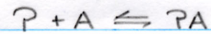


Ligand Binding to Macromolecules

A = ligand

P = protein



$$K = \frac{[PA]}{[P][A]} \quad \text{association constant}$$

or

$$K_d = \frac{[P][A]}{[PA]} \quad \text{dissociation constant}$$

~~~~~

Binding equations:

define  $\gamma$  = # moles of A bound per mole of P under a given set of conditions

( $\gamma \leq 1$  and is the "fractional saturation of the sites".)

↑ not true for multiple binding sites

$$\begin{aligned} \gamma &= \frac{\text{concentration of A bound to P}}{\text{total concentration of all forms of P}} \\ &= \frac{[PA]}{[P] + [PA]} \end{aligned}$$

from definition of  $K_d$ :

$$[PA] = \frac{[P][A]}{K_d}$$

Thus:

$$r = \frac{[P][A]/K_d}{[P] + ([P][A])/K_d}$$

or

$$r = \text{fractional saturation} = \frac{[A]}{K_d + [A]} \quad [1]$$

When  $r = 0.5$  (P half-saturated with A), then  $[A] = K_d$ .

Rearrangements of [1]:

$$(A) \quad \frac{1}{r} = 1 + \frac{K_d}{[A]} \quad (\text{double-reciprocal plot})$$

graph  $\frac{1}{r}$  against  $\frac{1}{[A]} \Rightarrow \text{slope} = K_d$

$$(B) \quad \frac{r}{[A]} = \frac{1}{K_d} - \frac{r}{K_d} \quad (\text{single reciprocal plot})$$

graph  $\frac{r}{[A]}$  against  $r \Rightarrow \text{slope} = -\frac{1}{K_d}$

Worked problems (note:  $\text{dm}^3 = \text{L}$ )

## Problems: 1:1 A:P binding

### Worked example

$\text{Mg}^{2+}$  and ADP form a 1 : 1 complex. In an experiment, the concentration of ADP was kept constant at  $80 \mu\text{mol dm}^{-3}$  and the concentration of  $\text{Mg}^{2+}$  varied. The following results were obtained.

|                                                           |      |      |      |      |      |      |
|-----------------------------------------------------------|------|------|------|------|------|------|
| Total $\text{Mg}^{2+}$ ( $\mu\text{mol dm}^{-3}$ )        | 20   | 50   | 100  | 150  | 200  | 400  |
| $\text{Mg}^{2+}$ bound to ADP ( $\mu\text{mol dm}^{-3}$ ) | 11.6 | 26.0 | 42.7 | 52.8 | 59.0 | 69.5 |

Determine the dissociation constant for MgADP under these conditions.

### Solution

At each value of the total  $\text{Mg}^{2+}$  concentration, the free  $\text{Mg}^{2+}$  concentration ( $[A]$  in the equations) can be evaluated simply by difference. The value of  $r$  is found by dividing the concentration of bound  $\text{Mg}^{2+}$  by the ADP concentration (i.e.  $80 \mu\text{mol dm}^{-3}$ ). We can convert the data into the correct form for graphical treatment.

|                                                                         |        |        |        |        |        |        |
|-------------------------------------------------------------------------|--------|--------|--------|--------|--------|--------|
| Total $\text{Mg}^{2+}$ ( $\mu\text{mol dm}^{-3}$ )                      | 20     | 50     | 100    | 150    | 200    | 400    |
| Bound $\text{Mg}^{2+}$ ( $\mu\text{mol dm}^{-3}$ )                      | 11.6   | 26.0   | 42.7   | 52.8   | 59.0   | 69.5   |
| Free $\text{Mg}^{2+}$ ( $\mu\text{mol dm}^{-3}$ )                       | 8.4    | 24.0   | 57.3   | 97.2   | 141.0  | 330.5  |
| $r$                                                                     | 0.145  | 0.325  | 0.534  | 0.660  | 0.738  | 0.869  |
| $\frac{1}{r}$                                                           | 6.90   | 3.08   | 1.874  | 1.515  | 1.356  | 1.151  |
| $\frac{1}{[\text{Mg}^{2+}]_{\text{free}}} (\mu\text{mol dm}^{-3})^{-1}$ | 0.1190 | 0.0417 | 0.0175 | 0.0103 | 0.0071 | 0.0030 |
| $\frac{r}{[\text{Mg}^{2+}]_{\text{free}}} (\mu\text{mol dm}^{-3})^{-1}$ | 0.0173 | 0.0135 | 0.0093 | 0.0068 | 0.0052 | 0.0026 |

The appropriate plots ( $1/r$  vs.  $1/[\text{Mg}^{2+}]_{\text{free}}$  and  $r/[\text{Mg}^{2+}]_{\text{free}}$  vs.  $r$ ) are shown in Figs. 4.1 and 4.2 respectively.

Of course we would not normally do both, but this is done here for the sake of completeness.

From both plots we obtain the result that  $K_d = 50 \mu\text{mol dm}^{-3}$  or  $50 \times 10^{-6} (\text{mol dm}^{-3})$ .† It is noticeable that in the ‘double reciprocal plot’ (Fig. 4.1) the experimental points are much more unevenly spaced than in the alternative plot (Fig. 4.2). This has led many workers to prefer the type of plot shown in Fig. 4.2 for the analysis of binding data, since it is rather easier in this case to draw the best straight line through the experimental points. In any experiment it is important to make a proper analysis of the distribution of errors in the method of plotting the data. This is also true in the analogous plots which are used in the analysis of enzyme kinetic data (see Chapter 10) and is discussed in the books by Cornish-Bowden mentioned in the reading list.

It is possible to simplify the experiment considerably if one component is present in a considerable excess over the other. For instance, suppose that P is present at a concentration of  $1 \mu\text{mol dm}^{-3}$  and [A] is varied from  $50 \mu\text{mol dm}^{-3}$  up to  $500 \mu\text{mol dm}^{-3}$ . Then, throughout the titration very little of the total A is actually bound to P and it is a very good approximation to write  $[\text{A}]_{\text{free}} = [\text{A}]_{\text{total}}$ . The equations would then become

$$\frac{1}{r} = 1 + \frac{K_d}{[\text{A}]_{\text{total}}} \quad \text{and} \quad \frac{r}{[\text{A}]_{\text{total}}} = \frac{1}{K_d} - \frac{r}{K_d}.$$

We often use this simplification in enzyme kinetic work. The substrate (S) of the enzyme is almost always greatly in excess over the enzyme concentration. (i.e.  $[\text{S}]_{\text{free}} = [\text{S}]_{\text{total}}$ .) In this type of work, we use the velocity of the enzyme catalysed reaction ( $v$ ) to give a measure of  $r$  (the amount of S bound to E) in the equations, since only the ES complex shows enzyme activity. We shall see in Chapter 10 that we do in fact plot  $1/v$  vs.  $1/[\text{S}]_{\text{total}}$  or  $v/[\text{S}]_{\text{total}}$  vs.  $v$  to obtain the *Michaelis constant* which characterizes the interaction of the enzyme with its substrate.‡

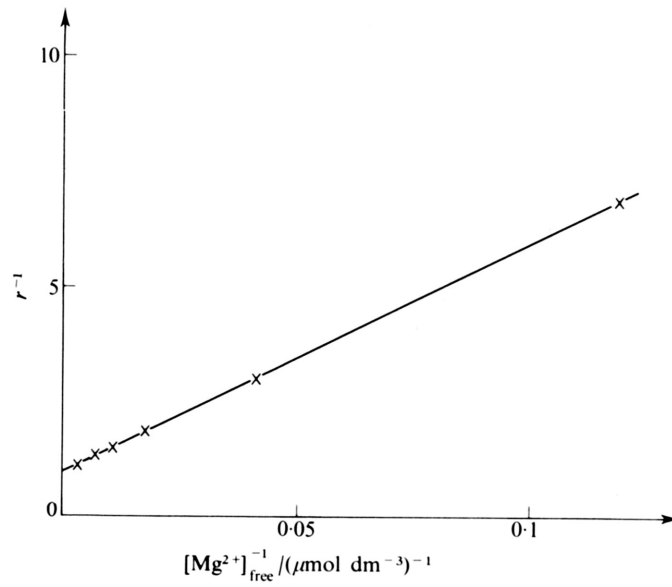
The simplification of the algebra which is achieved by setting  $[\text{S}]_{\text{free}}$  equal to  $[\text{S}]_{\text{total}}$  is illustrated in the following example.

### Worked example

Consider the equilibrium  $\text{E} + \text{S} \rightleftharpoons \text{ES}$ , and let  $K_s$  be the dissociation constant of the ES complex.

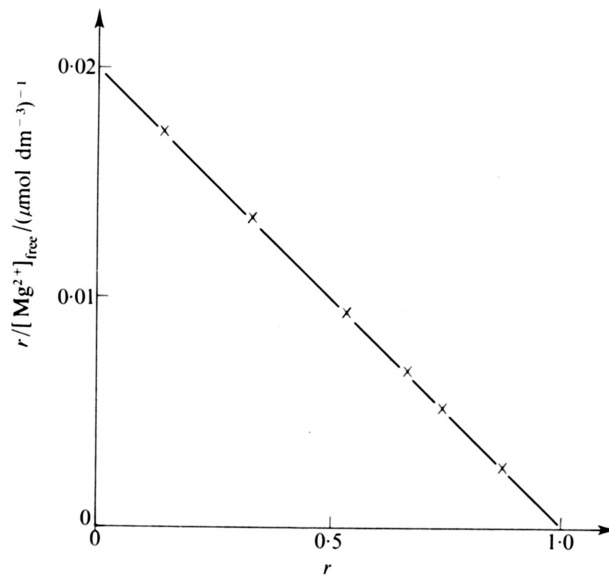
† Strictly speaking,  $K_d$  is dimensionless, as is pointed out earlier in Chapter 3. However, biochemists invariably quote units, i.e.  $K_d = 50 \mu\text{mol dm}^{-3}$ . Referred to a  $1 \text{ mol dm}^{-3}$  standard state, we could say  $K_d = 50 \times 10^{-6}$ . We shall adopt the convention of writing dissociation constants as for example,  $50 \times 10^{-6} (\mu\text{mol dm}^{-3})$  where the bracketed quantity refers to the standard state of a  $1 \text{ mol dm}^{-3}$  solution.

‡ *Cautionary note*: the Michaelis constant ( $K_m$ ) is not generally a true dissociation constant (see Chapter 10).



double-reciprocal plot

FIG. 4.1. Plot of binding data in 'Worked example' according to eqn (4.6).



single reciprocal plot

FIG. 4.2. Plot of binding data in 'Worked example' according to eqn (4.7).