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THEORY OF HAPTEN BINDING TO IgM: THE QUESTION OF REPULSIVE INTERACTIONS BETWEEN BINDING SITES

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Various workers in their studies of the binding of haptens to IgM have observed that at low hapten concentration IgM has an apparent valence of five or near five, while at high hapten concentration IgM has a valence of ten. A possible explanation for this is that there is an interaction between binding sites on the same $F(ab')_2$ region of the IgM molecule. In this paper the theory for such an interaction is presented and an expression for the apparent valence is derived. It is shown that the apparent valence depends on both the interaction between binding sites on the IgM molecule and on the width of the affinity distribution which characterizes the antiserum. A broad affinity distribution can give an apparent valence of five even when there is no interaction between sites, i.e., even when the ten binding sites on the IgM molecule are identical and independent.

The general properties of a Scatchard plot are also discussed. When there is no interaction between sites it is shown that the average affinity and the variance of the affinity distribution can be obtained from a Scatchard plot. To illustrate the theory, an antiserum with affinities characterized by a normal distribution is considered and a simple method is presented for determining σ, the parameter which measures the width of the normal distribution.

1. Introduction

In a recent study of the binding properties of IgM, Oriol and Rousset [1,2] suggested that there may be an interaction between binding sites on IgM molecules* This interaction was proposed to explain the observations that for some haptens IgM appears to have a valence of five at low hapten concentration and a valence of ten at high hapten concentration [1–7]. One possible explanation is that there is an interaction between binding sites on the same $F(ab')_2$ region of the molecule; when a monovalent hapten binds to IgM the probability of a second hapten binding to the adjacent site on the same $F(ab')_2$ region is reduced. Such an interaction would make the molecule appear to have a valence of five at low hapten concentration, but in the limit of high hapten concentration all ten sites would bind.

In this paper I present a theory for such binding and derive an expression for the apparent valence at low hapten concentration. I show that the apparent valence depends not only on the interaction between sites, but also on the width of the affinity distribution. A broad affinity distribution can give an apparent valence of five even when there is no interaction between sites. Thus, an apparent valence of five does not prove that IgM has other than ten identical, non-interacting binding sites.

* *Abbreviations*: IgM, immunoglobin M; IgG, immunoglobin G.

2. Theory

Consider a homogeneous IgM population where each IgM has ten identical sites and each site has an affinity K. When a monovalent hapten binds to a site where the adjacent site on the same $F(ab')_2$ region is occupied there is an interaction. We define the parameter α as follows:

$$\alpha = \exp(-\Delta G_I/RT), \tag{1}$$

where ΔG_I is the free energy change due to the interaction, T is the absolute temperature and R the gas constant. α is a number between zero and one; when $\alpha = 1$ there is no interaction and all ten sites bind independently, each with an affinity K. The smaller α is, the stronger the interaction is. When $\alpha = 0$ a maximum of five haptens can bind to a single IgM. α will depend on

the particular hapten, but I make the assumption that it will not depend on hapten concentration. (I consider in this paper only repulsive interactions. For example, they might be Coulomb repulsions between small charged haptens or steric hindrances between large haptens. Also I only treat monovalent haptens and therefore those interactions which lead to enhanced binding of antibodies by multivalent antigens are not considered. These latter interactions have been treated by Crothers and Metzger [8].)

In equilibrium the number of haptens bound per IgM is given by the expression [9]

$$r = 10\,x(1 + \alpha x)/(1 + 2x + \alpha x^2), \qquad (2)$$

where $x = Kc$ and c is the free hapten concentration. When $\alpha = 1$ eq. (2) reduces to the more familiar result, that $r = 10x/(1 + x)$.

Usually one has an antiserum with a distribution of affinities and only the average value of the number of haptens bound per IgM is determined. This average can be written as follows:

$$r = [\mathrm{Ab}]^{-1} \sum_i [\mathrm{Ab}]_i r_i\,, \qquad (3)$$

where [Ab] is the total antibody concentration and $[\mathrm{Ab}]_i$ is the concentration of those antibodies with affinity K_i. The sum is over all antibody populations. For small hapten concentrations eq. (2) can be expanded in a power series in x_i ($x_i = K_i c$) and r calculated from eq. (3). The details are presented in appendix 1 where, in the limit that c goes to zero, the following expression is obtained:

$$\lim_{c \to 0} r/c = 10\langle K\rangle - 2(\langle K^2\rangle/\langle K\rangle)(1 - \tfrac{1}{2}\alpha)r. \qquad (4)$$

The various moments of K_i are defined in the following way:

$$\langle K^n\rangle = [\mathrm{Ab}]^{-1} \sum [\mathrm{Ab}]_i K_i^n\,. \qquad (5)$$

Eq. (4) predicts that as c approaches zero a Scatchard plot (r/c versus r) will give a straight line. The derivation of eq. (4) requires that at least the first two moments of K_i exist. This means that the tail of the affinity distribution must drop off faster than the second power of K_i. If this is not the case, the slope of the Scatchard plot will go to infinity rather than a constant as c goes to zero. For example, a Sips distribution [10] will give an infinite slope as well as an infinite y intercept. The tail of the Sips distribution drops off so slowly that all the moments of K_i are infinite including the first moment. (The properties of the Sips distribution are reviewed in appendix 2.) Although it is often stated that the Sips distribution is very similar to the normal distribution [†], the normal distribution drops off much more rapidly at large K_i and all the moments exist. For a normal distribution the slope is finite as c goes to zero.

Eq. (4) indicates that one can determine the average affinity from the y intercept of a Scatchard plot whether or not there is an interaction. (The determination of the average affinity from the y intercept is equivalent to the method of Mukkur, Szewczuk and Schmidt for determining K_t [11]. Their K_t is identical to $\langle K\rangle$.) If there is no interaction one can also determine the ratio of the second moment to the first from the slope. Thus, when there is no interaction, one can determine both the average, $\langle K\rangle$, and the variance, $\langle K^2\rangle - \langle K\rangle^2$, of the distribution from the low hapten concentration portion of the Scatchard plot.

I define the apparent valence, n^*, as follows: The apparent valence equals the x intercept obtained by extrapolating the straight line portion of the Scatchard plot (low c) to c equals infinity. (See fig. 1.) From eq. (4) we have

$$n^* = 5(\langle K\rangle^2/\langle K^2\rangle)\,[1/(1 - \alpha/2)]\,. \qquad (6)$$

[†] By normal distribution I mean that the binding energies are given by a normal distribution and the affinities by a log normal distribution. This is the customary usage of the term normal distribution in immunology.

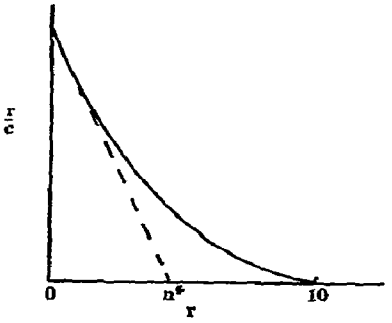

Fig. 1. A Scatchard plot with r being the number of haptens bound per IgM, c the free hapten concentration and n^* the apparent valence.

The quantity $\langle K\rangle^2/\langle K^2\rangle$ is a measure of the width of the distribution and is always less than or equal to one. Eq. (6) shows that the apparent valence depends on both the width of the distribution and the interaction between sites. Even if there is no interaction, $\alpha = 1$, one can still have an apparent valence of five if $\langle K\rangle^2/\langle K^2\rangle = 0.5$. The range of $\langle K\rangle^2/\langle K^2\rangle$ depends on the particular distribution. For example, for a uniform distribution it is between 1 and 0.75, and for a normal distribution between 1 and 0.

An equation similar to (4) can be derived for the high c limit. In the limit that c goes to infinity the following is obtained:

$$\lim_{c\to\infty} r/c = 10\alpha/\langle K^{-1}\rangle - (\alpha/\langle K^{-1}\rangle)\, r\,. \tag{7}$$

Eq. (7) states that as c goes to infinity a Scatchard plot should approach a straight line with a slope $\alpha/\langle K^{-1}\rangle$ provided the affinity distribution goes rapidly enough to zero that $\langle K^{-1}\rangle$ exist, i.e., faster than the first power of K.

3. An example – the normal distribution

To illustrate the properties of eq. (6) consider a normal distribution [12]

$$N(\Delta G) = (1/RT\sigma\sqrt{\pi})\exp[-(\Delta G-\Delta G_m)^2/(\sigma RT)^2], \tag{8}$$

where ΔG is the free energy of binding, T the absolute temperature, R the gas constant and σ a constant which is a measure of the width of the distribution. The distribution is normalized so that

$$\int_{-\infty}^{\infty} N(\Delta G)\, d\Delta G = 1. \tag{9}$$

The affinity is related to the free energy change in the standard way.

$$K = K_0 \exp(\Delta G/RT). \tag{10}$$

From eq. (8) all the moments of K can be calculated, the result being

$$\langle K^n\rangle = K_0^n \exp(n\Delta G_m/RT)\exp(n^2\sigma^2/4). \tag{11}$$

From eq. (10) it follows that

$$\sigma^2 = 2\ln(\langle K^2\rangle/\langle K\rangle^2). \tag{12}$$

If there is no interaction between sites and if the distribution is normal, one can determine σ from the apparent valence [$\sigma^2 = -2\ln(n^*/10)$]. I have determined n^* and then σ from the published curves of Oriol and Rousset [1] which describe the binding of dinitrophenylated haptens to IgM. In order to determine n^* the x intercept of the Scatchard plot must be known. This requires extrapolating the high c portion of the curve to $c = \infty$. Such a procedure tends to underestimate the value of the x intercept and therefore to overestimate σ. For the curves of Oriol and Rousset, assuming no interaction between sites, σ ranged from 0.8–2.5. These values for σ are of the same order as those reported for the binding of various haptens to IgG [12–15]. Thus, reasonable σ values will give the observed n^* values.

If there is an interaction between sites, it follows from eq. (6) that

$$\sigma^2 = 2\ln 5 - 2\ln[n^*(1-\alpha/2)]\,. \tag{13}$$

By taking $\alpha = 1$ an upper bound on σ is obtained and by taking $\alpha = 0$ a lower bound is obtained. If there is an interaction the range of σ determined in this paper will shift to lower values, corresponding to narrower distributions.

If the distribution is normal, then $\langle K\rangle$, σ and α can be easily determined from a Scatchard plot. The average value of K is given by the y intercept and α and σ can be determined from the slopes as c goes to zero (slope 1) and c goes to infinity (slope 2). From eqs. (4), (8) and (11) it follows that:

$$\text{slope } 1 = 2\langle K\rangle(1-\alpha/2)\exp(\sigma^2/2), \tag{14}$$

$$\text{slope } 2 = \alpha\langle K\rangle\exp(-\sigma^2/2), \tag{15}$$

from which it is easy to determine α and σ.

4. Discussion

The difficulty in deciding whether or not there is an interaction between adjacent sites on an IgM molecule lies in the fact that one is almost always dealing with an unknown distribution of affinities. If the distribution were known, then from a Scatchard plot one could determine not only whether there was an interaction, but also the interaction energy. Although mathematical procedures for obtaining the distribution of affinities

are available [16,17] they assume that there are no interactions between binding sites on the same antibody. If there are such interactions the distributions obtained by these procedures will be incorrect. One case where the distribution is known is where the IgMs are monoclonal products. For the Waldenström macroglobins [18,19] and the monoclonal equine anti-lactose IgM isolated by Kim and Karush [20,21], the Scatchard plots give straight lines. This appears to indicate that there is no interaction between binding sites. (A Scatchard plot of eq. (2) for $\alpha \neq 1$ is not a straight line even for a homogeneous affinity distribution.) An alternate interpretation has been suggested [1]. Since the affinity of the Waldenström macroglobins is small, large hapten concentrations are needed to detect binding. The Scatchard plots for the Waldenström macroglobins may not be the entire curve, but only the high c portion. If this is true the fact that the curve is straight does not rule out an interaction between sites. Instead it would mean that one is in the range of c values for which eq. (7) holds. However, the recent data of Tolleshaug and Hannestad [19] covers a wide range of hapten concentrations and makes this interpretation seem most unlikely.

Despite the very interesting and suggestive data on the binding of small haptens to IgM, there is no direct evidence for an interaction between binding sites. That in certain studies IgM has an apparent valence of five or near five at low hapten concentration may indicate the type of affinity distribution in the antiserum and have nothing to do with any interaction between sites. To obtain an apparent valence of five does not require an interaction between sites nor even an exotic affinity distribution (bimodal, etc.). An antiserum with a normal distribution can have an apparent valence of 5, or any value between zero and ten for that matter.

For the binding of large haptens to IgM steric hindrance has been observed [22,23]. Dextrans of different molecular weights were used to study the effect of hapten size on the binding of haptens to rabbit IgM and IgG. Steric hindrance was observed in the binding of dextran to IgM for a wide range of molecular weights. For IgG only for the highest molecular weight dextran, a dextran with a molecular weight of 1.9×10^6, was there any indication of steric hindrance. From this data it appears that the maximum distance between binding sites on the same $F(ab')_2$ region of an IgM is much smaller than the distance between binding sites on an IgG. This means that if there are interactions between binding sites when small haptens bind to antibodies, they are much more likely to occur in IgM than IgG. However, as we have pointed out, there is no evidence for such interactions.

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Appendix 1

If eq. (3) is expanded in a power series in x_i the following series is obtained:

$$r = (10/[\mathrm{Ab}]) \sum_i [\mathrm{Ab}]_i [x_i - 2x_i^2(1-\alpha/2) + \ldots], \quad (1\text{-}1)$$

$$r = 10[c\langle K\rangle - 2c^2\langle K^2\rangle(1-\alpha/2) + \ldots], \quad (1\text{-}2)$$

which follows from eq. (5).

When only the first two terms are kept eq. (1-2) can be rewritten as follows:

$$r = 10c\langle K\rangle[1 - 2c(\langle K^2\rangle/\langle K\rangle)(1-\alpha/2)]. \quad (1\text{-}3)$$

To the same order in the expansion the above equation can be written in the form

$$r = \frac{10c\langle K\rangle}{1 + (2c\langle K^2\rangle/\langle K\rangle)(1-\alpha/2)}. \quad (1\text{-}4)$$

From this equation, eq. (4) follows.

For large c eq. (7) can be obtained in a similar manner by expanding in powers of $1/x_i$.

For a given distribution one can calculate the moments from eq. (5) and investigate the convergence of the series for r, eq. (1-2). For the normal distribution, where the moments are given by eq. (11), one can show that the series for r is asymptotic.

Appendix 2

In terms of the affinity K, the Sips distribution [10] can be written as follows:

$$N(K) = \frac{\sin \pi a}{\pi K_0} \frac{(K/K_0)^{a-1}}{1 + 2(K/K_0)^a \cos \pi a + (K/K_0)^{2a}}, \quad (2\text{-}1)$$

where

$$\int_0^\infty N(K)\,dK = 1, \tag{2-2}$$

$$K = K_0 \exp(\Delta G/RT) = \exp[-(\Delta G_0 - \Delta G)/RT]. \tag{2-3}$$

If one uses eq. (2-1) to calculate the moments of ΔG, i.e., $\langle \Delta G^n \rangle$, they are all well defined. In particular $\langle \Delta G \rangle = \Delta G_0$ and $K_0 = \exp(-\langle \Delta G \rangle/RT)$. If one calculates r from eq. (2-1) one obtains the well-known result

$$r = (K_0 c)^a / [1 + (K_0 c)^a]. \tag{2-4}$$

However, if one uses eq. (2-1) to calculate the moments of K, i.e., $\langle K^n \rangle$, they are all infinite. For example, to calculate $\langle K \rangle$ one must evaluate the following integral

$$\langle K \rangle = \int_0^\infty K N(K)\,dK. \tag{2-5}$$

It is easy to see from eq. (2-1) that for large K the integral goes as K^{-a} where a is a positive number which is less than one. The integral therefore blows up.

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