Band-3 protein-mediated anion conductance of the red cell membrane

Slippage *vs* ionic diffusion

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The band 3 protein-mediated, valinomycin-induced KCl efflux continues to increase with increasing [KCl] when the Cl^-/Cl^- equilibrium exchange becomes saturated. This suggests the existence of a band 3-mediated component of Cl^- flux that contributes to the electrical conductance without being related to slippage; i.e., equilibration of the unloaded transport protein between the two membrane surfaces.

Band 3 protein *Anion transport* *Red cell membrane*

1. INTRODUCTION

Anion transport across the red cell membrane can be followed under two different experimental conditions:

(1) By measuring the flux with a radioactively labeled anion species while the anion distribution across the membrane is at equilibrium. This yields the permeability coefficient for anion equilibrium exchange.

(2) By measuring the net electrolyte efflux from the K^+-containing red cells into a medium that contains Na^+ as the principal cation species. The electrolyte efflux is induced by making the membrane selectively permeable for K^+ (e.g., by the addition of valinomycin). The ensuing K^+ loss generates a diffusion potential that drives an equivalent amount of anions across the membrane.

Applying the Goldman equation, it was possible to calculate from the net efflux of KCl the permeability coefficient for net Cl^- efflux. This coefficient was found to be about 4 orders of magnitude lower than the permeability coefficient for the Cl^-/Cl^- equilibrium exchange [1,2]. This difference indicates the existence of Cl^- movements across the membrane that do not contribute to the electrical conductance of the membrane and others that do, and shows that the former greatly exceed the latter.

Certain inhibitors of anion exchange, like SITS or H_2DIDS and their non-covalently binding analogues, that are known to react with a 1:1 stoichiometry with the anion transport protein in the red cell membrane [3,4], also produce a partial inhibition of the anion transport component that contributes to the conductance [5–7]. This suggests that the anion transport protein is not only responsible for the electrically 'silent' anion exchange but also for some of the net anion movements.

Currently, it is believed that the rate limiting step of the band 3 protein-mediated anion exchange involves the transition of a complex between the protein and the substrate from an outward-facing conformation to an inward-facing conformation, or vice versa. As long as this transition only takes place when an anion is bound, the protein mediates an exchange that does not contribute to the electrical conductance. However,

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when the unloaded form of the protein also undergoes the conformational change, net movements may occur that do contribute to the conductance (slippage) (review in [8,9]).

The occurrence of some slippage is to be expected for thermodynamical reasons since an exchange mechanism that operates at 100% efficiency is quite unlikely. Nevertheless, it would seem conceivable that there may also exist a mode of operation of the band 3 protein which allows Cl^- movements that contribute to the conductance but are not due to slippage.

The present note describes experiments that serve to clarify this question. The underlying rationale is the following:

Slippage involves conformational changes of the unloaded form of the transport protein. Increasing the substrate concentration should decrease this form and hence reduce conductive anion transport by slippage. At saturating anion concentrations, slippage should disappear altogether and no H_2DIDS-sensitive (i.e., band 3-mediated) component of conductive flux should be left [6,7,10].

The experiments presented below do not confirm this prediction and demonstrate the existence of an H_2DIDS-insensitive non-saturable flux component. Work based on the above ideas and first published in [10] is also underway in the laboratories of P. Knauf (Rochester) and R. Gunn (Atlanta).

2. EXPERIMENTAL ARRANGEMENTS AND RESULTS

Using the nystatin technique [11] human red cells were prepared with intracellular KCl at 50–400 mmol/l. The cells were then suspended in media in which [NaCl] were equal to the respective intracellular [KCl]. After addition of valinomycin to the medium, the ensuing net KCl efflux j_{KCl} was measured. This efflux depends on the P_K for the valinomycin-mediated K^+ transport and on the P_{Cl} for the naturally existing anion-transport pathway. P_K and P_{Cl} are usually defined by the Goldman equation which under the conditions existing in our experiments ($KCl_i = NaCl_o$), reduces to:

$$j_{KCl} = P_{Cl}\,[Cl^-]\,\ln\,[P_{Cl}/(P_{Cl} + P_K)]$$

where P_K and P_{Cl} represent, respectively, the permeability coefficients for K^+ and Cl^-. P_K was determined by measuring K^+/K^+ equilibrium exchange in experiments in which the KCl-loaded cells were suspended in media that contained KCl in place of NaCl. The P_K values were independent of KCl over the above concentration range. However, when H_2DIDS (an inhibitor of anion transport) was present in the media, the P_K values were found to be about 20% higher than in the absence of the agent (table 1).

Table 2 shows the relationship between the rate of KCl efflux and the (equal) salt concentrations in cells and medium. In the absence of H_2DIDS,

Table 1

K^+/K^+ equilibrium exchange, as measured by means of $^{42}K^+$ in the presence of 1×10^{-6} M valinomycin, 30°C, pH 6.8

[KCl] (mM)	H_2DIDS (μM)	$P_K \cdot 10^{-3}$ (min^{-1})	Flux ($mM \cdot cell^{-1} \cdot min^{-1}$)	$\pm \sigma_m$ ($n = 7$)
50	0	368.8	18.44	4.29
50	10	392.7	19.64	1.79
100	0	269.4	26.94	3.40
100	10	361.8	38.18	3.81
300	0	311.6	93.47	12.51
300	10	342.3	117.70	13.6

P_K, average for all KCl concentrations: (a) without H_2DIDS, $316.6 \times 10^{-3} \cdot min^{-1}$; (b) with H_2DIDS, $382.3 \times 10^{-3} \cdot min^{-1}$

Ratio (b)/(a) = 1.21

σ_m = standard error of mean

Table 2

Valinomycin-induced KCl efflux 1 × 10^{-6} M valinomycin, 30°C, pH 6.8

$[KCl]_i$ (mM)	Flux ± σ_m without H_2DIDS	$P_{Cl(1)}$	Flux ± σ_m + 10 μM H_2DIDS	$P_{Cl(2)}$	ΔP_{Cl}	$\Delta P_{Cl}/P_{Cl(1)}$
50	4.42 ± 0.35 (12)	0.0410[a]	1.89 ± 0.31 (12)	0.0100	0.031 [a]	0.76
100	6.07 ± 0.57 (8)	0.0223	2.41 ± 0.24 (8)	0.00575	0.0166	0.74
140	9.24 ± 2.00 (2)	0.0255	5.23 ± 1.44 (2)	0.01025	0.0153	0.60
150	8.96 ± 0.89 (12)	0.0208	3.95 ± 0.37 (10)	0.00645	0.0144	0.69
200	11.33 ± 1.93 (7)	0.0202	6.21 ± 0.98 (7)	0.00800	0.0122	0.60
250	13.55 ± 2.69 (3)	0.0178	8.13 ± 1.87 (3)	0.00850	0.0093	0.52
300	20.74 ± 2.03 (11)	0.0275	12.15 ± 1.35 (11)	0.0115	0.0160	0.58
400	23.20 ± 3.40 (10)	0.0209	15.99 ± 1.94 (10)	0.01125	0.0096	0.46
Av.	–	0.0214	–	0.00896	0.0133	0.62

[a] not included in the average

σ_m = standard error of the mean (no. expt in brackets)

$[KCl]_i = [NaCl]_o$ where i and o refer to inside and outside, respectively

Fluxes in $mM.cell^{-1}.min^{-1}$; permeabilities in min^{-1}

$\Delta P_{Cl} = P_{Cl(1)} - P_{Cl(2)}$

$P_{Cl(1)}$ and $P_{Cl(2)}$ were measured at $\Delta\Psi = -69.5$ mV and -85.4 mV, respectively

Contributions of the electrogenicity of the Na^+–K^+-pump to the total membrane potential could be of the order of magnitude of ~0.1 mV [15] and hence were neglected

≥100 mM KCl, this relationship becomes linear. If one assumes that this upper part of the curve represents flow by simple electro-diffusion, it is possible to calculate P_{Cl} values from the individual data points (table 2). It is apparent that except for the data point at 50 mM, the calculated values do not vary in a systematic manner with [KCl].

Parallel measurements were executed with sufficient H_2DIDS present in the external media to produce a virtually complete inhibition of the band 3-mediated anion exchange. Under these conditions, the rate of net KCl efflux is reduced but not completely inhibited. Again, P_{Cl} values were calculated from the data points (table 2), and again, no systematic variation with KCl concentration was observed.

Our data show that at high $[Cl^-]$ the net KCl flux, j_{KCl}, is linearly related to intracellular KCl. In our experimental arrangement there is no change of membrane potential over the $[Cl^-]$ range used, so j_{KCl} is dependent upon P_{Cl} and P_K only. In the absence of H_2DIDS (column 2 of table 2), we find $\Delta\Psi = -69.5$ mV and $P_{Cl} = 0.0214$ min^{-1}, in the presence of H_2DIDS (column 4 of table 2) we obtain $\Delta\Psi = 85.4$ mV and $P_{Cl} = 0.00895$ min^{-1}. In the presence and absence of H_2DIDS differing values of P_{Cl} and also P_K had to be used. The difference between H_2DIDS-insensitive and H_2DIDS-sensitive P_{Cl} is also represented in table 2. Except for the value at the lowest [KCl], this difference seems to be independent of the [KCl]. The percentage changes of the P_K values possibly decrease slightly with increasing $[Cl^-]$, suggesting that there exist some variations that are hidden in the scatter of the individual data points. It should be noted, however, that the scatter of the points is of necessity relatively large and thus this impression cannot be substantiated by statistical techniques.

It is instructive to compare the results in table 2 with the measurements of Cl^- equilibrium exchange, as taken from [12]. Fig.1b shows the relationship between net Cl^- movements in the presence or absence of H_2DIDS as a function of $[Cl^-]$. It is obvious that over the $[Cl^-]$ range in which Cl^- equilibrium exchange saturates (fig.1a)

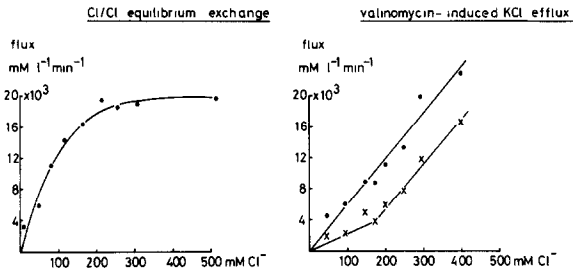

Fig.1. Comparison of concentration dependence of Cl^- equilibrium exchange and of valinomycin-induced net KCl efflux. Left: Data taken from [12] and recalculated for 30°C. On the scale employed in this panel, inhibition of the Cl^-/Cl^- equilibrium exchange by H_2DIDS would appear to be virtually complete. Right: Data from table 2; no H_2DIDS present; (×) H_2DIDS present; 30°C.

no signs of saturation become apparent.

The differences of the dependence on $[Cl^-]$ of the electrically 'silent' Cl^-/Cl^- exchange and the diffusive component of Cl^- flux prevent a simple quantitative evaluation of their relative contributions to the total Cl^- movements. It should be noted, however, that the ordinates of the two panels in fig.1, which depict the two flux components, differ by a factor of 1000.

3. DISCUSSION

It is evident from the above results, that we can divide the total Cl^- conductance into two fractions: an H_2DIDS-sensitive and an H_2DIDS-insensitive component. The H_2DIDS-insensitive flux, we will assume, is mediated by some pathway other than that provided by band 3. It shows no evidence of saturation in the concentration range used and may represent either an alternative ion-permeation route across the membrane or a contribution from OH^- or H^+ movements. The latter possibility is less likely since H^+ or OH^- movements are small and H_2DIDS-sensitive [5].

The existence of the H_2DIDS-sensitive component of Cl^- conductance clearly points to an involvement of the band 3 protein. This was anticipated on the basis of earlier studies with SITS [5,13] and the reversibly-acting H_2DIDS analogues DAS and APMB [13]. Like H_2DIDS, these compounds produce a partial inhibition of the Cl^- conductance. In addition, the reversibly-acting analogues show the same sidedness of their actions on Cl^- conductance and Cl^- exchange. Both inhibit Cl^- exchange at the extracellular membrane surface, but only APMB also inhibits at the cytoplasmic surface. The same-sidedness pertains to the inhibition of net Cl^- efflux [13]. The linear relationship between the inhibition of P_{Cl} and Cl^- equilibrium exchange in DIDS-treated cells, seen earlier, also strongly suggested the involvement of band 3 in Cl^- conductance [5].

It is evident from this work that anion penetration that is mediated by the band 3 protein is responsible for a portion of the Cl^- conductance. We had suggested that a 'slippage' in the Cl^- exchange apparatus could provide a conductance in [14]. However, one would expect that an increase in anion concentration reduces the frequency of slippage (section 1) since the proportion of unloaded transport protein molecules decreases. At complete saturation of all molecules, slippage should disappear completely and no longer contribute to net Cl^- efflux. Thus, if slippage would account entirely for the band 3-mediated Cl^- conductance, one should observe that the H_2DIDS-sensitive component of Cl^- conductance decreases with increasing $[Cl^-]$. Such decrease is suggested by the position of the data point at the lowest [KCl] in table 2. However, the absence of saturation of the H_2DIDS-sensitive component of Cl^- conductance with increasing Cl^- described above indicates that slippage cannot account completely for the Cl^- conductance that is mediated by the band 3 protein.

We are now able to dissect the anion flux through the red cell membrane into 4 components. By far the greatest contribution comes from the anion exchange component, mediated by band 3, which does not contribute to the conductance. The conductance can be accounted for by 3 components. Slippage and diffusive flow, both being mediated by band 3, whilst the remaining flow takes place via another pathway in the membrane separate from and parallel to the band 3-mediated pathway. Our experiments were not designed to determine slippage. However, they show that at high $[Cl^-]$, slippage is negligible as compared to a band 3-mediated diffusive component of Cl^- movements.

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