IMMUNOREACTIONS INVOLVING PLATELETS

II. Theoretical Analysis of the Model*

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In the preceding paper (see Discussion and Fig. 12) a specific model is outlined for the nature of the interaction between platelets, antibody, and quinidine leading to formation of a complex which may fix complement. Our object here is to derive some of the properties of this model using the methods of statistical mechanics. Equations describing these properties were used to calculate theoretical results for complement fixation when appropriate values were assigned for concentrations of reactants as they were used experimentally. A comparison of the theoretical and experimental results is also included in the preceding paper.

There are two cases to consider: (*1*) quinidine is bound on antibody, and the antibody-quinidine complex is then bound on platelets; and (*2*) quinidine is bound on platelets, in the neighborhood of the sites on platelets which then bind antibody. In either case the final antibody-quinidine-platelet complexes fix complement under conditions described in the model.

Case 1: Quinidine Bound on Antibody

We assume that each antibody molecule possesses three equivalent and independent sites for the binding of quinidine. Also, we assume that the platelets present in the system provide a total of B equivalent and independent sites for the binding of antibody; however, only antibody molecules with at least one quinidine attached can be bound.

Thus, whenever an antibody molecule is bound on a platelet, one quinidine is certainly attached to it and this 1-1 antibody-quinidine complex is considered a unit in counting possible states of bound antibody molecules. Additional quinidine molecules may be bound on the other two sites on the antibody; this binding is assumed to be "random" (*i.e.* as in Langmuir adsorption (reference 1)) and to have the same binding constant as for quinidine bound on antibody molecules in solution (in which there are three instead of two sites for "random" binding).

Antibody Molecules in Solution.—In order to investigate the equilibrium between bound antibody and antibody in solution, each of these states must be considered separately. Suppose that in a solution of volume V there are n_a antibody molecules with n_q quinidine mole-

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cules bound on the $3n_a$ sites available for such binding. The canonical ensemble partition function for this system is then (reference 2)

$$Q = \frac{1}{n_a!}[j_a^0 V]^{n_a} \frac{(3n_a)! j_q^{n_q}}{n_q!(3n_a - n_q)!} \tag{1}$$

in which j_a^0 and j_q are internal partition functions for antibody in solution and quinidine bound on antibody (including the energy of binding), respectively. We find ln Q, using Stirling's approximation, and then

$$-\frac{\mu_a}{kT} = \frac{\delta \ln Q}{\delta n_a} \tag{2}$$

$$-\frac{\mu_q}{kT} = \frac{\delta \ln Q}{\delta n_q} \tag{3}$$

in which μ_a and μ_q are chemical potentials. Equations (2) and (3) give

$$e^{\mu_a/kT} = \frac{c_a(1 - \alpha_q)^3}{j_a^0} \tag{4}$$

$$e^{\mu_q/kT} = \left[\frac{\alpha_q}{1 - \alpha_q}\right]\frac{1}{j_q} \tag{5}$$

in which $\alpha_q = n_q/3n_a$ and $c_a = n_a/V$. The conventional expression for the chemical potential of a dilute solute in solution is

$$\mu = \mu^0 + kT \ln c$$

with μ^0 representing a standard potential ($\mu = \mu^0$ when $c = 1$). Hence in equation (4) we write

$$j_a^0 = e^{-\mu_a^0/kT}$$

and we set the right side of equation (5) equal to $e^{\mu_q^0/kT}c_q$, in which c_q is the concentration of quinidine in solution, since quinidine bound on antibody is in equilibrium with quinidine in solution. Thus

$$e^{\mu_a/kT} = \frac{c_a e^{\mu_a^0/kT}}{(1 + k_q c_q)^3} \tag{6}$$

$$\frac{\alpha_q}{1 - \alpha_q} = k_q c_q \tag{7}$$

in which the "binding constant" $k_q = j_q e^{\mu_q^0/kT}$ and equation (7) has been used to eliminate α_q in equation (6). Equation (7) is essentially a Langmuir adsorption isotherm (references 1, 2).

Antibody Molecules Bound on Platelets.—Here we have a system of B sites for the binding of antibody, N_a molecules of antibody bound, N_a molecules of quinidine bound as a firm 1-1 complex, and a total of N_q molecules of quinidine bound (i.e., N_q-N_a molecules of quinidine bound "randomly"). The partition function in this case is

$$Q = \frac{3B! j_{aq}^{N_a}}{N_a!(B - N_a)!} \cdot \frac{(2N_a)! j_q^{N_q - N_a}}{(N_q - N_a)!(3N_a - N_q)!} \tag{8}$$

in which j_{aq} refers to the firm 1-1 antibody-quinidine complex. Using equations (2) and (3) again, we find

$$e^{\mu_a/kT} = \frac{N_a(3N_a - N_q)^3}{(B - N_a)(2N_a)^2(N_q - N_a)j_a} \tag{9}$$

$$e^{\mu_q/kT} = \frac{N_q - N_a}{(3N_a - N_q)j_q} \tag{10}$$

in which $j_a = j_{aq}/j_q$.

Antibody Equilibrium.—At equilibrium, the two chemical potentials in equations (9) and (10) must equal the same quantities in solution. Equations (6) and (9) give

$$\frac{k_a c_a}{(1 + k_q c_q)^3} = \frac{\theta_a}{1 - \theta_a} \cdot \frac{(1 - \theta_q)^3}{(2/3)^2(\theta_q - 1/3)} \tag{11}$$

in which $\theta_a = N_a/B$, $\theta_q = N_q/3N_a$, and $k_a = j_a e^{\mu_a^0/kT}$. Equation (10) can be rewritten as

$$k_q c_q = \frac{\theta_q'}{1 - \theta_q'} \tag{12}$$

in which $\theta_q' = (N_q - N_a)/2N_a$ (thus θ_q' is the fraction of "random" quinidine sites filled). We note that $\alpha_q = \theta_q'$, as expected from our assumption that the binding constant for "random" binding of quinidine on antibody is the same whether the antibody is in solution or on a platelet. If we use the relations

$$3\theta_q = 1 + 2\theta_q'$$

and

$$\theta_q' = \frac{k_q c_q}{1 + k_q c_q} \tag{13}$$

equation (11) becomes

$$k_a \theta_q' c_a = \frac{\theta_a}{1 - \theta_a}$$

or

$$\theta_a = \frac{k_a \theta_q' c_a}{1 + k_a \theta_q' c_a} \tag{14}$$

This is the equation for "random" binding of antibody with an *effective* binding constant $k_a\theta_q'$.

Case 2: Quinidine Bound on Platelets

We assume here that there are B sites on platelets for the binding of antibody molecules and at each of these B sites there are also three equivalent and independent adjacent sites (on platelets) for the binding of quinidine molecules. In order for an antibody to be bound, at least one of the three quinidine sites must be occupied. If an antibody is bound with one quinidine molecule two sites would be available for "random" binding of quinidine. The partition function is therefore

$$Q = \frac{3B!\, j_{aq}^{N_a}}{N_a!(B - N_a)!} \cdot \frac{(3B - N_a)!\, j_q^{N_q - N_a}}{(N_q - N_a)!(3B - N_q)!} \tag{15}$$

in which N_q is the total number of quinidine molecules bound, $N_q - N_a$ of which are bound on the $3B - N_a$ "random" sites. No quinidine is bound directly on antibody, either in solution or when antibody is bound on platelets. Equation (15) leads by the same procedures as in Case 1 to

$$k_a c_a = \frac{\theta_a (3 - \theta_a)}{(1 - \theta_a)(3\theta_q - \theta_a)} \tag{16}$$

$$k_q c_q = \frac{3\theta_q - \theta_a}{3(1 - \theta_q)} \tag{17}$$

in which $\theta_a = N_a/B$ and $\theta_q = N_q/3B$. If we define, for "random" sites, $\theta_q' = (N_q - N_a)/(3B - N_a)$, equations (16) and (17) become

$$k_a c_a = \frac{\theta_a}{(1 - \theta_a)\theta_q'} \tag{18}$$

$$k_q c_q = \frac{\theta_q'}{1 - \theta_q'} \tag{19}$$

These are the same as equations (14) and (12), respectively, so the two models lead to the same final equations (the formal definition of θ_q' is different but the physical significance is the same in the two cases). Hence we need no longer distinguish between the two cases.

Fixation of Complement, Case 1 or 2

It was assumed in the model that complement can be bound between antibodies only when each of two neighboring platelet sites is occupied with one antibody and two quinidine molecules. The probability that two neighboring antibody sites on a platelet are both occupied and further that each of the two antibody molecules has associated with it exactly two quinidine molecules (one "firm", one "random") is

$$\theta_a^2 [2\theta_q' (1 - \theta_q')]^2$$

If each antibody site has z nearest neighbor antibody sites, then the number of fixed complement molecules is, according to the model described in the preceding paper,

$$N_c = \frac{zB}{2} \theta_a^2 [2\theta_q' (1 - \theta_q')]^2 \tag{20}$$

Computational Equations.—We rewrite here some of the above equations in a form convenient for computational purposes. For example we shall use equation (13) in the form

$$\theta_q' = \frac{q}{1 + q} \tag{21}$$

$$q = k_q c_q$$

If c_p is the concentration of platelet sites (a number proportional to the concentration of platelets) and if c_a^0 is the initial concentration of antibody (before any is bound), we have at equilibrium

$$c_p = B/V, \qquad c_a = c_a^0 - \theta_a c_p. \tag{22}$$

By substituting equation (22) in equation (14) we can obtain

$$\theta_a = \frac{1 + \theta_q'(p + A) - [1 + 2\theta_q'(p + A) + \theta_q'^2(p - A)^2]^{1/2}}{2\theta_q' p} \tag{23}$$

$$p = k_a c_p, \qquad A = k_a c_a^0$$

That is, q, p, and A are quantities proportional to the concentrations of quinidine (initial and equilibrium concentrations assumed the same since quinidine is present in excess), platelets and antibody (initial), respectively. Equation (23), with use of equation (21), gives θ_a as a function of q, p, and A. Complement fixation, in terms of these same independent variables, is then, from equation (20),

$$C = p\,\theta_a^2\,[2\theta_q'(1 - \theta_q')]^2 \tag{24}$$

in which C (proportional to N_c) is defined by

$$C = 2k_a N_c/Vz$$

The analogous expression for the amount of antibody bound (proportional to N_a) is

$$\frac{k_a N_a}{V} = p\theta_a \tag{25}$$

SUMMARY

Theoretical equations have been derived, using the methods of statistical mechanics, for associations between platelets, antibody, quinidine, and complement, based on a model of an immunoreaction described in the preceding paper of this series. The two cases considered (*i.e.* the possibilities that quinidine can attach first to antibody or platelets) lead to the same final equations. The comparison of the theoretical results with experimental results is contained in the preceding paper.

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