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KINETICS OF THE PUMP-LEAK SYSTEM OF TRANSPORT IN THE OCULAR LENS,

DERIVED FROM CLASSIC ENZYME KINETICS AND DIFFUSION THEORY

BY

Ian McLean
Institute of Biological Sciences
Oakland University
Rochester, Michigan 48063

Richard Conley La Force
Mayo Foundation
Rochester, Minnesota 55901

V. Everett Kinsey
Institute of Biological Sciences
Oakland University
Rochester, Michigan 48063

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Culture techniques have proved to be useful for measuring the entrance and exit of substances into and out of the ocular lens. Interpretation of the results obtained by these methods has often been based on the general assumption that the lens functions as a pump-leak system (Kinsey and Reddy, 1963) (Kinsey and McLean, 1964) (Kinsey, 1965).

Expressed mathematically, the hypothesis states

$$\frac{d[S]_L}{dt} = K_p [S]_M + K_d \left([S]_M - [S]_L \right) \quad (1)$$

$$\frac{d[S]_M}{dt} = -\frac{Vol_L}{Vol_M} \frac{d[S]_L}{dt} \quad (2)$$

The meaning of the symbols and those used subsequently are shown in Table 1.

The first term on the right side of equation (1) describes active transport by the pump and the second term describes the leak by diffusion. The assumption is made that active transport involves a carrier and that diffusion occurs down a chemical gradient.

The purpose of the present study is threefold. First, to derive a relationship between the transfer coefficient of the pump, K_p , and the concentration of substrate and inhibitor ions in the media using classic Michaelis-Menten kinetics; second, to describe the leak, not only in terms of the chemical gradient but of the electric gradient and permeability characteristics of the lens as well; and, third, to outline a method whereby these relationships can be used to interpret lens culture data in terms of the various pump and diffusion parameters.

THEORY

The pump is believed to be in the epithelium (Kinsey and Reddy, 1965) and is described as an enzyme-substrate-inhibitor system using the mathematical treatment devised by Michaelis and Menten (1913).

$$\frac{d[ES]_{Ep}}{dt} = k_1 \left([S]_M [E - ES - EI]_{Ep} \right) - (k_2 + k_3) [ES]_{Ep} \quad (5)$$

$$\frac{d[EI]_{Ep}}{dt} = k'_1 \left([S]_M [E - ES - EI]_{Ep} \right) - (k'_2 + k'_3) [EI]_{Ep} \quad (6)$$

At steady state

$$\frac{d[ES]_{Ep}}{dt} = 0 \quad (7)$$

and

$$\frac{d[EI]_{Ep}}{dt} = 0 \quad (8)$$

By definition

$$K_m = \frac{k_2 + k_3}{k_1} \quad (9)$$

$$K_i = \frac{k'_2 + k'_3}{k'_1} \quad (10)$$

Solving equations (5) and (6) for K_m and K_i under steady state conditions it follows that

$$K_m = \frac{[S]_M ([E]_{Ep} - [ES]_{Ep} - [EI]_{Ep})}{[ES]_{Ep}} \quad (11)$$

$$K_i = \frac{[I]_M ([E]_{Ep} - [ES]_{Ep} - [EI]_{Ep})}{[EI]_{Ep}} \quad (12)$$

Solving equations (11) and (12) for $[ES]_{Ep}$ and $[EI]_{Ep}$, respectively, gives

$$[ES]_{Ep} = \frac{[E]_{Ep} [S]_M - [EI]_{Ep} [S]_M}{K_m + [S]_M} \quad (13)$$

$$[EI]_{Ep} = \frac{[E]_{Ep} [I]_M - [ES]_{Ep} [I]_M}{K_i + [I]_M} \quad (14)$$

Substituting equation (14) into equation (13) and simplifying gives

$$[ES]_{Ep} = \frac{[E]_{Ep} [S]_M K_i}{K_m K_i + K_i [S]_M + K_m [I]_M} \quad (15)$$

Since the velocity of the pump is analogous to the rate of breakdown of the enzyme substrate complex in the epithelium

$$K_3 [ES]_{Ep} = K_p [S]_M Vol_L \quad (16)$$

and

$$K_3 [E]_{Ep} = V_{max} \quad (17)$$

then in terms of substrate and inhibitor the transfer coefficient of the pump

$$K_p = \frac{V_{max} S}{Vol_L} \left(\frac{K_i}{K_m K_i + K_i [S]_M + K_m [I]_M} \right) \quad (18)$$

In the absence of an inhibitor, where $[I]_M = 0$

$$K_p = \frac{V_{max} S}{Vol_L} \left(\frac{1}{K_m + [S]_M} \right) \quad (19)$$

Analogously, in the absence of substrate where $[S]_M = 0$

$$K_p = \frac{V_{max_S} K_i}{Vol_L K_m} \left(\frac{1}{K_i + [I]_M} \right) \quad (20)$$

Equation (18) is the general expression for the pump in terms of the mechanism by which it is assumed to operate as a function of both concentration of the substrate and the inhibitor.

The pump-leak system, as previously formulated, took only chemical gradients into account (equation (1)) when describing the diffusion in and out of the lens (leak). The electric potential of approximately 45 mV, known to exist between the inside of the lens (negative) and the aqueous humor (Brindley, 1956; Sperelakis and Potts, 1959) must also affect the movement of ions in and out of the lens and tend to enhance the movement of cations into the lens and retard that of anions.

Both chemical and electrical forces can be taken into account when describing the leak by means of the Planck-Nernst equation where the diffusional flux of ions in the presence of an electrical field is given by

$$J = -D \left(\frac{\partial [S]}{\partial x} + H[S] \right) \quad (21)$$

At steady state the flux is constant across the membrane, consequently

$$\frac{\partial J}{\partial x} = 0 \quad (22)$$

If it is assumed that the electric field is also uniform,

$$\frac{\partial H}{\partial x} = 0 \quad (23)$$

Equation (21) can be integrated as follows:

Let

$$j = \frac{-J}{D} = \frac{\partial [S]}{\partial x} + H[S] \quad (24)$$

and let

$$y = [S] - j/H \quad (25)$$

then

$$\frac{\partial y}{\partial x} = \frac{\partial [S]}{\partial x} \quad (26)$$

from equation (24)

$$\frac{\partial [S]}{\partial x} = j - H[S] \quad (27)$$

then

$$\frac{\partial y}{\partial x} = j - H[S] \quad (28)$$

Since j and H are both independent of x

$$j = -Hy + H[S] \quad (29)$$

Substituting equation (29) into (28)

$$\frac{\partial y}{\partial x} = -Hy \quad (30)$$

and

$$\frac{\partial y}{y} = -H \partial x \quad (31)$$

Integrating both sides

$$\ln y = -Hx + C \quad (32)$$

and

$$y = Ce^{-Hx} \quad (33)$$

where C equals a constant of integration.

Substituting equation (25) into (33) produces an expression for j in terms of $[S]$ and H

$$[S] - \frac{j}{H} = Ce^{-Hx} \quad (34)$$

The constant of integration C and the value of the flux j can be explicitly evaluated by setting [S] equal to its boundary values at $x = 0$ and at $x = \Delta x$. At the outside surface of the membrane $x = 0$ and $[S] = \pi[S]_M$ where π equals the partition coefficient between membrane and media. Solving equation (34) at $x = 0$

$$\pi[S]_M - j/H = C \quad (35)$$

At the inside of the membrane

$$x = \Delta x \text{ and } [S] = \pi[S]_L$$

Solving for equation (34) at $x = \Delta x$

$$\pi[S]_L - j/H = C e^{-H\Delta x} \quad (36)$$

Substituting equation (35) into (36) produces

$$\pi[S]_L - j/H = \left(\pi[S]_M - j/H \right) e^{-H\Delta x} \quad (37)$$

and rearranging

$$j = \frac{\pi H}{1 - e^{-H\Delta x}} \left([S]_L - [S]_M e^{-H\Delta x} \right) \quad (38)$$

Substituting $-J/D$ for j

$$J = \frac{\pi D H}{1 - e^{-H\Delta x}} \left([S]_L - [S]_M e^{-H\Delta x} \right) \quad (39)$$

However,

$$H = \frac{nF}{RT} \frac{\partial v}{\partial x} \quad (40)$$

Integrating equation (40) between limits 0 and Δx

$$H\Delta x = \frac{nF_V}{RT} \quad (41)$$

Substituting into equation (39) gives

$$J = \frac{\frac{D\pi}{\Delta x} \frac{nF_V}{RT}}{1 - e^{-\frac{nF_V}{RT}}} \left([S]_M e^{-\frac{nF_V}{RT}} - [S]_L \right) \quad (42)$$

Since the rate of change of concentration in the lens due to diffusion is equal to $\frac{A}{Vol_L} J$, equation (1) may be rewritten in the form

$$\frac{d[S]_L}{dt} = K_p[S]_M + P\beta \left([S]_M - [S]_L \right) \quad (43)$$

where

$$P = \frac{\pi D A}{\Delta x Vol_L} \quad (44)$$

$$\beta = \frac{\frac{nFV}{RT}}{1 - e^{-\frac{nFV}{RT}}} \quad (45)$$

$$\text{and } \alpha = e^{-\frac{nFV}{RT}} \quad (46)$$

Thus influx by diffusion is $P\beta\alpha [S]_M Vol_L$

and outflux by diffusion is $P\beta [S]_L Vol_L$

The movement of labeled substrate, non-labeled substrate, and inhibitor ions in both the lens and media can be described from equations (2), (18), and (43) by six simultaneous differential equations which are as follows:

$$\frac{d[*S]_L}{dt} = \frac{V_{max_S} K_i [*S]_M}{Vol_L (K_m K_i + K_i [S]_M + K_m [I]_M)} + P_S \beta \left([*S]_M - [*S]_L \right) \quad (47)$$

$$\frac{d[*S]_M}{dt} = - \frac{Vol_L}{Vol_M} \frac{d[*S]_L}{dt} \quad (48)$$

$$\frac{d[S]_L}{dt} = - \frac{V_{max_S} K_i [S]_M}{Vol_L (K_m K_i + K_i [S]_M + K_m [I]_M)} + P_S \beta \left([S]_M - [S]_L \right) \quad (49)$$

$$\frac{d[S]_M}{dt} = - \frac{Vol_L}{Vol_M} \frac{d[S]_L}{dt} \quad (50)$$

$$\frac{d[I]_L}{dt} = \frac{V_{max_I} K_m [I]_M}{Vol_L (K_m K_i + K_i [S]_M + K_m [I]_M)} + P_I \beta \left([I]_M \alpha - [I]_L \right) \quad (51)$$

$$\frac{d[I]_M}{dt} = - \frac{Vol_L}{Vol_M} \frac{d[I]_L}{dt} \quad (52)$$

Equations 47 through 52 are based on the assumption that the lens functions as a pump-leak system, where the pump is described in terms of Michaelis-Menten kinetics and the leak in terms of the electrochemical gradients and the permeability properties of a passive membrane. They contain six unknowns, V_{max_S} , V_{max_I} , K_m , K_i , P_S , and P_I . These terms characterize the transport and passive diffusion of ions in ocular lenses.

From knowledge of the first four terms, i.e. those concerned with the pump, it is possible to deduce whether the same or different enzyme carriers are involved in transporting the substrate and the inhibitor and also whether inhibition is competitive or non-competitive.

By evaluating the last two coefficients, i.e. those concerned with the leak, it is possible to decide whether diffusion into the lens is compatible with diffusion across an uncharged membrane, since in this event P is constant with respect to concentration. The relative role of frictional^(D) and absorbtive(π) forces in the passive flux of ions in and out of the lens can also be determined from knowledge of the ionic radius and the magnitude of the permeability constant P and partition coefficient π .

The solution of equations 47 through 52 requires knowledge of the rate of change of concentration of labeled substrate, substrate, and inhibitor. Culture experiments usually provide information concerning only the rate of

change of concentration of the labeled substrate or inhibitor. The rate of change of concentration of the non-labeled species must, therefore, be calculated from data obtained with labeled substrate, a procedure that requires a number of successive approximations.

Under the condition that the concentration of non-labeled ions remains constant during the course of the experiment, equations (47) and (48) adequately describe movement of ions in the system. Substituting equation (18) into equation (47),

$$\frac{d[*S]_L}{dt} = K_p [*S]_M + P\beta \left([*S]_M \alpha - [*S]_L \right) \quad (53)$$

There are four unknowns α , β , P and K_p in equation (53). Values for α and β are determined by the transmembrane or fiber potential (equations (45) and (46)) which can be determined experimentally using microelectrodes. P is estimated from the slope of the tangent drawn at zero time to experimental data showing rate of efflux. Using the value for P so determined, a value for K_p is then found for a particular concentration of substrate by solving equations (48) and (53) using an analog computer to fit curves that show rate of accumulation of labeled substrate.

A more exact value of P is then determined using the above value for Kp, by solving the equations for the value of P which gives the best fit to the efflux data. The successive approximation is continued by determining a more accurate value for Kp ^{by} again fitting data showing the rate of accumulation.*

In this way, a relatively close approximation of Kp can be determined for a given concentration of either substrate or inhibitor in the media. Similar methods can be used to determine approximate values for Kp for other concentrations of substrate and inhibitor, thereby obtaining Kp as a function of the concentration of substrate or inhibitor. Assuming that the pump involves a carrier whose properties are limited by Michaelis-Menten

* Similarly, approximate values for Kp and P can be obtained without the use of an analog computer by solving the integrated form of equations (48) and (53) using the method of successive approximation which appear above.

$$[S]_L Q = \left(K_p + P\beta\alpha \right) \left([S]_{M_t=0} + \frac{Vol_L}{Vol_M} [S]_{L_t=0} \right) - \left[K_p [S]_{M_t=0} + P\beta \left([S]_{M_t=0} - [S]_{L_t=0} \right) \right] e^{-Qt} \quad (54)$$

where

$$Q = \left[\frac{Vol_L}{Vol_M} \left(K_p + P\beta\alpha \right) + P\beta \right] \quad (55)$$

kinetics, it is possible to describe K_p in terms of its components V_{max} , K_m and K_i , as indicated in equation (18).

A graphic method that involves the following linear transformation is employed to evaluate the coefficients. In the absence of an inhibitor, i.e. $[I]_M = 0$, equation (18) reduces to

$$K_p = \frac{V_{max} S}{Vol_L} \left(\frac{1}{K_m + [S]_M} \right) \quad (19)$$

Thus

$$\frac{1}{K_p Vol_L} = \frac{K_m}{V_{max} S} + \frac{[S]_M}{V_{max} S} \quad (56)$$

$\frac{1}{K_p Vol_L}$ is linearly related to $[S]_M$; when plotted, the slope is $\frac{1}{V_{max} S}$, the y intercept is $\frac{K_m}{V_{max} S}$ and the x intercept is $-K_m$.

Similarly, in the absence of substrate, i.e. when only tracer substrate ions are present, equation (20) states

$$K_p = \frac{V_{max} S}{Vol_L} \frac{K_i}{K_m} \left(\frac{1}{K_i + [I]_M} \right) \quad (20)$$

Thus

$$\frac{1}{K_p Vol_L} = \frac{K_m}{V_{max} S} + \frac{K_m [I]_M}{V_{max} S K_i} \quad (57)$$

In this case a plot of $\frac{1}{K_p Vol_L}$ against $[I]_M$ is linear and the slope is

$\frac{K_m}{K_i V_{max} S}$, the y intercept is $\frac{K_m}{V_{max} S}$ and the x intercept is $-K_i$.

Values of K_p obtained under the assumption that the concentration of the substrate and inhibitor do not change during the course of a culture experiment are used to determine the approximate values of V_{max} , K_m and K_i using the graphic method outlined above.

Approximate values for two additional coefficients V_{max_I} and P_I are then determined by treating data obtained with labeled inhibitor in a manner similar to that outlined for labeled substrate.

The approximate values for the six unknowns are now used to solve equations 47 through 52 using an analog computer. Because of the assumptions that are involved when determining the values for the coefficients, the curves that result give only approximate fits to the data. Small increments are made in the values of the parameters to improve the fit, thus providing values for the coefficients as exact as the data permit.

An alternate method of evaluating the six parameters might be considered.

Six experimental values of $[*S]_L$, for example, might be chosen at different times from smoothed experimental data and then substituted successively in equation (54). This would result in six simultaneous algebraic equations in six unknowns. These equations are transcendental but their solution can be found by numerical methods. The solution is the values of the six unknown parameters in the original equation.

The application of these methods to the system involved in transporting K, Rb and Cs in the rabbit lens will be described in a forthcoming paper.

SUMMARY

The pump-leak system of movement of ions in and out of the lens is described in terms of the parameters governing active transport and passive diffusion across membranes. The relation between the transfer coefficient of the pump and the concentration of the ion being transported (substrate) and inhibitor ion is derived from Michaelis-Menten kinetics. The leak is described in terms of the electrochemical gradient and permeability characteristics of the lens membrane. A method is outlined whereby these relationships can be used to interpret data from lens culture experiments in terms of the various parameters of the pump and leak.

TABLE 1

NOMENCLATURE

Symbol	Designation	Units
L	Lens	-
Ep	Epithelium of lens	-
M	Media	-
S	Substrate	-
I	Inhibitor	-
E	Enzyme carrier	-
[S]	Concentration of substrate	moles/L
[I]	Concentration of inhibitor	moles/L
[*S]	Concentration of labeled substrate	counts/min/ml
[E]	Concentration of total enzyme carrier	moles/L
[ES]	Concentration of enzyme substrate complex	moles/L
[EI]	Concentration of enzyme inhibitor complex	moles/L
[E-ES-EI]	Concentration of free enzyme carrier	moles/L
Kp	Transfer coefficient by active transport (pump)	hr ⁻¹
Kd	Transfer coefficient by diffusion (leak)	hr ⁻¹
K ₁	Rate constant of enzyme-substrate reaction	L/moles hr.
K ₂ & K ₃	Rate constants of enzyme-substrate breakdown	hr ⁻¹
K ₁ '	Rate constant of enzyme-inhibitor reaction	L/moles hr.
K ₂ ' & K ₃ '	Rate constants of enzyme-inhibitor breakdown	hr ⁻¹
Km	$\frac{K_2 + K_3}{K_1}$ = Michaelis-Menten constant for substrate	moles/L
Ki	$\frac{K_2' + K_3'}{K_1'}$ = Michaelis-Menten constant for inhibitor	moles/L
Vmax _S	Maximum velocity of pump for substrate	moles/hr/Lens

TABLE 1. (Continued)

Symbol	Designation	Units
$V_{\max_I}$	Maximum velocity of pump for inhibitor	moles/hr/Lens
J	Flux per unit area	moles/hr
D	Diffusion coefficient	cm^2/hr
H	Electric field strength = $\frac{nF}{RT} \cdot \frac{\partial v}{\partial x}$	dimensionless
v	Electric potential	volts
n	Charge	equivalents
F	Faraday constant	$\frac{96,500 \text{ coulombs}}{\text{equivalent}}$
R	Gas constant	Joules/degree
T	Absolute temperature	degrees, abs.
Δx	Thickness of "membrane"*	cm
A	Area	cm^2
Vol	Volume water in lens or media	cm^3
V	"Transmembrane" potential	volts
π	Partition coefficient between "membrane" and media	dimensionless
P	Permeability coefficient $\frac{D\pi A}{\Delta x \text{Vol}_L}$	hr^{-1}
α	$e^{-\frac{nFV}{RT}}$	dimensionless
β	$\frac{\frac{nFV}{RT}}{1 - \alpha}$	dimensionless
Q	$\frac{\text{Vol}_L}{\text{Vol}_M} \left(K_p + P\beta\alpha \right) + P\beta$	hr^{-1}

* The term "membrane" denotes the barrier in the lens across which movement occurs.

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