

PROTEIN FUNCTION: LIGAND BINDING - EXTRA QUESTIONS

1. These data relate the concentration of a ligand [L] and the extent of saturation of its binding site (θ) for two different proteins, A and B. Plot θ vs. [L] and determine whether the proteins exhibit hyperbolic or sigmoidal binding behavior.

[L] mM	θ for protein A	θ for protein B
0.10	2.2	0.3
0.35	7.2	1.0
0.80	15.0	3.0
1.80	29.0	9.0
3.00	40.0	25.0
4.50	50.0	50.0
5.75	56.0	76.0
8.00	64.0	90.0
13.00	74.0	97.0

2. The binding of a ligand (L) to a protein (P) is often a simple equilibrium, $P + L \rightleftharpoons PL$, which is characterized by the dissociation constant $K_d = [L][P]/[PL]$, where [L] is the concentration of unbound (free) ligand, [P] is the concentration of free protein (**empty binding sites**) and [PL] is the concentration of the protein-ligand complex (**occupied binding sites**). If $P_T = [P] + [PL]$ = total protein binding site concentration and $\theta = [PL]/P_T$, then $\theta = [L]/\{K_d + [L]\}$. However, a plot of θ vs. [L] is a hyperbola (see #1, protein A) and it is difficult to evaluate K_d from such a plot. Derive a linear transformation of $\theta = [L]/\{K_d + [L]\}$, and calculate what you need in order to replot the data from problem 1, **protein A**, on a linear plot. Determine K_d from the linear plot.
3. The binding of oxygen to hemoglobin involves an allosteric transition from a weak-binding form (T-state) to strong-binding form (R-state). One can evaluate the affinity of the R and T states for O_2 using a Hill plot in which the $\log\{\theta/(1-\theta)\}$ is plotted vs. $\log\{pO_2\}$, where θ = fractional saturation, and pO_2 = partial pressure of O_2 . Use these data to construct a Hill plot and determine the P_{50} (the pO_2 at which $\theta = 0.5$) for the T and R states.

pO_2 (torr)	θ	pO_2 (torr)	θ	pO_2 (torr)	θ
0.1	0.00315	2.88	0.24	12.88	0.969
0.35	0.0099	4.7	0.50	29.51	0.99
0.79	0.031	5.75	0.76	67.60	0.997
1.75	0.091	7.94	0.909		

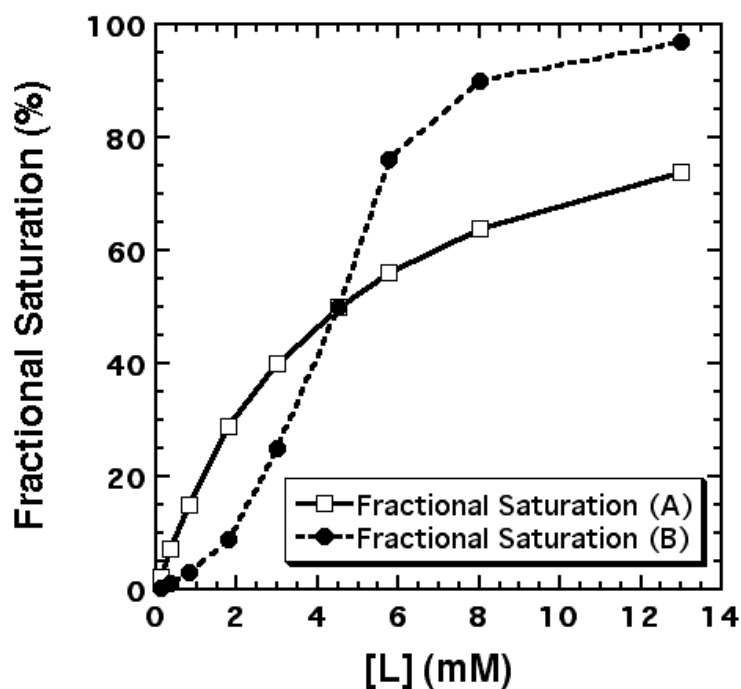
ANSWERS

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Answer:

See plot at the right. Protein A exhibits hyperbolic binding behavior; protein B exhibits sigmoidal binding behavior.

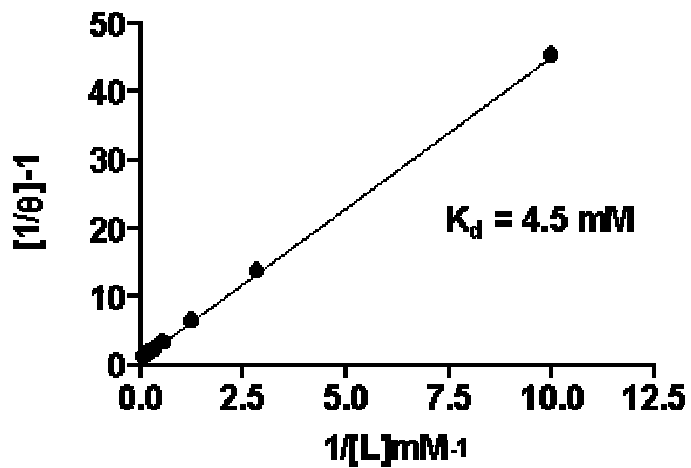


2. The binding of a ligand (L) to a protein (P) is often a simple equilibrium, $P + L \rightleftharpoons PL$, which is characterized by the dissociation constant $K_d = [L][P]/[PL]$, where [L] is the concentration of unbound (free) ligand, [P] is the concentration of free protein (**empty binding sites**) and [PL] is the concentration of the protein-ligand complex (**occupied binding sites**). If $P_T = [P] + [PL]$ = total protein binding site concentration and $\theta = [PL]/P_T$, then $\theta = [L]/\{K_d + [L]\}$. However, a plot of θ vs. [L] is a hyperbola (see #1, protein A) and it is difficult to evaluate K_d from such a plot. Derive a linear transformation of $\theta = [L]/\{K_d + [L]\}$, and calculate what you need in order to replot the data from problem 1, **protein A**, on a linear plot. Determine K_d from the linear plot.

Answer:

A linear transformation of this equation is $1/\theta = \{K_d/[L]\} + 1$, and a plot of $1/\theta$ vs. $1/[L]$ should be a straight line with slope = K_d . For this example,

$K_d = 4.5\text{mM}$.



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Answer:

The Hill plot is shown below, with both $\{\theta/(1-\theta)\}$ and pO_2 plotted on *logarithmic scales* instead of calculating $\log \{\theta/(1-\theta)\}$ and $\log \{pO_2\}$ for each point on the plot. By drawing an asymptote with a slope of 1.0 to the points at high values of θ and then extrapolating back to $\theta = 0.5$ (i.e., $\{\theta/(1-\theta)\} = 1$, so $\log \{\theta/(1-\theta)\}$ would be 0), one obtains P_{50} for the R-state. Using the same procedure with the points at low θ gives P_{50} for the T-state. (Units of pO_2 on plot are torr, i.e. mm Hg.)

