SIMPLE MODEL CAN EXPLAIN SELF-INHIBITION OF RED CELL ANION EXCHANGE

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ABSTRACT Ion translocation in red cell anion exchange is assumed to occur by means of an alternating access mechanism, in which a critical binding site for the transported ion alternates between two conformational states, each accessible from only one side of the membrane. If this alternating site is located within the transport protein at some distance from one or both surfaces of the membrane, an access channel is required to connect the alternating site to the adjacent bulk solution. This automatically leads to inhibition of transport at high concentrations of the transported ion because release of the ion from the alternating site can occur only via unoccupied channel sites.

INTRODUCTION

It is now established that anion exchange across the red cell membrane is catalyzed by an asymmetric protein molecule tightly anchored in the membrane (1), and the mobile carrier model for such exchange can therefore no longer be considered as tenable. A conceptually similar model that is applicable to membrane-embedded proteins, and in its simplest form leads to a similar equation for the transport rate, is an alternating access model of the kind first proposed by Patlak (2) and subsequently suggested for many transport systems (3, 4), including red cell anion exchange (5). In this model the protein is seen as alternating between two principal conformational states. Binding sites for the transported species are accessible only from one side of the membrane in one conformation and only from the opposite side in the other conformation. Transport stoichiometry is determined by restrictions on the kinetic transition state for the conformational alternation. In the case of red cell anion exchange, which has a 1:1 stoichiometry, only a single binding site is required, and the appropriate restriction is that the change from one conformational state to the other can occur at a significant rate only if the binding site is filled. The existence of two principal conformational states of this kind is reasonably well-established for ATP-driven Na, K, and Ca pumps (4). The ion translocating part of the protein is in these systems tightly coupled to an ATP processing region, such that ATP hydrolysis leads to thermodynamically uphill ion transport or, conversely, an ion concentration gradient can drive synthesis of ATP from ADP and P_i, but this difference in the overall catalyzed reaction does not mean that the translocation mechanism in these pump proteins need differ from the translocation mechanism for red cell anion exchange. On the contrary, the ability to prevent net unidirectional downhill diffusion is common to both systems, which makes it plausible that the translocation pathways may be similar. Moreover, exchange of the transported ion, without net ATP hydrolysis or synthesis, can be observed in these systems under appropriate experimental conditions.

A major difficulty with the alternating access model is the distance of ~40 Å that an ion has to travel to pass from one side of the membrane to the other. This creates no problem for a mobile carrier model, in which the carrier (with transported ion bound) is seen as lipid-soluble, so that it can diffuse across the hydrocarbon core of a lipid bilayer. It is, however, contrary to reasonable expectation to suppose that a protein embedded in the membrane can undergo a simple and rapid conformational change in which a binding site moves over such a large distance. Speculative models for appropriate conformational transitions have been proposed (6, 7), and they invariably involve a translational motion of only a few angstroms.

A simple way to circumvent this problem is by means of approach channels (4). The alternating site is seen as being located within the protein molecule, a considerable distance away from one or both membrane surfaces, and the postulated channels provide a connecting link between the alternating site and the external solutions. There is strong evidence for the existence of such an access channel in proton-translocating ATPases, and virtually all models for proton translocation in bacteriorhodopsin include access pathways of this kind. A general model for coupled transport proposed by Läuger (8), while phrased in somewhat different language, is essentially an alternating access model with connecting channels to both sides of the membrane.

The purpose of this paper is to derive formal equations for a model incorporating such channels. Even in its simplest form, such a model invariably leads to a rate equation of the type

$$V = \frac{Cx}{1 + Ax + Bx^2}, \quad (1)$$

for the initial rate in an experiment in which one ion concentration (x) is being varied under otherwise fixed conditions. The transport rate given by this equation goes to zero at both low and high x, passing through a maximum at $x = 1/B^{1/2}$. The model therefore automatically accounts for self-inhibition of transport at high ion concentrations, a phenomenon that has been observed experimentally in most red cell anion exchange studies that were carried to high ion concentrations (9–12). Eq. 1 is in fact formally identical to the equation derived by Dalmark (9) to describe self-inhibition for Cl^- isotope exchange at equilibrium. Dalmark's derivation was based, however, on a mobile carrier model, and the term in x^2 in the denominator was obtained by invoking an arbitrary modifier site of no direct relation to the transport process per se. Knauf (1) has previously suggested that the modifier site might be an approach site as here envisaged.

FORMAL RATE EQUATIONS

Fig. 1 illustrates the basic model. E and E' indicate the two conformational states of the protein. The alternating site is accessible from the inside of the cell in state E and from the outside in state E'. Access channels from both sides of the membrane are represented by single sites that the ions must occupy on the pathway to and from the alternating site. To keep the derivation as simple as possible, the following assumptions will be made: (a) The approach sites are assumed to be in rapid equilibrium with the adjacent bulk solution: equilibrium constants for binding of an ion L to the inside and outside channel sites, respectively, are designated by K_{Li} and K_{Lo}. (b) It will be assumed that there is no direct interaction between the approach sites and the alternating site, i.e., K_{Li} and K_{Lo} are taken as independent of the state of occupancy of the alternating site, and to have the same values in the E and E' states of the protein.

Fig. 2 shows the pathway in this model for exchange between intracellular L and extracellular M. The numbers assigned to the states indicate the convention used for labeling rate constants and equilibrium constants: k_{ij} refers to the rate constant for the transition $i \rightarrow j$ and $K_{ij} = k_{ij}/k_{ji}$

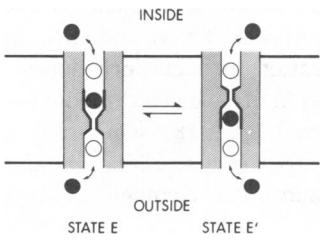

FIGURE 1 Schematic representation of the model. Shaded areas are the transmembrane domain of the transport protein. The alternating site is outlined in heavy lines and is shown occupied by an ion. Approach channels are represented by single sites between the alternating site and the two membrane surfaces. They are assumed to be in rapid equilibrium with the external solutions. Speculative structural models for how the movement of the alternating site might be accomplished have been suggested (6, 7).

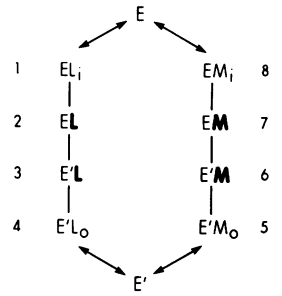

FIGURE 2 Reaction scheme for exchange between internal L and external M. Boldface L or M represents an ion bound to the alternating site. Subscripts i and o represent ions bound to the inward and outward facing approach sites. EL, E'L, EM, and E'M represent mixtures of several microstates, differing in occupancy of the approach sites (see text). Likewise, E, EL_i, and EM_i may have the outward facing approach site empty or occupied by L or M, and E', $E'L_o$, and $E'M_o$ may have the inward facing approach site empty or occupied.

is the corresponding equilibrium constant. Boldface symbols (L and M) are used to represent ions bound to the alternating site in state E or E'. Each state is an equilibrium mixture of several states differing in the occupancy of the approach sites, e.g., EL represents a mixture of ELO (approach sites empty), ELL_i, ELL_o, ELM_i, ELM_o, ELL_iL_o, ELL_iM_o, ELM_iM_o and ELM_iL_o. With the previous assumption of a lack of interaction between the approach sites and the alternating site, the rate constants for the conformational transitions 2 ↔ 3 and 6 ↔ 7 are the same for all these subspecies.

Occupancy of approach sites affects the overall kinetics in another way, unrelated to direct interaction between sites. The transition 1 → 2 in the scheme of Fig. 2 represents transfer of an ion from the inward-facing channel site to the alternating site, leaving the channel site unoccupied, and therefore the reverse transition, 2 → 1, can occur only if the inward-facing channel site is initially unoccupied. This means that the rate of the transition 2 → 1, expressed in terms of the total concentration of EL (regardless of the state of occupancy of channel sites) becomes

$$V(2 \rightarrow 1) = k_{21}\,[E\mathbf{L}] / (1 + y), \qquad (2)$$

where $y = K_{Li}\,[L(\text{in})] + K_{Mi}\,[M(\text{in})]$. Both transitions are independent of the state of occupancy of the outward-facing channel sites. A similar relation applies to the transition 7 → 8.

$$V(7 \rightarrow 8) = k_{78}\,[E\mathbf{M}] / (1 + y). \qquad (3)$$

On the other hand, transition 3 → 4 and 6 → 5 require that the outward-facing channel sites be vacant but their rates are independent of the state of occupancy of the inward-facing sites. This leads to

$$V(3 \rightarrow 4) = k_{34}\,[E'\mathbf{L}] / (1 + z) \qquad (4)$$

$$V(6 \rightarrow 5) = k_{65}\,[E'\mathbf{M}] / (1 + z), \qquad (5)$$

where $z = K_{Lo}\,[L(\text{out})] + K_{Mo}\,[M(\text{out})]$. It is these restrictions that lead to rate inhibition at high concentrations of L or M on either side of the membrane. Release of an ion from the alternating site can occur only via an unfilled channel site and will be impeded when the average degree of occupancy of the channel sites is high.

It is useful to define some additional equilibrium constants. The intrinsic affinities of L and M for the alternating sites can be expressed in terms of equilibrium constants for the reactions $E + L \rightleftharpoons E\mathbf{L}$, etc. These constants will be designated by boldface symbols $\mathbf{K}_L$, $\mathbf{K}_M$, $\mathbf{K}'_L$, and $\mathbf{K}'_M$, primes designating association with the alternating site in state E'. We have

$$\mathbf{K}_L = K_{12}\,K_{Li} \tag{6}$$

$$\mathbf{K}_M = K_{87}\,K_{Mi} \tag{7}$$

$$\mathbf{K}'_L = K_{43}\,K_{Lo} \tag{8}$$

$$\mathbf{K}'_M = K_{56}\,K_{Mo}. \tag{9}$$

The relative stabilities of the two conformational states E and E' (in their completely unliganded forms) can be expressed in terms of the equilibrium constant $K_p = [E']/[E]$. The following relations then apply

$$K_p = K_{23}\,\mathbf{K}_L / \mathbf{K}'_L = K_{76}\,\mathbf{K}_M / \mathbf{K}'_M. \tag{10}$$

Direct interconversion between unliganded E and E' is of course kinetically prohibited, but Eq. 10 provides a mandatory thermodynamic relationship between the equilibrium constants K_{23} and K_{76}, which apply to the conformational transitions (with ligand attached) on the reaction pathway. As noted before, it will be assumed here that there is no direct interaction between the approach sites and the alternating site, and this makes K_{23} and K_{76}, as well as the corresponding forward and reverse rate constants, independent of the state of occupancy of the channel sites.

Standard procedures (13–15) can now be used to derive steady state rate equations to fit any desired experimental circumstances. For example, if L is an anion present exclusively on the inside and M is present exclusively on the outside, and if $[M]$ is varied at constant $[L]$, then Eq. 1 with $x = [M]$ describes the initial rate as a function of $[M]$, with

$$1/C = \{1 + k_{67}/k_{65} + (k_{76}/k_{78})(1 + K_{Li}\,[L])\}/\mathbf{K}'_M\,k_{67} \tag{11}$$

$$A/C = k_{12}^{-1} + k_{23}^{-1} + k_{56}^{-1} + k_{67}^{-1} + k_{34}^{-1}\,(1 + K_{32}) + k_{78}^{-1}\,(1 + K_{76}) + (k_{21}^{-1} + k_{23}^{-1} + K_{32}/k_{34})/\mathbf{K}_L[L] + (1 + K_{76})K_{Li}[L]/k_{78} \tag{12}$$

$$B/C = (K_{Mo}/k_{34})\,(1 + K_{32} + K_{32}/\mathbf{K}_L[L]). \tag{13}$$

Extension of the model to allow for interaction between the channel sites and the alternating site would lead to a more complex rate equation than Eq. 1, e.g.,

$$V = \frac{C(x + Ex^2)}{1 + Ax + Bx^2 + Dx^3}, \tag{14}$$

without changing the principal feature, that V goes to zero at both high and low x.

Combining Eqs. 11 and 13 shows that the parameter B of Eq. 1 is proportional to the ratio k_{67}:k_{34}, and that B will therefore become very small and the inhibitory effect will essentially disappear if the rate constants for transfer between channel sites and alternating sites (here represented by k_{34}) greatly exceed the rate constants for the $E \rightleftharpoons E'$ transition (here represented by k_{67}). This is a general result: if exchange between channel sites and the alternating site is fast compared with the overall rate, then rapid equilibrium will be established between the alternating site and the bulk solution, and the channel sites cannot then exert a significant kinetic effect.

The equations for the coefficients of Eq. 1 become particularly simple for isotope flux of a single ionic species (L) at chemical equilibrium. The various states of the protein (apart from the minute fraction carrying the isotope) will then be at equilibrium. Furthermore, states 5 to 8 become chemically identical to states 1 to 4, and rate and equilibrium constants for the right-hand half of Fig. 2 can therefore be equated with corresponding parameters for the left-hand half. In the absence of a membrane potential, the equilibrium concentration of unlabeled L is the same on both sides of the membrane, and $x = [L]$ becomes the only concentration variable, with

$$1/C = (k_{21}^{-1} + k_{23}^{-1} + K_{32}/k_{32})\,(1 + K_p)/\mathbf{K}_L \tag{15}$$

$$A/C = (k_{21}^{-1} + k_{23}^{-1} + K_{32}/k_{34})\,(1 + K_{23}) + (\mathbf{K}_L/k_{12} + \mathbf{K}'_L K_{32}/k_{43})\,(1 + K_p)/\mathbf{K}_L \tag{16}$$

$$B/C = (\mathbf{K}_L/k_{12} + \mathbf{K}'_L K_{32}/k_{43})\,(1 + K_{23}). \tag{17}$$

A recent report (16) indicates that self-inhibition of Cl^- exchange occurs exclusively from the inside surface of the membrane, implying for the present model that a kinetically significant approach channel for Cl^- may exist only on that side. For this situation, assuming that the alternating site itself is now in rapid equilibrium with the extracellular solution in the E' state, Eqs. 15–17 become

$$1/C = (k_{21}^{-1} + k_{23}^{-1})\,(1 + K_p)/\mathbf{K}_L \tag{18}$$

$$A/C = (k_{21}^{-1} + k_{23}^{-1})\,(1 + K_{23}) + (1 + K_p)/k_{12} \tag{19}$$

$$B/C = (1 + K_{23})\mathbf{K}_L/k_{12}. \tag{20}$$

The foregoing equations for the coefficients can all be expressed in a variety of forms by use of Eqs. 6–10, e.g., we can replace K_{32}/k_{34} in Eqs. 15 and 16 by $\mathbf{K}_L/K_{Lo}K_pk_{43}$.

COMPARISON WITH EXPERIMENT

An interesting property of Eq. 1, which is independent of the values of the coefficients, is as follows: If x_p is the value of x at which V is a maximum, and x_1 and x_2 are the values of x for 50% maximal flux, then

$$x_1\,x_2 = x_p^2. \tag{21}$$

Moreover, if all coefficients are to be positive, it is necessary that $x_1/x_p < 0.27$ and $x_2/x_p > 3.7$. A consequence of these relations is that V must decrease relatively slowly with increasing x above x_p, and it will usually be impossible to verify by direct experiment that V actually goes to zero as x becomes very large. For example, if $x_p = 150$ mM and the lower concentration for half-maximal flux is 15 mM, then the upper concentration for half-maximal flux predicted by the equation would be 1.5 M.

Eq. 21 is valid not only for the x values that correspond to 50% of maximal flux, but for all fractions of the maximal flux, e.g., it applies where x_1 and x_2 represent the two values of x at which the flux is, say, 75% of its maximal flux. The equation therefore affords an initial criterion for testing the validity of Eq. 1 that can be applied to all experimental data near the flux maximum. Allowing for reasonable experimental uncertainty (see below for a specific example) most published data on initial rates of red cell anion exchange satisfy the criterion and probably obey Eq. 1 over the entire concentration range that was investigated. Phosphate isotope exchange (12) appears, however, to be an exception: the phosphate concentration (x_1) for half-maximal flux is slightly higher than the relation $x_1/x_p < 0.27$ permits, and there is indication of cooperative activation at very low phosphate concentration. These observations require an expanded form of the rate equation, such as Eq. 14. Alternatively, they could result from the procedure used for the experiments, which involved a decrease in ionic strength at low phosphate concentrations. This could have led to an appreciable Donnan potential across the membrane at low phosphate concentrations, with concomitant generation of a pH gradient across the membrane. The transport rate for phosphate is known to be very sensitive to pH (1, 5, 12).

As was noted in the Introduction, the equation used by Dalmark (9) to represent his measurements of Cl^- isotope flux at equilibrium is formally identical to Eq. 1. It provides an excellent representation of his results as a function of Cl^- concentration up to 500 mM Cl^-.

The number of parameters in the equations for A, B, and C for the present model is larger than the number of experimental coefficients, so that unique parameter values cannot be obtained. If only a single approach site (on the inside) is assumed, Eqs. 18–20 apply. Little generality is lost by setting the E/E' equilibrium constant (K_p) equal to unity, because Dalmark's experiments preclude distinction between the two sides of the membrane and can therefore give no information about the ratio $\mathbf{K}'_L/\mathbf{K}_L$, which is reciprocally related to K_p by Eq. 10. (Similarly, it would make no practical difference if we allowed for approach sites on both sides of the membrane and used Eqs. 15–17 for the analysis.) With the assumption of a single approach site and $K_p = 1$, the data yield unique values for k_{12}, for the sum of $\mathbf{K}_L$ and $\mathbf{K}'_L$, and for $(1/k_{21} + 1/k_{23})/\mathbf{K}_L$. The absolute magnitude of the observed flux sets an absolute lower limit on the rate constant for the conformational transition (k_{23}), which is obtained by setting $k_{21} = \infty$. The actual value of k_{23} need not be much higher than this minimal value: if, for example, we specify that K_{Li} should not exceed $\mathbf{K}_L$, then, by Eq. 6, k_{21} cannot be less than k_{12}, and this makes the contribution of $1/k_{21}$ to the sum of $1/k_{21}$ and $1/k_{23}$ relatively small. Table I lists a reasonable set of kinetic parameters based on two choices for the ratio $\mathbf{K}_L/\mathbf{K}'_L$.

Dalmark (9) also investigated Cl^- isotope flux at equilibrium in a mixture of Cl^- and another anion. The requisite modification of Eqs. 18–20 is given in terms of the functions

$$F_1 = 1 + (\mathbf{K}_M + K_p \mathbf{K}'_M)\,[M]/(1 + K_p) \qquad (22)$$

$$F_2 = 1 + K_{Mi}\,[M]/(1 + k_{21}/k_{23}), \qquad (23)$$

where $[M]$ is the concentration of the added ion. The expression for $1/C$ is multiplied by F_1F_2, the first term of the expression for A/C is multiplied by F_2, and the second term is multiplied by F_1. The expression for B/C remains unchanged. With $K_p = 1$ and k_{21} and k_{23} determined from the data in Cl^- alone, unambiguous values for the binding constants of the added ion are obtained. Where M is Br^-, Dalmark's data require that $K_{Mi} = 2\,K_{Li}$ and $(\mathbf{K}_M + \mathbf{K}'_M) = 2\,(\mathbf{K}_L + \mathbf{K}'_L)$. Dalmark obtained a similar result for his model: both the transport site and the modifier site had to be assigned twice the affinity for Br^- as for Cl^-.

Gunn and Fröhlich (10) have studied Br^- isotope flux in intact red cells at equilibrium, and have observed a peak flux near 100 mM Br^- and self-inhibition at higher concentrations. Their reported data, however, are not consistent with Eq. 1 over the entire concentration range studied: e.g., if their results at and above the peak position are fitted to Eq. 1, the data at low Br^- concentration are displaced to the right from the theoretical equation. The results can be brought into agreement with Eq. 1 if the

TABLE I
PARAMETERS FOR Cl^- EQUILIBRIUM SELF-EXCHANGE AT 0°C*

Uniquely determined parameters	
$\mathbf{K}_L + \mathbf{K}'_L\,(M^{-1})$	30
$k_{12}\,(s^{-1})$	5.1×10^3
$(k_{21}^{-1} + k_{23}^{-1})/\mathbf{K}_L\,(M)$	6.5×10^{-5}
k_{23} (absolute minimum, s^{-1})	5×10^2

	Selected values	
	$\mathbf{K}_L = \mathbf{K}'_L$	$\mathbf{K}_L = 4\,\mathbf{K}'_L$
$K_{Li}\,(M^{-1})$	10	10
$\mathbf{K}_L\,(M^{-1})$	15	24
$\mathbf{K}'_L\,(M^{-1})$	15	6
$k_{21}\,(s^{-1})$	3.4×10^3	2.1×10^3
$k_{23}\,(s^{-1})$	1.5×10^3	9×10^2

*Rate constants were obtained in molecular units by assuming the presence of 10^6 copies of the transport protein per cell. See text for other details.

concentration for half-maximal flux is moved from 19 mM Br^- (as reported) to 13.5 mM Br^-. If this is done, then the Br^- binding constants deduced from the data become about twice as large as the corresponding binding constants for Cl^-, in agreement with the values determined from Dalmark's data on the secondary effect of added Br^- on Cl^- equilibrium flux. The reduction in the Br^- concentration for half-maximal flux (implying uncertainty in the reported data) is not unreasonable. In an equilibrium experiment using resealed ghosts instead of intact cells, Gunn and Fröhlich observed half-maximal flux at 7 mM Br^-. It is also worth noting that the data in intact cells were obtained in the presence of a high concentration of acetate. Even a low affinity of this ion for the alternating site would lead to competition with Br^- and would raise the Br^- concentrations required both for peak and half-maximal rates, an effect that can be quantitated by use of Eqs. 22 and 23. Competition would, however, not affect the form of the rate equation, so that adjustment of the data per se (in this case at high [Br^-]) would still be necessary for adherence to Eq. 1.

The absolute magnitude of the flux for Br^- exchange is much lower than for Cl^-, and this leads to a much lower value for the rate constant k_{23}. However, k_{12} and k_{21} are also reduced, though not to the same extent. For example, with $\mathbf{K}_M = \mathbf{K}'_M$ (M representing Br^-), $k_{23} = 93\ s^{-1}$, $k_{12} = 690\ s^{-1}$, and $k_{21} = 440\ s^{-1}$, compared with the values of 1,500, 5,100, and 3,400 s^{-1} given for Cl^- in Table I.

The numerical data given in Table I and in the preceding paragraph would not be significantly altered if Eqs. 15–17 (approach channels from both sides) were used for the coefficients in place of Eqs. 18–20. The number of parameters would be increased, providing a wider choice of alternative assignments, but the significant physical features would not be changed thereby. One could, for example, now assign k_{12} the same value for Br^- exchange as for Cl^- exchange, but one could then not avoid assigning a lower value to k_{43}, the rate constant for passing from the channel site to the alternating site on the other side of the membrane.

DISCUSSION

Any mechanistic model leading to Eq. 1 or to a more extended rate equation such as Eq. 14 can fully account for self-inhibition of transport at high concentrations of a transported species. The advantage of the model used here is that the binding sites responsible for inhibition are introduced to fulfill a functional requirement on the translocation pathway, whereas the modifier sites that Dalmark (9) used to derive Eq. 1 have no obvious functional significance. However, no claim is made that the present model is the only possible model that incorporates a functional purpose.

An important feature of the present model is that it can lead to self-inhibition only if the rate of exchange of an ion between the postulated approach sites and the alternating site has the same order of magnitude as the rate of flip-flop of the alternating site itself. This is illustrated by the values for k_{12} and k_{21} in Table I: they are several orders of magnitude smaller than time constants expected for diffusional transfer and therefore require the existence of a relatively high free energy barrier for movement of the ion between the two sites. Furthermore, k_{12} and k_{21}, as well as k_{23}, are affected when data for Cl^- and for Br^- are compared. A possible reason for slow transfer is that a change in the hydration state of the ion may be involved.

The physical basis for the present model is that the translational displacement at an alternating access site is likely to be significantly smaller than the overall distance that an ion needs to traverse in passing from one side of a membrane to the other. In the case of the red cell anion exchange protein, there is direct structural evidence for a functional anion binding site deeply embedded in the domain of the membrane (17, 18), but the distance disparity here invoked might theoretically be expected to apply to all translocation processes that have an alternating access site as core feature of the translocation mechanism. Self-inhibition similar to that for red cell anion exchange has in fact been observed for Na^+ exchange fluxes in human fibroblasts (19), and there is evidence for similar inhibition in renal Na^+/H^+ exchange (20). It should be noted that failure to observe inhibition in any other exchange process does not necessarily indicate the absence of approach channels because of the kinetic requirement discussed in the preceding paragraph. Anion exchange across the red cell membrane (at least for Cl^- and Br^-) is a relatively rapid process. In systems where the overall ion translocation rate is slower, the principal reason for a lower rate may be that the rate of the conformational transition (analogous to the step 2 → 3 in the present scheme) is lower, e.g., in the case of ATP-coupled transport, because the conformational transition has to be coupled to changes in the ATP-processing part of the molecule. In that event it would become more difficult to meet the kinetic requirement that the rate of transfer between the approach sites and the alternating site must not greatly exceed the overall transport rate.

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REFERENCES

1. Knauf, P. A. 1979. Erythrocyte anion exchange and the band 3 protein: transport kinetics and molecular structure. *Curr. Top. Membr. Transp.* 12:249–263.

2. Patlak, C. S. 1957. Contributions to the theory of active transport. II. The gate type non-carrier mechanism and generalizations concerning tracer flow, efficiency, and measurement of energy expenditure. *Bull. Math. Biophys.* 19:209–235.
3. Tanford, C. 1983. Translocation pathway in the catalysis of active transport. *Proc. Natl. Acad. Sci. USA.* 80:3701–3705.
4. Tanford, C. 1983. Mechanism of free energy coupling in active transport. *Annu. Rev. Biochem.* 52:379–409.
5. Gunn, R. B. 1978. Considerations of the titratable carrier model for sulfate transport in human red blood cells. *In* Membrane Transport Processes. J. F. Hoffman, editor. Raven Press, New York. 1:61–77.
6. Tanford, C. 1982. Simple model for the chemical potential change of a transported ion in active transport. *Proc. Natl. Acad. Sci. USA.* 79:2882–2884.
7. Knauf, P. A. 1984. *In* Membrane Transport Disorders. T. Andreoli, J. F. Hoffman, S. G. Schultz, and D. D. Fanenstil editors. Plenum Publishing Corp., New York. Second ed. In press.
8. Läuger, P. 1979. A channel mechanism for electrogenic ion pumps. *Biochim. Biophys. Acta.* 552:143–161.
9. Dalmark, M. 1976. Effects of halides and bicarbonate on chloride transport in human red blood cells. *J. Gen. Physiol.* 67:223–234.
10. Gunn, R. B., and O. Fröhlich. 1979. Asymmetry in the mechanism for anion exchange in human red blood cell membranes. *J. Gen. Physiol.* 74:351–374.
11. Schnell, K. F., S. Gerhardt, and A. Schoppe-Fredenburg. 1977. Kinetic characteristics of the sulfate self-exchange in human red blood cells and red blood cell ghosts. *J. Membr. Biol.* 30:319–350.
12. Schnell, K. F., E. Besl, and R. v. der Mosel. 1981. Phosphate transport in human red blood cells: concentration dependence and pH dependence of the unidirectional phosphate flux at equilibrium conditions. *J. Membr. Biol.* 61:173–192.
13. King, E. L., and C. Altman. 1956. A schematic method of deriving the rate laws for enzyme-catalyzed reactions. *J. Phys. Chem.* 60:1375–1472.
14. Segel, I. H. 1975. Enzyme Kinetics. John Wiley & Sons, Inc., New York.
15. Hill, T. L. 1977. Free Energy Transduction in Biology. Academic Press, Inc., New York.
16. Knauf, P. A., and N. A. Mann. 1984. Location of the modifier site of the human erythrocyte anion exchange system. *Biophys. J.* 45 (2, Pt. 2):18*a*. (*Abstr.*)
17. Rao, A., P. Martin, R. A. F. Reithmeier, and L. C. Cantley. 1979. Location of the stilbenedisulfonate binding site of the human erythrocyte anion-exchange system by resonance energy transfer. *Biochemistry.* 18:4505–4516.
18. Macara, I. G., S. Kuo, and L. C. Cantley. 1983. Evidence that inhibitors of anion exchange induce a transmembrane conformational change in band 3. *J. Biol. Chem.* 258:1785–1792.
19. Villereal, M. L. 1981. Sodium fluxes in human fibroblasts: kinetics of serum-dependent and serum-independent pathways. *J. Cell. Physiol.* 108:251–259.
20. Ives, H. E., V. J. Yee, and D. G. Warnock. 1983. Mixed type inhibition of the renal Na^+/H^+ antiporter by Li^+ and amiloride. *J. Biol. Chem.* 258:9710–9716.