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Implications of the Alternating Access Model for Organic Anion Transporter Kinetics

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Abstract Many transport proteins, including the clinically important organic anion transporters (OATs), appear to function via an "alternating access" mechanism. In analyzing the kinetics of these transporters, the terms K_m and V_{max} are often treated in the field as denoting, respectively, the affinity of the substrate for the transporter and the turnover (conformational switch) rate of the substrate–transporter complex. In fact, the expressions for both these parameters have very complex forms comprising multiple rate constants from conformational switch as well as association/dissociation steps in the cycling of the transporter and, therefore, do not have straightforward physical meanings. However, if the rapid equilibrium assumption is made (namely, that the association/dissociation steps occur far more rapidly than the conformational switch steps), these expressions become greatly simplified and their physical meaning clear, though still distinct from the conventional interpretations. V_{max} will be a function of not just the rate of substrate–transporter complex turnover but also the rate of the "return" conformational switch and will vary largely with the slower of these two steps (the rate-limiting step). K_m will be seen to be related to substrate affinity by a term that varies inversely with the substrate–transporter complex turnover rate, essentially because the greater this rate, the greater the extent to which transporters will be distributed in a conformation inaccessible to substrate. Here, an intuitive approach is presented to demonstrate these conclusions. The phenomena of *trans*-stimulation and *trans*-inhibition are discussed in the context of this analysis.

Keywords Alternating access · Organic anion transporter · Michaelis–Menten kinetics · Substrate affinity · Turnover rate · *Trans*-stimulation · *Trans*-inhibition · K_m · V_{max} · K_d

Introduction

The alternating access model of transport postulates that the transporter alternates between two conformations: in one the substrate-binding site is accessible to the extracellular space (the "out-facing" conformation), and in the other, to the intracellular space (the "in-facing" conformation). Transmembrane transport occurs when substrate on one side of the membrane binds to a transporter molecule in the conformation "facing" that side, the conformational switch occurs and the substrate dissociates from the transporter into the space on the other side of the membrane (Fig. 1). It can be readily seen that such a mechanism could account for multiple modes of transport including both facilitated diffusion and exchange (Lemieux et al. 2004; West 1997).

Among the postulated alternating access transporters are the organic anion transporters (OATs), clinically important kidney proteins which are responsible for the urinary excretion of a great variety of important drugs (Burckhardt and Burckhardt, 2003; Eraly et al. 2003, 2004; Sweet 2005) and potentially also mediate transport of many physiological regulators (Eraly et al. 2003, 2004; Vallon et al. 2008). The OATs generally appear to function as exchangers—in the renal proximal tubule, e.g., cellular uptake of organic

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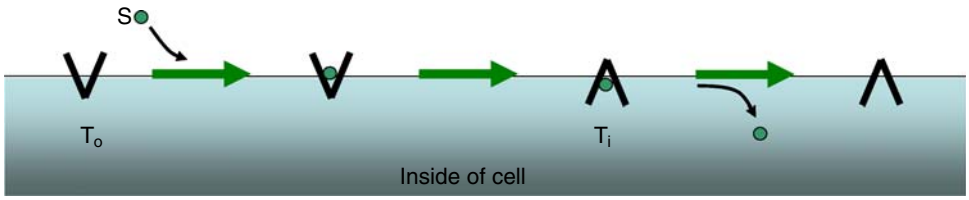

Fig. 1 The alternating access mechanism of transport. T_o, transporter in out-facing conformation; T_i, transporter in in-facing conformation; *S*, substrate. Entry of substrate into the cell is depicted; if the steps in this transport process were reversed, transport in the opposite direction would occur. Please refer to the text for details

anionic substrates from plasma/interstitial fluid is coupled to the exit of intracellular dicarboxylates (Burckhardt and Burckhardt 2003; Eraly et al. 2003, 2004; Sweet 2005). As with other transporters, it is customary to use the Michaelis–Menten equation to describe the transport kinetics of the OATs: $V = V_{max} * (S/[S + K_m])$, where V is the rate of transport at a given substrate concentration (S), V_{max} is the maximum rate of transport and K_m is the Michaelis–Menten constant, the concentration at which $V = V_{max}/2$. Within the organic anion transporter field, this equation appears to be frequently interpreted as follows (e.g., Kaler et al. 2007; Perry et al. 2006; Popp et al. 2005):

(1) V_{max} is equivalent to the product of the turnover (conformational switch) rate of the substrate–transporter complex and the total number (or concentration, depending on what units are being used to express transport rate) of transporter molecules.
(2) K_m is taken to correspond to, or at least closely approximate, the dissociation constant ("affinity"), K_d, of the substrate–transporter complex.
(3) Since V will be linearly related to the number of transporter molecules that are occupied by substrate, the term $(S/[S + K_m])$ denotes the proportional occupancy of the transporter—i.e., the proportion of maximum transport that is occurring (V/V_{max}) is the same as the proportion of all available transporter molecules that are occupied.

However, this understanding of the terms V_{max}, K_m and $S/(S + K_m)$ is, in fact, erroneous in the case of alternating access transporters. For such transporters, V_{max} is not directly related to the turnover rate of the substrate–transporter complex, K_m is not necessarily a close approximation of the dissociation constant of this complex and $S/(S + K_m)$ is not equivalent to the proportional occupancy of transporter molecules. This should perhaps not be unexpected since the conventional interpretation of the Michaelis–Menten equation relates principally to enzyme–substrate interactions in which, after the catalyzed reaction has taken place, the enzyme remains in a form that permits it to interact with additional substrate. By contrast, in the case of an alternating access transporter, after the catalyzed reaction (transport of substrate from one side of the membrane to the other) has taken place, the transporter is no longer in a form that permits it to interact with additional substrate on the original side of the membrane.

Results and Discussion

Kinetic Analysis of Alternating Access Transport

The entire alternating access cycle of transport (considering, e.g., the case of substrate/counterion exchange by organic anion transporters) can be diagrammed as comprising seven steps (Fig. 2): (1) substrate binding from plasma to the out-facing conformation of the transporter, A, to form complex B; (2) conformational switch of B resulting in the in-facing substrate–transporter complex, C; (3) substrate dissociation from C into the intracellular space, resulting in free in-facing transporter, D; (4) binding of an intracellular counterion (e.g., a dicarboxylate) to D to form complex, E; (5) switching of E, resulting in the out-facing counterion–transporter complex, F; and (6) counterion dissociation into the plasma, returning the transporter to its original state, A. There is also the possibility of conformational switch of uncomplexed transporter (A or D) (7).

Kinetic measurements are typically made during the initial phase of transport, while the intracellular concentration of substrate and extracellular concentration of counterion remain negligible; and it is to this phase that the terms K_m and V_{max} are ordinarily applied. Thus, in the analysis that follows it is this phase that is considered, and steps 3 and 6 in Fig. 2, dissociation of substrate into the intracellular space and of counterion into the extracellular space, are designated as irreversible. A total of 12 rate constants are accordingly specified, two each for the five reversible steps and one each for the two irreversible steps; these are indicated next to their associated steps in Fig. 2.

A kinetic model may now be set up in the standard manner (Stein 1986, 1989) as follows: The rate of transport of substrate (the rate at which substrate accumulates in the

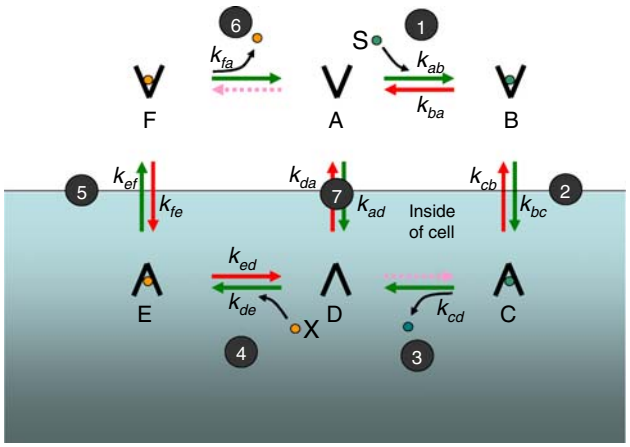

Fig. 2 Kinetic analysis of OAT-mediated transport. A–F, various transporter states: A, D, empty transporter; B, C, transporter complexed with substrate; E, F, transporter complexed with counterion; F, A, B, out-facing transporter states; C, D, E, in-facing transporter states; k_{ab}, k_{ba}, k_{bc}, etc., rate constants for the indicated transitional steps; *green-filled circles*, substrate; *gold-filled circles*, counterion; *S*, extracellular substrate concentration; *X*, intracellular counterion concentration. Please refer to the text for details

intracellular space), *V*, is equivalent to $[C] * k_{cd}$. At steady state the rate of formation of each of the six transporter states, A–F, is equivalent to the rate of its breakdown. This principle can be exploited to set up five independent equations, corresponding to the formation and breakdown of five of the transporter states. The equation for the sixth transporter state is redundant since the relative concentration of the sixth state can be deduced from those of the other five states. We can, however, state a sixth equation relating the concentrations of the transporter states to one another—their sum must, of course, remain equivalent to a fixed quantity, the total transporter concentration, *T*. These six equations can be readily solved (though the algebra is somewhat cumbersome) to express [*C*] (and therefore *V*) in terms of *T*, the rate constants, the extracellular substrate concentration, *S*, and the intracellular counterion concentration, *X* (the solution is provided in the supplementary material):

$$\begin{aligned} V = &\left\{Tk_{bc}k_{cd}k'_{da}/\left[k_{bc}\left(k_{cd}+k'_{da}\right)+k'_{da}\left(k_{cd}+k_{cb}\right)\right]\right\} \\ &* S/\left\{S+\left[k_{ad}+k'_{da}\right]\left[k_{ba}\left(k_{cd}+k_{cb}\right)+k_{bc}k_{cd}\right]/k_{ab}\right. \\ &\left.\left[k_{bc}\left(k_{cd}+k'_{da}\right)+k'_{da}\left(k_{cd}+k_{cb}\right)\right]\right\}, \text{ where } k'_{da} \\ &= \left\{k_{da}\left[k_{ef}k_{fa}+k_{ed}\left(k_{fe}+k_{fa}\right)\right]+Xk_{de}k_{ef}k_{fa}\right\}/ \\ &\left\{\left[k_{ef}k_{fa}+k_{ed}\left(k_{fe}+k_{fa}\right)\right]+Xk_{de}\left[k_{ef}+k_{fe}+k_{fa}\right]\right\} \end{aligned} \quad (1)$$

Britton (1966) previously provided a more general analysis of the kinetics of alternating access transporters by allowing for significant substrate concentrations on both sides of the membrane (as did other contemporaneous workers; reviewed by Stein 1986). The equation for net transport that was developed in that report becomes identical to Eq. 1 when the intracellular substrate concentration is set to zero.

Equation 1 is a complex formulation that is essentially unintelligible in the physical sense. If, however, we make the rapid equilibrium assumption (REA) (Segel 1993; Turner 1981)—namely, that the association/dissociation rate constants (k_{fa}, k_{ab}, k_{ba}, k_{cd}, k_{de}, k_{ed}; horizontal arrows in Fig. 2) are much greater than the conformational switch rate constants (k_{ef}, k_{fe}, k_{da}, k_{ad}, k_{cb}, k_{bc}; vertical arrows)—Eq. 1 will simplify as shown below to a form with a relatively clear physical meaning. (The REA seems to be a reasonable assumption since it is plausible that association/dissociation events would occur far more rapidly—i.e., have higher rate constants—than events involving conformational changes.)

Given the REA, the term for k_{da}' simplifies to $\approx (k_{da}k_{ed} + Xk_{de}k_{ef})/(k_{ed} + Xk_{de})$, which can be rewritten as $k_{da}' \approx (k_{da}K_{dX} + Xk_{ef})/(K_{dX} + X)$, where $K_{dX} = k_{ed}/k_{de}$, the dissociation constant of the counterion–transporter complex in its in-facing conformation. Moreover, under the REA, the term $X/(K_{dX} + X)$ is equivalent to the occupancy of the (inward-facing conformation of the) transporter by the counterion, which we will term Occ_x. The equation for k_{da}' can, accordingly, be further rewritten as

$$k'_{da} \approx k_{da}(1 - \ Occ_x) + k_{ef}(Occ_x) \quad (2)$$

Thus, k_{da}' can be seen to be essentially a weighted average of k_{da} and k_{ef}, such that when counterion is absent ($X = 0$), $k_{da}' = k_{da}$, the in-to-out switch rate of empty transporter; when it is saturating ($X >> K_{dX}$), $k_{da}' \approx k_{ef}$, the in-to-out switch rate of the counterion–transporter complex. Now, applying the condition that, in addition to the values of the conformational switch rate constants, the value of k_{da}' is negligible with respect to the association/dissociation rate constants (since k_{da}' is always in the range of k_{da} and k_{ef}), Eq. 1 simplifies to

$$\begin{aligned} V \approx &\left(Tk_{bc}k'_{da}/\left[k_{bc}+k'_{da}\right]\right) \\ &* S/\left(S+k_{ba}\left[k_{ad}+k'_{da}\right]/k_{ab}\left[k_{bc}+k'_{da}\right]\right) \end{aligned} \quad (3)$$

I present below an intuitive approach to deriving Eq. 3, based on considerations of how changing substrate concentrations affect the relative distribution of the in-facing and out-facing conformational states of the transporter, and discuss the physical meaning and implications of this equation.

Inhibition by a Competing Substrate

What proportion of alternating access transporter molecules on a cell's membrane would be expected to face outward (in the conformation accessible to extracellular substrate)? It can be readily appreciated that if the switch

from the in-facing conformation to the out-facing conformation (in-to-out switch) tends to occur more rapidly than the out-to-in switch, the majority of transporter molecules will be out-facing (and vice versa). Let the proportion of transporter molecules that are out-facing be A_o and the proportion that are in-facing be A_i (outside and inside "availability," respectively) (Fig. 3a). It is apparent that (at steady state) the ratio of out-facing to in-facing OAT molecules will be the same as the ratio of the in-to-out to out-to-in rates—in the absence of substrate and counterion, this would be equivalent to k_{da}/k_{ad} (using the same terminology as in Fig. 2) so that

$$A_o = k_{da}/(k_{da} + k_{ad}) \text{ and, similarly, } A_i = k_{ad}/(k_{da} + k_{ad}) \quad (4)$$

When counterion is present, the in-to-out switch rate will be given by k_{da}', the "weighted" average, as described above, of the switch rates of the "empty" and counterion-loaded transporter, k_{da} and k_{ef}, respectively. Note that, although k_{da}' will vary with counterion concentration, for our purposes it can be treated as a constant. This is because we are dealing with the initial phase of transport, during which this concentration can be treated as essentially having a fixed value. The presence of the counterion also potentially introduces a new path—F-to-E in Fig. 2—for out-to-in switching. However, under the REA, the contribution of this path would be negligible during the initial phase of transport (the rate of F-to-E switch events would be negligible in comparison to A-to-D switch events)

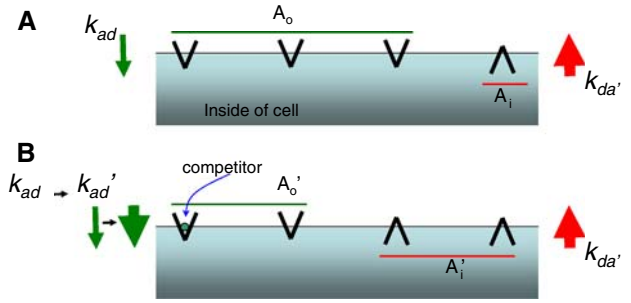

Fig. 3 **a** Relationship between conformational switch rates and the distribution of conformational states. A_o, proportion of transporter molecules that are in the out-facing conformation (outside availability); A_i, proportion that are in the in-facing conformation (inside availability); k_{ad}, out-to-in conformational switch rate; k_{da}', in-to-out switch rate (in presence of counterion). When $k_{da}' > k_{ad}$ (as depicted), A_o will be greater than A_i. Please refer to the text for details. **b** Alteration in the distribution of conformational states in the presence of a competitor. Substrates, including competitors, will affect the distribution of conformational states to the extent that the switch rate of the substrate–transporter complex is different from that of the empty transporter. A_o', A_i' and k_{ad}' denote the outside availability, inside availability and overall out-to-in conformational switch rate, respectively, in the presence of a competitor. When $k_{ad}' > k_{ad}$ (as depicted), $A_o' < A_o$ so that the degree of inhibition will be greater than that expected on the basis of just the physical occupancy of the transporter by the competitor. Please refer to the text for details

because of the rapid and irreversible dissociation of counterion from F; thus, the out-to-in switch rate would remain essentially equivalent to k_{ad}. In sum then, in the presence of counterion

$$A_o = k_{da}'/(k_{da}' + k_{ad}) \text{ and, similarly, } A_i = k_{ad}/(k_{da}' + k_{ad}) \quad (4a)$$

Now, consider the uptake into a transporter-expressing cell of a substrate that is present at trace concentrations (tracer). A competing substrate will decrease tracer uptake due to its physical occupancy of the transporter. However, it will also alter uptake based on how it affects the rate of switch from the out-facing to the in-facing conformation. If it increases this rate (i.e., if the out-to-in rate of the competitor–transporter complex is greater than that of the empty transporter), it will decrease A_o relative to basal conditions and, therefore, inhibit tracer uptake to a degree greater than that expected on the basis of its occupancy alone (Fig. 3b). Conversely, a competitor substrate that *decreases* the out-to-in rate of the OAT will increase A_o and, therefore, inhibit tracer uptake to a degree *less* than that expected on the basis of its occupancy alone. (As above, under the REA, this competitor substrate would not significantly affect the overall "return" [in-to-out] rate during the initial phase of transport—i.e., the rate of C-to-B switch events would be negligible in comparison to D-to-A and E-to-F switch events due to the rapid and irreversible dissociation of substrate from C.) Compounds conferring high out-to-in rates are, of course, transported more rapidly (they are of greater "efficacy") than are compounds conferring low out-to-in rates. Thus, at any given level of OAT occupancy, more efficacious compounds will inhibit tracer uptake to a greater degree than less efficacious compounds. This intuition can be mathematically formulated as follows.

Let us define the outside availability of the transporter in the presence of the competitor substrate as A_o'. Let the proportion of outside-available transporter molecules that are occupied by the competitor (proportional occupancy) be Occ_i. Finally, let the proportional inhibition of tracer uptake (the ratio of the decrease in uptake in the presence of the competitor to the uptake in its absence) be Inh. From these definitions it follows that $Inh = 1 - (A_o'/A_o)(1 - Occ_i)$. (This can be seen by considering that A_o'/A_o represents the fold-change in outside availability in the presence of the competitor and that $1 - Occ_i$ represents the proportion of outside-available transporters that are unoccupied by the competitor and therefore free to interact with the tracer.) Making the REA, Occ_i is given by $I/(I + K_{dI})$, where I is the extracellular concentration of the competitor (inhibitor) and K_{dI} is the dissociation constant of the competitor–transporter complex (in its out-facing conformation). Substituting into the above relationship and simplifying, we have

$$\text{Inh} = 1 - (A_o'/A_o)(K_{dI}/[I + K_{dI}]) \quad (5)$$

Let the overall out-to-in rate in the presence of the competitor be k_{ad}' (Fig. 3b; this overall rate encompasses the rates of both the competitor–transporter complex and the empty transporter [the contribution of the tracer–transporter complex can be ignored as negligible]). Then, from Eq. 4a, it follows that

$$A_o'/A_o = (k_{ad} + k_{da}')/(k_{ad}' + k_{da}') \quad (6)$$

Let k_{bc} be the out-to-in rate of the competitor–transporter complex (to stay consistent with Fig. 2). Then, k_{ad}' will be a weighted average of k_{ad} and k_{bc} (when the competitor is present at trace concentrations, $k_{ad}' \approx k_{ad}$, and when the competitor is saturating, $k_{ad}' \approx k_{bc}$). Specifically, $k_{ad}' = k_{ad} * (1 - \text{Occ}_i) + k_{bc} * (\text{Occ}_i)$ or

$$k_{ad}' = (k_{bc} * I + k_{ad} * K_{dI})/(I + K_{dI}) \quad (7)$$

Substituting Eq. 7 into 6 and then into 5, and simplifying, we have

$$\text{Inh} = I/\{I + K_{dI} * [(k_{ad} + k_{da}')/(k_{bc} + k_{da}')]\} \quad (8)$$

This equation fits our expectations as outlined in the first paragraph of this section: When $k_{bc} = k_{ad}$ (i.e., when there is no difference between the out-to-in switch rate of the competitor–transporter complex and that of the empty transporter), $\text{Inh} = I/(I + K_{dI})$—i.e., the proportional inhibition is equivalent to the proportional occupancy by the competitor. When $k_{bc} > k_{ad}$ (the out-to-in switch rate of the competitor–transporter complex is greater than that of the empty transporter, as when the competitor is itself an efficacious substrate), $\text{Inh} > I/(I + K_{dI})$—inhibition is greater than occupancy; conversely, when $k_{bc} < k_{ad}$ (as with an inefficacious substrate), inhibition is less than occupancy.

It should be noted here that the latter scenario ($k_{bc} < k_{ad}$) would result in the phenomenon of *trans*-inhibition: The rate of efflux of an intracellular counterion (present at a fixed concentration) will vary directly with the inside availability of transporter, A_i. When the out-to-in switch rate of a substrate–transporter complex is less than that of the empty transporter, the extracellular presence of that substrate will result in a decrease in A_i and, therefore, in decreased efflux of intracellular counterions (*trans*-inhibition of efflux).

In the case of the OATs, this does not appear to be merely a theoretical consideration. Firstly, substantial OAT-mediated efflux of intracellular substrate has been observed in the absence of obvious counterions in the extracellular fluid (e.g., Apiwattanakul et al. 1999; Bakhiya et al. 2003), indicating that there is a significant degree of switching of the empty transporter and, therefore, that OATs, in at least some circumstances, are not obligate exchangers. Moreover, *trans*-inhibition has been observed for such well-characterized OAT substrates as estrone sulfate (Bakhiya et al. 2003) and indomethacin (Apiwattanakul et al. 1999), as well as for the OAT inhibitor, probenecid (Chatsudthipong and Dantzler 1991, 1992; Dantzler et al. 1995). Indeed, one would generally expect the degree of *trans*-inhibition or *trans*-stimulation due to various compounds to correlate with their corresponding rates of substrate–transporter turnover and, therefore, with their relative V_{max} values. (The equations presented below readily reveal that there would be a linear relationship between the V_{max} of a substrate and initial tracer efflux in the presence of saturating concentrations of that substrate.)

Relationship Between Substrate Concentration and Transport Rate

Let us now consider the overall rate of transport. (For convenience, our assessment will be of the same competitor substrate from the previous section, enabling the designations for the terms of the equations to remain consistent, with the exception that S, rather than I, is used to designate its concentration.) Under the REA, the rate of transport, V, is determined by the overall rate of out-to-in switch events of substrate-loaded transporter molecules (since the subsequent dissociation of substrate into the intracellular space occurs very rapidly). This overall rate is equivalent to the product of the rate constant of the out-to-in switch of the substrate–transporter complex (k_{bc}) and the concentration of this complex in its out-facing conformation. The latter concentration will be a product of the concentration of total outside-available transporter molecules in the presence of the substrate ($T * A_o'$) and occupancy (Occ_s) so that

$$V = k_{bc} * T * A_o' * \text{Occ}_s \quad (9)$$

Substituting $k_{da}'/(k_{ad}' + k_{da}')$ for A_o' (as in Eq. 4a), $(k_{bc} * S + k_{ad} * K_{dS})/(S + K_{dS})$ for k_{ad}', where $K_{dS} = k_{ba}/k_{ab}$, the dissociation constant of the substrate–transporter complex in its out-facing conformation (Eq. 7), and $S/(S + K_{dS})$ for Occ_s (under the REA), simplifying and rearranging yields

$$V = T[k_{bc} * k_{da}'/(k_{bc} + k_{da}')] * S/\{S + K_{dS}[(k_{ad} + k_{da}')/(k_{bc} + k_{da}')]\} \quad (10)$$

Substituting k_{ba}/k_{ab} for K_{dS} yields Eq. 3.

Equation 10 has, of course, the same form as the Michaelis–Menten equation, with K_m having the value $K_{dS}[(k_{ad} + k_{da}')/(k_{bc} + k_{da}')]$ and V_{max}, the value $T(k_{bc} * k_{da}'/[k_{bc} + k_{da}'])$. This value for V_{max} can also be derived directly from Eq. 9: When substrate is saturating,

$V = V_{max}$, Occ = 1 and $k_{ad}' = k_{bc}$ so that $A_o' = k_{da}'/(k_{bc} + k_{da}')$; substituting these values into Eq. 9, we arrive again at the same formulation as above.

Thus, it can be seen that V_{max} is not related directly to the out-to-in rate of the substrate–transporter complex, k_{bc}, but is a nonlinear function of k_{da}', the return rate of the transporter, as well. (The term $[k_{bc} * k_{da}'/(k_{bc} + k_{da}')]$ is, in fact, precisely the overall "average" rate that is expected for a cyclical process with a forward step of rate k_{bc} and a return step of rate k_{da}': The total time taken to complete a cycle is $(1/k_{bc}) + (1/k_{da}') = (k_{bc} + k_{da}')/(k_{bc} * k_{da}')$; the overall rate of the cycle is the reciprocal of this sum, $[k_{bc} * k_{da}'/(k_{bc} + k_{da}')]$.) Thus, when the value of k_{da}' is very low relative to k_{bc}, $[k_{bc} * k_{da}'/(k_{bc} + k_{da}')] \approx k_{da}'$ so that V_{max} would be "responsive" to changes in k_{da}' but not to changes in k_{bc} (and vice versa) (Fig. 4a). This matches our intuition—the slower step in a two-step process is rate-limiting; changes in the rate of the slower step will affect the overall process rate far more than changes in the faster step.

Equation 10 also reveals the distinction between K_m and K_{dS}. As discussed in the section on inhibition, this distinction arises because, as substrate concentration increases, the availability of transporter that is free to interact with additional substrate molecules changes not only due to occupancy of transporter by substrate but also due to redistribution of the conformational states of the transporter. In the event that $k_{bc} > k_{ad}$, K_m would always be less than K_{ds} (Fig. 4b). In this circumstance, introduction of substrate would lead to a decrease in the availability of out-facing transporters so that as the concentration of substrate molecules increased, they would "use up" transporter molecules to a greater degree than expected on the basis of occupancy alone. The case of $k_{ad} \approx 0$—i.e., the conformational switch rate of the free transporter being negligible in comparison to that of the bound transporter, as would be the case for an obligate exchanger—represents an extreme case of this scenario. Conversely, when $k_{bc} < k_{ad}$, K_m would always be greater than K_{ds}. In general, the ratio of K_m to K_{ds} would decrease in proportion to the efficacy of a substrate and, thus, would tend to vary inversely with its V_{max}.

However, the magnitude of this effect is determined by the relative value of k_{da}'—when k_{da}' has a very high value relative to k_{bc} and k_{ad}, the term K_{dS} $(k_{ad} + k_{da}')/(k_{bc} + k_{da}') \approx K_{dS}$ and is therefore not appreciably modified by changes in the value of k_{bc} (Fig. 4b). This can be understood by seeing that under these circumstances the vast majority of transporter molecules will necessarily be out-facing so that the out-facing proportion can be only minimally affected by changes in out-to-in switch rates. In all cases though, the proportion of maximum transport achieved at a certain concentration of substrate will be identical to the degree of competitive inhibition of tracer uptake due to that concentration (compare Eqs. 8 and 10)—since either parameter is determined by the quantity of transporter that is "used up," by virtue either of actual occupancy by substrate or of redistribution to the unavailable conformation.

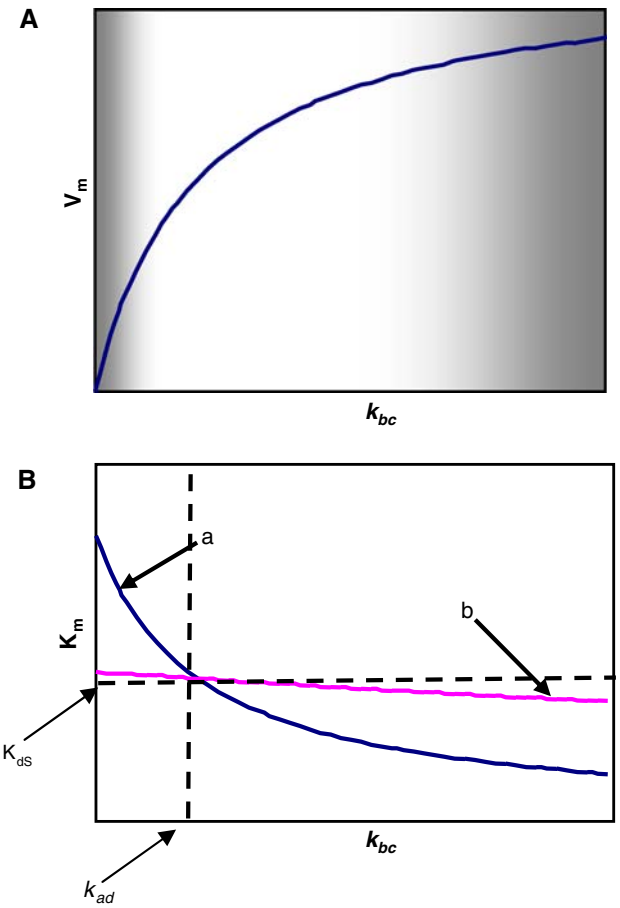

Fig. 4 **a** Theoretical relationship between V_{max}, the maximum rate of transport, and k_{bc}, the out-to-in switch rate of the substrate–transporter complex, for a fixed value of k_{da}', the return rate of the transporter. When $k_{bc} << k_{da}'$ (*left-hand portion of the graph, shaded*), V_{max} will increase essentially linearly with k_{bc}; when $k_{bc} >> k_{da}'$ (*right-hand portion of the graph, shaded*), V_{max} will plateau. Please refer to the text for details. **b** Theoretical relationship between K_m, the Michaelis–Menten constant, and k_{bc} for fixed values of k_{ad}, the out-to-in switch rate of the empty transporter, and k_{da}'. When $k_{bc} < k_{ad}$, $K_m > K_{dS}$, the dissociation constant of the out-facing substrate–transporter complex, and when $k_{bc} > k_{ad}$, $K_m < K_{dS}$ (curve *a*). However, the magnitude of this effect is determined by the relative value of k_{da}'; when $k_{da}' >> k_{bc}$ and k_{ad}, K_m will deviate only minimally from K_{dS} (curve *b*). Please refer to the text for details

It should be noted that, in at least some cases (Kaler et al. 2007; Truong et al. 2007), very large differences in V_{max} (up to approximately 1,000-fold) have been observed for the entry of different substrates into OAT-expressing cells. (V_{max} will, of course, vary with transporter expression levels and therefore will not necessarily be comparable across different experiments; in the above-

referenced studies, all comparisons of V_{max} values were based on measurements made in the same experiment, using the same batch of OAT cRNA–microinjected oocytes.) This suggests that for these cases it is the out-to-in, and not the return, step that is rate-limiting; i.e., the value for k_{da}' is much greater than the various k_{bc} values of the different substrates. (Specifically, Eq. 10 implies that in order to observe a 1,000-fold difference in V_{max} for the uptake of two substrates, the k_{bc} value of the less efficacious substrate would have to be less than 1/1,000 of the value of k_{da}'.) Thus, it follows from Eq. 10 that, in these particular contexts, K_m might indeed be essentially equivalent to substrate affinity, K_{dS}—and therefore the term $S/(S + K_m)$ to proportional occupancy, $S/(S + K_{dS})$—and V_{max} might indeed be directly related to the turnover rate of the substrate–transporter complex, k_{bc}.

Also, it has been argued very recently on thermodynamic grounds that asymmetric cycling of free transporter, absent an exogenous energy source, violates energy conservation laws (Naftalin 2008). In this context it should be emphasized that the analysis presented here does not necessitate asymmetry of transporter cycling. Asymmetric distribution of out-facing and in-facing states of transporters would occur whenever $k_{da}' \neq k_{ad}'$, which would be the case for a "symmetric" transporter ($k_{da} = k_{ad}$) whenever substrate and/or counterion concentrations were not equivalent across the membrane. This state of affairs is likely to generally prevail in physiological environments. For instance, in the kidney proximal tubule, there exist independent (and energy-requiring) mechanisms to keep intracellular concentrations of OAT substrates and extracellular concentrations of OAT counterions at a low level – substrates taken up from plasma into the tubular cell by OATs are promptly pumped into the urine by efflux pumps, while dicarboxylate counterions in plasma are rapidly taken up into cells via the action of Na^+ gradient driven cotransporters (Burckhardt and Burckhardt 2003; Dantzler and Wright 2003; Wright and Dantzler 2004).

Conclusions

The precise expressions for K_m and V_{max} of OATs and other presumed alternating access transporters have very complex forms comprising multiple rate constants from conformational switch as well as association/dissociation steps in the cycling of the transporter. However, if one makes the REA, these expressions become greatly simplified and their physical meanings clear. V_{max} is seen to be a function of the rate constants for the two conformational switch steps (out-to-in and in-to-out), with its value largely varying with the rate of the slower of the two steps (the rate-limiting step). K_m is seen to be related to K_d by a factor that would tend to vary inversely with the V_{max} (efficacy) of the substrate in question. This is because efficacious substrates cause a decrease in the availability of transporter molecules that are in the appropriate conformation to interact with substrate and, therefore, "use up" transporter molecules to a greater degree than expected merely on the basis of their physical occupancy of the transporter.

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