Multiple binding site equilibria : Equivalent

If binding is very tight, then # binding sites can be determined by direct titration.

If binding is weak, equilibrium equations apply.

For case where one mole of P can bind up to n moles of A:

[2] $$r = \frac{n[A]}{K_d + [A]}$$ ($n = 1$ for only one binding site)

the n sites are assumed to be equivalent and independent (i.e., free energy of binding the same for each site).

K_d = average dissociation constant

Rearranging [2]:

(A) $$\frac{1}{r} = \frac{1}{n} + \frac{K_d}{n[A]}$$

plot $1/r$ against $1/[A]$ $\Rightarrow$ slope = K_d/n

y-intercept = $1/n$

(the Hughes-Klotz equation)

(B) $r/[A] = \frac{n}{K_d} - \frac{r}{K_d}$

plot $r/[A]$ against r $\Rightarrow$ slope $= -1/K_d$

x-intercept $= n$

(the Scatchard equation)

Worked example

In an experiment the concentration of an enzyme is kept constant at 11 μmol dm^{-3}, and the concentration of inhibitor [I] varied. The following results were obtained.

$[I]_{total}$(μmol dm^{-3})	5·2	10·4	15·6	20·8	31·2	41·6	62·4
$[I]_{free}$(μmol dm^{-3})	2·3	4·8	7·95	11·3	18·9	27·4	45·8

Determine the dissociation constant for the enzyme-inhibitor complex and the number of inhibitor binding sites on the enzyme.

Solution

At each value of $[I]_{total}$ we can evaluate $[I]_{bound}$ by subtraction; r is obtained by dividing $[I]_{bound}$ by the concentration of enzyme (i.e. 11 μmol dm^{-3}). The following table can be constructed:

$[I]_{total}$ (μmol dm^{-3})	5·2	10·4	15·6	20·8	31·2	41·6	62·4
$[I]_{free}$ (μmol dm^{-3})	2·3	4·8	7·95	11·3	18·9	27·4	45·8
$[I]_{bound}$ (μmol dm^{-3})	2·9	5·6	7·65	9·5	12·3	14·2	16·6
r	0·264	0·510	0·695	0·864	1·118	1·291	1·510
$\frac{1}{r}$	3·793	1·964	1·438	1·158	0·894	0·775	0·663
$\frac{r}{[I]_{free}}$ (μmol dm^{-3})$^{-1}$	0·115	0·106	0·087	0·076	0·059	0·047	0·033
$\frac{1}{[I]_{free}}$ (μmol dm^{-3})$^{-1}$	0·435	0·208	0·126	0·088	0·053	0·036	0·022

The two binding plots are shown in Figs. 4.3 and 4.4 respectively. From the 'double reciprocal plot' we find that the intercept on the y axis is 0·5, so that $n = 2$. The slope of the line is 7·6 so that $K_d = 15{\cdot}2 \times 10^{-6}$ (mol dm^{-3}).

From the 'Scatchard' plot (Fig. 4.4), again we find that $n = 2$ and the value of K_d is $15{\cdot}2 \times 10^{-6}$ (mol dm^{-3}). It is also clear that the sites are equivalent and independent, since, otherwise a curved plot would be expected.

As in the previous example (Mg^{2+} and ADP) we find that the Scatchard plot has a more even spacing of the experimental points, than does the 'double reciprocal'. (However, this need not always be the case.)

It is important to note that in order to determine the number of binding sites n accurately it is essential to cover as wide a range of the total saturation curve as possible. Roughly, the required range is the region

Problems:
Multiple equivalent binding sites;
*n*A and P

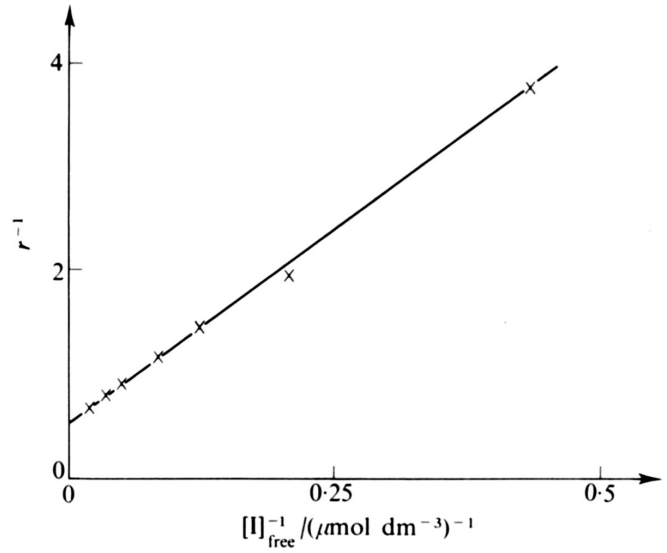

FIG. 4.3. Plot of binding data in 'Worked example' according to eqn (4.9).

double reciprocal plot
(Hughes-Klotz plot)

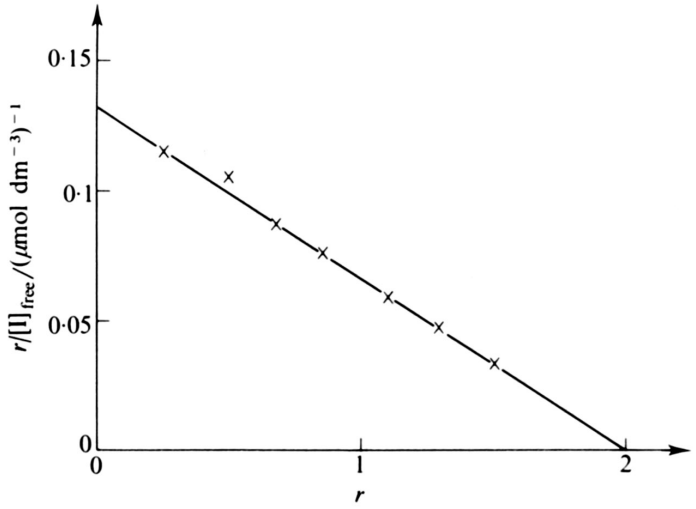

FIG 4.4. Plot of binding data in 'Worked example' according to eqn (4.10).

single reciprocal plot
(Scatchard plot)