ION-BINDING MEASUREMENT BY ISOTOPIC EXCHANGE

C. M. Cho, J. Greco and E. Kaplan

Veterans Administration Hospital, Hines, Illinois
Loyola University, Stritch School of Medicine, Hines, Illinois
University of Illinois College of Medicine, Chicago, Illinois

In this paper we describe a method for determining the fraction of a diffusible ion in a compartment bound to a nondiffusible substance. This can be accomplished without separating the ion-binding substance from its equilibrium solution.

A theoretical consideration (*1*) leads us to the possibility of determining a fraction of the bound form of an ion in a sample by isotopic exchange. The method is based on the kinetic measurement of isotopic exchange by the continuously monitoring system developed in this laboratory (*2*). Total labile quantities must be determined by a separate method in order to calculate the quantities in bound and free forms.

Ion-binding characteristics of protein have been studied by equilibrium dialysis (*3,4*) and by ion-selective membrane electrodes (*5–7*).

The equilibrium-dialysis method requires knowledge of Donnan distribution and the chemical determination of the oppositely charged ion from the ion of prime interest. The average charge of the ionic species must also be known before computation of the electrostatically interacting quantities can be accomplished.

The membrane electrode method requires knowledge of ionic-activity coefficients, bi-ionic potential in the multi-ionic system and the availability of a truly permi-selective membrane.

The proposed method using isotopic exchange at equilibrium does not require the separation of a particular ion under investigation into bound and free forms for quantitative estimation. It has particular advantage in the study of an adsorption isotherm or binding of an ion in the presence of many ions.

THEORETICAL CONSIDERATION

The system to be considered is a tri-compartmental model at equilibrium

$$a \underset{k_1}{\overset{k_1}{\rightleftharpoons}} b \underset{k_2}{\overset{k_2}{\rightleftharpoons}} c$$

where a, b and c refer to the phases and k_1 and k_2 are the rates of exchange at equilibrium. If a and b are labeled while c is not labeled and the size of c is "infinitely" large, the change in the radioactivity in a and b can be expressed as

$$A \underset{\lambda}{\overset{\mu}{\rightleftharpoons}} B \xrightarrow{k} C$$

where A and B are the radioactivity and μ, λ and k are rate constants. The rate constants are related to k_1 and k_2 by the following relations

$$\mu = \frac{k_1}{M_a} \qquad \lambda = \frac{k_1}{M_b} \qquad k = \frac{k_2}{M_b}.$$

M_a and M_b are the concentrations of the ion in respective phases expressed in terms of total sample volume containing both phases a and b. In this paper phases a and b refer to bound and free forms of the ion under investigation and c is an "infinite" sink containing unlabeled ion which has an electrochemical potential identical to that present in phase b (b and c are in equilibrium); a and b are separated from c by a membrane. The rate equations for A and B will be

$$\frac{dA}{dt} = \lambda B - \mu A \tag{1}$$

and

$$\frac{dB}{dt} = \mu A - (k + \lambda)B. \tag{2}$$

The solutions are

$$A = \frac{A_0}{\rho_2 - \rho_1}(\rho_2 e^{\rho_1 t} - \rho_1 e^{\rho_2 t}) \tag{3}$$

and

$$B = \frac{B_0}{\rho_2 - \rho_1}\left[(k + \rho_2)e^{\rho_1 t} - (k + \rho_1)e^{\rho_2 t}\right] \tag{4}$$

where

$$\rho_1 = -\frac{1}{2}\left\{(\mu + \lambda + k) - \left[(\mu + \lambda + k)^2 - 4k\mu\right]^{1/2}\right\}$$

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and $$\rho_2 = -\frac{1}{2}\left\{(\mu+\lambda+k) + \left[(\mu+\lambda+k)^2 - 4k\mu\right]^{1/2}\right\}.$$

A_0 and B_0 are the initial activities of the ion in phases a and b, respectively. In obtaining Eqs. 3 and 4, the relationship that the initial specific activity in both phases are identical, $\lambda B_0 = \mu A_0$, was used. When $\frac{\mu A_0}{k_1}$ and $\frac{\lambda B_0}{k_1}$, the initial specific activities in both phases, are not equal, then a fraction of the ion under investigation in phase a does not participate in the process of isotopic exchange, and Eqs. 1 and 2 do not apply. If attention is directed to that fraction of the ion that is isotopically exchangeable and M_a is defined for that fraction, then μA_0 can always be made equal to λB_0. In this paper all definitions are based on these labile fractions only.

If phases a and b are separated from c and the radioactivity emitted from a and b is measured simultaneously, the registered activity will be proportional to A + B where

$$(A+B) = \frac{1}{2}(A_0+B_0)\left\{(e^{\rho_1 t} + e^{\rho_2 t}) + \frac{k_1 + k_2 f_a (f_a - f_b)}{[(k_1+k_2f_a)^2 - 4k_1k_2f_af_b]^{1/2}}(e^{\rho_1 t} - e^{\rho_2 t})\right\}. \quad (5)$$

ρ_1 and ρ_2 are expressed as

$$\rho_1 = -\frac{1}{2}\frac{M_a+M_b}{M_aM_b}\left\{k_1 + f_ak_2 - \left[(k_1+k_2f_a)^2 - 4k_1k_2f_af_b\right]^{1/2}\right\} \quad (6)$$

$$\rho_2 = -\frac{1}{2}\frac{M_a+M_b}{M_aM_b}\left\{k_1 + f_ak_2 + \left[(k_1+k_2f_a)^2 - 4k_1k_2f_af_b\right]^{1/2}\right\}.$$

In obtaining Eqs. 5 and 6 the relationships that

$$\frac{A_0}{A_0+B_0} = f_a = \frac{M_a}{M_a+M_b} \text{ and } \frac{B_0}{A_0+B_0} = f_b = \frac{M_b}{M_a+M_b}$$

were used. f_a and f_b in these equations are the fractions of the ion in phases a and b, respectively.

If an experimental condition is such that k_2 is very small, the over-all rate of isotopic exchange is governed by k_2 and is membrane-controlled. A proper choice of k_2 being rate-limiting can be experimentally accomplished. If this condition is fulfilled, then log (A + B) plotted against time will always become linear. Therefore the term $e^{\rho_2 t}$ does not contribute to the experimental semi-log plot. Under this condition Eq. 5 can be approximated as

$$\ln(A+B) \cong \ln(A_0+B_0) - f_b\frac{k_2}{M_b}t. \quad (7)$$

The exact form of Eq. 7 can be obtained from Eqs. 1 and 2 by putting

$$\frac{A_0}{B_0} = \frac{A}{B} = \frac{M_a}{M_b} = k'. \quad (8)$$

The sum of the two becomes

$$\frac{d}{dt}(A+B) = (1+k')\frac{dB}{dt} = -kB.$$

Therefore

$$A = A_0 e^{-\frac{k}{1+k'}t}, \qquad B = B_0 e^{-\frac{k}{1+k'}t} \quad (9)$$

and

$$\ln(A+B) = \ln(A_0+B_0) - f_b\frac{k_2}{M_b}t. \quad (10)$$

Equations 7 and 10 become identical.

Equation 10 was derived on the basis that the specific activity of the ion under investigation in both bound and free phases is identical during isotopic exchange despite the fact that the total specific activity is continuously diminishing. This condition is approached whenever the rate-limiting process of the isotopic exchange exists at k_2.

If the sample does not contain phase a (i.e. a standard solution alone), the measurable rate constant will provide information concerning k_2 at the concentration of M_b. The value of f_b, the fraction of the ion in a free state, can then be obtained by taking the ratio of the two rate constants, one with and the other without ion-interacting substance. For practical application to determine the free fraction,

$$f_b = \frac{R(\text{sample})}{R(\text{standard})}$$

and the bound fraction

$$f_a = 1 - f_b$$

where R(standard) and R(sample) are the measured rate constants of isotopic exchange for a standard (not containing binding substance) and a sample (containing a binding substance).

DISCUSSION

The kinetic equations derived above hold true only if there is no gradient of specific activity within or outside the sample. The condition can be attained

by rigorously shaking the sample and "infinite" sink. The appearance of multi-exponential curves for a non-mixed system has previously been discussed (*1,8,9*).

In obtaining the value of f_b, the membrane characteristic k_2 was assumed to be unaffected by the kind of sample and the presence of ion-interacting substance within the membrane container. If, however, the value of k_2 is affected by the sample, the ratio of the two rate constants does not provide the information concerning f_b. A proper choice of the membrane should be made to eliminate the possible error due to this effect.

In situations in which the concentration of the ion in phase c, M_c, can only be approximated to that of b, M_b, the electro-chemical potential of the ion in two phases, is not equal. This does not complicate the measurement of f_b if proper estimates of M_b by chemical analysis of the total concentration, $M_a + M_b$, in the sample is made. If M_c is chosen in such a manner that no net flux of the ion from phase c to b takes place, then the measured rate constant is generally not significantly different from the "true" rate constant as measured when the electro-chemical potential of the ion in b and c is identical. This confirms the fact that the diffusion constant and self-diffusion constant of an ion are known to be approximately the same across an uncharged membrane in solutions of moderate concentration. For exact determination of the bound form of an ion whose diffusion and self-diffusion constants are greatly different, one should establish equilibrium conditions before any kinetic study of isotopic exchange. Equilibrium applies also to ions other than those to be measured.

The proposed method has particular advantage in the study of adsorption isotherm or the binding of an ion in the presence of many other ions. The method does not require the separation of ion-interacting substance from its equilibrium solution.

SUMMARY

A method to determine the fraction of an ion to ion-binding substance in aqueous solution is presented. The principle of the method is based on altering the isotopic-exchange constant across a rate-limiting membrane due to interaction of the diffusible ion with ion-binding substance. The relationship between the degree of alteration in the isotopic-exchange constant and the fraction of the bound form was derived.

The proposed method does not require the separation of a particular ion under investigation into bound and free forms for quantitative estimation. The presence of other ions does not interfere.

Some limitations concerning the alteration in the membrane permeability due to interaction between ion-binding substance and membrane and the right choice of dialyzate concentration are also discussed.

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