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SPECIFIC CATION MODULATION OF ANION TRANSPORT ACROSS THE HUMAN ERYTHROCYTE MEMBRANE

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Summary

The specific modulation by three cations, Ca^{2+}, Mg^{2+}, and tetracaine of the equilibrium exchange of SO_4^{2-} across the erythrocyte membrane was investigated. While external calcium had no effect on SO_4^{2-} exchange, internal calcium, and external calcium in the presence of 10 μM A23187 were found to be potent inhibitors of the exchange reaction. The apparent inhibition constants (K_I) for Ca^{2+} were calculated to be 6.1 μM and 5 μM for the above two conditions, respectively.

Unlike Ca^{2+}, Mg^{2+} was shown to be a weak activator of SO_4^{2-} exchange with an apparent dissociation constant of 3.6 μM. Competition experiments demonstrated that the Ca^{2+} and Mg^{2+} sites associated with anion transport are distinct and noninteracting.

Tetracaine, a cation at neutral pH, was also found to be an inhibitor of SO_4^{2-} exchange with an apparent K_I of 0.8 mM. Although tetracaine was observed to displace calcium from non-specific sites on the erythrocyte membrane, it showed no effect on the apparent inhibition constant of Ca^{2+} for SO_4^{2-} exchange. Thus, the Ca^{2+} and tetracaine sites also appear to be independent. The difficulty of situating three mutually independent sites on a single subunit protein, i.e., band 3, is considered.

Using the experimental data obtained from five individuals, the concentration of free calcium in the red cell cytoplasm was calculated to range from 0.2 to 0.7 μM. This concentration was sufficient to reduce SO_4^{2-} exchange only 3—8%. It was concluded that calcium inhibition of anion exchange, and, hence, impairment of CO_2 transport, may be physiologically significant only in senescent cells and in certain types of anemia where calcium concentrations are significantly increased.

Abbreviations: EGTA, ethyleneglycol bis(α-aminoethylether)-*N*,*N*′-tetraacetic acid; TES, *N*-Tris-(hydroxymethyl)methyl-2-aminoethane sulfonic acid.

Introduction

To facilitate the transport of CO_2 from the tissues to the lungs the red blood cell is equipped with an anion transport system which catalyzes the rapid exchange of anions (e.g., HCO_3^- for Cl^-) across the red cell membrane. In addition to the predominance of an electrically neutral exchange of anions [1], anion transport in erythrocytes is characterized by a high degree of anion selectivity [1], a pH optimum which depends on the valence of the anion [1,2], inhibition by highly specific covalent and noncovalent inhibitors [3,4], an extremely high activation energy of 30—35 kcal/mol [5], and substrate saturation at high concentrations of monovalent anion [1]. These observations suggest that anion exchange is not via free diffusion but instead is catalyzed by a specific structure or structural complex in the erythrocyte membrane.

The nature of the structure or structures involved in anion exchange in erythrocytes is currently a matter of considerable scientific activity. The transmembrane protein band 3 is very likely involved since it is the binding site for several highly specific inhibitors of the exchange reaction [6]. For several years a hydrophobic domain has also been implicated in the transport scheme [3,5—8] and this hypothesis has received considerable support from recent calorimetric studies of Snow, Brandts and Low [9] which implicate the participation of a crystalline phospholipid phase. The extremely strong association of band 3 with band 4.2 and other integral membrane proteins also raises the possibility that an interaction of band 3 with other polypeptides might be important for transport [10,11]. Thus, at present, the precise identity of all possible components of the anion transport system remains obscure.

One method for obtaining information about the relative complexity of a macromolecular structure is to study specific ligand binding by that structure. While a number of investigations have been concerned with anion binding to the transport system [1,12] no investigations to date have focused on cation binding. In this initial project we have taken a kinetic approach to the study of cation binding and have found that calcium is a potent non-competitive inhibitor of anion exchange in erythrocytes. We further demonstrate that the apparent inhibition constant (K_I) of calcium for the transport system is sufficiently small that its modulation of anion exchange may be observed even at the low intracellular calcium concentrations reported for normal eruthrocytes. We also demonstrate that Mg^{2+} and tetracaine interact either directly or indirectly with the transport system at sites which are distinct from the binding site for Ca^{2+}, and that they are, respectively, an activator and an inhibitor of anion exchange.

Methods

Materials. A23187 was a generous gift of Dr. Robert L. Hamill of Lilly Research laboratories, Indianapolis, Ind; tetracaine, ethyleneglycol bis(β-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA), ethylendiamine tetraacetic acid (EDTA), *N*-Tris-(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES) were purchased from Sigma Chemical Co.; fresh human blood was obtained from the Central Indiana Regional Blood Bank with citrate-dextrose solution

added, and stored at 3°C until used. Unused blood was discarded 21 days after withdrawl, when it was officially outdated. All chemicals were reagent grade and used without further purification.

Assay buffers. The assay buffers contained 5 mM Na_2SO_4, 10 mM TES, 120 mM KCl, and divalent cation buffers prepared to give a final EDTA or EGTA concentration of 8.81 mM. This buffer strength was determined experimentally to be sufficient to maintain the Ca^{2+} and Mg^{2+} concentrations at the desired values when erythrocytes were present at 10% hematocrit. The total osmolarity was then adjusted to 330 mosM with NaCl. The pH was adjusted to 7.0 at 36°C with NaOH and the pH of all solutions was monitored frequently to ensure no pH changes occurred during the course of an experiment. For calculation of the free concentration of divalent cation at pH 7.0, the following $\log_{10}$ of the apparent association constants were used: Ca^{2+}-EGTA, 6.68; Ca^{2+}-EDTA, 7.27; Mg^{2+}-EGTA, 1.6; Mg^{2+}-EDTA, 5.37 [13]. The divalent cation buffers were prepared as described by Amos et al. [13].

We determined that 120 mM KCl prevented the net efflux of K^+ which has been observed in Ca^{2+}-treated red blood cells [14]. This value agrees with the findings of Lake et al. [15], but is somewhat higher than the 75 mM KCl used by Ferreira and Lew [16] to offset K^+ leakage. However, unless such a high concentration of K^+ was used we observed some non-linearity in our rate plots, which was probably due to the co-transport of SO_4^{2-} with K^+.

Measurement of SO_4^{2-} exchange. Erythrocytes from human blood were washed four times at 3°C in 10 mM TES buffer, pH 7.0 at 36°C, containing 5 mM Na_2SO_4, 120 mM KCl and 30 mM NaCl, pH 7.0 at 36°C. The cells were then suspended at 10% hematocrit in the assay buffer containing the desired concentration of free Ca^{2+} or Mg^{2+}, and tracer amounts of $^{35}SO_4^{2-}$ were added. The divalent cation ionophore, A23187, was then introduced to reach a final concentration of 10 μM and the suspension was incubated in a shaker bath at 36°C for 90 min. This incubation period was shown to allow sufficient time for equilibration of both $^{35}SO_4^{2-}$ and divalent cation. The erythrocytes were then separated from the solution by centrifugation and washed twice in the same assay buffer at 1°C containing no $^{35}SO_4^{2-}$.

The assay was initiated by injecting 0.5 ml of the washed erythrocytes into 9.5 ml of the divalent cation assay buffer at 36°C and under agitation in the shaker bath. The efflux of $^{35}SO_4^{2-}$ was monitored as a function of time by removing 0.7-ml aliquots of cell suspension, centrifuging rapidly in a microfuge, and adding 0.2 ml of the supernatant to 15 ml of Bray's solution to be counted. The first order rate constant for SO_4^{2-} exchange was calculated according to the equation

$$\ln \frac{y_\infty - y_t}{y_\infty} = -kt$$

where y_t and y_∞ represent the radioactivity in the supernatant at time t and at infinite time, respectively, and where k is the first order rate constant. At the low Ca^{2+} concentrations employed in this study, the red cell volume remained essentially unchanged; thus, flux was not routinely calculated.

In studies with tetracaine, the local anesthetic was dissolved at the desired concentration in the final assay solution, the pH was readjusted to 7.0 at 36°C,

and the solution was brought to 36°C in the shaker bath before the washed erythrocytes were added.

For preparation of resealed erythrocyte membranes the method of Bodemann and Passow [17] was used. In all cases the unhemolyzed cells and also the erythrocytes membranes which did not reseal were removed and discarded.

Results

Because of its extraordinary calcium binding capacity, the red cell membrane may act as a calcium sink for large quantities of intracellular ionized calcium [18]. Thus, in order to obtain accurate information on the effect of calcium on anion transport it was necessary to use calcium-EGTA buffers which maintained the concentration of free calcium at a constant, predetermined value. When little or no calcium buffering was used, elevated apparent inhibition constants of calcium for anion transport were obtained. Only at total EGTA concentrations greater than 2 mM was the apparent inhibition constant independent of the total EGTA concentration. For this reason all experiments subsequently described employ divalent cation buffers with total EGTA or EDTA concentration set at 8.81 mM.

Fig. 1A shows the first order plots of the rate of sulfate exchange across the

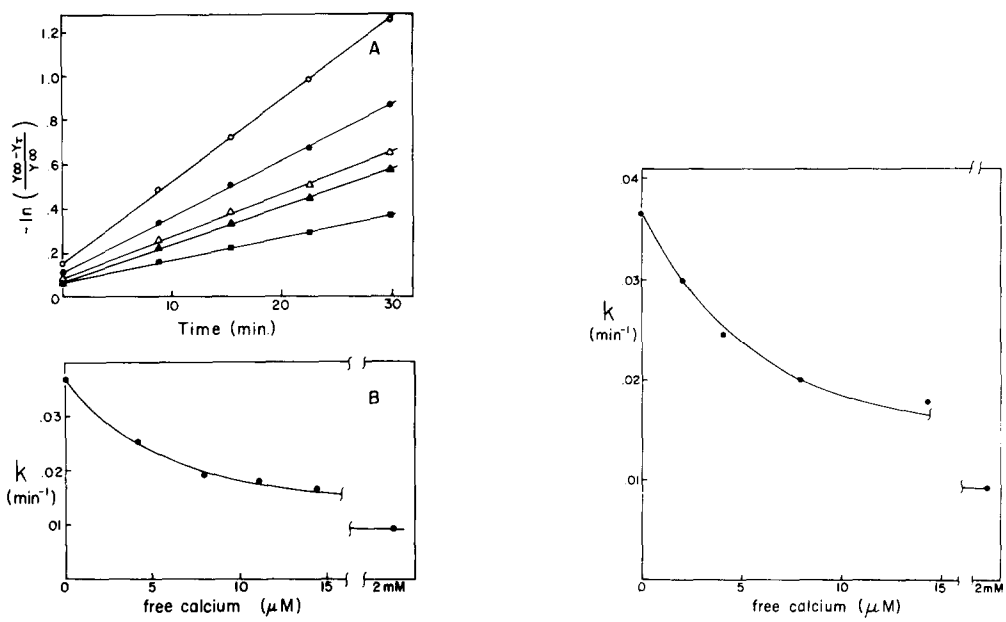


Fig. 1. The effect of free Ca^{2+} on SO_4^{2-} exchange across the erythrocyte membrane. Whole human erythrocytes were incubated in the presence of 10 μM A23187 and different Ca^{2+}-EGTA buffers as described in Methods. A. First order plots of the rate of SO_4^{2-} exchange experimental data of the following Ca^{2+} concentrations: ○, 0 μM (8.81 mM EGTA); ●, 4.11 μM; △, 7.92 μM, 11.07 μM (deleted for clarity of graph); ▲, 14.32 μM; ■, unbuffered 2 mM Ca^{2+}. B. Variation of the first order rate constant (k) with free calcium concentration.

Fig. 2. Variation of the rate constant of SO_4^{2-} exchange across resealed erythrocyte membranes containing different concentrations of internal free calcium.

membrane of whole human erythrocytes treated with 10 μM A23187 and various concentrations of free calcium. As free Ca^{2+} is increased from 0 to approx. 15 μM a decrease in the rate constant for SO_4^{2-} exchange is observed. This decrease in SO_4^{2-} exchange begins to level off above 15 μM free Ca^{2+} and reaches a constant exchange rate at approx. 2 mM Ca^{2+} (Fig. 1B). In the absence of the Ca^{2+} ionophore, A23187, Ca^{2+} concentrations up to 2 mM have no effect on SO_4^{2-} exchange. These results suggest that Ca^{2+} inhibits SO_4^{2-} exchange across the erythrocyte membrane and that the Ca^{2+} binding site is accessible to Ca^{2+} only in the presence of ionophore, A23187.

That the ionophore was required only for Ca^{2+} transport across the red cell membrane and did not otherwise participate in the inhibition of SO_4^{2-} exchange was demonstrated by incorporating the respective Ca^{2+} buffers into resealed erythrocyte membranes in the absence of A23187, according to the method of Bodemann and Passow [17]. The results of these experiments (Fig. 2) demonstrate that internal free Ca^{2+} is an inhibitor of SO_4^{2-} exchange even in the absence of A23187. The apparent inhibition constant for Ca^{2+} (see below) is essentially unchanged regardless of the mode of introduction of Ca^{2+} into the cell cytoplasm.

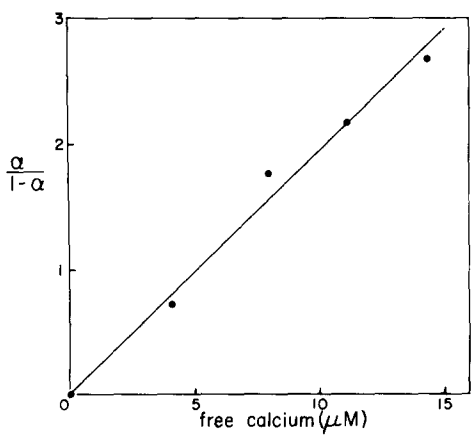
Fig. 1A demonstrates that Ca^{2+} inhibition is incomplete even at saturating concentrations of the inhibitor, and this behavior may be interpreted in one of two ways. First, it may suggest that the transporter-sulfate complex is still capable of catalyzing SO_4^{2-} exchange when all Ca^{2+} binding sites are fully occupied. This would imply that free SO_4^{2-} can bind to the Ca^{2+}-saturated transporter and, therefore, that Ca^{2+} is a non-competitive (hyperbolic) inhibitor of SO_4^{2-} exchange. Alternatively, the incomplete inhibition by Ca^{2+} could suggest the existence of two transport systems, a Ca^{2+}-sensitive system responsible for 75% of the equilibrium exchange, and a Ca^{2+}-insensitive transporter responsible for 25% of SO_4^{2-} exchange. Since there is no evidence for two distinct anion exchange pathways, the former explanation appears to be currently more plausible.

We have determined the apparent inhibition constant (apparent K_I) for calcium by assuming that the fractional saturation (α) of the Ca^{2+} binding sites involved with anion transport can be obtained from the kinetic data in Fig. 1 using the following equations:

$$\alpha = \frac{k_o - k_c}{k_o - k_\infty} \quad \text{and} \quad K_I = \frac{(1 - \alpha)[Ca^{2+}]}{\alpha},$$

where k_o and k_∞ represent the rate constants for SO_4^{2-} exchange in the presence of 8.81 mM EGTA and 2 mM Ca^{2+}, respectively, and k_c is the rate constant at any intermediate calcium concentration. The apparent K_I obtained from the reciprocal of the slope of the plot in Fig. 3 (using data from Fig. 1) is 5 μM, and the apparent K_I from the data obtained in the experiment with resealed ghosts is 6.1 μM (see Fig. 2).

Since the concentration of ionized Ca^{2+} in red cells is thought to be maintained below 1 μM the question arises as to whether Ca^{2+} inhibition occurs to any extent in red cells with normal calcium concentrations. To answer this question we compared the rate of SO_4^{2-} exchange in control erythrocytes with erythrocytes treated with 10 mM EGTA and 10 μM A23187. The results



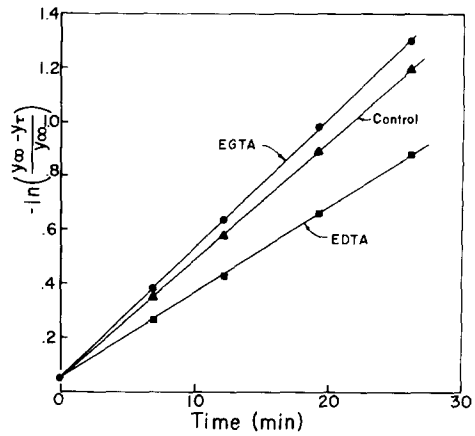


Fig. 3. Replot of the inhibition data from Fig. 1 as described in the text. The apparent dissociation constant for the Ca^{2+}-transporter complex is the reciprocal of the slope.

Fig. 4. The effect of EGTA and EDTA on the rate of SO_4^{2-} exchange across the erythrocyte membrane. Whole human erythrocytes were incubated at 36°C, pH 7 for 90 min in assay buffer containing 10 μM A23187 and either 10 mM EGTA or 10 mM EDTA. The control contained no A23187 and no chelating agent. The rate of SO_4^{2-} exchange was then determined as described in Methods.

shown in Fig. 4 were typical of results from five different donors. In all cases normal erythrocytes showed a rate of SO_4^{2-} exchange 3—8% below the rate for EGTA-treated erythrocytes, suggesting that physiological concentrations of free Ca^{2+} do modify anion exchange to some extent.

It is also possible to use this data and the data in Fig. 1A or Fig. 3 to estimate the concentration of free Ca^{2+} present in normal erythrocytes. For example, a reduction in the rate constant of 3—8% corresponds in Fig. 1A to an internal free Ca^{2+} concentration of 0.2 to 0.7 μM. Thus, for the five donors tested, internal free Ca^{2+} probably ranged between 0.2 and 0.7 μM, a value consistent with estimates using other techniques [19,20].

The effect of EDTA on SO_4^{2-} exchange (Fig. 4) was somewhat surprising in view of the fact that both EGTA and EDTA strongly complex Ca^{2+}. However, since EDTA is a strong chelator of several other divalent cations it seemed possible that the removal of some other divalent cation was inhibitory to anion exchange. For this reason we determined the effect of Mg^{2+} on SO_4^{2-} exchange, and these results are shown in Fig. 5. As the concentration of free Mg^{2+} is increased a weak, yet definite, activation (approx. 50%) of SO_4^{2-} exchange is observed which reaches a maximum at approximately 20 μM Mg^{2+}. The apparent dissociation constant obtained from this data is 3.6 μM. Since intracellular concentrations of Mg^{2+} are two orders of magnitude greater than this value [20], the binding site for Mg^{2+} should remain saturated under normal physiological conditions.

Since Mg^{2+} is an activator of SO_4^{2-} exchange even in the absence of Ca^{2+}, its mechanism of activation cannot be a direct result of Ca^{2+} displacement. This, however, does not necessarily preclude the possibility that the binding sites for Ca^{2+} and Mg^{2+} are identical. To determine if each divalent cation interacts with the anion transport structure at a unique site we compared Ca^{2+} inhibition

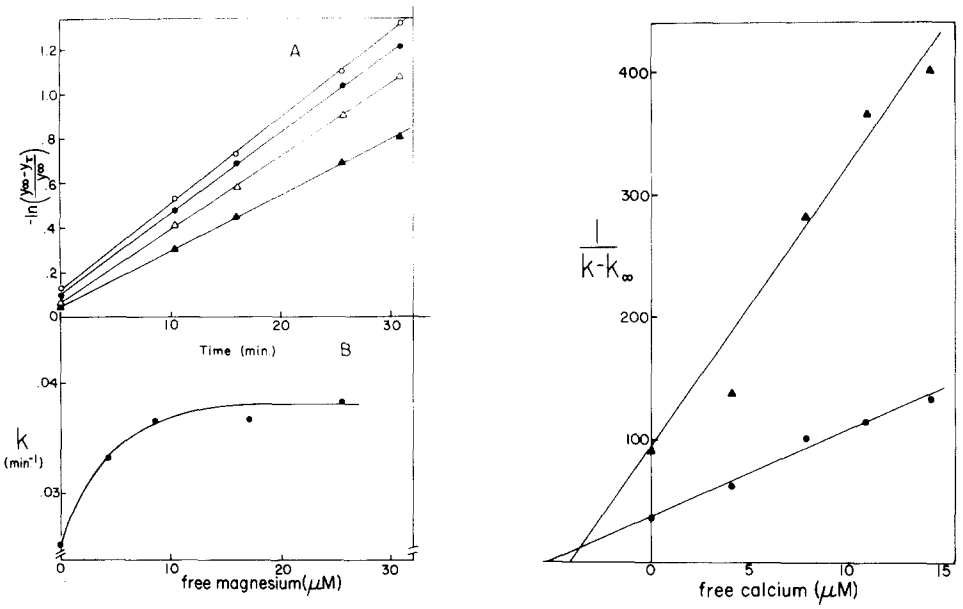


Fig. 5. The effect of different concentrations of free Mg^{2+} on the rate of SO_4^{2-} exchange across the erythrocyte membrane. Whole human erythrocytes were incubated in the presence of 10 μM A23187 and different Mg^{2+}-EDTA buffers as described in Methods. A. First order plots of the rate of SO_4^{2-} exchange at the following Mg^{2+} concentrations: ○, 0 μM (8.81 mM EDTA); ●, 4.27 μM; △, 8.53 μM and 17.03 μM; ▲, 25.60 μM. B. Variation of the rate constant (k) with free Mg^{2+} concentration.

Fig. 6. Dixon type replot of the rate data for SO_4^{2-} exchange in the presence (▲) and absence (●) of 1 mM tetracaine. The x-intercept is taken as the apparent dissociation constant of the Ca^{2+}-transporter complex.

of SO_4^{2-} exchange in the presence of sufficient Mg^{2+} to saturate the Mg^{2+} site (Figs. 1 and 3) and in the absence of Mg^{2+}. For maximal removal of intracellular Mg^{2+}, erythrocytes were incubated in isotonic buffer (pH 7.0) at 10% hematocrit and 36°C for 1.5 h in the presence of 5 μM A23187 and 10 mM EDTA. This treatment was shown previously to extract all removable Mg^{2+} from the cell. The cells were then washed twice with 40 vols. of isotonic buffer without EDTA and incubated for an additional hour in the appropriate Ca^{2+} buffers containing 5 μM A23187. The rate of SO_4^{2-} exchange and the apparent K_I were determined as usual (not shown) and compared with the data in Figs. 1 and 3. Although the above treatment considerably depressed the rate of SO_4^{2-} exchange, the observed apparent K_I for Ca^{2+}, approx 5.5 μM, was not significantly different from the value observed for erythrocytes containing excess Mg^{2+}. These results suggest that Mg^{2+} does not compete with Ca^{2+} for its binding site on the anion transport system.

One final group of cations which exerts a significant effect on anion transport are the tertiary amine local anesthetics. These compounds inhibit anion exchange in erythrocytes with roughly the same relative potencies required to block impulse conduction in nerves [21]. The anesthetics (e.g., dibucaine, tetracaine, lidocaine and procaine) have also been shown to displace calcium from binding sites on the inner surface of the erythrocyte membrane at the same respective concentrations where inhibition of anion transport occurs

(Low and Davio, unpublished). This competition between Ca^{2+} and, for example, tetracaine, for non-specific sites on the erythrocyte membrane raises the possibility that the two cationic inhibitors of anion transport might also occupy the same site on the anion transport complex. This hypothesis was tested by determining the effect of Ca^{2+} on the rate of SO_4^{2-} exchange in the presence and absence of 1 mM tetracaine. (We determined the apparent K_I of tetracaine for anion transport to be 0.8 mM in the presence of 10 mM EGTA and 10 μM A23187 and, therefore, 1 mM tetracaine should be sufficient to occupy over 50% of the tetracaine binding sites associated with anion transport.) In order to avoid superposition of points, the data from this experiment were analyzed using a modified Dixon plot (Fig. 6), where the apparent K_I for calcium is obtained from the x-intercept. As expected, tetracaine reduced the rate of sulfate exchange at all calcium concentrations. However, the local anesthetic had no significant effect on the apparent K_I of Ca^{2+} which decreased slightly from approx. 5 μM to approx. 4 μM in the presence of tetracaine. Thus, although 1 mM tetracaine displaces roughly 25% of total membrane calcium (unpublished observations), it does not appear to have access to the calcium binding site on the anion transport structure. It therefore follows that the inhibitory sites for calcium and tetracaine are distinct.

Discussion

This study presents evidence that three cations, Ca^{2+}, Mg^{2+}, and tetracaine modify anion transport from three distinct sites on the erythrocyte membrane. The calcium binding site is most likely located on the cytoplasmic surface of the membrane since the site is inaccessible to external calcium in the absence of the ionophore, A23187. Since intracellular free Ca^{2+} concentrations are apparently maintained below 1 μM, this binding site should be less than 15% occupied in healthy human erythrocytes. However, internal calcium concentrations are significantly increased in several types of anemia, in senescent red cells, and possibly also in red cells which have been stored for extended periods in acid citrate-dextrose medium under normal blood bank conditions [19,22–24]. Thus, in certain situations enhanced levels of intracellular calcium may be an important determinant of the rate of anion exchange in red cells. It is interesting to speculate, for example, that the 8-fold rise in internal calcium concentration in sickle cells [22] might significantly reduce the rate of bicarbonate-chloride exchange (inhibit CO_2 transport) and thereby contribute to the anemia.

Non-specific calcium binding to the red cell membrane appears to occur to both protein and phospholipid components [18,20]. While our evidence is still tenuous, two observations suggest that the inhibitory site for the Ca^{2+}-anion transport system interaction is located on a protein. Firstly, the reported dissociation constants for Ca^{2+}-phospholipid interactions are between one and two orders of magnitude higher than the inhibition constant we observed for the Ca^{2+}-anion transport interaction [25–27]. Even the high affinity of the Ca^{2+}-phosphatidylserine complex (log apparent dissociation constant −4.07 [25], −4.03 [26]) is roughly seventeen times too weak to account for the Ca^{2+}-anion transport structure interaction. Calcium-protein dissociation constants

are, however, commonly in the range of the K_I for Ca^{2+}, and thus this type of interaction can more easily account for the calcium effect. Secondly, tetracaine competes strongly with calcium for the anionic head group region of negatively charged phospholipids [28]. Thus, tetracaine readily displaces calcium from its binding site on monomolecular films [29] phospholipid bilayer vesicles [28, 30] and from non-specific sites on the cytoplasmic surface of the red cell membrane (unpublished observations). However, we observed no displacement of calcium from its inhibitory site when tetracaine was equilibrated with red cells. Hence, this site may be separate from the lipid bilayer domain of the membrane. With these considerations in mind it is interesting to note that a 40 000 dalton segment of the anion transport protein, band 3, is exposed on the cytoplasmic side of the red cell membrane and that this segment is highly enriched in glutamic acid [31].

The results of this investigation are also supported by several recent calorimetric studies of red cell membrane structure. Two major endothermic transitions of the red cell membrane have been identified with structural components required for anion transport [9]. The B_2 transition is associated with a structural change in band 3, the anion transport protein [32]. The C transition appears to be largely phospholipid in character and has been shown to be physically associated with band 3 [9,32]. Furthermore, compounds which modify the C transition are invariably inhibitors of anion exchange [9]. Thus, it is not surprising that tetracaine reduces the enthalpy of the C transition and shifts it to lower temperature. On the other hand, Ca^{2+} significantly broadens the B_2 transition and increases its melting temperature [33]. These results thus corroborate our observation that Ca^{2+} and tetracaine interact with the anion transport system at two distinct sites and that the Ca^{2+} site may reside on or near a protein. It should be mentioned, however, that the above effects occur at Ca^{2+} and tetracaine concentrations approximately 10 times higher than the apparent K_I values which we report for these substances.

Finally, it is interesting that no significant interaction exists among the three cationic binding site. Thus, calcium binding is essentially unaffected by tetracaine binding, and in fact, their inhibitory effects are nearly additive. Since both cations are non-competitive inhibitors of anion exchange (for tetracaine, see ref. 21) this implies that each inhibitor distorts a different segment of the anion exchange pathway and that these modifications are highly localized, i.e., that a distortion of one segment does not significantly alter the other. This constraint, of situating three non-interacting sites on a single subunit structure (i.e., band 3) is quite formidable, yet may be easily accomodated if structural components in addition to band 3 are required for anion transport [9].

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References

1 Wieth, J.O., Dalmark, M., Gunn, R.B. and Tosteson, D.C. (1973) in Erythrocytes, Thrombocytes, Leucocytes (Gerlach, E., Moser, K. and Deutsch, E., eds.), pp. 71—76, Georg Thieme Publishers, Stuttgart

2 Deuticke, B. (1970) Naturwissenschaften 13, 172—179
3 Cabantchik, Z.I. and Rothstein, A. (1972) J. Membrane Biol. 10, 311—330
4 Ho, Michael K. and Guidotti, G. (1975) J. Biol. Chem. 250, 675—683
5 Dalmark, M. and Wieth, J.O. (1972) J. Physiol. 224, 583—610
6 Rothstein, A., Cabantchik, Z.I. and Knauf, P. (1976) Fed. Proc. 35 (1), 3—10
7 Rassow, H. and Schnell, K.F. (1969) Experientia 25/5, 460—468
8 Passow, H. (1969) Progr. Biophys. Mol. Biol. 19, 425—467
9 Snow, J.W., Brandts, J.F. and Low, P.S. (1978) Biochim. Biophys. Acta, in press
10 Yu, J. and Steck, T.L. (1975) J. Biol. Chem. 250, 9176—9184
11 Wang, K. and Richards, F.M. (1974) J. Biol. Chem. 249, 8005—8018
12 Shami, Y., Carver, J., Ship, S. and Rothstein, A. (1977) Biochem. Biophys. Res. Commun. 76, 429—436
13 Amos, W.B., Routledge, L.M., Weis-Fogh, T. and Yew, F.F. (1976) in Calcium in Biological Systems, (Duncan, C.J., ed.), S.E.B. Symposia XXX, p. 273—301
14 Lew, V.L. (1971) Biochim. Biophys. Acta 233, 827—830
15 Lake, W., Rasmussen, H. and Goodman, D.B.P. (1977) J. Membrane Biol. 32, 93—113
16 Ferreira, H.G. and Lew, V.L. (1976) Nature 259, 47—49
17 Bodemann, H. and Passow, H. (1972) J. Membrane Biol. 8, 1—26
18 Forstner, J. and Manery, J.F. (1971) Biochem. J. 124, 563—571
19 Schatzman, H.J. (1975) Curr. Topics Membranes Transp. 6, 125—168
20 Harrison, D.G. and Long, C. (1968) J. Physiol. 199, 367—381
21 Gunn, R.B. and Cooper, Jr., J.A. (1975) J. Membrane Biol. 25, 311—326
22 Eaton, J.W., Skelton, T.D., Swofford, H.S. and Jacob, H.S. (1973) Nature 246, 105—106
23 Rumsby, M.G., Trotter, J., Allan, D. and Mitchell, R.H. (1977) Biochem. Soc. Trans. 5, 126—128
24 La Celle, P.L., Kirkpatrick, F.H. and Udkow, M. (1973) in Erythrocytes, Trhombocytes, Leucocytes (Gerlach, E., Moser, K., Deutsch, E. and Wilmanns, W., eds.), Georg Thieme Publishers, Stuttgart
25 Barton, P.G. (1968) J. Biol. Chem. 243, 3884—3890
26 Hendrickson, H.S. and Fullington, J.G. (1965) Biochem. 4, 1599—1605
27 Puskin, J.S. (1977) J. Membrane Biol. 35, 39—55
28 Papahadjopoulos, D. (1972) Biochim. Biophys. Acta 265, 169—186
29 Hauser, H. and Dawson, R.M.C. (1968) Biochem, J. 109, 909—916
30 Ohnishi, S. and Ito, T. (1974) Biochemistry 13, 881—887
31 Steck, T.L., Ramos, B. and Strapazon, E. (1976) Biochem. 15, 1154—1161
32 Brandts, J.F., Taverna, R.D. and Lysko, K.A. (1978) Biochim. Biophys. Acta, in press
33 Brandts, J.F., Lusko, K., Schwartz, A.T., Erickson, L., Carlson, R.B., Vincentelli, J. and Taverna, R.D. (1975) Colloq. Int. C.N.R.S. No. 246, 169—175