Introduction to Equilibrium Dialysis

Equilibrium dialysis is a specific application of the general phenomenon of dialysis that is important for the study of the binding of small molecules and ions by proteins. It is one of several methods currently available but its attractive feature continues to be its physical simplicity.

The objective of an equilibrium dialysis experiment is usually to measure the amount of a ligand bound to a macro-molecule. This is typically done through an indirect method because in any mixture of the ligand and macro-molecule, it is difficult to distinguish between bound and free ligand. If, however, the free ligand can be dialyzed through a membrane, until its concentration across the membrane is at equilibrium, free ligand concentration $C_{L\ (f)}$ and the following data can be measured:

Temperature (absolute)	T
Concentration of binding component, e.g. protein	$C_{P\ (o)}$
Starting concentration of ligand	$C_{L\ (o)}$
Final concentration of free ligand	$C_{L\ (f)}$

From which the following parameters can be derived directly:

Concentration of bound ligand	$C_{L(b)}$
Free fraction (of ligand)	f
Bound fraction (of ligand)	b
Degree of binding or saturation fraction	r

Data obtained from several experiments at a range of temperatures and with varying initial concentration of ligand can provide other binding parameters:

Association constant	K
Number of binding sites	n
Binding capacity	N

Further, the thermodynamics of the binding reaction can be derived:

Change of free energy	ΔG
Enthalpy change	ΔH
Entropy change	ΔS

Since equilibrium exists, the value $C_{L\ (f)}$ is the same on both sides of the membrane. *(Note: where charged species are involved the Gibbs-Donnan effect can upset the equilibrium unless moderately concentrated salts are in solution; say 0.6% NaCl).*

Hence: $$C_{L\ (o)} = C_{L\ (f)} + C_{L\ (f)} + C_{L\ (b)}*$$

**It is essential to correct this equation to take account of any ligand which might be bound to the membrane.*

$$C_{L\ (b)} = C_{L\ (o)} - 2 \times C_{L\ (f)}$$

The free fraction f is given by:

$$f = \frac{C_{L\ (f)}}{C_{L\ (o)} - C_{L\ (f)}}$$

The bound fraction b is: $b = 1 - f$

The degree of binding or saturation fraction r is:

$$r = \frac{C_{L\ (b)}}{C_{P\ (o)}}$$

If the protein concentration is known, the Scatched plot can be used to determine binding constants and the number of binding sites. If the protein concentration is unknown, the absolute number of binding sites is replaced by binding capacity N.

In the former case, values of r would be plotted on the abscissa against $r/C_{L\ (f)}$ on the ordinate. If only one class of binding sites is present, the Scatched plot results in a straight line with slope equal to $-K$ see Fig. 1.

The intercept on the abscissa give the value n. If two classes of binding sites are involved, the plot takes the form of an hyberbola. In this case, the asymptotes have slopes equal to $-K$ for each class of site, and their intercepts on the abscissa give the two values for n. The intercept between the curve and the abscissa is equal to the sum of the two values for n, see Fig. 2.

The free energy change is obtained simply by substituting the appropriate values in the following equation:

$$\Delta G = -RT\ln K$$

Where R is the gas constant.

ΔH can be obtained from a graph based upon an integrated form of the van/t Hoff equation.

$$\ln K = \frac{-\Delta H}{RT} + C$$

In this case a plot $\ln K$ versus $1/T$ has a slope of $-\Delta H/R$. Once a value for ΔH has been found it can be substituted into: $\Delta G = sH - T\Delta S$ to obtain a result for the entropy change ΔS.

Fig. 1.

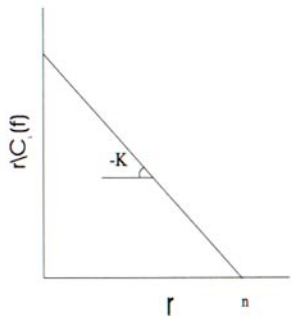
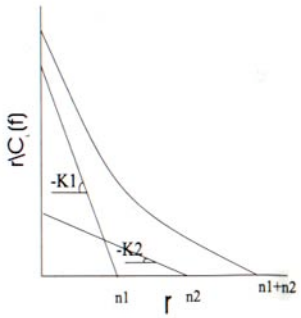


Fig. 2.



Scatchard, G., Am. N.Y. Acad. Sci. ***51****, 660-672 (1949)*
Rosenthal, H.E., Anal. Biochem. ***20****, 525-532 (1967)*
Weder, H.G., Schildknecht, J., Lutz, R.A. and Kesselring, P., Eur. L. Biochem. ***42****, 475-481 (1974)*
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