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EFFECT OF STAPHYLOCOCCAL ALPHA-HEMOLYSIN UPON ANION TRANSPORT IN THE RABBIT ERYTHROCYTE

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Equilibrium exchange of SO_4^{2-} was measured prior to and during hemolysis in rabbit erythrocytes exposed to staphylococcal α-hemolysin. The anion-transport protein of the rabbit erythrocyte has also been identified. Equilibrium exchange of SO_4^{2-} was measured by both efflux and influx of $^{35}SO_4^{2-}$. The rate of influx of SO_4^{2-} in rabbit erythrocytes exposed to α-hemolysin was twice that of the untreated cells. The rate of SO_4^{2-} efflux was unchanged by α-hemolysin. Inhibition of anion exchange with 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) did not inhibit hemolysis, therefore, the increased influx of SO_4^{2-} may occur through a DIDS-insensitive pathway.

Introduction

Staphylococcal α-hemolysin causes leakage of K^+ and Rb^+ upon binding to rabbit erythrocytes. Release of K^+ from rabbit erythrocytes during the prelytic lag phase has been demonstrated repeatedly [1–3]. Cassidy and Harshman [4] have demonstrated the prelytic release of $^{86}Rb^+$ and have shown that it closely parallels the binding of α-hemolysin to the erythrocyte.

Close correlations have been obtained between binding of α-hemolysin and hemolysis [4,5]. Human erythrocytes are 100-times less sensitive to α-hemolysin than rabbit erythrocytes, and bind correspondingly less hemolysin. This correlation between binding and hemolysis suggests that a specific receptor exists for α-hemolysin in the rabbit erythrocyte membrane. Barei and Fackrell [5] demonstrated the presence of both high- and low-affinity binding on the basis of Scatchard plots. Treatment of erythrocytes with heat-inactivated hemolysin (toxoid) will protect the cells against lysis by α-hemolysin [5], probably by saturation of the specific binding sites.

Pretreatment of rabbit erythrocytes with various proteases reduces both the capacity of the cells to bind α-hemolysin and the hemolytic sensitivity of the cells to α-hemolysin [4,6]. This observation suggests the involvement of a membrane protein in the binding of α-hemolysin. Previously, we identified band 3 of the rabbit erythrocyte membrane as a receptor for staphylococcal α-hemolysin [6]. Band 3 has been identified as the anion-transport protein of human erythrocytes [7].

The possibility that anion transport may be disrupted upon binding of α-hemolysin to the rabbit erythrocyte has not been examined, perhaps because of the relatively high permeability to anions of the erythrocyte membrane in its natural state. To examine this possibility we studied exchange of SO_4^{2-} rather than that of chloride because it is 1/10000 as fast as chloride exchange

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Abbreviations: DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; SITS, 4-acetimido-4′-isothiocyano-2,2′-stilbenedisulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

[8,9]. The relatively slow rate of SO_4^{2-} exchange allowed us to measure release or uptake of $^{35}SO_4^{2-}$ during the lag phase and during hemolysis.

Materials and Methods

Reagents and Buffers. Tritiated dihydro-2,2′-diisothiocyano-4,4′-distilbenedisulfonic acid ($[^3H_2]$-H_2DIDS) and dihydro-2,2′-diisothiocyano-4,4′-distilbenedisulfonic acid (H_2DIDS) were gifts from M. Ramjeesingh and A. Rothstein, The Hospital for Sick Children, Toronto, Canada. $Na_2{}^{35}SO_4$ (2 mCi/ml) and Bray's solution were from New England Nuclear, Boston, MA. 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS), 4-acetimido-4′-isothiocyano-2,2′-stilbenedisulfonic acid (SITS), bovine serum albumin and Hepes were from Sigma Chemical Co., St. Louis, MO.

The sulfate/chloride buffer, used for all SO_4^{2-} exchange experiments, consisted of 130 mM NaCl, 5 mM KCl, 10 mM Na_2SO_4, 5 mM Hepes and 5 mM D-glucose (pH 7.4) with 0.5 M NaOH [10]. Phosphate-buffered saline consisted of 145 mM NaCl and 10 mM sodium phosphate buffer (pH 7.2).

Sulfate efflux. The method used was essentially that of Rakitzis et al. [10]. All steps were carried out at 37°C unless stated otherwise. Fresh rabbit blood was collected from the ear of a New Zealand white rabbit into sulfate/chloride buffer supplemented with 1 mM EDTA. This blood was centrifuged at $2000 \times g$ for 5 min and the plasma and buffy coat were removed. The erythrocytes were washed three times with 5 vol. sulfate/chloride buffer. The washed cells were resuspended to 6% hematocrit in sulfate/chloride buffer and equilibrated at 37°C for 2 h with gentle shaking. The equilibrated cells were centrifuged at $2000 \times g$ for 5 min and the supernatant was discarded. The cells were resuspended at 16% hemocrit in sulfate/chloride buffer containing aqueous $Na_2{}^{35}SO_4$ (1 μl per 1 ml of packed cells) and loaded in this medium for 90 min with gentle shaking. The loaded cells were centrifuged at $2000 \times g$ for 5 min and the supernatant was removed by aspiration. The cells were resuspended in sulfate/chloride buffer to 10% hematocrit. A volume of the 10% suspension of cells was pipetted into sulfate/chloride buffer containing the desired concentration of α-hemolysin or inhibitor to give a final hematocrit in the flux medium of 0.5%. A 0.5-ml aliquot of the cell suspension was removed at various times and pipetted into 0.5 ml ice-cold sulfate/chloride buffer. This sample was immediately centrifuged at $10000 \times g$ for 3 min at 4°C and the supernatant was removed and saved. Portions of the supernatants were retained for hemoglobin measurements (absorbance at 541 nm) and the remainder of the supernatants, after precipitation of the protein with 2.5% (w/v) trichloroacetic acid, was added to 10 ml Bray's solution and counted in a Beckman LS-3150P liquid-scintillation counter to determine $^{35}SO_4^{2-}$ content.

Sulfate influx. The method of Young and Ellory [11] was used for sulfate influx determinations. 100 μl sulfate/chloride buffer containing 0.5 μCi $^{35}SO_4^{2-}$ and the desired concentration of α-hemolysin was added to several 1-ml centrifuge tubes (one tube for each sample) and warmed to 37°C. 100 μl of a 10% suspension of prewarmed rabbit erythrocytes in sulfate/chloride buffer were added to each tube so that the final suspension contained 5% erythrocytes. The time of addition of the cells to the buffer containing $^{35}SO_4^{2-}$ was considered time zero. At each sampling time, 1000 μl ice-cold sulfate/chloride buffer containing 1 mM SITS, a noncovalent inhibitor of anion transport, was added to one of the tubes to stop the influx. Samples were centrifuged at $15000 \times g$ for 20 s at 4°C in a microcentrifuge and the supernatants were removed and saved for determination of hemoglobin. The cells were washed twice in ice-cold sulfate/chloride buffer containing 1 mM SITS. Packed cells were lysed by resuspension in 500 μl of 1% Triton X-100 in sulfate/chloride buffer and the lysate was deproteinized with 2.5% (w/v) trichloroacetic acid. Samples were then centrifuged at $15000 \times g$ for 10 min. 200 μl deproteinized lysates were added to 10 ml Bray's solution for liquid-scintillation counting.

Labeling of rabbit erythrocyte membranes with $[^3H_2]H_2$DIDS. Rabbit erythrocytes were labeled with $[^3H_2]H_2$DIDS using the method of Ship et al. [12]. Labeled membranes were dissolved directly in SDS-polyacrylamide gel electrophoresis sample buffer [13] and electrophoresed on 8% acrylamide tube gels [13]. The tube gels were sliced into 2-mm slices and each slice was swelled in 90%

NCS tissue solubilizer (Amersham) prior to liquid-scintillation counting.

Preparation of α-hemolysin. *Staphylococcus aureus* strain Wood 46 was inoculated into Dolman-Wilson medium [14] and incubated at 37 °C with shaking in an atmosphere of 10% CO_2 and 90% air for 36 h. The supernatants of the cultures were harvested by centrifugation at 8000 × *g* for 15 min and frozen immediately. Culture supernatants were used as the source of α-hemolysin. All hemolytic activity of these supernatants could be neutralized by monospecific anti-α-hemolysin.

Heat inactivation of α-hemolysin. Staphylococcal α-hemolysin was heated to 60 °C at pH 4 for 5 min [15].

Hemolytic titration of α-hemolysin. The 50% endpoint method of Lo and Fackrell [16] was performed in Microtitre equipment. The reciprocal of the dilution of toxin that gave 50% hemolysis was defined as one hemolytic unit (HU).

Results

Binding of $[^3H_2]H_2$DIDS to the rabbit erythrocyte membrane

Exposure of intact rabbit erythrocytes to $[^3H_2]H_2$DIDS resulted in the labeling of two polypeptides. A major peak of radioactivity appeared at 90 kDa, corresponding to band 3 in Coomassie blue-stained SDS-polyacrylamide gel electrophoresis gels (Fig. 1). A much smaller peak appeared at approx. 40 kDa. The small peak contained less than 5% $[^3H_2]H_2$DIDS.

Effect of DIDS and SITS upon SO_4^{2-} exchange

The effects of DIDS and SITS upon SO_4^{2-} efflux in human erythrocytes are shown in Fig. 2. The covalently binding inhibitor, DIDS, inhibited the rate of exchange 98.5% at a concentration of 195 μM. SITS, a noncovalent inhibitor, inhibited the rate of exchange 82.6% at the same concentration (Fig. 2).

Effect of heat-inactivated hemolysin upon SO_4^{2-} release

Previously, we demonstrated that heat-inactivated α-toxin could bind to erythrocytes but was not hemolytic [6]. The binding of heat-in-

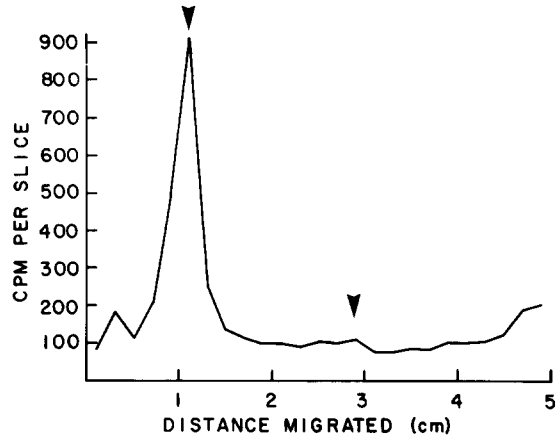

Fig. 1. SDS-polyacrylamide gel electrophoretogram and labeling profile of rabbit erythrocyte membrane proteins of erythrocytes labeled with 10 μM $[^3H_2]H_2$DIDS. The arrows point to band 3 and the minor band.

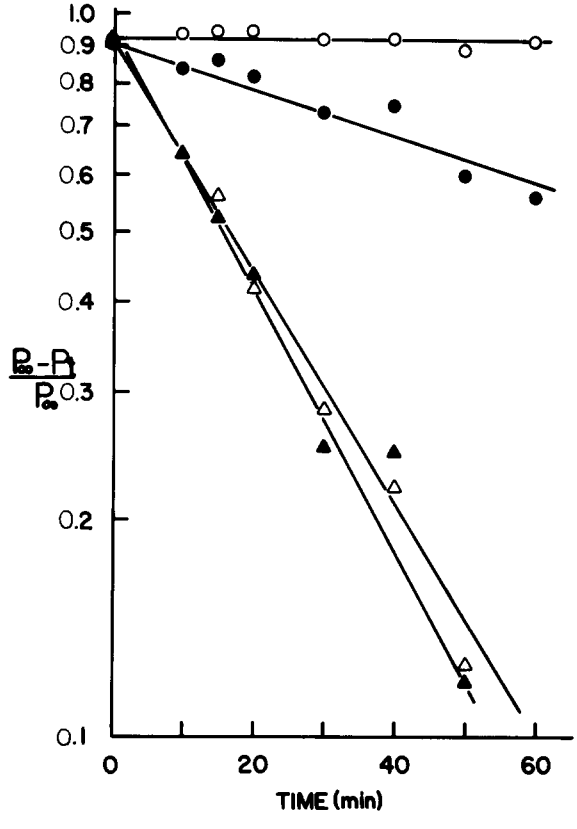

Fig. 2. Sulfate efflux from rabbit erythrocytes. ▲, Control cells; ○, cells exposed to 195 μM DIDS; ●, 195 μM SITS; △, heat-inactivated α-hemolysin (equivalent to 2 HU active hemolysin).

activated α-hemolysin to rabbit erythrocytes does not have a pronounced effect upon the rate of exchange (Fig. 2). The rate of exchange with inactivated hemolysin bound to the membrane was only 12% less than that of the untreated cells.

Effect of α-hemolysin upon SO_4^{2-} release

Exposure of rabbit erythrocytes to α-hemolysin increased the rate of $^{35}SO_4^{2-}$ release over that of the untreated cells (Fig. 3). The release of SO_4^{2-} preceded the release of hemoglobin. This increased rate of SO_4^{2-} release in the presence of α-hemolysin was assumed to be the result of both anion transport and hemolysis. Therefore, to measure only the SO_4^{2-} released that was due to hemolysis, cells were loaded with $^{35}SO_4^{2-}$ exposed to DIDS to inhibit anion exchange, and then exposed to hemolysin. The data presented in Fig. 4 show that the SO_4^{2-} release in DIDS-labeled cells exposed to the hemolysin began slowly during the prelytic lag phase and increased once hemolysis began. This observation suggests that the SO_4^{2-} released during the prelytic phase was via the DIDS-sensitive pathway and was not a result of membrane damage due to the hemolysin. If the rate of SO_4^{2-} release in DIDS-treated cells exposed to the hemolysin is subtracted from the rate of release from the cells not labeled with DIDS but exposed to the hemolysin, the rate of release due to transport during lysis can be obtained. When this was done, little

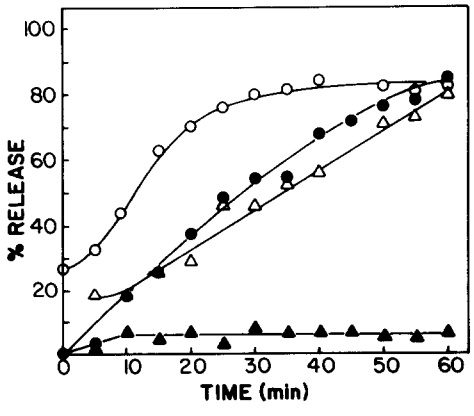

Fig. 3. Release of SO_4^{2-} and hemoglobin from toxin-treated and untreated cells. Toxin-treated cells were exposed to 2 HU of toxin in the flux medium. ○, SO_4^{2-} release from toxin-treated cells; ○, hemoglobin release from toxin-treated cells; ●, SO_4^{2-} release from untreated cells; ▲, hemoglobin release from untreated cells.

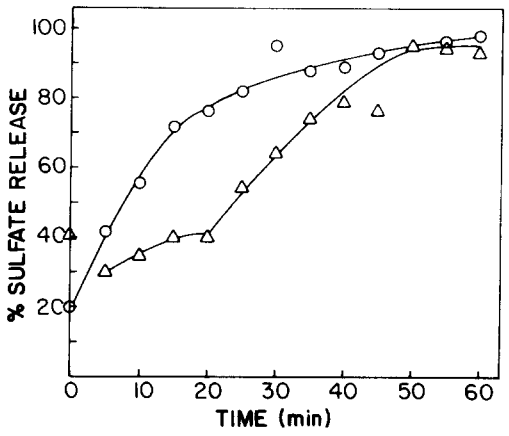

Fig. 4. Loss of SO_4^{2-} from cells exposed to α-hemolysin in media with DIDS (△) and without DIDS (○). In both cases, the flux medium contained 2 HU of α-hemolysin.

difference in the rates is observed. The rate of exchange from red cells exposed to α-toxin and DIDS was 6.66 amol/cell per min whereas, the exchange rate from toxin-treated cells was 29.3 amol/cell per min. The difference in the rates is 23.6 amol/cell per min. This difference reflects the rate of exchange through band 3 in toxin-treated cells, and is comparable with the exchange rate of 20.7 amol/cell per min for untreated red cells.

Effect of α-hemolysin upon SO_4^{2-} efflux during hemolysis

To confirm that the rate of efflux in cells about

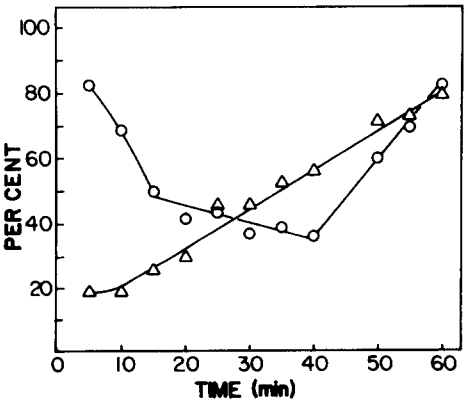

Fig. 5. Residual SO_4^{2-} in intact erythrocytes upon exposure to α-hemolysin. Data taken from Fig. 3. The residual SO_4^{2-} per cell (○) was calculated by the formula: $(P_\infty - P_t)/(100 - \%$ lysis) P_∞. Values for % lysis (△) were obtained from hemoglobin measurements.

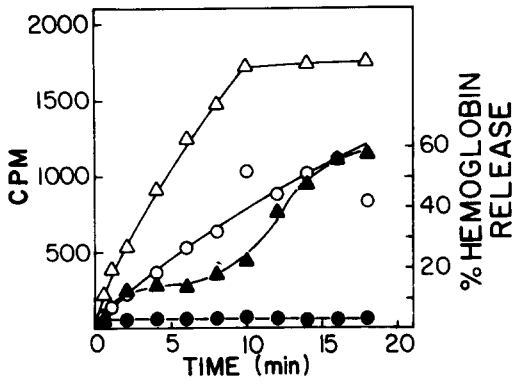

Fig. 6. Influx of SO_4^{2-} into toxin-treated and untreated erythrocytes. ○, SO_4^{2-} influx of untreated cells; △, SO_4^{2-} influx of toxin-treated cells; ●, hemoglobin release of untreated cells; ▲, hemoglobin release of toxin-treated cells.

to lyse is identical to that of cells not exposed to α-hemolysin, the net efflux was measured. To do this, the percentage of the initial SO_4^{2-} remaining in the cells ($P_\infty - P_t/P_\infty$) was expressed as a fraction of the percent cells unlysed and plotted versus time (Fig. 5). The initial $^{35}SO_4^{2-}$ content of the intact cells decreased as it did in the cells not exposed to hemolysin. However, in the later stages of hemolysis, the SO_4^{2-} content of the intact cells increased, suggesting that SO_4^{2-} accumulated in the cells.

Effect of α-hemolysin upon influx of SO_4^{2-}

Measurement of entry of $^{35}SO_4^{2-}$ into rabbit erythrocytes exposed to α-hemolysin demonstrated that SO_4^{2-} enters these cells at a rate double that of the unexposed cells (Fig. 6). This entry occurred during the prelytic lag period and stopped once hemolysis began.

Discussion

The rate of SO_4^{2-} exchange in rabbit erythrocytes, and its inhibition by DIDS and SITS, suggest similar mechanisms of transport in rabbit and human erythrocytes. $[^3H_2]H_2$DIDS bound predominantly to a 90 kDa polypeptide, corresponding to rabbit erythrocyte band 3 in Coomassie blue-stained SDS-polyacrylamide gel electrophoresis gels. This suggests that band 3 is the anion-transport protein of rabbit erythrocytes. Jennings and Adams-Lackey [17] have previously demonstrated the binding of $[^3H_2]H_2$DIDS to rabbit-erythrocyte band 3 and a 40–50 kDa polypeptide that was identified as a lactate transport protein.

In a series of papers [5,6,16,18], we demonstrated that heat-inactivated α-hemolysin (toxoid) retains its ability to bind to its receptor although it is no longer hemolytic. When rabbit erythrocytes were exposed to heat-inactivated α-hemolysin, the rate of SO_4^{2-} exchange only decreased by 12%. The binding of heat-inactivated hemolysin may exert a minimal effect upon anion transport. Heat denaturation of native α-hemolysin seems to cause a loss of all of the protein's functions except its ability to bind to the receptor. α-Hemolysin apparently possesses a heat-labile 'active site' and a heat-stable binding site. The inability of toxoid to influence the flux of SO_4^{2-} supports the observation of Cassidy and Harshman [4] that binding of the toxin is separate from the subsequent events leading to lysis of the erythrocyte.

Release of SO_4^{2-} by rabbit erythrocytes exposed to α-hemolysin was a result of both transport (i.e., equilibrium exchange), and hemolysis. Since release occurs by two mechanisms, the rate of release due to hemolysis was subtracted from the combined rates to obtain the rate due to transport during hemolysis. When this was done, no change in the transport rate was seen upon addition of α-hemolysin. The release of SO_4^{2-} during the prelytic lag phase was inhibited upon the addition of DIDS, suggesting that the prelytic release of SO_4^{2-} was via the DIDS-sensitive component of anion exchange.

We have demonstrated that the efflux of SO_4^{2-} is not changed by α-hemolysin; therefore, the increased influx results in an accumulation of SO_4^{2-} in the cells. This accumulation occurred during the prelytic lag phase and stopped once hemolysis began. This is similar to the influx of sodium into human erythrocytes upon exposure to α-hemolysin [19]. It is tempting to think that accumulation of anions leads to expansion of the cell and, eventually, to osmotic lysis but the causal relationship, in the case of α-toxin, remains to be demonstrated.

Anion transport in human erythrocytes is accomplished by a one-for-one exchange [20]. An increase in rates of influx, without an accompanying increase in efflux rate, is a very unusual result. Influx of SO_4^{2-} was measured in a medium con-

taining high activity of $^{35}SO_4^{2-}$ as compared to the medium in which efflux was measured. Resealing of the lysed erythrocytes could possibly trap $^{35}SO_4^{2-}$ in the cells, resulting in an apparent increase in the net influx. This situation could not occur in the efflux experiments, as the released $^{35}SO_4^{2-}$ was diluted 95.5%. However, the increased rate of influx occurred during the prelytic lag phase, before hemolysis of the cells and, before any possible resealing could occur. Also, we have attempted to reseal ghosts derived from erythrocytes lysed by α-hemolysin using the method of Steck [21] but were unsuccessful. Fussle et al. [22] were also unable to reseal rabbit erythrocyte ghosts but were able to reseal ghosts prepared from human and sheep erythrocyte membranes.

Whether the accumulation of SO_4^{2-} prior to lysis occurred via band 3 or whether it occurred via a 'pore' in the membrane has not been determined. DIDS did not inhibit hemolysis and may not have inhibited the accumulation of SO_4^{2-}. The lack of effect of DIDS suggests that the usual anion exchange mechanism is not involved in the accumulation of anions. It is possible that the net accumulation of SO_4^{2-} occurred through a DIDS-insensitive component of the net anion flux since 20–30% of the net chloride flux is not inhibited by DIDS [23]. However, it is important to note that that the SO_4^{2-}-transporting form of the carrier is not involved in net anion flux [24]. It remains possible that binding of α-hemolysin to band 3 results in a conformational change in band 3 causing a radical change in either the exchange flux or net flux of anions. Conclusions as to the role of anion transport in the mode-of-action of α-hemolysin must be made cautiously because of the lack of information regarding anion transport in the rabbit erythrocyte.

The release of CrO_4^{2-} and PO_4^{2-}, as well as glucose, from artificial lipid spherules exposed to α-hemolysin has been demonstration by Weissman and co-workers [25]. These observations led them to suggest that α-hemolysin interacted with the phospholipid of the erythrocyte through its inner hydrophobic amino acids. Several groups have suggested that α-hemolysin imbeds itself into the phospholipid of the erythrocyte forming an ion channel leading to osmotic lysis [22,26–29]. Although Cassidy and co-workers [30] confirmed the release of markers from liposomes, they found no correlation between the lipid composition of the erythrocytes and their sensitivity to α-toxin.

This finding of Cassidy et al. [30], that liposomes prepared from human erythrocyte lipids are disrupted by the same concentration of α-hemolysin as those prepared from rabbit erythrocyte lipids, even though rabbit erythrocytes are 100-times more susceptible, suggests that more than lipid is involved in hemolysis. The studies with liposomes involved concentrations of α-hemolysin more than 100-times those required to lyse rabbit erythrocytes (0.1–0.2 μg/ml) [31]. Thus, the lysis of liposomes may be a result of nonspecific binding to the lipid, causing lysis through a different mechanism.

Cassidy and Harshman [4] found a correlation between binding of ^{125}I-labeled α-hemolysin to erythrocytes of various species and the hemolytic sensitivity of the erythrocytes. Barei and Fackrell [5] found a correlation between receptor number and hemolytic sensitivity. They determined the receptor number to be 125 980 per rabbit erythrocyte and postulated the presence of a finite number of high-affinity binding sites and a relatively large number of low-affinity sites. Two binding mechanisms were proposed, specific and nonspecific, both mechanisms leading to hemolysis.

Band 3 may act simply as a receptor for α-hemolysin. After binding to band 3, the hemolysin may insert itself into the membrane and act either as a nonspecific or specific pore, or as a carrier. Using concentrations of α-hemolysin greater than the hemolytic concentration for rabbit erythrocytes, Bhakdi et al. [29] and Fussle et al. [22] have demonstrated the appearance of hexamers of α-hemolysin on both erythrocyte membranes and deoxycholate detergent micelles. They have proposed that these hexamers form a cylinder with a central aqueous pore which disrupts the permeability barrier of the cell. Hexamers of α-hemolysin have also been observed in other laboratories [23–25], including this laboratory. We, however, are unable to detect the hexamers at low, but lytic, concentrations of 1–2 HU. Two possible explanations for this inability to detect the hexamers are possible. Perhaps only a few hexamers are required to cause hemolysis of a cell or, two modes-of-action exist. The latter possibility may involve hexamer forma-

tion at high hemolysin concentrations and disruption of the ion-transport function of band 3 at low concentrations.

Fussle et al. [22] demonstrated the release of marker molecules of diameter less than 30 Å from resealed human erythrocyte membranes exposed 30 μg/ml α-hemolysin. This release of marker molecules was demonstrated to be through hexamers of α-hemolysin imbedded in the membrane. Alteration of SO_4^{2-} exchange, in rabbit erythrocytes, by 1 or 2 HU (0.1 μg/ml) of α-hemolysin illustrates a different action of the hemolysin. It is very unlikely that a pore which allows flow of molecules 30 Å in diameter would allow SO_4^{2-} ions to enter, but not exit, the rabbit erythrocyte. The activity of α-hemolysin at low concentrations is probably one of disruption of the usual mechanism of anion exchange upon binding to band 3.

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References

1 Madoff, M.A., Cooper, L.S. and Weinstein, L. (1962) J. Bacteriol. 87, 145–149
2 Cooper, L.Z., Madoff, M.A. and Weinstein, L. (1964) J. Bacteriol. 87, 127–135
3 Cooper, L.Z., Madoff, M.A. and Weinstein, L. (1964) J. Bacteriol. 87, 136–144
4 Cassidy, P. and Harshman, S. (1976) Infect. Immun. 13, 982–986
5 Barei, G.M. and Fackrell, H.B. (1979) Can. J. Microbiol. 25, 1219–1226
6 Maharaj, and Fackrell, H.B. (1980) Can. J. Microbiol. 26, 524–531
7 Cabantchik, Z.I. and Rothstein, A. (1974) J. Membrane Biol.1 5, 207–226
8 Schnell, K.F., Gerhardt, S., Lepke, S. and Passow, H. (1973) Biochim. Biophys. Acta 318, 474–477
9 Schnell, K.F., Gerhardt, S. and Schoppe-Fredenburg, A. (1977) J. Membrane Biol. 30, 319–350
10 Rakitzis, E.T., Gilligan, P.J. and Hoffman, J.F. (1978) J. Membrane Biol. 41, 101–115
11 Young, J.D. and Ellory, J.C. (1982) in Red Cell Membranes – A Methodological Approach (Ellory, J.C. and Young, J.D., eds.), pp. 119–133, Academic Press, Toronto
12 Ship, S., Shami, Y., Breuer, W. and Rothstein, A. (!977) J. Membrane Biol. 33, 311–324
13 Laemmli, U.K. (1970) Nature (Lond.) 227, 680–685
14 Dolman, C.E. and Wilson, R.J. (1940) Can. J. Public Health 31, 68–71
15 Dalen, A.B. (1976) Acta Path. Microbiol. Scand. Sect. B 84, 326–332
16 Lo, C.Y. and Fackrell, H.B. (1979) Can. J. Microbiol. 25, 686–692
17 Jennings, M.L. and Adams-Lackey, M. (1982) J. Biol. Chem. 257, 12866–12871
18 Lo, C.Y., Fackrell, H.B. and G.M. Barei (1982) Can. J. Microbiol. 28, 1127–1132
19 Sengers, R.C.A. (1970) Antonie van Leeuwenhoek, 36, 57–65
20 Harris, E. and Pressman, B. (1967) Nature (Lond.) 216, 918–920
21 Steck, T.L. (1974) In Methods in Membrane Biology (Korn, E.D., ed.), Vol. 2, pp. 245–281, Plenum, New York
22 Fussle, R., Sucharit, B., Sziegoleit, A., Tranum-Jensen, J., Kranz, T. and Wellensiek, H. (1981) J. Cell Biol. 91, 83–94
23 Knauf, P.A., Fuhrmann, F., Rothstein, S. and Rothstein, A. (1977) J. Gen. Physiol. 69, 363–386
24 Knauf, P.A., Law, F. and Marchant, P.A. (1983) J. Gen. Physiol. 81, 95–126
25 Weissman, G., Sessa, G. and Bernheimer, A.W. (1966) Science 154, 772–774
26 Freer, J.H., Arbuthnott, J.P. and Bernheimer, A.W. (1968) J. Bacteriol. 95, 1153–1168
27 Freer, J.H., Arbuthnott, J.P. and Billcliffe, B. (1973) J. Gen. Microbiol. 75, 321–332
28 Bernheimer, A.W., Kim, K.S., Romsen, C.C., Antanavage, J. and Watson, S.W. (1972) Infect. Immun. 6, 636–642
29 Bhakdi, S., Fussle, R. and Tranum-Jensen, J. (1981) Proc. Natl. Acad. Sci. USA 78, 5475–5479
30 Cassidy, P.S., six, H.R. and Harshman, S. (1974) Biochim. Biophys. Acta 332, 413–423
31 Harshman, S. (1979) Mol. Cell. Biochem. 23, 143–152