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The diagram methods for the evaluation of exchange fluxes in membrane transport systems

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In this paper theoretical methods for the evaluation of fluxes of ligand exchange processes in a transporter-mediated membrane transport system are studied. The exchange process of a transport system is defined as a set of reactions of the transporters in the membrane that do not result in a complete turnover and must include the following consecutive sequence of steps: the binding of ligands from bath 1 and a subsequent release of bound ligands to bath 2 followed immediately by a binding of ligands from bath 2 and a subsequent release of bound ligands to bath 1. Thus, unlike the ordinary one-way cycles, the completion of an exchange process does not result in a net transport of ligands across the membrane. However, since it exchanges the ligands between the two baths, the exchange process of a transport system is closely related to the operational tracer flux of labelled ligands in the system. In this paper, both the numerical and the analytical procedures for the evaluation of exchange fluxes in any given biochemical diagram are discussed. In particular, we show that the exchange fluxes of a given kinetic diagram, like one-way cycle fluxes, can be expressed analytically in terms of the rate constants of the diagram with the use of either the original diagram or an expanded diagram. The diagram methods presented in this paper should be very useful in analyzing the mechanisms of transporter-mediated transport systems when tracer flux data are available.

1. Introduction

The transport (active or passive) of nutrients across cell membranes is usually catalyzed by specific membrane proteins (the so-called transporters). In general, the kinetic properties of these transport systems can be studied by modelling a transporter with a finite number of discrete states undergoing first- or quasi-first-order reactions [1–4]. For example, the facilitated transport of a ligand across a membrane can be represented by the simple four-state model shown in fig. 1a. In active transport systems where the transport of one ligand is coupled to the transport of another, the kinetics may become rather complicated as illustrated in fig. 2a. The kinetic schemes in figs. 1b and 2b are called the 'biochemical kinetic diagram' (or just the diagram) of the transport system. As can be seen from these diagrams, one common property of all transporter-mediated transport systems is that the kinetic diagrams of these systems contain at least one cycle. With given rate constants, the operational steady-state flux of transport of ligands can be computed from the state probabilities of the diagram [1–4]. However, a more useful approach to the analysis of transport properties is to study the rate of net cycle completions of each individual cycle (cycle fluxes) in the diagram. For example, individual cycle fluxes of an active transport system are very useful in the theoretical study of force-flux relations, degree of coupling, thermodynamic efficiency, etc. [2–4].

The individual cycle fluxes of a diagram would not have been very useful, if they were not readily

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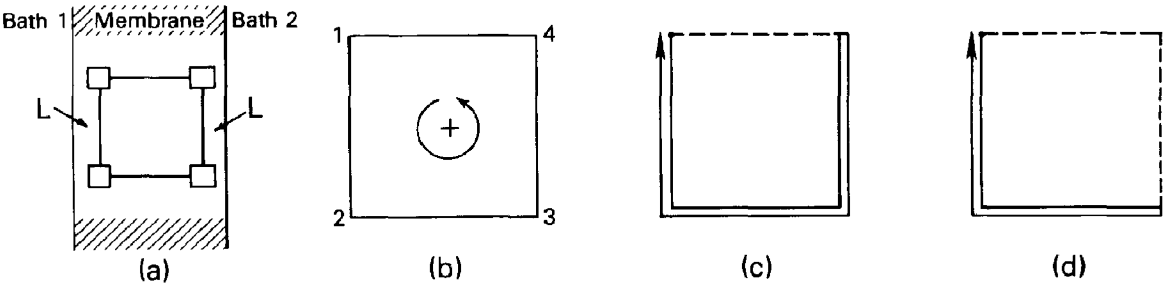

Fig. 1. (a) An illustrative ligand transport model. (b) The corresponding biochemical diagram of the model. Each line represents a pair of first-order (or quasi-first-order) rate constants. The + sign indicates the direction of the flow of ligand from bath 1 to bath 2. (c) The paths of the operational exchange process of the system. The end points of this process are states 1 and 4. The exchange steps are in 1 ⇄ 2 and 3 ⇄ 4. (d) The paths of the non-operational exchange process of the system.

calculable. With given rate constants, the state probabilities of the system at steady state can be obtained by solving the set of kinetic differential equations at infinite time. This method is not applicable to the calculation of net cycle fluxes. However, like the state probabilities, each cycle flux in a diagram can be expressed analytically in terms of rate constants using the diagram method of Hill [3–5]. For example, the net cycle flux of a particular cycle κ in a diagram can be expressed explicitly as

$$J_\kappa = (\pi_+ - \pi_-)\Sigma_\kappa/\Sigma \tag{1}$$

where Σ is the sum of directional diagrams for all states, π_+ and π_- the product of rate constants around the cycle in the + and − directions, respectively, and Σ_κ the sum of all feed-in parts of the cycle flux diagrams of κ. Both Σ_κ and Σ as well as π_+ and π_- in eq. 1 are functions of rate constants of the diagram. Thus, with given rate constants, the net fluxes of each cycle in a diagram can be obtained and the properties of the transport system can be studied quantitatively.

Besides the state probabilities and the net cycle fluxes, the fluxes of one-way cycles and exchange processes are also very important in studying transport mechanisms, because they are closely related to the tracer flux frequently measured in membrane transport studies. For example, in the simple ligand transport system in fig. 1a, if ligands in bath 1 are all labelled and those in bath 2 are not, then the initial increase of labelled ligands in bath 2 will not depend on the net cycle flux of the system, but on the flux of the one-way cycle in the + direction, 1 → 2 → 3 → 4 → 1, and the flux of the ligand exchange process, 1 → 2 → 3 → 4 → 3 → 2 → 1. In this exchange process, a transporter in state 1 can pick up a labelled ligand from bath 1, cross the membrane, release the labelled ligand to bath 2, pick up an unlabelled ligand from bath 2 (labelled ligands in bath 2 are negligible), cross the membrane, and release the ligand to bath 1 to return to state 1. Therefore, unlike the one-way

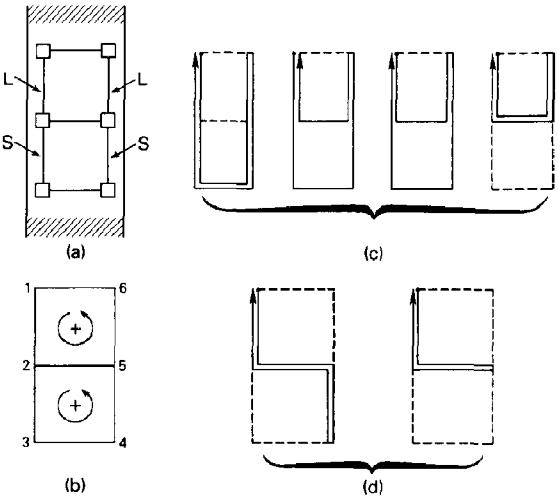

Fig. 2. (a) A model for the coupled transport of ligands L and S. (b) The corresponding biochemical diagram. (c) The four operational exchange processes for ligand L. (d) Two illustrative non-operational exchange processes of the system.

cycle, this process does not change the total number of ligands (labelled plus unlabelled) in the two baths. However, similarly to the one-way cycle in the + direction, it increases the number of labelled ligands in bath 2 by one. This is why exchange fluxes as well as one-way cycle fluxes are important in studying the mechanisms of a transport system with tracer data.

As discussed in a number of recent papers [5–8], each factor on the right-hand side of eq. 1 represents a one-way cycle flux (of different directions). Very recently, Hill [10] has shown that one-way cycle fluxes of any given diagram can also be obtained from an 'expanded' diagram derived after a diagram expansion procedure. Thus, analytical expressions for all one-way cycle fluxes of a given diagram can be derived readily either using the original or the expanded diagram. In contrast to the case of one-way cycles, the fluxes of exchange processes on a diagram have not been well studied. In studying the relation between the driving force and the flux ratio of an active transport model, Blumenthal and Kedem [9] discovered a diagram-expansion method in the derivation of the tracer exchange flux of the model (see section 3). In this paper, another procedure for the evaluation of general exchange fluxes based on the original diagram is presented. Since the original diagram is simpler than the expanded one, the present method is easier to use.

We begin in section 2 with a discussion on the formal definition and the numerical evaluation of the flux of a general exchange process in a biochemical kinetic diagram (the numerical method is useful in checking the diagram method). The diagram-expansion method of Blumenthal and Kedem is then reviewed in section 3. In section 4, the method based on the original diagram is presented. Finally, the limitation of the diagram methods is discussed in section 5.

2. The exchange processes of a diagram

As mentioned before, the kinetics of a transporter can be described by a graph (or diagram) specified by a set of points and arcs (or lines). Each point in the graph represents a state of the transporter and each arc between any two points represents the existence of a pair of forward and backward transitions. At steady state, each transporter in the transport system constantly undergoes transitions (a random walk) among the states on the diagram and through the completion of a particular walk sequence ligands may be transported from one bath to the other (a cycle completion) or exchanged between the two baths (an exchange process). To determine what walk sequence corresponds to an exchange process, it is necessary to define a few terms related to the graph theory.

A path in a graph is a non-empty sequence $[S_1, S_2, \ldots, S_n]$ of points (states) on the graph. S_1 and S_n are respectively denoted as the initial and terminal states of the path. A path is elementary if it does not meet the same point twice. That is, the states of an elementary path are all distinct. Thus, the path of a long random walk on a (finite) biochemical diagram (such as in figs. 1b and 2b) is expected to be non-elementary. When a state is met by the path the second time, the path is said to have made a 'return' to that state. For example, in the sequence 12342, the path has made a return to state 2. In general there are two kinds of return: immediate and non-immediate. An immediate return occurs when the path returns to the state just preceding the present one, such as in 121, 1232, etc. If the returned state is not the preceding one, the return is called non-immediate (such as in 12342, 12341, etc.). Thus, an elementary path is a path without any immediate or non-immediate return.

The arcs along a path are called the track of the path. The track of an elementary path that makes a non-immediate return to the initial point is the 'cycle' discussed above. That is, a cycle is a cyclic path that, apart from the coincidental initial and final points, does not meet the points twice.

Let the track of the elementary path $P = [S_1, S_2 \ldots, S_{n-1}, S_n]$ be denoted as T and that of $P' = [S'_1, S'_2, \ldots, S'_{m-1}, S'_m]$ as T'. Then, the composite track, $T + T'$, is called an exchange process, if

$$S_1 = S'_m,\ S_2 = S'_{m-1},\ S_{n-1} = S'_2,\ S_n = S'_1. \qquad (2)$$

That is, an exchange process is formed by two elementary paths with the first arc of the first path coinciding with the last arc of the second path and vice versa. The points S_1 (or S'_m) and S_n (or S'_1) are referred to as the 'end points' of the exchange process. The arcs between S_1 and S_2 and S_{n-1} and S_n are called the 'exchange steps' of the exchange process. These exchange steps are where the transporters bind ($S_1 \rightarrow S_2$ and $S_n \rightarrow S_{n-1}$) and release ($S_2 \rightarrow S_1$ and $S_{n-1} \rightarrow S_n$) the ligands in an actual transport system. One must note that the exchange process defined here is rather general in that it includes those that are not related to actual ligand exchange at all. For example, both fig. 1c and d represent exchange processes of the diagram in fig. 1b. However, only the former is a true ligand exchange process. An exchange process is called 'operational' if its exchange steps involve actual ligand binding and ligand desorption (fig. 1c) and 'nonoperational' otherwise (fig. 1d).

For convenience, an exchange process is referred to as 'simple' if path P' is exactly the reverse of P and as 'complex' if not. Thus, a complex exchange process contains at least one internal loop. For example, those in fig. 1c and d are simple exchange processes and as shown in fig. 2c there are two simple and two complex operational exchange processes for the system in fig. 2a with respect to ligand L.

The formal definition for an exchange process described above can be implemented easily into a computer simulation program. The key point is to recognize that an exchange process is composed of two independent elementary paths. Thus in Monte Carlo simulations both the immediate and the non-immediate returns have to be eliminated from the complete random walk record (for reduction to an elementary path, see ref. 5) and the elimination has to be carried out separately for each path. Other than this complication, the computer algorithm is very similar to that discussed in ref. 5. The results of a computer simulation on the diagram in fig. 1b are given below.

3. The evaluation of exchange fluxes using an expanded diagram

Recently, Hill [10–13] has reported the use of expanded diagrams in the evaluation of steady-state properties of diagrams, such as the one-way cycle fluxes, the mean first passage time to absorption, etc. In this section, the diagram expansion procedure discussed by Blumenthal and Kedem [9] in the evaluation of exchange fluxes will be reviewed. Before doing so, let us define the exact meaning of an expanded diagram.

Consider the isomeric reaction between states A and B (a diagram) with rate constants k and k':

$$\mathrm{A} \underset{k'}{\overset{k}{\rightleftarrows}} \mathrm{B} \tag{3}$$

If $N_{\mathrm{A}}(t)$ and $N_{\mathrm{B}}(t)$ are the number of molecules of A and B at time t, then $N_{\mathrm{A}}(t)$ and $N_{\mathrm{B}}(t)$ obey the differential equations:

$$\frac{\mathrm{d}N_{\mathrm{A}}}{\mathrm{d}t} = -kN_{\mathrm{A}} - k'N_{\mathrm{B}} \tag{4a}$$

$$\frac{\mathrm{d}N_{\mathrm{B}}}{\mathrm{d}t} = kN_{\mathrm{A}} - k'N_{\mathrm{B}}. \tag{4b}$$

Now consider the following set of reactions (also a diagram):

$$\mathrm{A}' \underset{k'}{\overset{k}{\rightleftarrows}} \mathrm{B} \overset{k}{\leftarrow} \mathrm{A}'' \tag{5}$$

where k and k' are the same as those in eq. 3. The kinetic differential equations for this system are:

$$\frac{\mathrm{d}N_{\mathrm{A}'}}{\mathrm{d}t} = -kN_{\mathrm{A}'} + k'N_{\mathrm{B}} \tag{6a}$$

$$\frac{\mathrm{d}N_{\mathrm{A}''}}{\mathrm{d}t} = -kN_{\mathrm{A}''} \tag{6b}$$

$$\frac{\mathrm{d}N_{\mathrm{B}}}{\mathrm{d}t} = k(N_{\mathrm{A}'} + N_{\mathrm{A}''}) - k'N_{\mathrm{B}} \tag{6c}$$

It is easy to show that eqs. 6 become identical to eqs. 4 when $N_{\mathrm{A}'}$ and $N_{\mathrm{A}''}$ are substituted with an N_{A} defined as

$$N_{\mathrm{A}'} + N_{\mathrm{A}''} = N_{\mathrm{A}} \tag{7}$$

That is, if only the sum of A′ and A″ (not the individual A′ and A″) is considered, the kinetic property of eq. 5 is exactly the same as that of eq. 3. In other words, if A′ and A″ are regarded as the substates of A (so that eq. 7 is satisfied), eq. 5 is kinetically equivalent to eq. 3 and is referred to as the expanded diagram of eq. 3.

If the transition B → A is put back, the right-hand side of eq. 5 is simply the mirror image of the left-hand side (if the primes on the As are neglected). In other words, eq. 5 can be obtained from eq. 3 by duplicating the part of the diagram that contains A and rate constants k and k' about the 'pivot' point B followed by removing the k' (the transition from the pivot point to the nearest point; henceforth referred to as the 'outbound' transition for brevity) from the expanded diagram. This procedure is called a 'one-pivot' expansion procedure. When a diagram is expanded by duplicating (the diagram) about two pivot points followed by removing an outbound transition from either the duplicated or the original line at each pivot point, the process is referred to as a two-pivot expansion procedure. Like one-pivot expansion, a two-pivot expansion also generates a diagram that is kinetically equivalent to the original diagram.

The procedure described below can be used to evaluate certain exchange fluxes of a diagram. The key of the procedure is a two-pivot diagram expansion operation just mentioned above. The procedure contains the following four steps:

(1) Identify the paths of all exchange processes (each contains two elementary paths, see above) characterized by the end points and the exchange steps.

(2) Duplicate the part of the diagram that contains the exchange paths using the two 'end' states of the exchange processes as the two pivot points. The tracks of all exchange processes of the system are therefore expanded into two populations connected by the two end points, forming cycles (see fig. 3a).

(3) Delete the outbound rate constant of each duplicated path at one pivot point and delete the outbound rate constant of each original path at the other pivot point (see fig. 3a).

(4) Each exchange process of the original diagram can be identified as a one-way cycle in the expanded diagram and its flux can be derived using the cycle flux formula in eq. 1.

This procedure applies to any exchange process in a diagram where the two 'exchange steps' of the exchange process are either absent simultaneously or present as a pair in any cycle of the diagram. For example, the procedure applies to both the operational exchange process in fig. 1c and the non-operational exchange process in fig. 1d, but not to the processes in fig. 2d. We have checked the procedure with several diagrams, but will only discuss one example here (see table 1 for another example). We consider the exchange process in fig. 1c and wish to show that the flux obtained from the procedure presented above is the true exchange flux of the system. In this case, the end points are states 1 and 4 and the exchange steps are 1 ⇄ 2 and 4 ⇄ 3.

Table 1

Analytical expressions for the terms on the right-hand sides of eqs. 16 and 17 for the exchange processes in fig. 1c and d

$$\begin{aligned}\Sigma = {} & \alpha_{43}\alpha_{32}\alpha_{21} + \alpha_{41}\alpha_{32}\alpha_{21} + \alpha_{41}\alpha_{23}\alpha_{34} + \alpha_{41}\alpha_{21}\alpha_{34} \\ & + \alpha_{43}\alpha_{32}\alpha_{12} + \alpha_{41}\alpha_{32}\alpha_{12} + \alpha_{41}\alpha_{12}\alpha_{34} + \alpha_{43}\alpha_{12}\alpha_{23} \\ & + \alpha_{41}\alpha_{12}\alpha_{23} + \alpha_{12}\alpha_{23}\alpha_{34} + \alpha_{14}\alpha_{43}\alpha_{21} + \alpha_{14}\alpha_{43}\alpha_{23} \\ & + \alpha_{14}\alpha_{43}\alpha_{32} + \alpha_{14}\alpha_{21}\alpha_{32} + \alpha_{14}\alpha_{23}\alpha_{34} + \alpha_{14}\alpha_{21}\alpha_{34}\end{aligned}$$

	Fig. 1c	Fig. 1d
π_{ex}	$\alpha_{12}\alpha_{23}\alpha_{34}\alpha_{43}\alpha_{32}\alpha_{21}$	$\alpha_{12}\alpha_{23}\alpha_{32}\alpha_{21}$
Σ'	$(\alpha_{32}\alpha_{21} + \alpha_{23}\alpha_{34} + \alpha_{21}\alpha_{34})\Sigma$	$(\alpha_{21} + \alpha_{23})\Sigma$
Σ'_a	1	$\alpha_{41} + \alpha_{43}$
Σ_1	1	$\alpha_{41} + \alpha_{43}$
Σ_2	1	$\alpha_{41} + \alpha_{43}$
Σ_{ex}	$\alpha_{32}\alpha_{21} + \alpha_{23}\alpha_{34} + \alpha_{21}\alpha_{34}$	$(\alpha_{21} + \alpha_{23})(\alpha_{41} + \alpha_{43})$
J_{ex}	$\dfrac{\alpha_{12}\alpha_{23}\alpha_{34}\alpha_{43}\alpha_{32}\alpha_{21}}{(\alpha_{32}\alpha_{21} + \alpha_{23}\alpha_{34} + \alpha_{21}\alpha_{34})\sum}$	$\dfrac{\alpha_{12}\alpha_{23}\alpha_{32}\alpha_{21}(\alpha_{41} + \alpha_{43})}{(\alpha_{21} + \alpha_{23})\sum}$

When the above procedure is applied to the diagram in fig. 1b, the expanded diagram in fig. 3a is obtained, which contains three one-way cycles cycles I–III in fig. 3b). Cycle III has the appearance of an exchange process. We wish to show that the flux of this one-way cycle is indeed equal to the flux of the exchange process in fig. 1c. The fluxes of these three cycles can be expressed explicitly as:

$$J_I = \alpha_{12}\alpha_{23}\alpha_{34}\alpha_{41}\sum\nolimits_I / \sum\nolimits' \tag{8a}$$

$$J_{II} = \alpha_{14}\alpha_{43}\alpha_{32}\alpha_{21}\sum\nolimits_{II} / \sum\nolimits' \tag{8b}$$

$$J_{III} = \pi_{ex}\sum\nolimits_{III} / \sum\nolimits' \tag{8c}$$

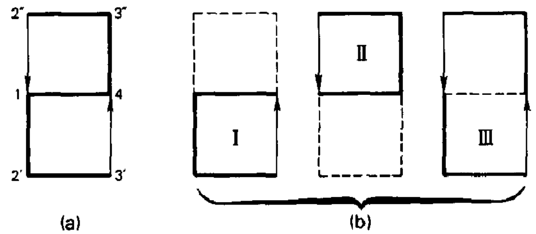

Fig. 3. (a) The expanded diagram of the exchange process in fig. 1c. Thin lines indicate single rate constants and heavy lines double rate constants. (b) The three-cycle diagrams of the expanded diagram. Cycle III has the paths of an exchange process.

where α_{ij} is the rate constant between states i and j of fig. 1b, the Σ_i ($i =$ I–III) the sums of the feed-in parts of the cycle diagrams in fig. 3b, Σ' the sum of directional diagrams of all states of fig. 3a, and π_{ex} the product of rate constants of the exchange paths in fig. 1c. From the diagrams in fig. 3, it is easy to show that

$$\sum\nolimits_{I} = \sum\nolimits_{II} = \alpha_{32}\alpha_{21} + \alpha_{23}\alpha_{34} + \alpha_{21}\alpha_{34} \tag{9}$$

$$\sum\nolimits_{III} = 1 \tag{10}$$

$$\pi_{ex} = \alpha_{12}\alpha_{23}\alpha_{34}\alpha_{43}\alpha_{32}\alpha_{21} \tag{11}$$

$$\sum' = (\alpha_{32}\alpha_{21} + \alpha_{23}\alpha_{34} + \alpha_{21}\alpha_{34})\sum \tag{12}$$

where Σ, as given in table 1, is the sum of directional diagrams of all states of the original diagram in fig. 1b.

Now let us consider the physical meaning of fig. 3a. It is easy to see that the expanded diagram in fig. 3a is exactly the complete kinetic diagram of the transport system in fig. 1a under the condition that only labelled ligands are present in bath 1 and only the unlabelled in bath 2. In this case, ligand-bound transporters can be classified into two groups: one bound with labelled ligands (2′ and 3′) and the other with unlabelled ligands (2″ and 3″), as shown in the diagram. That transitions 4 → 3′ and 1 → 2″ are absent in the diagram is due to the fact that no labelled ligands are in bath 2 and no unlabelled ligands are in bath 1. The steady-state flux of labelled ligands (the tracer flux, J_{tr}) flowing into bath 2 in this system is equal to the transition flux of the binding of labelled ligands from bath 1 or the desorption of labelled ligands to bath 2:

$$J_{tr} = \alpha_{12} p_1 - \alpha_{21} p_{2'} = \alpha_{34} p_{3'} = J_I + J_{III} \tag{13}$$

where the ps are the steady-state probabilities. The last equality comes from the fact that a transition flux can be expressed in terms of participating cycle fluxes (see refs. 2–4).

As mentioned in section 1, if all ligands in bath 1 are labelled while those in bath 2 are not, then the steady-state tracer flux from bath 1 to bath 2 in fig. 1a is equal to the sum of the one-way cycle flux in the + direction (J_+) of the cycle in fig. 1b and the flux of the exchange process (J_{ex}) in fig. 1c:

$$J_{tr} = J_+ + J_{ex}. \tag{14}$$

J_+ can be obtained from eq. 1 and fig. 1b as

$$J_+ = \alpha_{12}\alpha_{23}\alpha_{34}\alpha_{41} / \sum. \tag{15}$$

With eqs. 9 and 12, it is easy to see that the J_+ in eq. 15 is exactly equal to the J_I in eq. 8a. From eqs. 13 and 14, the exchange flux of fig. 1c (J_{ex}) is then exactly identical to the flux of cycle III (J_{III}) in eq. 8c.

Thus, in general the flux of an exchange process in an arbitrary diagram can be expressed analytically as

$$J_{ex} = \pi_{ex} \sum\nolimits'_a / \sum' \tag{16}$$

where π_{ex} is the product of rate constants along the two exchange paths, Σ'_a is the sum of the feed-in parts of the cycle diagrams of the exchange process in the expanded diagram, and Σ' the sum of directional diagrams of all states of the expanded diagram. Explicit expressions of these terms for the exchange processes in fig. 1c and d are listed in table 1.

As a numerical check, a Monte Carlo stimulation for the process in fig. 1c was carried out with the following rate constants: $\alpha_{12} = 10$, $\alpha_{21} = 2$, $\alpha_{23} = 3$, $\alpha_{32} = 3$, $\alpha_{34} = 1$, $\alpha_{43} = 0.01$, $\alpha_{41} = 0.01$, $\alpha_{14} = 0.01$. As shown in table 2, the simulated

Table 2

State probabilities, cycle fluxes, and the exchange flux for the system in fig. 1a evaluated by the diagram and the Monte Carlo methods

	Diagram method [a]	Monte Carlo method [b]
p_1	0.005358	0.005382
p_2	0.022175	0.022172
p_3	0.019015	0.019014
p_4	0.953427	0.953432
J_+	0.009499	0.009499
J_-	0.000019	0.000019
J_{ex}	0.005182	0.005179

[a] The values of J_+, J_- and the ps were evaluated using the usual diagram method of Hill [3]. The value of J_{ex} was calculated from the formula in table. 1.

[b] The total number of transitions in the simulation was 100×10^6.

exchange flux was found to agree with that calculated from eq. 8c.

4. The evaluation of exchange fluxes using the original diagram

In section 3, we presented the derivation of analytical exchange fluxes (as a function of the rate constants of the system) through the use of an expanded diagram. In this section we show that the same formula can also be obtained from the original diagram without going through the diagram expansion procedure of the previous section. To do so, we need to define some new diagrams.

If there exists a line (arc) between the two end points of the exchange processes, the line is called the 'axis' of the exchange processes. If no single line exists, draw a line between them and refer this line as the axis of the exchange processes. Then, the 'directional diagrams of the axis' are defined as the diagrams that contain the maximum number of directed lines flowing into the axis without forming any cycle. For example, the directional diagrams of the axis of the exchange process in fig. 1c are shown in fig. 4a.

If one of the two elementary paths of an exchange process is added to the axis, the combined diagram is an ordinary cycle. The diagram with all directional feed-in parts of this cycle is called the 'cycle diagram of the axis'. There are two sets of cycle diagrams of the axis for each exchange process (because each exchange process contains two elementary paths). If the exchange process is simple, the two cycle diagrams of the axis are identical. Otherwise, the two are different. For example, the two cycle diagrams of the axis of fig. 1c are identical as shown in fig. 4b.

With these new terms, the flux of each exchange process of an arbitrary diagram can now be expressed explicitly as

$$J_{ex} = \pi_{ex} \sum_1 \sum_2 / \left(\sum_{ex} \sum \right) \tag{17}$$

where π_{ex} is the product of rate constants along the two paths of the exchange process (eq. 11), Σ the sum of directional diagrams of all states of the (original) diagram, Σ_{ex} the sum of the feed-in parts of all directional diagrams of the axis, and Σ_1 and Σ_2 the sums of the feed-in parts of all cycle diagrams of the axis of the two exchange paths.

Again we will use the exchange process in fig. 1c to confirm eq. 17. From fig. 4, it is easy to see that

$$\sum_1 = \sum_2 = 1 \tag{18}$$

$$\sum_{ex} = \alpha_{32}\alpha_{21} + \alpha_{23}\alpha_{34} + \alpha_{21}\alpha_{34}. \tag{19}$$

With these equations and the Σ' (eq. 12), the exchange flux in eq. 17 can easily be shown to be identical to the J_{III} in eq. 8c.

As another example, each individual term on the right-hand sides of eqs. 16 and 17 for the exchange process in fig. 1d (a non-operational

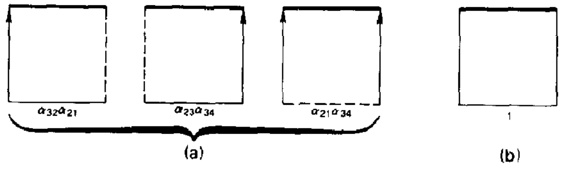

Fig. 4. (a) The directional diagrams of the axis of the exchange process in fig. 1c. The axis is represented by a heavy line. The product of rate constants of the feed-in part of each diagram is indicated by each diagram. (b) The cycle diagram of the axis for the two paths. There is no feed-in part on the diagram. The weight for this diagram is therefore equal to 1.

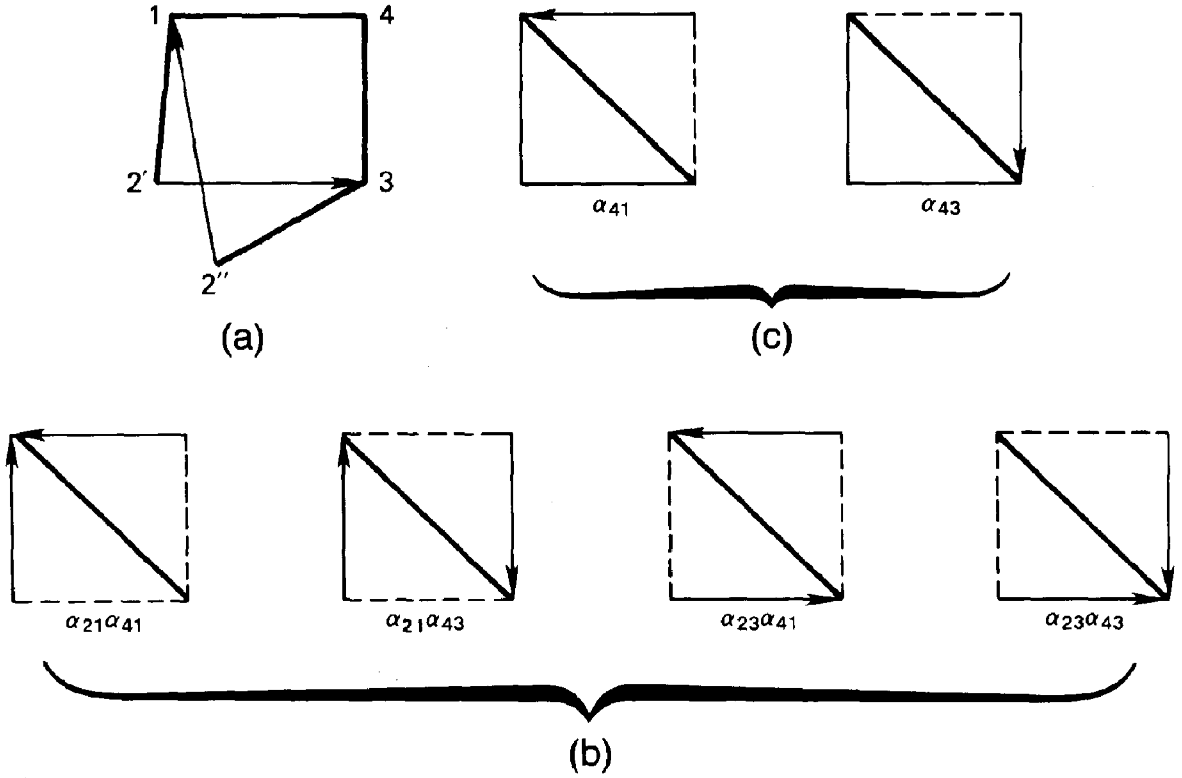

Fig. 5. (a) The expanded diagram of the exchange process in fig. 1d. (b) The directional diagrams of the axis. (c) The cycle diagrams of the axis for the two paths.

exchange process) is listed in table 1, along with that for the exchange process in fig. 1c. The corresponding expanded diagram and the directional and cycle diagrams of the axis of this non-operational exchange process are shown in fig. 5.

5. Discussion

The purpose of this paper is two-fold: (1) To present the formal definition of a general exchange process on a biochemical diagram so that exchange fluxes of a membrane transport system can be evaluated numerically using Monte Carlo simulation; (2) To show that the fluxes of certain exchange processes on a biochemical diagram can be expressed analytically as a function of the rate constants of the diagram using either the original diagram (eq. 17) or an expanded diagram derived after a simple diagram expansion procedure (eq. 16). The methods should prove useful in the analysis of transport mechanisms when experimental tracer fluxes are available.

If one is interested only in the analytical expression of an exchange process, the method based on the original diagram is preferred because the diagram is simpler than the expanded one. On the other hand, if one is interested only in numerical values, then the method based on the expanded diagram is better. This is due to the fact that exchange processes become one-way cycles in the expanded diagram and their fluxes can be calculated from the state probabilities which in turn can be obtained by solving a set of linear algebraic equations. The same situation occurs in the use of expanded diagrams in the calculation of one-way cycle fluxes as discussed by Hill [10].

Although the original goal of this work was to study the operational ligand exchange flux, the exchange processes defined here in section 2 are very general in that they include non-operational as well as operational exchange processes (see fig. 1c and d). That is, the exchange steps of an exchange process may or may not involve the actual ligand binding and ligand desorption reactions. Since a computer simulation algorithm does not depend on the nature of the exchange steps, numerical computer calculations are applicable to any exchange process. In contrast, the diagram methods presented in sections 3 and 4 are applicable only to certain exchange processes. In general, whether diagram methods apply depends on the positions of the two exchange steps of the exchange process in the diagram. If the two exchange steps of the exchange process under consideration always appear simultaneously as a pair in the cycle in which they are present, the diagram methods are applicable. For example, they are applicable to the exchange processes in figs. 1c, d and 2c, but not to those in fig. 2d. Since the binding and desorption reactions of ligands from baths 1 and 2 always appear as a pair in any cycle where they are present, the diagram methods are always applicable to operational (actual) ligand exchange processes.

Finally, it is worth noting that the completion of an exchange process or a one-way cycle on a diagram is identical to the return to the starting point of a random walk on the diagram. That is, the calculation of one-way cycle and exchange fluxes can be treated as a random walk problem. The main difference between this and the ordinary random walks is that in this case the walk space is finite (a two-dimensional lattice), not infinite. In addition, we are interested in the distribution of first-passage times of completing certain walk sequences in the present case, instead of the spatial distributions often discussed in the ordinary random walk problems. In this and other [7–13] studies, the mean first-passage times for the completion of cycles and exchange processes have been derived. In another paper, we will report the studies of other stochastic properties of the system, such as the distribution of the first-passage times, the completion of processes other than the one-way cycles and the exchange processes discussed in this paper, etc.

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