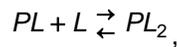
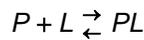


Ligand binding to an homo-oligomeric protein, cooperativity, macro and micro dissociation constants

The binding of ligand molecules to an homo-oligomeric protein is not complicated, but care is required because important differences exist in interpretation of dissociation constants between the equations written in terms of concentrations of oligomer (macro dissociation constants) and the equations written in terms of concentrations of the monomers (micro dissociation constants). The most straightforward formulation for describing the binding of ligand molecules to a homo-oligomeric protein lead to experimental values for the dissociation constants that suggest the existence of cooperativity in ligand binding when, in fact, there is none. Here the binding to a homo-dimeric protein will be considered, first in terms of concentrations of dimers, then in terms of monomers. This will reveal how the equilibrium dissociation constants in each formulation must be interpreted. For our discussion, let P represent the concentration of a dimeric protein and L the concentration of ligand. Let K_1 be the equilibrium dissociation constant for the binding of the first molecule of ligand to P and K_2 be the constant for the second molecule. The relevant equilibria are:



and the equilibrium equations are:

$$K_1 = \frac{P \times L}{PL}$$

$$K_2 = \frac{PL \times L}{PL_2}.$$

Since total protein equals unliganded plus singly liganded plus doubly liganded,

$$P + PL + PL_2 = P_t.$$

Solving these three equations for P , PL , and PL_2 gives

$$P = \frac{K_1 \times K_2 \times P_t}{K_1 \times K_2 + K_2 \times L + L^2}$$

$$PL = \frac{K_2 \times L \times P_t}{K_1 \times K_2 + K_2 \times L + L^2}$$

$$PL_2 = \frac{L^2 \times P_t}{K_1 \times K_2 + K_2 \times L + L^2}$$

These three quantities can be used to obtain the equations for various quantities of interest. For example, the amount of subunits with bound ligand will be $2 \times PL_2 + PL$, which is

$$\frac{(2L^2 + K_2 \times L) \times P_t}{K_1 \times K_2 + K_2 \times L + L^2}.$$

We might at first think that the situation with no cooperativity would have $K_1 = K_2$, but this is not the case due to the fact that there are two sites to which L can bind to P and two subunits of PL_2 from which L can dissociate to form PL. To understand more clearly what is going on, let us view the situation from the viewpoint of concentrations of monomers. Biochemists call the equilibrium constants that are used in the former description macro dissociation constants, and the equilibrium constants that will be used in the equations written in terms of monomer concentrations as micro dissociation constants. Although in many situations upper case K 's are used to refer to equilibrium constants and lower case k 's are used refer to rate constants, in this discussion, let lower case k 's refer to micro dissociation constants.

Although we are to express the micro dissociation constants in terms of monomer concentrations, we need to distinguish the cases of a ligand binding to a monomer in an unliganded dimer, which is described by the k_1 micro dissociation constant and ligand binding to a singly liganded dimer. This latter is described by the k_2 micro dissociation constant. The concentration of monomers in unliganded dimers is simply $2 \times P$. Thus

$$k_1 = \frac{2 \times P \times L}{PL}$$

Since

$$K_1 = \frac{P \times L}{PL}$$

$k_1 = 2 \times K_1$ or $K_1 = k_1 / 2$. Similarly, because there are two monomers with a bound ligand in PL_2 ,

$$k_2 = \frac{PL \times L}{2 \times PL_2}$$

and $k_2 = K_2 / 2$, or $K_2 = 2 \times k_2$.

Suppose there were no cooperativity or anticooperativity in the binding of ligand. In this case, $k_1 = k_2$ and we obtain the very important result that in the absence of cooperativity, $K_1 = \frac{1}{4}K_2$. Cooperativity in binding exists when $K_1 > \frac{1}{4}K_2$ and anticooperativity is present when $K_1 < \frac{1}{4}K_2$.

The binding equations can be written in terms of micro dissociation constants merely by replacing the macro dissociation constants with their equivalents in terms of micro dissociation constants. For example, the previous equation for the amount of subunits with bound ligand yields

$$\text{Bound subunits} = \frac{(2L^2 + 2k_2 \times L) \times P_t}{k_1 \times k_2 + 2 \times k_2 \times L + L^2}$$

To check the derivations, consider the case of no cooperativity. In this situation it doesn't matter whether or not the protein is dimerized. Thus we can consider the solution to consist of monomers. In this case, we know that the binding is described by a simple Michaelis-Menton binding curve,

$$\text{Subunits bound} = \frac{2 \times L \times P_t}{k + L}$$

Now, let us check whether the substitution of $k_1 = k_2$ in the equation for bound subunits yields the same.

$$\text{Subunits bound} = \frac{(2L^2 + 2k_1 \times L) \times P_t}{k_1 \times k_1 + 2 \times k_1 \times L + L^2}$$

$$= \frac{2 \times L \times (L + k_1)}{(L + k_1)^2} \times P_t$$

$$= \frac{2 \times L \times P_t}{k_1 + L}$$

Thus proving the point.

The free energy of the binding reaction must be independent of whether we express the dissociation constants in macro or micro form. Let us check that this is the case. The ΔG of the binding reaction is the sum of the ΔG 's of the first and second binding reactions. $\Delta G = \Delta G_1 + \Delta G_2$. In terms of macro dissociation constants,

$$\Delta G = -RT \ln K_1 - RT \ln K_2$$

In terms of micro dissociation constants

$$\Delta G = -RT \ln k_1 - RT \ln k_2$$

but since $k_1 = 2 \times K_1$ and $k_2 = \frac{1}{2} \times K_2$ the second ΔG equation becomes

$$\Delta G = -RT \ln 2 \times K_1 - RT \ln \frac{1}{2} \times K_2$$

$$= -RT (\ln 2 + \ln K_1 - \ln 2 + \ln K_2)$$

$$= -RT \ln K_1 - RT \ln K_2$$