Sidebar

crosslinking — a theoretical approach

The problem at hand was to calculate the equilibrium concentration of crosslinks formed when BPO-specific IgE antibodies on basophil surfaces are exposed to the monovalent and bivalent synthetic penicillin allergens $(BPO)_1$ and $(BPO)_2$. We began by constructing a model consisting of all the binding reactions that can occur in this situation. Crucial to the calculations is knowledge of the equilibrium constants for these binding reactions. Although the model includes an infinite number of reactions, some reasonable assumptions reduce to a manageable number the equilibrium constants that must be known.

First, we let K be the equilibrium constant for the binding between a monovalent allergen and a "monovalent antibody." (A monovalent antibody does not, of course, exist but is useful as a theoretical construct because the equilibrium constant for its reaction with a monovalent allergen is indicative of the basic strength of the forces between the binding sites.) Consider now the simplest of the reactions depicted in the accompanying figure, those initial reactions in which a $(BPO)_1$ or $(BPO)_2$ allergen binds to one of the two sites on a free antibody (that is, an antibody bound through its Fc region to the cell surface but with each of the binding sites in its Fab regions free). The equilibrium constant for the reaction when $(BPO)_1$ is involved is 2K since the antibody offers two possible binding sites. Similarly, the equilibrium constant for the reaction involving $(BPO)_2$ is simply 4K because in this case the allergen also offers two possible binding sites. We assume that the equilibrium constants for these two reactions are unaffected if the free antibody is replaced by a chain of crosslinked antibodies with a free binding site on the antibody at each end of the chain.

Considering next the binding of a $(BPO)_1$ or $(BPO)_2$ allergen to the single available site on the products of the initial reactions, we assume that the equilibrium constants for these reactions are also related to K by appropriate statistical factors.

Next, we let K_x be the equilibrium constant for the basic crosslinking reaction, the binding of the complex containing one antibody and one $(BPO)_2$ allergen, each with a free binding site, to a free antibody. Again we assume that the equilibrium constant is unaffected if the free antibody is replaced by a chain of crosslinked antibodies with a free binding site on the antibody at each end.

Finally, a ring containing i antibodies is formed when a free BPO group of a $(BPO)_2$ allergen bound to one end of a chain of i crosslinked antibodies binds to the free site on the antibody at the other end of the chain. We assume that for $i \geq 2$ the equilibrium constant J_i for such a reaction is inversely proportional to i^2. Therefore, $J_i = 4J_2/i^2$ for ≥ 2. We let J_1 be the equilibrium constant for formation of the "ring" consisting of a single $(BPO)_2$ allergen spanning the sites on a single antibody.

Armed with the four equilibrium constants K, K_x, J_1, and J_2, we can calculate the equilibrium concentrations of all possible reaction products, (Reasonable estimates for the magnitudes of these constants can be obtained from various experimental data,) We will not present details of the calculations but rather the general concepts on which they are based.

The accompanying figure shows that seven complexes contain one antibody. The equilibrium concentrations of each of these complexes can be expressed as a function of K, J_1, and the $(BPO)_1$ and $(BPO)_2$ concentrations multiplied by the equilibrium concentration of free antibody. Therefore, W_1, the total equilibrium concentration of complexes containing one antibody, is obtained simply by adding together the equilibrium concentration of each of the complexes, We find that

$$W_1 = X f\left([(\mathrm{BPO})_1],[(\mathrm{BPO})_2],K,J_1\right) \quad ,$$

where

$$f = \left\{1 + K[(\mathrm{BPO})_1] + 2K[(\mathrm{BPO})_2]\right\}^2$$

$$+ 4KJ_1[(\mathrm{BPO})_2] \quad .$$

In these equations $[(BPO)_1]$ and $[(BPO)_2]$ are the concentrations of $(BPO)_1$ and $(BPO)_2$, respectively, and X, the only unknown, is the equilibrium concentration of free antibody.

The accompanying figure also shows that seven complexes contain two antibodies. We can express the concentration of each of these complexes as a function of K, J_2, $[(BPO)_1]$, and $[(BPO)_2]$ multiplied by the concentration X_2 of the crosslinked chain containing two antibodies with a free binding

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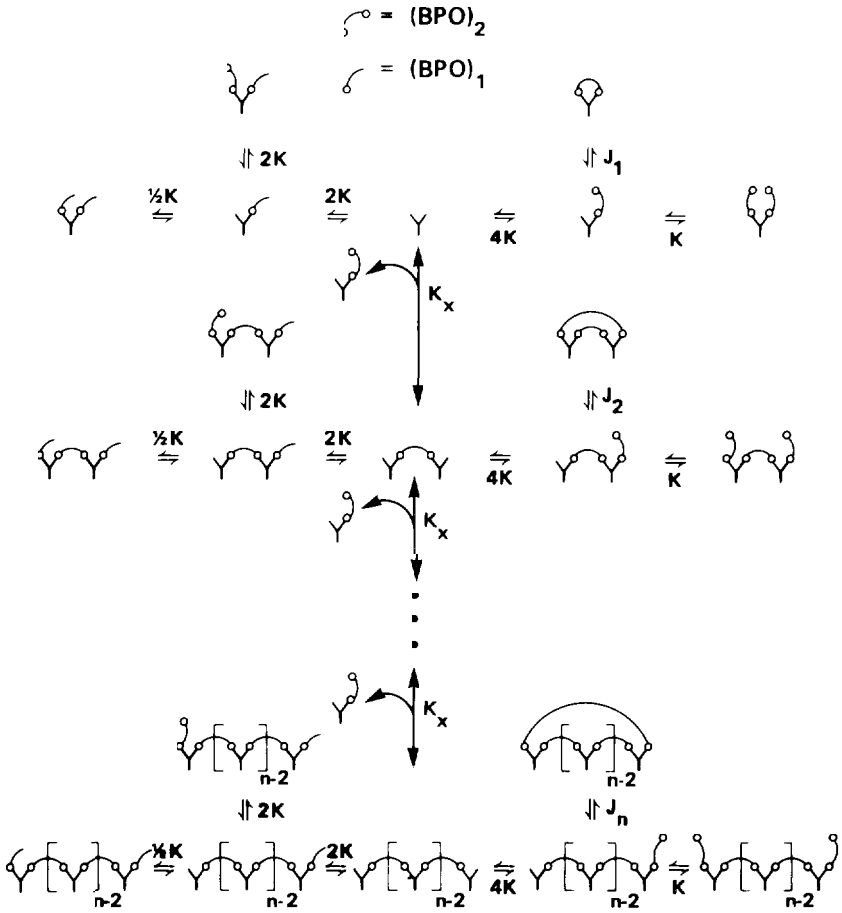

Shown here are all the binding reactions that can occur when BPO-specific IgE antibodies on basophils are exposed to the monovalent and bivalent synthetic penicillin allergens $(BPO)_1$ and $(BPO)_2$. The equilibrium constants for each reaction are also given.

site on the antibody at each end. But X_2 is in turn a function of K, $[(BPO)_2]$, and X, namely

$$X_2 = 4KK_x[(BPO)_2]X^2 \quad .$$

We can continue this process iteratively and develop a general expression for W_n, the equilibrium concentration of complexes containing n antibodies, as a function of $[(BPO)_1]$, $[(BPO)_2]$, K, K_x, J_n, and X:

$$W_n = X^n\{4KK_x[(BPO)_2]\}^{n-1}$$

$$\times f([(BPO)_1],[(BPO)_2],K,J_n) \quad .$$

The conservation law for total antibody concentration X_T leads to the equation

$$X_T = \sum_{n=1}^{\infty} nW_n \quad .$$

When we express all the W_n in this infinite series in terms of X, the infinite series can be summed, and we obtain an algebraic equation that can be solved for X. By substituting the solution for X into the expression for a particular W_n or for the equilibrium concentration of a particular complex, we can compute values for these expressions.

Because of the central role of crosslinks in the activation and desensitization of basophils, we are particularly interested in X_{poly}, the fraction of antibodies incorporated at equilibrium into complexes containing more than one antibody, that is, in the fraction of crosslinked antibodies. An expression for X_{poly} is easily derived since

$$X_{poly} \equiv \sum_{n=2}^{\infty} nW_n = (X_T - W_1)/X_T \quad .$$

Since this calculation was first presented, much theoretical work has been done on both the equilibrium and the kinetic theory of binding of multivalent antigens to antibodies on cell surfaces. As a result primarily of the work of Alan Perelson (Los Alamos), Charles DeLisi (National Institutes of Health), and Catherine Macken (Lincoln College, New Zealand), much progress has been made toward understanding the bonding of these more complicated antigens. ■