The Competition Dialysis Method. A Possible Application: The Determination of Association Constants for Complexes of Tightly Bound or Labile Apoproteins

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The competition dialysis is based on the competition for a ligand between a macromolecule and an excess of another molecule able to form a diffusible complex with this ligand. The procedure used the Colowick's cell and is proposed for determining association constants of complexes which contain tightly bound or labile apoproteins.

The measurement of the rate of dialysis has been developed by Colowick and Womack [1] for determination of the association constant of a macromolecule-ligand complex. The method is rapid, but is not reliable if the macromolecule used at the beginning of the measurements is already saturated by the ligand. Many metalloproteins are denatured when their metal is removed by the usual procedures. In order to avoid this difficulty, the time interval between the removal of the ligand and the measurements has to be reduced to several minutes. The competition dialysis method is expected to fulfill this requirement. The method is based on the competition between the macromolecule and an excess of a chelating agent able to form a diffusible chelate with the ligand. The repartition of the ligand between the complex and the chelate depends on their respective association constant and on the concentration of the total macromolecule and of the total chelating agent. If the chelate has a lower association constant and the chelating agent a higher total concentration, the latter binds a fraction of the total ligand which increases with increasing concentrations of the total ligand, while the fraction incorporated in the complex decreases. This property is used in the following procedure. In the apparatus described by Colowick and Womack the solution in the upper chamber initially contains an excess of the chelating agent and trace amounts of the labeled ligand. The number N_F of cpm is measured. Then the protein and successive amounts of unlabeled ligand are added according to the procedure of Colowick and Womack. The number N_i of cpm counted in the effluent corresponds to the part f_i of the ligand which is not protein bound and which is calculated according to the relation:

$$f_i = N_i/N_F .$$

The association constant is obtained from a Scatchard-type [2] plot of the values determined by Eqns (7) and (8) of the methematical appendix. One can expect that the major limit of the method is the low level of the radioactivity measured. Furthermore, some conditions given in the mathematical appendix must be respected.

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Mathematical Appendix

In a solution containing a substrate, a binding protein and an excess of diffusible binding molecules respectively in total concentration S, P, X, the total substrate concentration equals the sum of the concentrations S' of the free substrate, SP of the protein bound substrate, and SX of the chelate bound substrate:

$$S = S' + SX + SP . \tag{1}$$

The repartition of the total substrate in its three forms S', SX, SP, is ruled by the set of equations:

$$\frac{SP}{S'(P-SP)} = K_{SP} , \tag{2}$$

$$\frac{SX}{S'(X-SX)} = K_{SX} . \tag{3}$$

Therefore:

$$S' = \frac{SX}{(X-SX)K_{SX}} \tag{4}$$

and

$$\frac{SP}{S'} = \frac{SP}{SX}(X-SX)K_{SX} . \tag{5}$$

The association constant K_{SP} is determined from a Scatchard-type plot of a series of values SP_i/S'_i values [2]. For each total concentration S_i of substrate we have, if $SX_i \gg S'_i$:

$$SX_i = f_i S_i . \tag{6}$$

Therefore:

$$SP_i = (1-f_i)S_i \tag{7}$$

and according to (5), (6), and (7):

$$\frac{SP_i}{S'_i} = \frac{(1-f_i)}{f_i}(X - f_i S_i)K_{SX} . \tag{8}$$

Eqn (6) is valid only if $SX_i \gg S'_i$, *i. e.* for comparatively negligible concentrations of free ligand. In order to meet this condition, the chelate is chosen and added in such a way that $X \gg S$ and $K_{SX} \cdot X > 10^2$. Moreover, the following relation is recommended:

$$K_{SP}/K_{SX} \cong X/P . \tag{9}$$

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[1] S. P. Colowick and F. C. Womack, J. Biol. Chem. **244**, 774 [1969].

[2] G. Scatchard, Ann. N. Y. Acad. Sci. **51**, 660 [1949].