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Topical Review

The "Tunneling" Mode of Biological Carrier-Mediated Transport

Otto Fröhlich
Department of Physiology, Emory University School of Medicine, Atlanta, Georgia 30322

Introduction

This article deals with a new mode of mediated transport whose existence finds support on experimental and theoretical grounds. It has been postulated to explain certain observations on anion transport kinetics in erythrocytes and is consistent with the expectations of theoretical considerations. This new mode of mediated transport may well provide a functional link between biological channels and carriers. To begin, the term "tunneling" used for this process needs to be clarified: tunneling is used to mean the movement of a substrate along a narrow, channel-like pathway within a biological carrier protein, and that during this movement the substrate traverses the protein without it undergoing any significant conformational changes. Note that this definition does not imply a quantum-mechanical mechanism. Quantum-mechanical tunneling has been observed for electrons in chemical and biological reactions (*see* DeVault, 1984) and for some proton transfer reactions involving hydrogen bonds (*see* Bell, 1980). It has been examined as a potential mechanism for proton movements in biological proteins (Sarai & Devault, 1986), although it has not been documented in these systems. Most importantly, one can safely assume that quantum-mechanical events occur with an essentially zero probability for massive ionic particles such as chloride.

In order to set a frame of reference for this review, the terms "channel-type" and "carrier-type" kinetics or behavior need to be defined. The prototypes of a channel or carrier may be best represented by the ionophores gramicidin and valinomycin, respectively (Läuger, 1980). The major kinetic difference between these is seen after a substrate molecule or ion has been bound on one side (the *cis* side) of the membrane, translocated across the membrane, and released on the opposite side (the *trans* side). In a simple channel, another ion can immediately bind and be transported in the same direction, i.e., from the *cis* to the *trans* side. A carrier, on the other hand, has to reset itself before it can transport another ion in the same direction. In the case of the mobile carrier this resetting is the return diffusion ("translocation") of the carrier. In the overall reaction cycle, the carrier mediates exchange transport only when resetting occurs in the loaded state and net transport (in the *cis*-to-*trans* direction) when resetting occurs in the unloaded state.

These concepts are also applicable for biological channels and carriers. A large biological carrier protein typically spans the thickness of the lipid bilayer. Rather than diffusing from one side of the bilayer to the other, it undergoes conformational changes that result in a kinetically equivalent process of the bound substrate, alternatingly gaining access to the two aqueous compartments.

Given these definitions, the tunneling process is to be categorized as a channel-type behavior of a carrier protein since by definition it does not require the conformational change of the protein that is associated with the translocation of the transport site. Tunneling-type behavior has been observed with a transport system that can be considered a prototype biological carrier: the anion exchanger of the human erythrocyte. In terms of the physiological purpose

Key Words Band 3 · anion transport · anion conductance · transport kinetics · capnophorin

of mediating a one-for-one exchange of chloride and bicarbonate, tunneling constitutes a molecular slip in the coupling mechanism. This nonideality may help elucidate the kinetic and possibly also the physical mechanisms of carrier-mediated transport. The accumulating experimental evidence suggests that it might well be worth a closer look. This article therefore will review the theoretical considerations, experimental evidence and possible structural implications of this hypothetical transport mechanism.

Theoretical Considerations

It is generally understood that substrate transport by a membrane protein does not occur by means of a gross protein movement such as rotation through the plane of the membrane. Rather, the protein undergoes conformational changes that result in the substrate (and binding site) accessibility changing from one side of the membrane to the other. The physical nature of this translocation reaction is a matter of speculation. For example, the alternating accessibility of a site might be the result of a physically moving substrate binding site (i.e., an amino acid residue or a group of residues), possibly accompanied by altering affinities. Alternatively, the substrate might change positions in the protein while a conformational change "fixes" its new location and, therefore, its access to the aqueous compartment. There are a good number of descriptions and cartoons in the literature depicting the different notions of how transport by a biological carrier might occur. Early cartoons of erythrocyte anion exchange showed the translocation step as a swinging-door-type motion of an anion-binding group in a channel (Cabantchik, Knauf & Rothstein, 1978) or as a process involving two gate-type structures, one each near the entry and exit of a protein channel (Gunn, 1978). In the latter model, the two gates act in synchrony such that only one gate is open at any time. This alternates the accessibility of a fixed central binding site; the primary movement is accomplished by the surrounding protein structure. This lock-carrier model was fashioned after the early double-gated models of carrier-mediated transport of Patlack (1957) and Jacquez (1964). In contrast, more recent models suggest the relative motions of several positively and negatively charged amino acid residues along the transport pathway (Passow et al., 1980; Wieth, Bjerrum & Anderson, 1982; Brock, Tanner & Kempf, 1983; Macara & Cantley, 1983). These residues are assumed to break and reform salt bridges during translocation and at the same time to permit (or force) the anion to move through the protein. All these sketches and models have a channel structure in common for at least part of the transport pathway (*see also* Tanford, 1985).

While it is not easy to deduce from these models a realistic physical mechanism of carrier-mediated transport, a channel-like structure for transport proteins is reasonable. Very important contributions to this notion came from the theoretical considerations by Läuger and his coworkers (Läuger, Stephan & Frehland, 1980; Läuger, 1980, 1985). In their work on theoretical aspects of ion permeation through channels, first with rigid and then with fluctuating potential energy barriers, they proposed a minimal model that incorporated both carrier- and channel-like properties. This model is both simple and elegant. It is a channel, possessing two major potential energy barriers of differing heights and one central binding site (Fröhlich, 1984*b*; Fig.). If these barriers are small and fixed in height, then the model describes the simplest channel possible. Its conductance or net transport rate is determined by the height of the higher of the two potential energy barriers. However, if these barriers fluctuate in their height in a particular manner, namely such that as one barrier is lowered, the other barrier is raised, the model yields two different conformational states. With respect to their orientation in a cell membrane, these two states can be labeled "inward-facing" and "outward-facing" or *cis*-state and *trans*-state. With the additional assumption that the higher of the two barriers is "infinitely" high, the model becomes kinetically indistinguishable from a carrier and is equivalent to the double-gated lock-carrier model. The rate of substrate transport in this model is determined by the rate with which the two conformational states interconvert (steps 1 and 2 in Fig.).

This model may also describe a hybrid situation which is part carrier and part channel (*see* Fig.). In this situation, there are the two conformational states in which neither of the two major potential energy barriers is infinitely high. The interconversion between the two conformational states (steps 1 and 2) gives rise to carrier-kinetics. Interconversion between the unloaded conformations (step 1) is part of the carrier-type net transport cycle (in the order of the steps 3, 2, 4 and 1 or reverse), as described in the introduction, and interconversion between the substrate-loaded conformations (step 2) determines the exchange properties of the channel/carrier (involving the steps 3, 2, and 4 and reverse). On the other hand, while the protein resides in either conformational state with its "fixed" barrier profile, a substrate can also move through it (steps 5 or 6), and the rate of this channel-type net transport mode is determined by the height of the highest of the two barriers. This last process, i.e., substrate passage

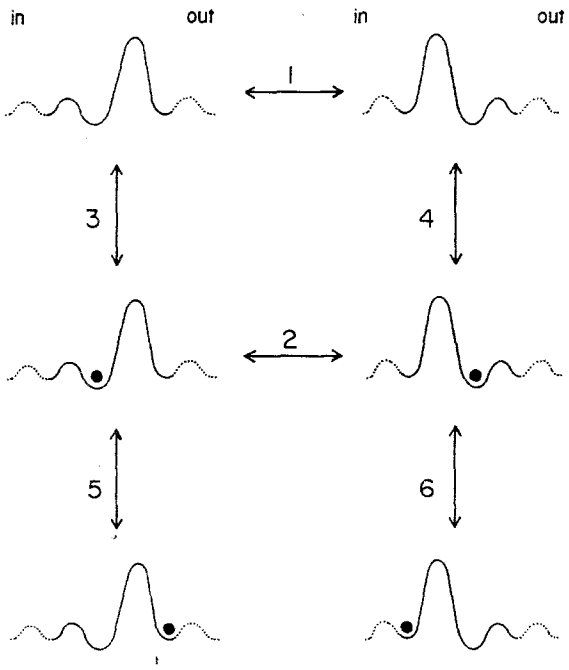

Fig. Representation of the different transport modes of the tunneling carrier model. The two different conformational states of the transporter (inward-facing on the left and outward-facing on the right-hand side) differ in the relative position of the large and the small barrier (solid lines) and thus in the access of the substrate (solid circle) to the central binding site. The dotted lines show additional barriers defining possible pre-binding sites, which are not considered in the simple model but which might have to be included as expansion towards a more realistic model. Reactions *1* and *2* represent the conformational changes that lead to carrier-type net and exchange transport. Reactions *3* and *4* represent the loading and unloading reactions of intracellular or extracellular substrate (solid sphere) to the main binding site. Reactions *5* and *6* are the tunneling steps through the inward-facing and the outward-facing state, respectively. Note that during these reactions only the substrate moves, but the barrier profile and thus the conformational state of the protein does not change

without a change in barrier height, is the tunneling process.

It is this type of passage that is examined here as a possible general feature of protein-mediated transport. Obviously, this scheme is an oversimplification. All mechanistic ignorance is put into the height of the potential energy barriers. However, it might be useful to consider these barrier heights as parameters whose physical interpretation is to be attempted at a later stage. Note then, e.g., that tunneling could occur through the *cis*-state or through the *trans*-state in either direction. Also, the relative tunneling rates could be different for the two states if the major barrier heights differ. Furthermore, the potential energy barrier profile within each state is by definition asymmetric with respect to the central plane of the bilayer. It is therefore quite possible, if not expected, that the tunneling rates in the two directions would differ. In the case of an ionic substrate this would mean a rectifying current-voltage relationship.

Fundamental Observations

Several experimental observations have been made with the anion transport system of the human erythrocyte membrane which are not consistent with classical carrier kinetics but are consistent with the tunneling hypothesis. The anion transporter, also known as band 3 protein or as capnophorin, behaves kinetically like a prototype carrier, mediating rates of monovalent anion exchange up to three orders of magnitude faster than that of anion net transport (Tosteson, 1959; Hunter, 1972). Still, band 3-mediated anion net transport is the major conductance pathway of the red cell membrane, rendering the membrane anion-selective and clamping the membrane potential to the Cl equilibrium potential (Vestergaard-Bogind & Lassen, 1974). Considering the large discrepancy between unidirectional (tracer) and net (conductive) permeabilities for chloride, the latter authors referred to the chloride conductance as a "diminutive slip in the coupling mechanism." Since around that time the carrier concept was introduced in kinetic studies of anion transport, it was widely presumed that the anion conductance would exhibit carrier kinetics, as well. This led to the common use of the term "slippage" for carrier-type anion net transport involving the translocation of the unloaded carrier.

In order to study anion net transport, one has to increase the cation permeability of the membrane until it no longer limits the rate of anion net flux for electrostatic reasons. This was achieved by several laboratories through the use of the ionophore valinomycin (Hunter, 1971, 1974, 1977; Kaplan & Passow, 1974; Knauf et al., 1977; Kaplan, Pring & Passow, 1980; Knauf & Law, 1980) or gramicidin (Cass & Dalmark, 1979) and opened the way for more detailed studies of the mechanism of the anion conductance.

Kaplan, Knauf and their coworkers (Kaplan et al., 1980, 1983; Knauf & Law, 1980; Knauf, Law & Marchant, 1983*a*) examined the rates of Cl net efflux in the presence of valinomycin and a K concentration gradient. When they measured Cl net efflux under conditions in which both the intracellular and extracellular Cl concentrations were varied in unison, they found a nearly linear dependence on Cl over a wide range, up to several hundred millimolar. This observation is not compatible with the notion of a classical carrier mechanism for anion

transport, for the following reason: At low substrate concentrations, the rate of carrier-mediated net transport is expected to increase because of an increased fraction of inward-facing loaded transport sites which can be translocated in the outward direction. At the same time, however, the fraction of outward-facing unloaded sites that are available for subsequent inward translocation decreases. Above a certain substrate concentration, a decreased rate of inward translocation of unloaded sites should in turn lead to a decrease of net transport in the efflux direction. At high, saturating substrate concentrations, carrier-kinetic net flux ("slippage") is expected to decrease to zero and only exchange transport should occur. From chloride exchange experiments it is known that at least at 0°C, physiological chloride concentrations nearly saturate the exchange mechanism (Gunn & Fröhlich, 1979) so that at least above 150 mM Cl one would expect a significant decrease of the chloride conductance. The observation that the chloride conductance increases at even high anion concentrations instead of decreasing therefore is not compatible with a simple carrier-kinetic scheme.

An equivalent observation was made by Fröhlich and coworkers (Fröhlich, Leibson & Gunn, 1983; Fröhlich, 1984*a*) who measured Cl net efflux as a function of the extracellular Cl concentration (at fixed Cl_i). In the framework of carrier kinetics, this approach would test the rate of inward translocation of the unloaded anion transporter, since for any rapidly exchanged (i.e., translocated) anion the unloaded half-cycle limits the overall rate of net efflux. Changing Cl_o results in shifts ("recruitment") of the anion transporter between the inward-facing and the outward-facing state: at Cl_o = 0, all transporters are in the outward-facing, unloaded state, and with increasing Cl_o they are converted into the inward-facing (and mainly loaded) state (*see* Jennings, 1980; Knauf, 1982; Fröhlich & Gunn, 1986). Indeed, a decreased rate of net efflux was observed with increasing Cl_o which one could tentatively explain with a decreased occurrence of the outward-facing, unloaded state of the transporter. However, this decrease did not proceed to zero, contradicting the expectation of the carrier scheme that the probability of that state approaches zero. Instead, the stilbene-inhibitable efflux approached the nonzero value observed with symmetrical Cl concentrations. Again, the conclusion was that chloride net efflux, at least at high extracellular anion concentrations, did not involve a transport half-cycle of the unloaded anion transporter. As explanation, the different laboratories invoked very similar channel-like concepts, albeit by different names: "barrier transit" (Knauf & Law, 1980; Knauf et al., 1983*a*), "electrodiffusion" (Kaplan et al., 1980, 1983), or "tunneling" (Fröhlich et al., 1983).

Other Evidence

These observations established tunneling as a prime candidate for anion net transport at physiological chloride concentrations (i.e., exchange site-saturating conditions). Further studies by Fröhlich (1984*a*) showed that also under nonsaturating conditions (at zero Cl_o) net transport by the slippage mode is not detectable within the experimental error limits. At zero Cl_o, virtually all anion transporter molecules are in the unloaded, outward-facing form and where one would expect the maximal contribution from the return translocation of the unloaded form. The virtual absence of slippage was demonstrated in two ways: qualitatively by studying net efflux of other anions such as bromide and nitrate and quantitatively by studying chloride net efflux as function of Cl_i. In the first test, bromide and nitrate net efflux into anion-free media was considerably faster than chloride net efflux. One would have expected very similar rates for the slippage mode that was rate limited by the same anion-independent inward translocation step of the unloaded transporter. The second test permitted one to divide the rate of net efflux into two components: the first, slippage component that is postulated by the carrier scheme, was expected to be saturated at low millimolar concentrations of Cl_i[1] and to become Cl_i independent at higher Cl_i. The second, tunneling component would be activated by increasing Cl_i with a lower apparent affinity and increase throughout the Cl_i range. Since the data could only be collected in a range of Cl_i where the carrier-kinetic component was saturated, slippage would be detectable only as a Cl_i-independent offset to any Cl_i-dependent tunneling component. When the data were extrapolated to zero Cl_i, they did not reveal a Cl_i-independent component and thus led to the conclusion that the rate of inward translocation of the unloaded anion transporter was negligibly slow compared to the rate of the presumed tunneling process.

This conclusion was recently confirmed in sul-

[1] For Cl net efflux by the slippage mode one would expect a very high apparent transport affinity (small $K_{1/2\text{-in}}$) for Cl_i since the low probability of inward translocation of the unloaded transport site would easily be matched by a few rapidly outward-translocated, Cl_i-loaded sites, even at very low levels of site saturation (Fröhlich & Gunn, 1986). This argument is the same as that previously used by Jennings (1980, 1982) for Cl-SO_4 and Cl-PO_4 exchange.

fate net transport experiments (King, Fröhlich & Gunn, 1986; King & Fröhlich, 1988).[2] Sulfate exchange and net transport are much slower than monovalent anion transport (Knauf et al., 1977). Thus with sulfate as anion, inward translocation of the unloaded transporter is no longer negligibly slow compared to other modes of net transport and should be more readily detectable: assuming that approximately 1 million copies of band 3 per red cell participate in transport (*see* Knauf, 1979; Passow, 1986), it was estimated to be about 1 sec^{-1}, compared to 30–40 anions sec^{-1} for the rate of Cl tunneling under comparable conditions (at 20°C, pH = 7.0, $V_m = -90$ mV, no extracellular transportable anions).

These studies have not only provided evidence in favor of a process that could be explained by the hypothetical tunneling mechanism, they have also provided an initial characterization of this process. First, there is the observation mentioned previously that the rates of net efflux differ into media containing zero and high anion concentrations. This means that the two conformational states which prevail under these two conditions have different tunneling rates, outward tunneling through the outward-facing state being about 10 times more rapid than through the inward-facing state. Second, activation of chloride net efflux by intracellular chloride shows little saturation up to several hundred millimolar (Kaplan et al., 1983; Knauf et al., 1983*a*), also when Cl_o is kept constant and only Cl_i is varied (Fröhlich, 1984*a*).[3] This low apparent affinity for entry into the tunneling pathway exists for the outward as well as the inward-facing state. Third, the anion selectivity is different for the rates of net transport ($NO_3 > I > F > Br > Cl > SO_4$) and for exchange ($Cl = NO_3 > Br = F > I > SO_4$) (Knauf et al., 1983*a*; Fröhlich, 1984*a*; King & Fröhlich, 1988;[4] *see also* Knauf, 1979; Passow, 1986), but the selectivity is similar for net efflux into anion-free media and into media containing the respective anions (Fröhlich, 1984*a*). Fourth, the activation energy of net efflux (both into high and zero-Cl_o media), 60–70 kJ mol^{-1}, is much lower than that of exchange, 110–120 kJ mol^{-1} (Fröhlich et al., 1983; O. Fröhlich, *unpublished observations*). This would be consistent with the notion that a less complex process is required for net flux by tunneling than for exchange with its protein conformational changes. Finally, there is preliminary evidence of a weak pH dependence of the Cl conductance at high Cl concentrations (Knauf et al., 1983*a*) and that this conductance depends on the membrane potential, i.e., that net efflux is a superlinear function of V_m (Knauf & Marchant, 1977).

More recent studies have provided additional evidence supporting the notion that anion net transport does not involve the conformational changes required for carrier kinetics. In these studies several inhibitors of anion transport were examined. The rationale behind these experiments is that a noncompetitive inhibitor, which by definition does not interfere with the binding of the transported substrate, should inhibit carrier-type transport by inhibiting the translocation conformational change. A tunneling substrate, however, should not be affected by this inhibitor. Accordingly, Knauf et al. (1983*a*) used the photo-activatable inhibitor NAP-taurine, which binds irreversibly to the anion transporter. They found that Cl exchange was inhibited by NAP-taurine binding to a larger extent than was Cl net efflux. The observation that net efflux was only partially inhibited complicates its interpretation, but it could be explained by a partial blocking effect of the sulfonate group of NAP-taurine if this residue was located near the anion binding site or elsewhere in the tunneling pathway. Simpler, more easily interpreted results were obtained recently with the reversibly binding, noncompetitive phloretin (Fröhlich, Bain & Weimer, 1986, 1988).[5] At concentrations that inhibited Cl exchange, phloretin had no detectable effect on Cl net efflux, neither into Cl-free nor into Cl-containing media (i.e., independent of the conformational state through which tunneling took place). In contrast, the reversibly binding, competitively inhibiting stilbene disulfonate, DNDS, inhibited Cl net efflux under both experimental conditions (Fröhlich et al., 1983). This is consistent with the notion that DNDS binds to the outward-facing anion binding/transport site and not only locks the transporter into this state but also blocks the passage of tunneling anions. Since DNDS and phloretin are mutually exclusive inhibitors of Cl exchange (Fröhlich & Gunn, 1987),

[2] King, P.A., Fröhlich, O. 1988. The effects of phloretin on sulfate exchange and net flux in human erythrocytes (*submitted*).

[3] This is not necessarily expected for tunneling through the simple, two-barrier, single-site model described in Fig. The apparent transport affinity of the inward-facing state, which is 20–60 mM for Cl exchange (Gunn & Fröhlich, 1979; Hautmann & Schnell, 1985) should be similar to the apparent loading affinity onto the tunneling mechanism since both share the inward-facing binding site. However, such a discrepancy should not be surprising because of the basic nature of the simple model, which at a later time will have to be extended to a more complex and realistic model.

[4] *See* footnote 2, above.

[5] Fröhlich, O., Bain, D. Weimer, L. 1988. The effect of phloretin and DNDS on chloride net transport in erythrocytes (*submitted*).

this permitted an additional test for the action of phloretin: phloretin, which by itself has no effect on Cl net flux, should keep DNDS from binding and thus from blocking Cl tunneling. Indeed, it was found that phloretin restored Cl net efflux that had been inhibited by DNDS. This observation also demonstrated that phloretin's noneffect on net efflux in the absence of DNDS was not due to an inability to bind to the anion transporter. These data therefore strongly support the hypothesis that there is a mode of substrate net flux through a carrier protein that does not require a conformational change and thus behaves more like the movement through a channel than through a carrier.

Alternative Explanations

While the above observations are consistent with the tunneling hypothesis and thus support it, they do not prove it. As alternative explanations, one could invoke the existence of additional, channel-like pathways in the erythrocyte membrane, in parallel with the anion exchange pathway. Such parallel pathways could reside in the anion transport protein or in another membrane protein. One would be inclined to exclude the latter possibility in view of the observation that the stilbene disulfonates which inhibit anion exchange, also blocked the anion conductance, but possible cross-reactivities of these inhibitors with other transport pathways should not be neglected. One might therefore search in the band 3 protein for a conductance pathway that is not congruent with the anion exchange pathway. Alternatively, one might conceive that a channel-like pathway is formed between band 3 monomers in a dimer or tetramer complex. Or, as a third possibility, one might suppose that on rare occasions and for very brief periods band 3 converts into a "classical" anion-selective channel.

There is little doubt that the band 3 protein is responsible for the anion conductance of the red cell membrane. Knauf and coworkers (1977) have established a clear correlation between the rates of anion exchange and net transport, by titrating red cells with different amounts of the irreversibly binding stilbene derivative DIDS and by measuring the rates of exchange and net flux at different extents of DIDS exposure. They found linear relationships between the degrees of inhibition of exchange and of net transport for both chloride and sulfate. Interestingly, complete inhibition of chloride exchange corresponded to only 65% inhibition of chloride net efflux, indicating the presence of a stilbene-insensitive Cl net efflux pathway. For sulfate, on the other hand, over 95% of net efflux could be inhibited under comparable conditions. The linear relationship clearly demonstrated that the transport pathways for exchange and net transport had the same chemical reactivity with the irreversibly binding DIDS. However, one could still argue that the similarity of reactivities of the two pathways was coincidental and that such a correlation might not hold under the more specific conditions of reversible binding.

This potential criticism was recently examined in studies of the effects of the reversible DNDS on chloride net efflux (Fröhlich et al., 1986, 1988).[6] The rationale was to compare its mode of inhibition with that previously established for chloride exchange (Fröhlich, 1982). As expected from chloride exchange, DNDS acted as a competitive inhibitor of chloride net efflux, possessing a higher inhibitory potency at lower extracellular chloride concentrations. Additional evidence in favor of the exchange mechanism as the mediating pathway of the chloride conductance came from the above-mentioned observation that phloretin reduced the inhibitory potency of DNDS for net Cl flux (Fröhlich et al., 1988)[7] in the same way as it competed with DNDS for binding to the exchange mechanism (Fröhlich & Gunn, 1987).

This study also examined the hypothesis that the conductance pathway might not be located within a band 3 molecule but instead is mediated by a narrow channel that is formed by the association of band 3 molecules. This hypothesis is based on the observation that band 3 resides in the erythrocyte membrane in the form of dimers or tetramers (*see* Jennings, 1984; Cuppoletti et al., 1985). The observed stilbene sensitivity of the anion conductance would then have to be explained by allosteric effects of stilbene binding on the oligomeric arrangement. There is, however, a testable prediction of this oligomer channel hypothesis which, for the sake of simplicity, will be discussed here for the case of a dimeric arrangement. Since the anion transporter can be shifted by the anion concentration gradient between the inward-facing and the outward-facing state, one would have to assume the existence of three different states of the dimer: the homo-dimer states in which both monomers are in the same, inward-facing or outward-facing conformation, and the hetero-dimer state where one monomer is inward-facing and the other is outward-facing. Since the outward-facing conformation has a considerably higher conductance than the inward-facing conformation (Knauf et al., 1983*a*; Fröhlich et al., 1983; Fröhlich, 1984*a*), the question arises as

[6] *See* footnote 5, p. 193.

[7] *See* footnote 5, p. 193.

to the conductance of the hypothetical hetero-dimeric state. A theoretical analysis revealed that, except in the case where the hetero-dimer's conductance equals the mean of the two homo-dimer conductances, one would expect a nonhyperbolic dependence of the chloride conductance on Cl_o, with either positive or negative apparent cooperativity. The data gathered for this purpose revealed no significant deviation from simple hyperbolic behavior. This means either that the tunneling rate through the hypothetical hetero-dimer equals the mean of the tunneling rates of the two homodimers, or that single band 3 molecules are the functional units responsible for net transport.

A third possible mechanism which one could invoke to explain the anion conductance is the presence of "classical" anion-selective channels. Given the similarity of the inhibitory patterns of exchange and net flux (DIDS titration, DNDS affinities, DNDS-phloretin interaction) one would then be compelled to postulate either that a band 3-like protein forms these channels or that occasionally a band 3 protein molecule switches into a state that possesses a large conductance. One has to keep in mind here that the anion conductance of the membrane of one red cell is about 30 pS. This means that approximately one permanently open channel would be sufficient to account for this conductance. Alternatively, if "normal" band 3 with its one million copies were to form such channels, it would have to convert to the channel form with a very low probability or its open lifetime would have to be very short.

Nevertheless, there are studies which indicate that band 3 could form channels (Freedman & Miller, 1983; Benz, Tosteson & Schubert, 1984; Galvez, Jennings & Tosteson, 1984). Benz et al. (1984) have reported that purified band 3, when incorporated into artificial lipid bilayers, increased their macroscopic conductance with a fourth-power dependence on the concentration, as if a tetramer of the protein were responsible for this conductance. Furthermore, they resolved single-channel events of magnitude of around 50 pS. However, these conductances were strikingly nonselective for cations and anions, and their open lifetime was in the range of seconds to minutes. One must therefore conclude that somewhere during the isolation or reconstitution procedure the protein underwent a major modification away from its physiological state, or that these observed conductance increases were due to contaminants. A more applicable observation was recently reported by Schwarz and Passow (1986). In patch-clamp experiments on intact red cells they observed single-channel events that exhibited anion selectivity as judged by diffusion potentials. Furthermore, these events were not observed with cells that had been pretreated with irreversible stilbene disulfonate inhibitors. However, this report was preliminary and gave no value for the single-channel conductance so that comparison is difficult between the observed single-channel events and the earlier artificial bilayer data, both of which will also have to be compared to the macroscopic anion net fluxes measured with cell suspensions. Further experiments will be needed to test whether single-channel data can account for the macroscopic fluxes, i.e., for the hyperbolic decrease of net efflux with increasing Cl_o, and for the nearly linear increase with increasing Cl_i. In the first case one should expect to observe a channel whose single-channel conductance and/or life-time decreases tenfold by raising the *trans*-chloride concentration from zero to 20 mM, while in the second case raising the *cis*-chloride concentration up to several hundred millimolar should linearly increase the single-channel conductance (or life-time).

Related Phenomena

Most of the experiments on band 3-mediated net transport have focused on net transport of anions. However, there are observations that suggest that at times band 3 might also transport cations.[8] Expanding on older reports in the literature (e.g., La Celle & Rothstein, 1966; Donlon & Rothstein, 1969; Morel, 1973), Jones and Knauf (1985) showed that in the absence of cationophores red cells lose KCl when they are placed into the media at low chloride concentrations in which salt was replaced by sucrose. They demonstrated that these fluxes are inhibited by DIDS. Furthermore, by titrating with different amounts of the irreversibly binding DIDS, they showed that the extent of anion exchange inhibition paralleled the extent of inhibition of KCl loss, and that the DIDS inhibition of KCl loss was not a secondary consequence of the inhibition of the anion conductance. The loss of cations was not specific to K^+; Na^+ and Rb^+ could utilize this pathway as well. It therefore appears that the band 3 protein at times can mediate the permeation of cations, although the mechanism that causes the cation permeability is not clear. In the framework of this

[8] There is still another way cations can be transported by band 3, namely as ion pairs with carbonate (Funder, Tosteson & Wieth, 1978). Li and Na can combine with carbonate to form $LiCO_3^-$ or $NaCO_3^-$, respectively, and are transported as negatively charged complexes in the same way as other anions. This transport mode, however, is of less concern here since it appears to occur via the carrier-like exchange mode with other anions.

discussion, the observed Cl_o dependence is noteworthy: Cl_o inhibited cation loss with an ID_{50} of 2–5 mM, very similar to the value obtained for Cl net efflux. Since with increasing Cl_o (but constant Cl_i) the fraction of transporters in the outward-facing, unloaded state decreases from unity to less than 1%, and since at $Cl_o \simeq 4$ mM half of the anion transporters are in that outward-facing state, one is tempted to associate the cation permeability increase with the recruitment of the transporter into the outward-facing state. However, Jones and Knauf (1985) found evidence against this notion in the insensitivity of the cation movements to niflumic acid, which is believed to bind preferentially to the outward-facing state of the anion transporter (Knauf & Mann, 1984). Despite the nearly saturating chloride concentration (Cl_o = 12 mM), niflumic acid should therefore recruit the transporter into the higher-conducting outward-facing state and thus increase the cation permeability; however, no increase was observed (Knauf, Mann & Kalwas, 1983*b*). Jones and Knauf (1985) therefore attributed the cation permeability changes to possible local influences of the ionic strength or the transmembrane potential.

The band 3 protein also appears to transport cations under ionically normal but cellularly pathological conditions. It has been known for several years that in the deoxygenated state sickle erythrocytes had an increased cation permeability (e.g., Tosteson, Carlsen & Dunham, 1952; Berkowitz & Orringer, 1985). Joiner and Dew (1986) recently demonstrated that the increased permeability for Na and K is mediated by the anion transporter since it can be inhibited by the same inhibitors as anion transport: DNDS inhibited with an affinity of about 10 μM, and titrating the cells with different amounts of DIDS reduced cation movements to the same extent as Cl-SO_4 exchange. One might note, however, that the band 3-mediated cation movements differ by nearly two orders of magnitude: the low Cl_o-stimulated fluxes are in the range of mmol (kg Hb)$^{-1}$ min^{-1}, whereas the deoxy fluxes were in the range of mmol (kg Hb)$^{-1}$ hr^{-1}. To which extent they are related and how hemoglobin could modify the transport behavior of band 3 is at the present time unknown.

As a final point one might mention the anion permeability of the erythrocyte membrane in the presence of inhibitors. Band 3-mediated fluxes are generally defined as the fluxes that are inhibited by the stilbene disulfonate inhibitors. However, there is a considerable fraction of the anion permeability (up to 35% at high Cl_o; Knauf et al., 1977; Fröhlich et al., 1983) that is not inhibited by reversibly bound DNDS or irreversibly bound DIDS, and the anion selectivity (in terms of transport rates) of this pathway is similar to that of the stilbene-sensitive net transport pathway: $NO_3 > F > Br > Cl$ (Knauf et al., 1983*a*; Fröhlich, 1984*a*). This sequence is qualitatively different from the sequence known for other erythrocyte transport systems such as cation-anion cotransport. Also, quantitatively the transport rates are several orders of magnitude higher than the cotransport rates. The question therefore is whether the stilbene-insensitive net flux occurs on band 3 as well, possibly through a pathway on the protein different from the stilbene-sensitive tunneling pathway, or whether another, unrelated protein pathway is involved. From the differing pH dependencies of the two net flux components Knauf et al. (1983) and the differing potential dependence (Freedman & Novak, 1987), one might suspect a separate pathway. These observations could also be explained by a separate tunneling pathway through band 3 that utilizes a portion of the transport mechanism that is not occluded in the presence of bound stilbenes.

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