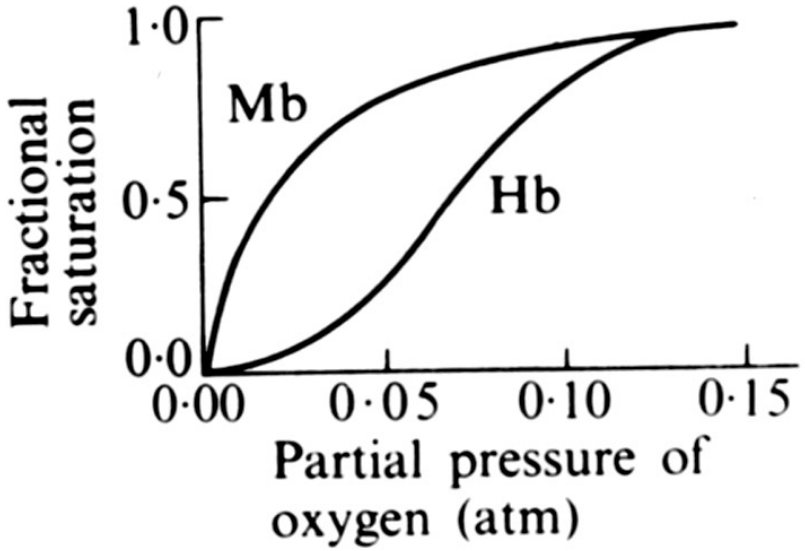
Multiple binding site equilibria: Non-equivalent
(Non-equivalent ligand sites on a macromolecule)

Non-linear binding plots: ligand binding sites are not equivalent

e.g. O_2 binding to hemoglobin — tetrameric protein (4 subunits)
sigmoidal binding curve vs
hyperbolic for myoglobin — monomeric protein (one subunit)

Hb displays positive cooperativity
(binding of first O_2 enhances the binding of subsequent O_2)

Type of binding can be recognized from inspection of saturation curve, double-reciprocal plot, or Scatchard plot.



O_2 saturation curves for myoglobin (Mb) and hemoglobin (Hb)

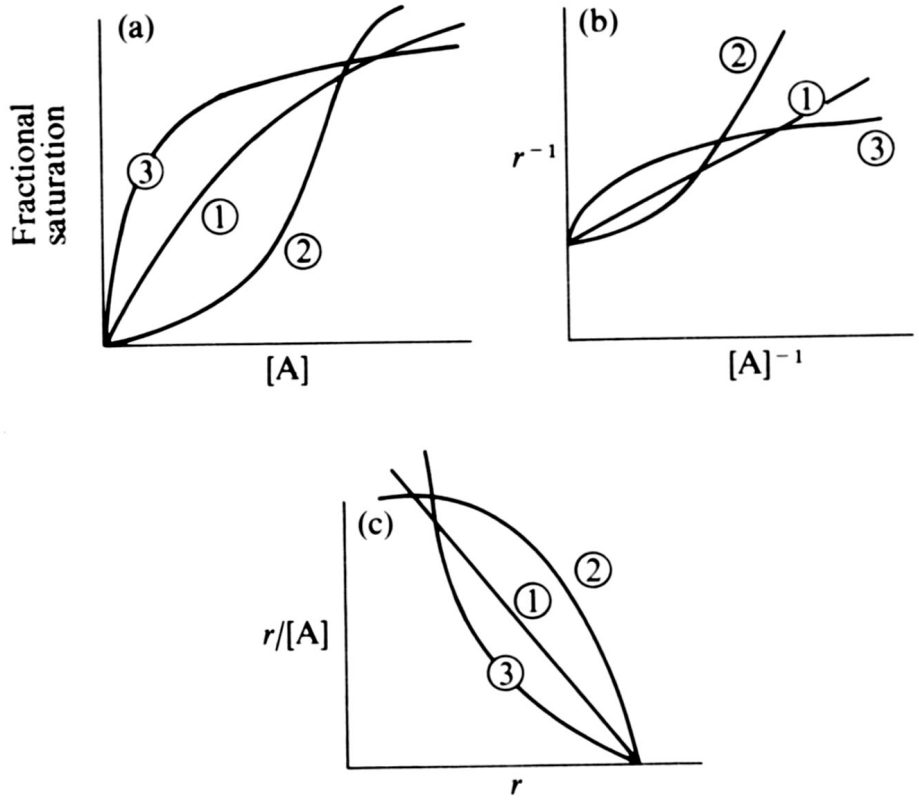


FIG. 4.6. Typical plots for binding data (a) saturation curve, (b) double reciprocal plot, (c) Scatchard plot. r represents the moles of A bound per mole of macromolecule and [A] represents the concentration of free ligand. Curves ①, ②, and ③ are typical for hyperbolic binding, positive co-operativity, and negative co-operativity respectively. Note in Fig. 4.6(a) it is difficult to distinguish between curves 1 and 3 hence the use of other plots.

Oxygen Binding by Myoglobin

$$Mb + O_2 \rightleftharpoons MbO_2$$

$$K = \frac{[Mb][O_2]}{[MbO_2]}$$ dissociation constant

fractional saturation, Y_{O_2} = fraction of O_2-binding sites occupied by O_2

$$Y_{O_2} = [MbO_2] / [Mb] + [MbO_2]$$

where $[Mb] + [MbO_2]$ = total concentration of Mb molecules in solution

$$Y_{O_2} = [O_2] / K + [O_2]$$

$[O_2]$ best represented as pO_2 (partial pressure)

$$Y_{O_2} = pO_2 / K + pO_2$$

if $P_{50} = pO_2$ when $Y_{O_2} = 0.5$, then substituting $\Rightarrow K = P_{50}$

thus: $$Y_{O_2} = pO_2 / P_{50} + pO_2 = \Theta$$

The Hill Equation

Protein E with n subunits:

$$E + nS \rightleftharpoons ES_n$$

(assumes infinite cooperativity)

$$K = \frac{[E][S]^n}{[ES_n]} = \text{dissociation constant} \qquad [1]$$

The fractional saturation, Y_S, is defined as the fraction of S binding sites occupied by S:

$$Y_S = \frac{n[ES_n]}{n[E] + n[ES_n]} \qquad [2]$$

Combining [1] and [2]:

$$Y_S = \frac{[E][S]^n/K}{[E](1 + [S]^n/K)}$$

or

$$Y_S = \frac{[S]^n}{K + [S]^n} = \text{Hill equation} \qquad [3]$$

describes the degree of saturation of a multi-subunit protein as function of ligand concentration

n } a non-integer parameter related to degree of cooperativity among interacting ligand binding sites = Hill constant

n ⇒ increases with degree of cooperativity

$n = 1$ ⇒ parabola (myoglobin) ⇒ non-cooperative

$n > 1$ ⇒ positively cooperative

$n < 1$ ⇒ negatively cooperative

Rearrange [3]:

$$\frac{Y_S}{1 - Y_S} = \frac{[S]^n}{K}$$

taking log of both sides:

$$\log\left(\frac{Y_S}{1 - Y_S}\right) = n \log [S] - \log K$$

plot $\log\left(\frac{Y_S}{1 - Y_S}\right)$ vs. $\log S$ ⇒ slope = n

x-intercept = $\log K / n$

Hill plot

For hemoglobin:

$$\log\left(\frac{Y_{O_2}}{1 - Y_{O_2}}\right) = n \log pO_2 - \log P_{50}$$