Comments to the Editor

Alternating Carrier Models and the Energy Conservation Laws

INTRODUCTION

Membrane proteins such as transporters, exchangers, and cotransporters, have traditionally been represented by different versions of the mobile carrier model as illustrated in Fig. 1 *A*. For a number of transporters, the apparent affinities for intracellular and extracellular substrates have been shown to be different. This has been accounted for in transport activity models by creating an asymmetry in the rate constants for the binding and debinding reactions on each side of the membrane. Care must be taken to adjust the rate constants of the other reactions in the transport cycle in such a way that the microscopic reversibility principle is respected. In an article (1) recently published in the *Biophysical Journal*, R. J. Naftalin argues that a model of the type shown in Fig. 1 *A*, presenting an asymmetry between the intracellular and extracellular binding constants, violates the energy conservation laws. We don't agree with this conclusion.

THE CARRIER MODEL AND THE ALTERNATING ACCESS MECHANISM

In the carrier model, a transporter is pictured as a molecule whose binding site(s) is exposed to one side of the membrane at a time, where it can bind or release a substrate molecule. Even though some ionophores are believed to function according to this ''ferry boat'' mechanism, a multi-transmembrane segment protein is more likely to function with an alternating access mechanism as illustrated in Fig. 1 *B*, an assumption borne out by the recent crystallographic structures of transporters in different orientations (2–5). In this case, a binding site is alternatively exposed to each side of the membrane through a conformational change. In kinetic modeling, the two mechanisms are indistinguishable as they can be represented by the same number of states linked by the same number of rate constants (Fig. 1 *C*).

THE PROBLEM

In the Naftalin article, the author analyzes several transport models displaying asymmetry in binding affinities for intracellular and extracellular substrates. In the case of the four-state transporter model (Fig. 1 *C*), the difference in the intracellular and extracellular binding affinities (k_{12}/k_{21} vs. k_{43}/k_{34}) can be compensated for by an asymmetric distribution of the free (k_{14} vs. k_{41}) or of the loaded (k_{23} vs. k_{32}) transporter with respect to the orientation of the binding site. When the rate constants are adjusted in such a way that the microscopic reversibility constraint is satisfied,

$$k_{12} \times k_{23} \times k_{34} \times k_{41} = k_{14} \times k_{43} \times k_{32} \times k_{21}, \quad (1)$$

there is no net flux when the neutral solute ''*G*'' has equal concentrations on each side of the membrane ($G_{in} = G_{out}$).

According to the author of the *Biophysical Journal* article, a thermodynamic contradiction arises when, in addition to the microscopic reversibility constraint, the ''phase equilibrium condition'' is implemented. If one considers the two sides of the membrane as two different phases, the phase equilibrium condition requires that: ''all the chemical potentials of mobile components between connected phases must be equal'' (see Eq. 15 in the Naftalin article (1)). This notion comes from the thermodynamic treatment of fluid phase equilibrium as explained in the reference (6) cited in the Naftalin article. Although one can visualize how the principle of phase equilibrium can be used with the carrier mechanism (Fig. 1 *A*), it is much more difficult to do with a physically realistic model of the alternating access mechanism (Fig. 1 *B*). Nevertheless, as the two models are kinetically equivalent, the conclusion that this type of model violates the energy conservation laws needs to be considered.

Let's illustrate the problem by taking one of the specific examples used by the author of the *Biophysical Journal* article. In the case of the kinetic model presented in Fig. 1 *C*, assume that the specific affinity for the binding reaction of extracellular solute ''*G*'' is 10 times larger than for intracellular solute. This can be accounted for by having

$$\frac{k_{21}}{k_{12}} = \frac{1}{10}\frac{k_{34}}{k_{43}}. \quad (2)$$

Let's assume that $k_{23} = k_{32}$ and that the microscopic reversibility constraint (Eq. 1) can be met by adjusting the rate constants for the free carrier reorientation as follows:

$$k_{14} = 10 \times k_{41}. \quad (3)$$

When $G_{in} = G_{out}$, there will be no net flux between each pair of consecutive states. This will lead to an asymmetry

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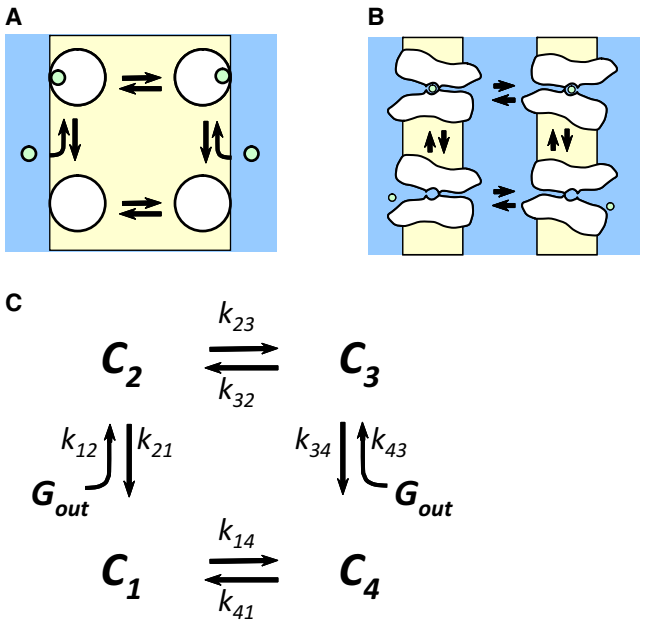

FIGURE 1 Different kinetic representations of the transporter mechanism. Panel *A* represents the mobile carrier model that was implicitly used in the *Biophysical Journal* article on which we are commenting (1). Panel *B* represents the alternating access model, which is more relevant to the known crystal structure of several transporters. Both mechanisms can be represented by the same kinetic mechanism shown in panel *C*.

between the probabilities of finding the transporter in state C1 vs. C4 (see Fig. 1 *C*). In a kinetic system, probabilities can be represented as equivalent concentrations of a transporter in a given state (in this case, C_{C1} and C_{C4}) and one can write

$$C_{C1} \times k_{14} = C_{C4} \times k_{41} \tag{4}$$

and, considering Eq. 3,

$$\frac{C_{C1}}{C_{C4}} = \frac{k_{41}}{k_{14}} = \frac{1}{10}. \tag{5}$$

The principle of phase equilibrium stipulates that the chemical potential of the transporter in state C1 (in a phase corresponding to the external leaflet of the membrane) should be identical with the chemical potential of the transporter in state C4 (present at the internal leaflet of the membrane):

$$\mu_{C1} = \mu_{C4}. \tag{6}$$

According to the author, Eq. 6 implies that the activity of the carrier in state C1 and C4 must be equal,

$$a_{C1} = a_{C4}. \tag{7}$$

If the concentration of the carrier in state C4 is larger than in state C1 (see Eq. 5), Eq. 7 implies that drastically different activity coefficients must be used in each case and the author concludes that "… no energetic benefit can be derived from the asymmetric distribution of the free carrier". Furthermore,

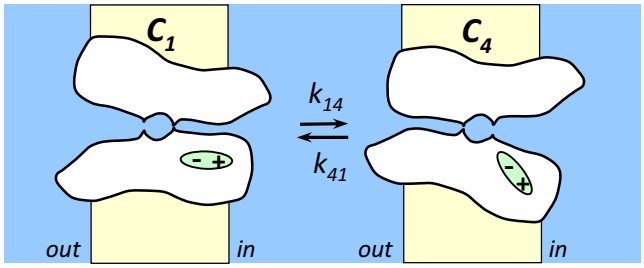

FIGURE 2 Example of a transporter adopting two different configurations associated with charge displacement within the membrane electrical field. In this case, the probability of finding the transporter in state C1 or C4 will be a function of the membrane potential.

according to the author, maintaining such an asymmetric distribution of the free carrier concentrations would require the presence of an exogenous source of energy.

OUR POINT OF VIEW

First, asymmetric distribution of the free carrier is perfectly possible for both the carrier model and the alternating access model. For example, let's consider the two conformations of the free carrier in the alternating access model (Fig. 2). The probability of a carrier being found in either of these two states will vary depending on the Gibbs free energy associated with each conformation. This difference in free energy may come from numerous sources including a change in the interaction of different protein segments with themselves, with the membrane or with the aqueous environment. For example, in Fig. 2, we have depicted a reorientation of a dipole moment in the membrane electrical field. In the case of the Na/glucose cotransporter (SGLT1) and many other Na-coupled transporters (NaPiII, GAT1, Na-K/ATPase…), the reorientation of the free carrier is accompanied by a charge displacement that can be clearly monitored as a pre-steady-state transient current.

At equilibrium, this difference in Gibbs free energy would create a difference in the probability of finding the protein in each of the two configurations until the chemical potentials associated with states C1 and C4 (μ_1 and μ_4, respectively) became equal:

$$\mu_1 = \mu_4. \tag{8}$$

The chemical potential is defined as

$$\mu = \mu_0 + RT \ln a, \tag{9}$$

where a is the activity of the substance considered, R and T have their usual meaning, and μ_0 is the standard free energy. A difference in the activities associated with two states in equilibrium can be established if a difference in μ_0 exists. This standard free energy contains terms representing all types of interactions that may stabilize the protein in one conformation or the other. In general, the possibility that the μ_0 values associated with the two states are equal would

be fortuitous if the conformational change between C1 and C4 is significant.

Even if the possibility of different standard energies is actually acknowledged in the Naftalin article (see Eq. 18 in the Naftalin article (1)), the author has assumed that the activity of any ''mobile component'' must be equal in all phases to which this component has access. As shown by comparing Eqs. 8 and 9, this is only true if the standard energies (μ_0) in the different phases are equal. This notion is discussed in a reference (6) cited by the author himself: ''The condition that the activities must be equal holds only for the special case where the standard states in all phases are the same'' (6). As an asymmetry is expected in the standard energy associated with states 1 and 4 (Fig. 1 *C*), the activities or concentrations of states 1 and 4 do not have to be equal and the apparent contradiction is removed.

THE CONCLUSION

In conclusion, there is nothing wrong with proposing an asymmetric model to account for asymmetry in the apparent binding affinities for the extra or intracellular substrate as long as the microscopic reversibility constraint is satisfied.

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