SHORT COMMUNICATIONS

BBA 73020

Facilitated diffusion in the chloride shift in human erythrocytes

Considerable evidence has been accumulated indicating the presence of a carrier mechanism, or facilitated diffusion, in the permeability of various types of cells, including erythrocytes, to different non-electrolytes. The criteria most frequently used to demonstrate this mechanism are data showing saturation kinetics and competitive inhibition[1].

The fact that erythrocytes have a relatively high permeability to anions has led several authors to suggest that possibly these ions cross the membrane of these cells by a special mechanism[2–4]. Recent data obtained from studies of erythrocyte-anion permeability have been interpreted by several authors as being consistent with the hypothesis that only simple diffusion is involved or that the regulation of anion permeability in erythrocytes is mediated by certain biochemical processes[5–7]. TOSTESON[8] studied halide exchange across the red-cell membrane measuring isotope flux. He concluded that probably only simple diffusion was involved in the movement of these anions but the process was not as simple as diffusion in an aqueous solution. He suggested that additional studies should be made before the possibility of halide exchange diffusion could be definitely excluded.

Since the exchange of Cl^- for HCO_3^- across the red-cell membrane is of such importance to many vertebrates, a better understanding of any details concerning this process is of interest. The present experiments were designed to test the possibility of saturation kinetics and/or competitive inhibition with the chloride shift.

JACOBS[9] has studied the permeability of various cells to NH_4Cl and has proposed that this substance crosses the membrane in the form of molecules of NH_3 with a subsequent exchange of Cl^- for OH^-. It has been shown that HCO_3^- greatly accelerates the movement of NH_4Cl across the erythrocyte membrane[10]. The fact that the catalytic effect of HCO_3^- is inhibited by sulfanilamide[11] supported the suggestion that the movement of NH_4Cl across the erythrocyte membrane depends in part on the chloride shift. Since under most circumstances the movements of NH_3 and CO_2 are rapid in comparison with the anion exchange[9], measurements of the rate of osmotic volume changes of erythrocytes in solutions of NH_4Cl have been used to study the anion permeability of these cells. Anion shifts can also be studied using solutions of Na_2SO_4 (ref. 12). In this case one SO_4^{2-} exchanges for two Cl^- and thus brings about an osmotic shift in water.

In the present experiments human blood was drawn by venipuncture and heparinized. It was used immediately or after 1–3 days in the refrigerator with no difference in the results. Immediately before an experiment the blood was centrifuged ($\pm$ 1000 $\times$ *g*) and the plasma and buffy layer were removed by aspiration. The cells were washed three times in 1% NaCl (buffered with Tris to pH 7.4 in some experiments* and unbuffered in others). Volume changes were measured using a densi-

* All of the solutions buffered with Tris contained 6.05 g Tris *plus* 3.45 ml conc. HCl per liter.

meter[13]. All of the solutions used in these measurements were buffered to pH 7.4 with Tris buffer. To demonstrate the rate of swelling with different concentration gradients of NH_4Cl, increasing volumes of a 5 M NH_4Cl in 1% NaCl solution were added to different cell suspensions in 1% NaCl *plus* $6 \cdot 10^{-4}$ M $NaHCO_3$ in the densimeter. Typical results can be seen in Fig. 1. The initial downward deflection of the pen resulted from the rapid exit of water from the cells due to the hypertonicity of the added NH_4Cl solution. The initial, short, rapid upward deflection of the pen resulted from the rapid entrance of NH_3 molecules. The subsequent upward movement of the pen resulted from the exchange of Cl^- outside for HCO_3^- inside. The equilibrium position of the pen above its initial level was a dilution effect resulting from the addition of the small volume of NH_4Cl *plus* a shift in the equilibrium volume[14]. It can readily be seen that the rate of swelling is not what would be expected if simple diffusion alone were involved.

The most satisfactory method encountered to demonstrate a reversible inhibi-

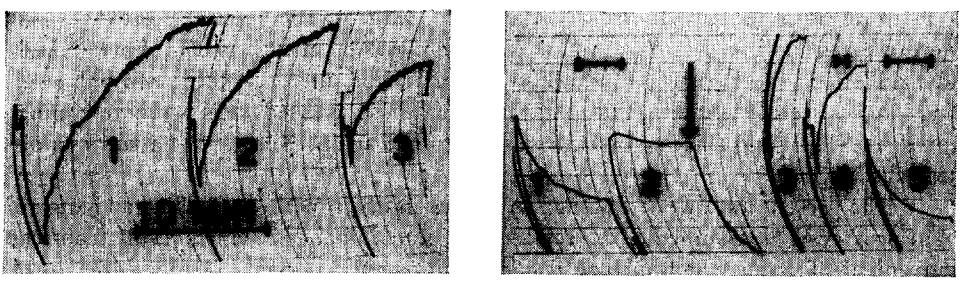

Fig. 1. The rate of swelling of human erythrocytes in NH_4Cl. Washed cells in $6 \cdot 10^{-4}$ M $NaHCO_3$ in 1% NaCl to which were rapidly added: (1) 0.6 ml of 5 M NH_4Cl in 1% NaCl; (2) 0.2 ml of 5 M NH_4Cl in 1% NaCl; (3) 0.1 ml of 5 M NH_4Cl in 1% NaCl. Total volume of solution in the densimeter in all experiments, 10 ml. All solutions buffered with Tris to pH 7.4. Temperature, 31°.

Fig. 2. The effect of SO_4^{2-} on the permeability of human erythrocytes to NH_4Cl. (1) The rate of shrinking in $6 \cdot 10^{-4}$ M $NaHCO_3$ in 1.575% NaCl of cells previously equilibrated in 0.17 M NH_4Cl. (2) The rate of shrinking in $6 \cdot 10^{-4}$ M $NaHCO_3$ in 1.575% NaCl of cells previously equilibrated in 0.17M NH_4Cl in 1% NaCl and subsequently in a solution containing 0.03 M Na_2SO_4, 0.17 M NH_4Cl and 1% NaCl. At the arrow the rate of movement of the paper in the recorder was changed from "per minute" to "per hour". (3) Rate of swelling in 0.3 M glycerol in 1% NaCl of cells equilibrated in Na_2SO_4 and NH_4Cl as described in 2 above. (4) Swelling of cells suspended in $6 \cdot 10^{-4}$ M $NaHCO_3$ in 1% NaCl to which was added 0.1 ml of 5 M NH_4Cl. This is comparable to Curve 3 in Fig. 1 except that in the present experiment the cells had been equilibrated as in 2 above and then washed first in 1.575% NaCl and secondly in 1% NaCl. (5) Shrinking of cells in $6 \cdot 10^{-4}$ M $NaHCO_3$ in 1.575% NaCl. This is comparable to Curve 1 of this figure except that in the present experiment the cells had been equilibrated as in 2 above, then washed as in 4 above and then reequilibrated in 0.17 M NH_4Cl in 1% NaCl. The black horizontal lines indicate 1 min except for the second part of Curve 2 (to the right of the arrow) where it is 1 h. A slower rate of movement of the paper in the recorder was used for the Curve 4. All solutions buffered with Tris to pH 7.4. Temperature, 31°.

tion in the presence of an additional anion was to equilibrate the cells first in NH_4Cl in 1% NaCl and subsequently expose these same cells to a small volume of a much lower concentration of Na_2SO_4 in 1% NaCl. An equal volume of 1% NaCl was added to the control cells. The rate of shrinking was then measured when these cells were put in a NaCl solution. However, as the NH_4Cl left the cells the internal osmotic

pressure decreased and water also left the cells so that they shrank; on the other hand, as one SO_4^{2-} left the cells two Cl^- entered[12], the effect of this being to increase the volume of the cell. A decrease in rate of shrinking of the cells might then simply result from these two opposing factors. This problem could be minimized by selecting concentrations of the two substances such that the two opposing osmotic changes would be of different orders of magnitude. This system had the advantage that the rate of shrinking, which was being measured, did not depend directly on the more slowly moving SO_4^{2-} as has been the case in many previous studies (*e.g.*, TOSTESON[8]). Data from a typical experiment are presented in Fig. 2. The difference in the rate of shrinking in the absence (Curve 1) and in the presence (Curve 2) of SO_4^{2-} is obvious. The fact that the total volume change was no less in the presence of SO_4^{2-} indicates that the osmotic changes resulting from movement of this ion could not have been the predominant factor.

One might suggest that the SO_4^{2-} had a non-specific effect on the membrane. To test this possibility, cells containing NH_4Cl and Na_2SO_4 were added to a solution of glycerol in 1% NaCl. The subsequent swelling as the glycerol entered (Curve 3) is quite normal. To demonstrate the reversibility of the sulfate effect, the cells were washed first in 1.575% NaCl solution and then in 1% NaCl. When NH_4Cl was added to a suspension of these washed cells in 1% NaCl, normal swelling curves were obtained (Curve 4). Finally, these washed cells were re-equilibrated in NH_4Cl and the rate of exit was measured again. Normal behavior was observed (Curve 5).

The present data were obtained by a very indirect method in which a change in light scattering is assumed to be related to volume changes, which result from the movement of water which in turn results from osmotic pressure changes due to the movement of molecules and ions across the cell membrane. Movements of NH_3 and CO_2 as well as an anion exchange are involved. Previous workers (*e.g.*, JACOBS[9] and EDLEBERG[15]), however, have assumed that the anion exchange is rate limiting throughout most of the period of volume change and there is considerable evidence to support this view. For example, by using different concentrations of butanol, JACOBS[9] has shown that the initial rapid portion of the swelling curve, obtained when erythrocytes were placed in a solution of NH_4Cl, was changed but little but the main portion of the curve could be inhibited to varying degrees depending on the concentration of the alcohol. His interpretation of these observations was that the initial, alcohol-insensitive portion of the curve resulted from the movement of molecules and that the major, slower, alcohol-sensitive portion of the curve resulted from the exchange of anions.

A number of observations in the literature suggests that simple diffusion may not be the only factor involved in the movement of anions across the red-cell membrane. The inhibition of the anion exchange in the presence of narcotics[9,12,17] *plus* possible pH optimum[12] and a possible temperature optimum[16] led DAVSON[4] to suggest that "some sort of complex formation" (carrier?) might be involved in anion permeability of erythrocytes. LEFEVRE AND MCGINNISS[18] showed that the ratio of tracer exchange to net movement was much smaller in a system involving carrier-mediated diffusion than in one in which only simple diffusion was involved. A comparison of the rapid exchange rate in TOSTESON's experiments[8] with the much slower rates found in studies such as this one of net movement might suggest

carrier-mediated kinetics. The present data certainly can be reconciled with such a suggestion.

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Received April 7th, 1967

Biochim. Biophys. Acta, 135 (1967) 784–787

BBA 73022

Investigation into the permeability of yeast cells to phosphate

Some doubt exists in the literature as to whether yeast cells are permeable to phosphate. Several authors have found that phosphate does not escape or only slowly escapes from yeast cells[3,4,6]. LEGGETT AND OLSEN[5], however, hold that yeast cells are completely permeable to phosphate. The free space for phosphate was about 80% of the cell volume. A rapid release of phosphate occurred from cells previously loaded with ^{32}P, provided they were washed with a solution containing unlabelled phosphate. One of us[1] has also observed a rapid outflow of phosphate from yeast cells, also suggesting that the free space available for phosphate in these cells is relatively extensive.

Cells of *Saccharomyces cerevisiae*, Hansen Delft II, which have a low phosphate content, were aerated in 0.1 M sodium succinate buffer (pH 4.5) at 25° for one day in order to exhaust the internal substrate. No bacterial contamination occurred under our experimental conditions. Determinations of the free space of phosphate ($^{32}P_i$) were conducted according to the method of CONWAY AND DOWNEY[2]. Corrections for intercellular water were made with the help of [*carboxy*-^{14}C]dextran having an