Asymmetries Generated by Diffusion and Reaction, and Their Bearing on Active Transport Through Membranes

(model/theoretical/Michaelis–Menten)

R. ARIS AND K. H. KELLER

Department of Chemical Engineering, University of Minnesota, Minneapolis, Minn. 55455

Communicated by Bryce Crawford, Jr., January 10, 1972

ABSTRACT  A model is presented that suggests that a natural, stable asymmetry may arise on either side of a membrane, even when the bulk conditions on either side of the membrane are the same and the two faces of the membrane are indistinguishable. Equations are derived on the assumption of Michaelis–Menten kinetics with substrate inhibition to describe the properties of such a model membrane.

The purpose of this note is to draw attention to a class of problems of simultaneous diffusion and reaction in membranes that is worth exploring as being possibly related to the theory of active transport* through biological membranes. Many theories of active transport [see, for example, Glynn (4), Mitchell (7), or Stein (11)] presume a significant difference between the two faces of a membrane in some factor, such as the rate of the ATP reaction, that will affect the binding of a solute or group to the carrier species. We wish to point out that just such an asymmetry may arise naturally and be maintained stably, even when the bulk conditions on either side of the membrane are the same and the two faces of the membrane are otherwise physically indistinguishable.

The underlying model was suggested by examples given by M. Marek in a seminar on asymmetrical solutions of symmetrical reaction problems. Similar solutions have been found for more complicated equations by Pismen and Kharkats (9) and Horn, Jackson, Martel, and Patel (5), but these do not really pertain to the biological situation.

Let $s$ be the concentration of the substrate $S$ whose reaction $S \rightarrow P$ differentiates the two sides of the membrane. The reaction $S \rightarrow P$ is catalyzed by an enzyme that is located on the two surfaces and obeys Michaelis–Menten kinetics with substrate inhibition, so that the velocity of the reaction, $v$, is given by

$$v = v(s) = k \left( 1 + \frac{K_m}{s} \right)$$

[1]

where $v =$ reaction rate per unit area of membrane,

$k =$ rate constant,

$K_m =$ Michaelis constant,

$K_s =$ inhibition constant.

* We define active transport as the movement of a species across a membrane from a reservoir of lower to one of higher chemical activity in a situation in which the energy required for the transport is supplied by a coupling between transport and metabolism.

Let suffixes $O$ and $a$ distinguish the conditions at the two surfaces of a membrane of thickness $a$ and let $s_f$ be the concentration of $S$ in the bulk fluid at some distance from either side. We assume that the substrate can diffuse to some degree through the thickness of the membrane (with diffusion coefficient $D$), but that it only reacts at the two surfaces, since the enzyme is localized there. If $k_r$ is a mass-transfer coefficient to the surface so that $k_r(s_f - s)$ is the rate at which substrate is transferred to the surface from the bulk fluid, then a balance at each face, expressing the fact that what reaches the surface from the bulk fluid either reacts there or is transferred to the other surface by diffusion, gives two equations:

$$x = O: \quad k_r(s_f - s_O) = v_O + D(s_O - s_a)/a \quad [2]$$

$$x = a: \quad k_r(s_f - s_a) = v_a + D(s_a - s_O)/a \quad [3]$$

With $v_O = v(s_O)$ and $v_a = v(s_a)$ given by Eq. [1], Eq. [2] and [3] can be solved for $s_a$ and $s_O$.

Consider first the symmetrical solutions $s_O = s_a = s$, for which the last terms vanish in Eq. [2] and [3]. To see this in a form that will be more useful for the general asymmetrical problem, the equations may be rewritten as

$$F(s) \equiv s_f - s - \frac{k_r}{k_r} \left( \frac{1 + K_m}{s} + \frac{s}{K_s} \right) = 0 \quad [4]$$

$F(s)$ is proportional to the difference between the rate of transfer of $S$ from bulk to surface and its rate of disappearance by reaction at the surface. It must, of course, be zero for a steady state to prevail. Eq. [4] is a cubic which, as O’Neill, Lilly, and Rowe have shown in another context (8), may well have three real roots in the range $O \leq s \leq s_f$ for suitable values of the other parameters. Fig. 1 shows a typical case for which there are three solutions, $s_1$, $s_{II}$, and $s_{III}$. From its definition, it can be seen that when $F(s)$ is positive, the concentration of $S$ near the surface will tend to increase and when $F(s)$ is negative, the opposite effect will occur. Thus, perturbations in the value of $s$ in the vicinity of $s_{II}$ will tend to grow so that the solution $s_o = s_a = s_{II}$ is unstable. However, the other two solutions are stable.

Indeed, in an analogous situation, Degn (2) has shown experimentally that in an open system, an enzyme-catalyzed reaction that exhibits the kinetic behavior postulated in this note does have two stable steady-states. In his system, the required dynamic balance occurs between the rate of supply of substrate to the system from its surroundings and the rate of disappearance of substrate through homogeneous reaction,
in accordance with an expression similar to Eq. [1]. Several workers (1, 6, 12) have noted that such a phenomenon may play an important role in processes of cellular differentiation.

Returning to Eq. [1], [2], and [3], we see that they may be written as

\[
F(s_0) = r(s_0 - s_a)
\]

\[
F(s_a) = r(s_a - s_s),
\]

or, by adding and subtracting them,

\[
F(s_0) + F(s_a) = 0, \quad [5]
\]

\[
\{F(s_a) - F(s_0)\}/[s_a - s_s] = 2r, \quad [6]
\]

\[r = (D/k_a) = (1/k_a)/(a/D)\] is the ratio of the diffusional resistance of the concentration boundary layer adjacent to the membrane to the diffusional resistance of the membrane itself. If \(r = 0\), the two sides of the membrane are not in communication with one another and behave independently. The possible solutions are that \(s_a\) and \(s_s\) can be any pair of \(s_0, s_1, s_2\), or of \(s_1, s_2, s_3\); of course, only the symmetric pairs \((s_1, s_2), (s_1, s_2), (s_1, s_2)\), and the asymmetric pairs \((s_1, s_3), (s_2, s_3)\), \((s_3, s_0)\) are stable. The asymmetric pairs are the ones of interest, since they differentiate the two sides of the membrane.

If \(r\) is small [certainly less than half the greatest slope of \(F(s)\)], Eq. [5] and [6] will still be satisfied by the symmetric pairs \((s_1, s_2)\) and \((s_3, s_3, s_0)\), but it is evident that it is also possible to find three other sets of points that are solutions to Eq. [5] and [6] by virtue of the fact that the points are equidistant above and below the abscissa, and that the slope of the chord joining them is \(2r\). These solutions will be stable as long as neither of the end points in any pair is too close to \(s_0\). The extent of the unstable region in the vicinity of \(s_0\) depends upon the reaction and transfer parameters of the system. Stability can be assured if \(F'(s) < 0\) at both ends.

Typical symmetric and asymmetric solutions that are stable are shown in the lower parts of Fig. 1. From Eq. [1] and [4], it can be seen that the rate of reaction is proportional to \(\{s_f - s_0\} - F(s)\) which, graphically, corresponds to the vertical distance between the curve \(F(s)\) and the broken line \(s_f - s\). The rate is, therefore, large if the concentration at the site of reaction is near \(s_f\) and small if it is near \(s_{1f}\).

If the reaction that differentiates the two sides of the membrane is, for example, the ATP \(\rightarrow\) ADP reaction, then in the lower part of Fig. 1 the availability of energy is much greater on the left side of the second membrane than on the right and vice-versa for the third membrane shown. This kind of differentiation is fundamental to several theories of active transport (4, 7, 10, 13) that postulate a direct or indirect coupling of an ATPase to the translocation of other groups or solutes. Indeed, the assumption of substrate inhibition is consistent with the evidence of Garrahan and Glynn (3) on the inhibitory effect of increased ATP concentration of Na+ exchange.

Note that the actively transported solute is not the substance that is reacting asymmetrically at the two membrane faces. Because the model for the development of the asymmetry does not fix the nature of the transport coupling, it is consistent both with those theories that postulate vectorial chemical reaction within the membrane and those that rely on more conventional membrane diffusion phenomena (7).

It is clear, however, that the proposed model is not adequate by itself to explain all of the characteristics of any one active transport system. Nevertheless, it does suggest the importance of considering the dynamic balance between surface reactions and transport to (or from) the membrane surfaces to assess the origins of steady-state asymmetries in membrane behavior. The simple Michaelis–Menten model with substrate inhibition discussed here is only one of many that can lead to multiple stable steady-states. Szilard (12), for example, postulated a kinetic scheme involving deactivation of a repressor substance by the reaction product that also leads to two stable steady-states. One goal of future efforts should be to evolve schemes that are consistent with the available experimental observations of specific transport systems that, in cases such as that of Na+/K+ transport, are extensive (3, 4, 10, 13).

The remaining theoretical questions that would seem worthy of every effort to solve concern the transient conditions that give rise to the stable, asymmetric steady-states. There are also possibilities other than the perfectly symmetric external conditions that have been assumed here. For example, the cubic nature of the reaction rate curve implies that under the correct circumstances a very small difference between the values of \(s_f\) on the two sides of the membrane may lead to vastly different energy releases at the two faces, and that this difference can be maintained in a stable fashion. Such models might relate not only to the often observed specific and irreversible directionality of active transport, but also to the large variations in apparent membrane permeability sometimes observed with relatively small environmental perturbations. The possible relation of this latter phenomenon to biological control mechanisms makes it an area of particular interest.

Dr. John DeSimone of the Department of Chemical Engineering and Materials Science, University of Minnesota, offered several helpful suggestions during the preparation of this note that are gratefully acknowledged.