HOW CHANNEL-LIKE IS A BIOLOGICAL CARRIER?

Studies with the Erythrocyte Anion Transporter

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Monovalent anion transport across the human erythrocyte membrane occurs almost exclusively as a 1:1 exchange transport of intracellular for extracellular anions. Even when the membrane is made highly cation-permeable, the rate of chloride exchange is several orders of magnitude faster than the rate of chloride net transport. This high ratio of unidirectional flux to net flux kinetically defines the red cell anion transporter as a carrier-type transporter. (For comparison, a channel-type transporter would exhibit a ratio of, at most, unity.) A carrier-type transporter has to undergo conformational changes that result in the translocation of empty or substrate-loaded transport sites across the membrane, and in a channel-type transporter the substrate is able to move through a quasi-static structure without the need for a conformational change of the protein.

We and others have tested whether the carrier concept is applicable to anion net transport across the red cell membrane (1, 2). We found that a simple carrier-type kinetic scheme in which net transport is mediated by the return conformational change of the unloaded transporter cannot completely account for the concentration dependence of chloride net efflux from red blood cells. The data can be explained, however, by a model which describes the anion transporter as a specialized channel of very low conductance that can undergo rapid conformational changes between two states, thus exhibiting carrier-type exchange kinetics (1).

THEORY AND EXPERIMENTAL TEST

The simplest model one can envision for the proposed specialized conformational channel is the single-site, two-barrier, two-state model of Läuger (3), shown in Fig. 1. The two states correspond to the transport protein conformations in which the anion binding/transport site faces the cell interior (C_i) or exterior (C_o). The net transport mode derived from carrier-type behavior is the slippage mode, named after the occasional slipping of the unloaded transporter conformation as compared to the rapid conformational change of the loaded transporter (4). In this mode anions do not cross the higher barrier, and translocation of the anion is achieved only by the conformational changes of the protein. The net transport mode derived from channel-type behavior is the tunneling mode. In this mode the anion migrates through the anion transport protein, in either conformational state, crossing both barriers but not involving a conformational change of the protein (1).

Our strategy to examine the existence and relative contributions of the slippage and tunneling modes to net transport was to test three specific predictions of the slippage process available from our knowledge of the kinetic modeling of chloride exchange transport (5). It involved measuring the dependence of net efflux on the extracellular anion concentration, on the chemical nature of the transported anion, and on the intracellular anion concentration. All three tests are based on the assumption that the translocation conformational change of the unloaded transport site (the slippage step) is much slower than that of the loaded transporter.

In the first test one would expect the chloride net efflux by slippage to decrease to zero with increasing extracellular chloride concentration, $[Cl_o]$. Increasing $[Cl_o]$ reduces the number of empty outward-facing transport sites available for the return slippage reaction. We found that

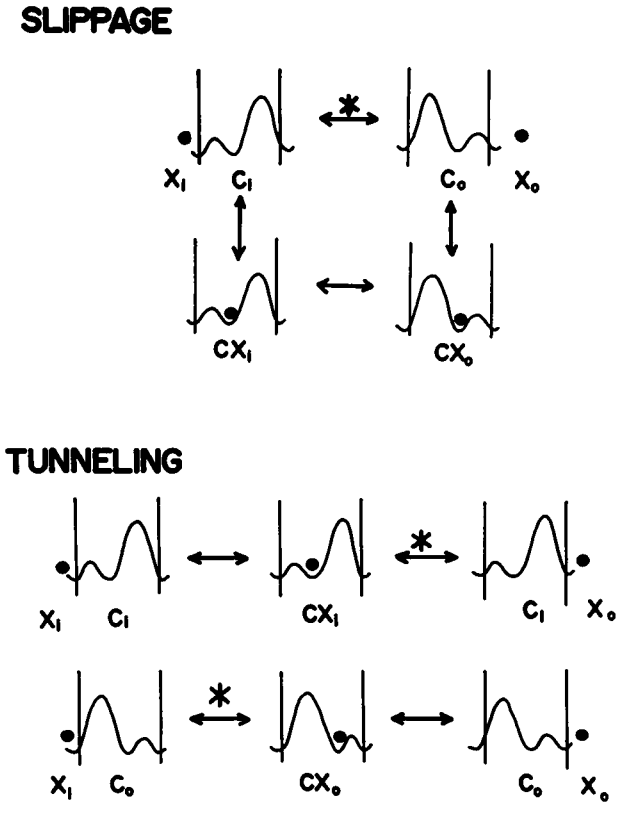

FIGURE 1 Modes of net transport mediated by the two-state channel of Läuger (3). The reactions marked by an asterisk are the slippage or tunneling reaction steps that underlie the two different modes.

DNDS-sensitive chloride net efflux from valinomycin- or gramicidin-treated red cells was hyperbolically inhibited by extracellular chloride with an inhibitor constant of ~3 mM, which was the same as the $K_{1/2}$ of exchange transport (1). However, the inhibition was not 100%, even at full saturation, indicating the presence of another net efflux component besides the slippage component. We tentatively attributed the $[Cl_o]$-inhibitable component to the slippage process and the $[Cl_o]$-independent component to the tunneling process (1).

The second test involved measuring the rate of bromide and nitrate net efflux as a function of the extracellular bromide/nitrate concentration. If the extracellular anion-inhibitable component were due only to slippage, it should have the same magnitude for all anions, because it is rate-limited by the slippage reaction of the unloaded transporter. Table I shows that both net efflux components depend on the chemical nature of the transported anion. This indicates that the $[Cl_o]$-inhibitable component is not solely due to the slippage mechanism.

In the third test, the dependence of chloride net efflux at $[Cl_o] = 0$ on the intracellular chloride concentration, $[Cl_i]$, was measured in nystatin-dialyzed red cells. The net efflux component due to slippage should be fully activated by low values of $[Cl_i]$, since at $[Cl_o] = 0$ nearly all transporter molecules are in the unloaded, outward-facing state that can undergo the slippage reaction. We found that chloride net efflux depended linearly on $[Cl_i]$ between 50 and 300 mM. Both curves could be extrapolated to zero flux at $[Cl_i] = 0$ within the error limits. This extrapolated flux value quantitates the relative contribution of slippage to overall chloride net efflux at $[Cl_o] = 0$. The data therefore indicate that the slippage process contributes virtually nothing to chloride net transport across the red cell membrane.

The data can, however, be explained by the proposed tunneling mechanism. One would expect the rate of outward tunneling to depend on the intracellular anion concentration. Also, the dependence of net efflux on the extracellular anion concentration is easily explained by assuming that the rate of outward tunneling is different for the two conformational states. At $[Cl_o] = 0$ the transporter exists nearly exclusively in the outward-facing conformation with the relatively higher tunneling rate. As $[Cl_o]$ is increased, more of the transporter molecules are loaded with chloride and switch to the inward-facing conformation with a lower rate of tunneling. At $[Cl_o] = 0$ one measures the rate of tunneling of the outward-facing conformation; at high $[Cl_o]$ one measures primarily the rate of the inward-facing conformation.

TABLE I

DNDS-SENSITIVE EFFLUX RATES FROM GRAMICIDIN-TREATED RED CELLS AT ZERO AND HIGH EXTRACELLULAR ANION CONCENTRATION (ESTIMATED MEMBRANE POTENTIAL −83mV).

Anion	0 mM	120 mM	Anion-inhibitable component
Chloride	55	7	48
Bromide	180	20	160
Nitrate	290	60	230

units are mmoles (kg Hgb)$^{-1}$ min^{-1}

The proposed kinetic model of the red cell anion transporter as a specialized two-conformation channel shed new light on the mechanism of this biological carrier. The "single channel conductance" of this channel-like structure is extremely low, corresponding to a turnover number of only 5–15 ions s^{-1} (at 25°C and 100 mV), and it is ~fivefold different for the two conformations. The slippage rate of the empty transporter is even slower (<1 s^{-1}). On the other hand, the rate of the conformational change is increased dramatically when the anion binding site is loaded (~13,000 s^{-1} at 25°C), thus accounting for the high rate of chloride exchange transport. The concept of tunneling may not only apply to the anion transporter but more generally to other carrier-type transporters, including pumps.

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