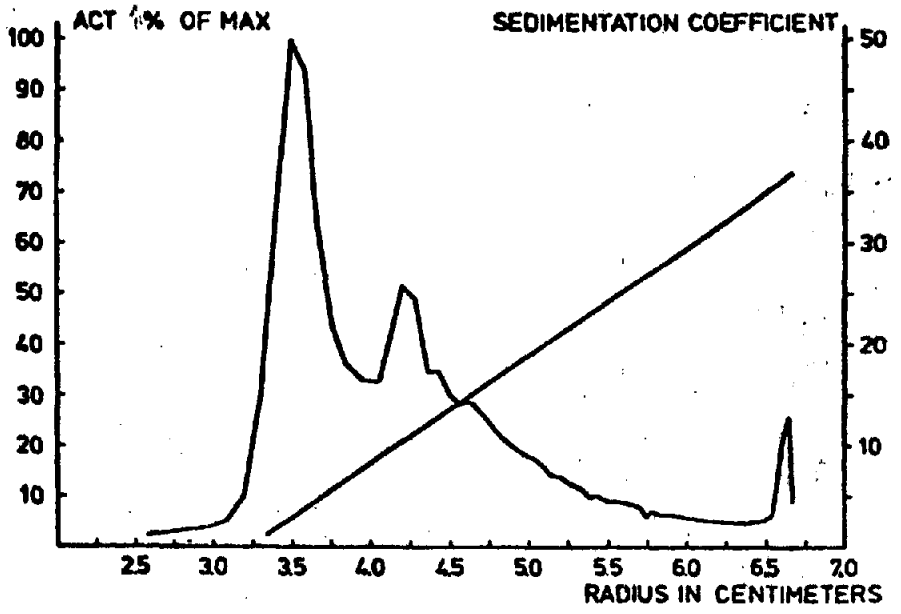

Figure 3. Zonal centrifugation effluent pattern of a centrifugation of immune complexes, performed as described in the section EXPERIMENTAL METHODS. The x-axis has been recalculated to show radius in the rotor. The y-axis is percent of maximum value.

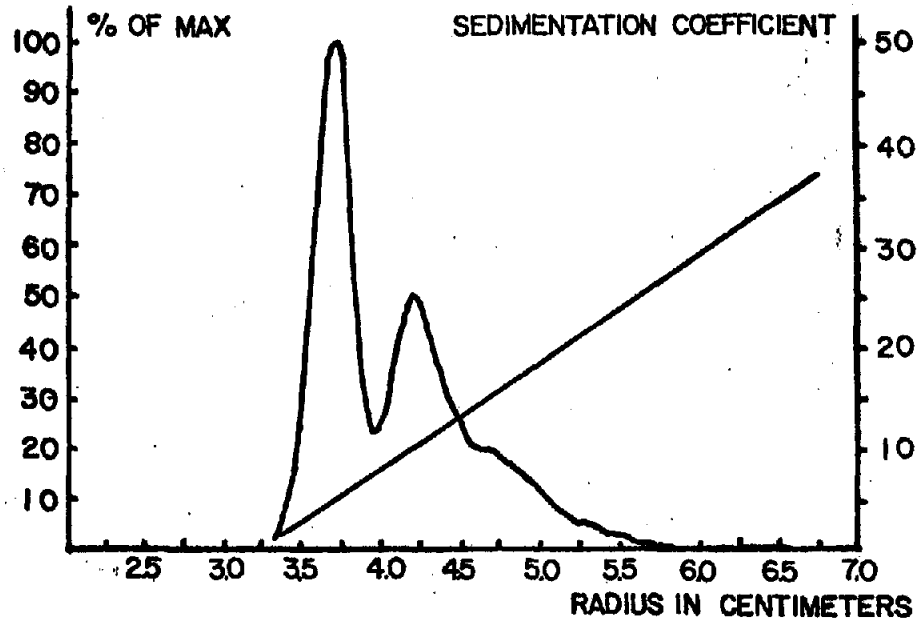

Figure 4. Simulation of the experiment shown in Fig. 3. Used concentrations of antigen and antibody were 0.36 and 0.48 mg/ml, respectively. The association constant was taken as 10^6 1/M.

Table 1. Calculated physical data for immune complexes formed between human serum albumin and rabbit anti-human serum albumin immunoglobulin G. The calculations were based on a molecular weight of 140,000 for rabbit IgG, and 66,000 for human serum albumin.

Complex	Molecular Weight(K)	Sedimentation Coefficient(S)	Diffusion Coefficient (Ficks)	Position (cm)	Width (cm)
AgAb	206	8.9	3.52	4.08	0.25
Ag_2Ab	272	10.5	3.21	4.23	0.29
$AgAb_2$	346	12.3	2.96	4.40	0.25
Ag_2Ab_2	412	13.8	2.79	4.54	0.34
Ag_3Ab_2	478	15.3	2.66	4.68	0.34
$AgAb_3$	486	15.3	2.64	4.68	0.34
Ag_2Ab_3	552	16.5	2.53	4.80	0.26
Ag_3Ab_3	618	18.3	2.44	4.97	0.38
Ag_4Ab_3	684	19.2	2.36	5.05	0.38
$AgAb_4$	626	17.9	2.43	4.93	0.30
Ag_2Ab_4	692	19.2	2.35	5.05	0.38
Ag_3Ab_4	758	20.3	2.28	5.15	0.43
Ag_4Ab_4	824	21.4	2.22	5.26	0.34
Ag_5Ab_4	890	22.6	2.16	5.37	0.32
$AgAb_5$	766	20.4	2.27	5.16	0.34
Ag_2Ab_5	832	21.7	2.21	5.28	0.39
Ag_3Ab_5	898	22.6	2.15	5.36	0.32
Ag_4Ab_5	964	24.2	2.10	5.52	0.47
Ag_5Ab_5	1030	24.9	2.06	5.58	0.41
Ag_6Ab_5	1096	25.8	2.01	5.67	0.32

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