INFECTION AND IMMUNITY, May 1988, p. 1249–1253
0019-9567/88/051249-05$02.00/0

Vol. 56, No. 5

The Cytolytic Toxin Aerolysin Must Aggregate To Disrupt Erythrocytes, and Aggregation Is Stimulated by Human Glycophorin

WILLIAM J. GARLAND AND J. THOMAS BUCKLEY*

Department of Biochemistry and Microbiology, University of Victoria, Box 1700, Victoria, British Columbia, Canada, V8W 2Y2

Received 12 November 1987/Accepted 4 February 1988

The hole-forming toxin aerolysin was shown to aggregate after binding to erythrocytes at 37°C. Although the protein also bound and aggregated at 4°C, hole formation was not observed, indicating that aggregation preceded penetration of the lipid bilayer. Aggregation, but not binding, could be blocked by pretreatment of the toxin with diethyl pyrocarbonate, a histidine-reactive reagent. This resulted in inactivation of the toxin. Incubation of aerolysin with glycophorin purified from human erythrocytes caused aggregation and complete inactivation. Erythrocytes which lacked glycophorin were less sensitive to the toxin. Proaerolysin, the inactive precursor of aerolysin, also bound to erythrocytes; however, it did not aggregate, nor did it aggregate when preincubated with glycophorin. The protoxin could be activated by treatment with trypsin even after it had bound to erythrocytes. Activation could also be achieved by reaction of proaerolysin with a variety of other proteases, each of which brought about a similar reduction in protein molecular weight. The activated protein was resistant to further proteolysis. These results indicate that aggregation is a necessary step in hole formation and that the sites on aerolysin required for binding and for aggregation and hole formation are separate.

Many bacteria release polypeptides which disrupt eucaryotic cells by breaching their plasma membranes (see reference 8 for a recent review). Not only do these cytolytic toxins often make important contributions to the virulence of the bacteria which release them, but their study may help us to understand a variety of membrane-associated phenomena. For example, all of them undergo a transformation from a water-soluble to an insoluble membrane-bound form. Some of the toxins, such as *Staphylococcus aureus* delta-hemolysin (16), are small polypeptides similar in structure and properties to bee venom melittin (15). These have hydrophobic regions which probably have a direct disruptive effect on lipid bilayers (13). Others, including the oxygen-labile proteins such as streptolysin O, also appear to disrupt the lipid bilayer directly (1). They bind selectively to membrane cholesterol and aggregate to form large lesions (23). In contrast, alpha-toxin from *S. aureus* and aerolysin from *Aeromonas hydrophila* bind to receptors on the cell surface and form discrete holes approximately 3 nm in diameter. The receptor for aerolysin in the rat membrane have been identified as glycophorin (19), and Bernheimer and Avigad (7) have suggested that this protein is the receptor for alpha-toxin as well, since it inhibits hemolysis induced by this toxin in vitro. Other evidence that alpha-toxin binds to band 3, the anion transport protein (25), has been challenged by the observation of Harshman and Bondurant (18) that Friend virus-infected murine erythroblasts, which lack band 3, are nevertheless susceptible to hemolysis.

It has been generally accepted that insertion of the protein complex into the lipid bilayer and aggregation to form protein-lined channels follow receptor binding by toxins like aerolysin and alpha-toxin. Even in the case of alpha-toxin, however, there is little direct evidence that aggregation is necessary for hole formation, and a recent report suggests that *Escherichia coli* hemolysin, which like aerolysin and alpha-toxin is a large protein which forms 3-nm holes in membranes, may be capable of doing so without aggregating (5). Bashford et al. (2) have argued that alpha-toxin-induced permeability changes do not require the formation of protein-lined channels, but rather that membrane damage occurs by some other mechanism common to other toxins as well as to hemolytic viruses and complement.

The gene for aerolysin has been cloned and sequenced (21, 22). The protein is exported from *A. hydrophila* as an inactive protoxin which is subsequently activated by removal of about 25 amino acids from the C terminus (20). The protoxin can be purified and converted to aerolysin under controlled conditions. This provides an important advantage in studying the mechanism of action of the cytotoxin, especially because, like alpha-toxin, mature aerolysin aggregates spontaneously and irreversibly and it is therefore difficult to store and to maintain as an aggregate-free preparation. Here we examine the activation of the protoxin and compare its interaction with erythrocytes with that of the toxin.

MATERIALS AND METHODS

Source of erythrocytes. The erythrocytes used in most of the experiments were obtained from outdated human blood supplied by the Canadian Red Cross. Cells used in the studies of glycophorin sensitivity to hemolysis were kindly provided by J. J. Moulds of Gamma Biologicals, Houston, Tex. These included En (a−) cells, which completely lack glycophorin A (17), En^a (KT) (Mi VIII/Mi VIII) cells, which contain glycophorin A with two amino acid substitutions (10), and S-s-U- cells, which lack glycophorin B (11). Rat blood was obtained by heart puncture.

Purification of proaerolysin. Proaerolysin was purified by affinity chromatography after protein precipitation from culture supernatants of *A. hydrophila* with ammonium sulfate as described earlier (20). The precipitate was redissolved in 20 mM Tris (pH 7.4) and passed directly onto an immunoaffinity column containing Affi-Gel 10 (Bio-Rad) to which a monoclonal antibody to aerolysin had been attached (9). The column was washed with several volumes of 1.5 M NaCl in 20 mM phosphate (pH 6.5), and the protoxin was eluted with

* Corresponding author.

TABLE 1. Binding of ^{125}I-labeled aerolysin and proaerolysin to rat erythrocytes

Toxin	Binding[a] at temp:	
	4°C	37°C
Aerolysin	58	100
Aerolysin, trypsin-treated erythrocytes	6	0
Proaerolysin	60	54
Proaerolysin, trypsin-treated erythrocytes	4	0

[a] Averages of triplicate determinations which did not differ by more than 5% were used in these calculations. Results are expressed as percentages of aerolysin binding at 37°C.

50 mM sodium citrate (pH 2.5) directly into 0.2 M MOPS (morpholinepropanesulfonic acid; pH 8.7). Proaerolysin purified in this way migrated as a single band on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. To minimize irreversible aggregation and inactivation of the mature toxin, which occurs spontaneously at concentrations of 0.1 mg/ml or higher, aerolysin was produced immediately before use by treatment of proaerolysin (approximately 1 mg/ml) with 0.01 mg of trypsin per ml at room temperature for 15 min. In some experiments, trypsin inhibitor was added to a concentration of 0.5 mg/ml after incubation.

Isolation of glycophorin. Human erythrocyte membranes were prepared by the method of Dodge et al. (12), and glycophorin was isolated by extraction with lithium diiodosalicylate as described by Marchesi and Andrews (26).

Iodination of proaerolysin. Radiolabeling was carried out with Iodogen, using the methods of Markwell and Fox (27) as we have described earlier (19). Specific activities of approximately 2×10^9 dpm/mg of protein were obtained.

Chemical modification of aerolysin. Histidine residues in aerolysin were modified by treating a 0.5-mg/ml solution with 0.1 M diethyl pyrocarbonate in 0.15 M NaCl–20 mM Tris (pH 7.4) for 20 min at room temperature. Sulfhydryl residues were modified by incubating aerolysin with 0.05 M *N*-ethylmaleimide or 5 mM 5,5′-dithiobis(2-nitrobenzoic acid) for 30 min in the same buffer at room temperature (24).

Binding and aggregation assays. Binding of radiolabeled aerolysin was measured in polycarbonate centrifuge tubes containing approximately 2×10^7 rat erythrocytes in 0.8 ml of 0.1% albumin–0.3 M sucrose–0.15 M NaCl–20 mM Tris (pH 7.4). Iodinated protein (approximately 5 μg) was added, and the reaction mixture was incubated at the required temperature as described in Results. Sucrose was present to minimize hemolysis which could otherwise have occurred when cells were incubated with active toxin (19). Under these conditions, approximately 2×10^5 molecules of active aerolysin were bound to each untreated cell. Where indicated, cells were pretreated with trypsin as described previously (19). After centrifugation, cells were dispersed in 15 μl of sample buffer and counted, and the proteins were separated by SDS-polyacrylamide gel electrophoresis after treatment for 3 to 5 min at 100°C.

General procedures. Protein was measured by the Lowry procedure as modified by Markwell et al. (28). Proteins were separated by polyacrylamide gel electrophoresis in SDS as described by Neville (29). Unlabeled proteins were stained with Coomassie blue, and radioiodinated proteins were detected by autoradiography. Titers were measured as described by Bernheimer and Avigad (6), using 0.1-ml volumes of 0.8% human erythrocytes after serial dilution of 0.1-ml samples with 20 mM Tris (pH 7.4)–0.85% NaCl containing 0.1% albumin. Results are expressed as $\log_2$ values of the highest dilutions resulting in 100% cell hemolysis. Ten micrograms of pure, freshly prepared aerolysin measured this way gave a titer of 10 against normal human erythrocytes. In some cases, 0.2% suspensions of cells were incubated with approximately 100 ng of aerolysin or proaerolysin in 1.0 ml of Tris-buffered saline. Erythrocyte hemolysis was determined by measuring hemoglobin release spectrophotometrically at 410 nm after sedimentation of intact and disrupted cells by centrifugation.

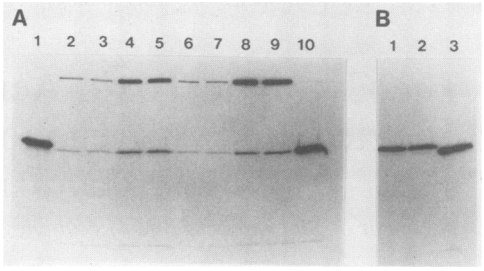

FIG. 1. Binding of ^{125}I-labeled aerolysin and proaerolysin to rat erythrocytes. (A) Binding of aerolysin. Lane 1, Aerolysin; lanes 2, 3, 6, and 7, aerolysin incubated without cells at 0°C (lanes 2 and 3) and 37°C (lanes 6 and 7); lanes 4, 5, 8, and 9, cells incubated with aerolysin at 0°C (lanes 4 and 5) and 37°C (lanes 8 and 9); lane 10, proaerolysin. (B) Binding of proaerolysin. Lanes 1 and 2, Cells incubated with proaerolysin at 37°C; lane 3, proaerolysin alone.

RESULTS

Binding of aerolysin and proaerolysin to erythrocytes. Similar amounts of aerolysin and proaerolysin bound to rat erythrocytes at 4°C (Table 1). Binding of aerolysin but not proaerolysin was higher at 37°C, perhaps as a result of aggregation and insertion of the active toxin. Pretreatment of the cells with trypsin resulted in greatly reduced binding not only of active toxin, as we have shown earlier (19), but also of proaerolysin. Most of the aerolysin bound to cells migrated as an aggregate upon electrophoresis, whether binding was carried out at 0 or 37°C (Fig. 1), whereas none of the proaerolysin aggregated under the same conditions. Proaerolysin was unable to lyse cells at any temperature, and in spite of the fact that it could bind and aggregate, aerolysin was completely unable to cause lysis at 0°C (Table 2). Cell disruption was also prevented by treating aerolysin with diethyl pyrocarbonate (Table 2 and Fig. 2), which specifically blocks histidine residues in proteins (24), whereas treatment with sulfhydryl-reactive *N*-ethylmaleimide had no effect. Both diethyl pyrocarbonate- and *N*-ethylmaleimide-

TABLE 2. Effect of various treatments on aerolysin activity

Toxin	Treatment	Hemolytic titer[a]
Aerolysin	None	9
	0°C incubation	0
	Diethyl pyrocarbonate	0
	N-Ethylmaleimide	9
	5,5′-Dithiobis(2-nitrobenzoic acid)	9
	Preincubation with glycophorin[b]	2–3
Proaerolysin		0–1

[a] Titers of approximately 5 μg of proaerolysin or aerolysin were determined before or after treatment as described in the text.

[b] Aerolysin was preincubated with a fivefold excess of human glycophorin.

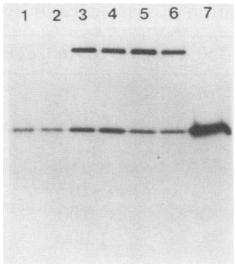

FIG. 2. Effect of diethyl pyrocarbonate and *N*-ethylmaleimide on ^{125}I-aerolysin binding to rat erythrocytes. Lanes 1 and 2, Diethyl pyrocarbonate-treated aerolysin; lanes 3 and 4, *N*-ethylmaleimide-treated aerolysin; lanes 5 and 6, untreated aerolysin; lane 7, ^{125}I-aerolysin alone.

treated toxin were able to bind to rat erythrocytes. Aerolysin treated with *N*-ethylmaleimide aggregated to the same extent as aerolysin but, as with proaerolysin, there was no trace of aggregate formation by bound diethyl pyrocarbonate-treated aerolysin.

Association of glycophorin with aerolysin action. Preincubation of aerolysin with an equal amount of human glycophorin led to a very large reduction in hemolytic activity (Table 2). This is due to irreversible aggregation of aerolysin in the presence of glycophorin (Fig. 3). The purified glycoprotein had no effect on the aggregation state of proaerolysin. Human En (a−) erythrocytes, which completely lack glycophorin A, and En^a (KT) cells, which contain glycophorin A with amino acid modifications at positions 49 and 52 (10), were lysed by the toxin; however, the concentration required for lysis was more than twofold higher than that required for control cell lysis (Table 3).

Effect of temperature shift on hemolysis rate. The results in Fig. 4 illustrate the effect of changing incubation temperature on the rate of erythrocyte hemolysis by aerolysin. Conditions were chosen so that there was a lag of nearly 30 min at 37°C before lysis started. Changing the incubation temperature to 37°C from 0°C after 30 min resulted in nearly

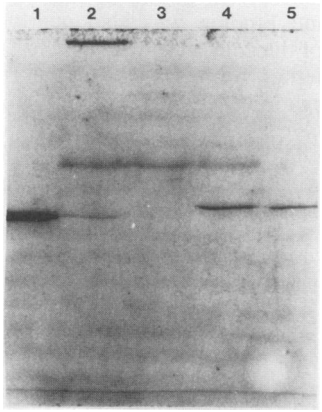

FIG. 3. Aggregation of aerolysin by human glycophorin. Aerolysin or proaerolysin was incubated at room temperature with or without an equal amount of human glycophorin (25 μg/25 μg) in 0.2 ml of phosphate-buffered saline. After 25 min loading buffer was added, and the samples, containing 2 to 5 μg of protein, were immediately boiled and electrophoresed in 12% gels. Lanes 1, Aerolysin alone; lane 2, aerolysin plus glycophorin; lane 3, glycophorin alone; lane 4, proaerolysin plus glycophorin; lane 5, proaerolysin alone.

TABLE 3. Action of aerolysin on erythrocytes with surface glycoprotein modifications

Cell type[a]	Titer
Controls	10
S-s-U-	10
En (a−)	8–9
En^a (KT) (Mi VIII/Mi VIII)	8–9

[a] Titers were determined in duplicate with 0.8% cell suspensions. Several control cell samples were used and treated exactly like the aberrant cells; all of them gave titers of 10. Approximately 10 μg of aerolysin was used for each titration.

immediate hemolysis, indicating that the rate-determining step had occurred at the lower temperature. No hemolysis was ever observed when the temperature was maintained at 0°C throughout the experiment.

Trypsin activation of proaerolysin after binding to erythrocytes. Addition of trypsin to cells preincubated with proaerolysin resulted in hemolysis after a much shorter delay than was required when the corresponding amount of activated toxin was added directly to cells (Fig. 5). This indicates not only that the protoxin can be activated after it has bound to the cells, but also that binding of the protoxin, and not aggregation, is the rate-limiting step during the preincubation.

Activation of proaerolysin by other proteases. A variety of proteases which are specific for neutral or basic amino acids were capable of converting proaerolysin to active toxin (Table 4). The protease of *S. aureus*, which is specific for acidic amino acids, was the only protease tested which did not activate proaerolysin. Although lower concentrations of proteases specific for basic amino acids were required for activation, SDS-polyacrylamide gel electrophoresis showed that all of the proteases, except the *S. aureus* protease, converted proaerolysin to active forms with molecular weights very similar to those of trypsin-activated toxin or active toxin isolated from *A. hydrophila* culture supernatants (20). A product slightly smaller than active aerolysin was produced by *S. aureus* protease (Fig. 6).

DISCUSSION

There are at least three stages in the disruption of erythrocytes by aerolysin. The first step is binding, which is rate limiting at low toxin concentrations since human cells pre-

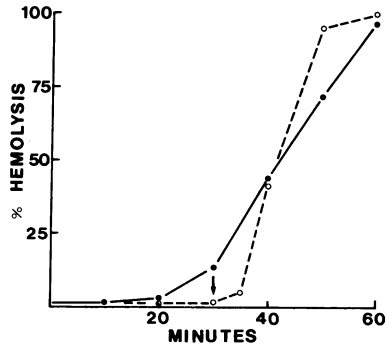

FIG. 4. Effect of temperature shift on rate of hemolysis of human erythrocytes. Symbols: ●, cells incubated with aerolysin at 37°C from time 0; ○, cells incubated with aerolysin at 0°C for 30 min. The arrow marks where the temperature was shifted to 37°C. One of two very similar experiments.

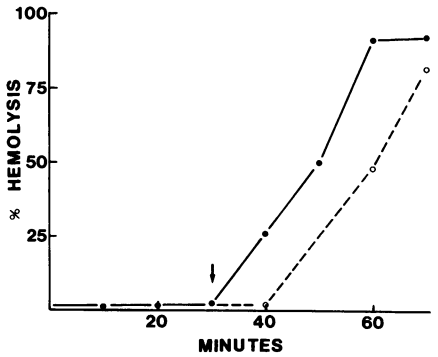

FIG. 5. Trypsin activation of aerolysin after binding to human erythrocytes. Symbols: ●, cells incubated with aerolysin from time 0; ○, cells incubated with the same concentration of proaerolysin for 30 min. The arrow marks the time of addition of trypsin. One of two very similar experiments.

incubated with toxin at 0°C were disrupted with virtually no delay when the temperature was raised to 37°C (Fig. 4). The second stage is aggregation of the toxin. Both aerolysin and its precursor can bind to erythrocytes; however, only the active form of the toxin can aggregate. Aggregation must occur rapidly and after binding, because cells preincubated with protoxin were lysed almost immediately when they were treated with trypsin, without the lag observed when there was no preincubation (Fig. 5). Aggregated aerolysin is stable enough to withstand boiling in the SDS-mercaptoethanol sample buffer used in electrophoresis. Aggregation is nevertheless not due to covalent modification of the protein, as dissociation to monomeric toxin occurred in high concentrations of urea (not shown here). The aggregate migrated with an apparent molecular weight near 275,000 (determined in low-percentage gels; data not shown), about six times the size of monomeric aerolysin (21). The size of the aggregate was the same regardless of whether it was formed spontaneously in concentrated solution, by incubation with purified glycophorin, or on the surface of the erythrocyte (unpublished data). Thus, if glycophorin or other membrane proteins aggregate with aerolysin, they may be dissociated by boiling with SDS and reducing agent. Clearly aggregation is an essential step in cell disruption, as the toxin was inactivated when aggregation was prevented by treatment with diethyl pyrocarbonate. In addition, the inability to aggregate accounts for the inactivity of the protoxin.

Treatment of the protoxin with proteases specific for neutral or basic amino acids yielded products with molecular weights and activities very similar to those of trypsin-activated aerolysin, which we have shown is produced by removal of approximately 25 amino acids from the C terminus of proaerolysin (20). Presumably the proteolysis of purified proaerolysin removes a small domain of the protein which prevents aggregation, mimicking an event which occurs after export of the protoxin from the producing cell (20). The rest of the proaerolysin molecule and aerolysin are resistant to proteolysis, as loss of activity and smaller breakdown products were not observed except after denaturation of the protein or after prolonged treatment with high protease concentrations. Treatment of proaerolysin with *S. aureus* protease resulted in a polypeptide which is not only inactive, but also slightly smaller than aerolysin produced by the other proteases. Whether inactivity is due to removal of an essential portion of the C terminus or whether it is due to the acidic amino acid left at the carboxy terminus after *S. aureus* protease treatment is not known. At least one histidine appears to be involved in aggregation (and therefore hole formation), as this step was completely blocked by reaction with diethyl pyrocarbonate.

TABLE 4. Activation of proaerolysin by various proteases

Protease	Protease concn[a] (μg/ml)	Hemolytic titer
Trypsin	10	10
Chymotrypsin	100	10
Submaxillary Arg-C	10	10
Clostripain	10	10
Thermolysin	100	10
Proteinase K	100	10
S. aureus protease	1,000	1
Control		1

[a] Approximate concentrations required for maximum titers. Proaerolysin (100 μg/ml) was incubated for 10 min with the proteases.

Although the results presented here have established that aerolysin must both bind and aggregate, the next stage, which results in cell disruption, is much less clear. Several observations suggest that the toxin aggregates before it enters the lipid bilayer, as apparently occurs during pore formation by complement (30). First, aerolysin is a soluble, hydrophilic protein (22), and our preliminary observations indicate it will not penetrate lipid monolayers with surface pressures higher than 15 mN/m. It can be argued therefore that aggregation is necessary to generate a conformation or to expose a domain which will allow penetration of the bilayer. In addition, the results in Fig. 1 suggest that aggregation occurs at 4°C. If monomeric aerolysin has entered the membrane at this temperature, its movement should be as restricted as the movement of erythrocyte membrane proteins (14), and lateral migration to form aggregates would be unlikely.

The transmembrane protein glycophorin may have dual roles to play in the action of aerolysin. We have shown earlier that in the rat erythrocyte membrane, but not in human erythrocytes, glycophorin acts as a receptor for toxin binding and that the portion of the glycophorin molecule involved in binding can be removed by treatment with trypsin (20). Proaerolysin bound in similar amounts to rat erythrocytes, and binding was also nearly eliminated by prior exposure of the cells to trypsin (Table 1). In addition to acting as a receptor for aerolysin in the rat, glycophorin stimulates aggregation of the toxin in vitro. It may also affect aggregation in vivo, as human cells which lack glycophorin A and those which contain a modified form are more resistant to toxin action. The effect of glycophorin on aerolysin aggregation may not be specific. Thus, other

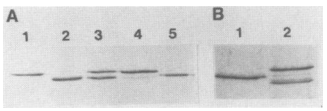

FIG. 6. Electrophoretic comparison of chymotrypsin and *S. aureus* protease-treated proaerolysin. (A) Chymotrypsin-treated proaerolysin. Lane 1, Proaerolysin; lane 2, 0.1 mg of chymotrypsin per ml; lane 3, 0.1 mg of chymotrypsin per ml; lane 4, 0.001 mg of chymotrypsin per ml; lane 5, trypsin-treated aerolysin. (B) Lane 1, Trypsin-treated aerolysin; lane 2, *S. aureus* protease-treated proaerolysin (1 mg/ml). Incubation conditions were the same as those described in Table 4. After 10-min incubations with the proteases, phenylmethylsulfonyl fluoride was added to a final concentration of 0.2 mM, and the samples were electrophoresed.

membrane proteins such as glycophorin B and the anion transporter could also stimulate aerolysin aggregation, which would account for the observation that glycophorin A-deficient cell sensitivity was only reduced twofold. Aggregation of alpha-toxin is stimulated by a variety of amphipathic molecules, including detergents (3) and lipoproteins (4) as well as glycophorin (7). Experiments with artificial lipid systems and purified membrane proteins should help to establish further details of the mechanism of hole formation by both of these toxins.

ACKNOWLEDGMENTS

This work was supported by the British Columbia Health Care Research Foundation and the Natural Sciences and Engineering Research Council.

LITERATURE CITED

1. **Alouf, J. E.** 1980. Streptococcal toxins (streptolysin O, streptolysin S, erythrogenic toxin). Pharmacol. Ther. **11**:661–717.
2. **Bashford, C. L., G. M. Alder, G. Menestrina, K. J. Micklem, J. J. Murphy, and C. A. Pasternak.** 1986. Membrane damage by hemolytic viruses, toxins, complement and other cytotoxic agents. J. Biol. Chem. **261**:9300–9308.
3. **Bhakdi, S., R. Fussle, and J. Tranum-Jensen.** 1981. Staphylococcal alpha toxin: oligomerization of hydrophilic monomers to form amphiphilic hexamers induced through contact with deoxycholate detergent micelles. Proc. Natl. Acad. Sci. USA **78**: 5475–5479.
4. **Bhakdi, S., R. Fussle, G. Utermann, and J. Tranum-Jensen.** 1983. Binding and partial inactivation of S. aureus alpha toxin by plasma low density lipoprotein. J. Biol. Chem. **258**: 5899–5904.
5. **Bhakdi, S., N. Mackman, J.-M. Nicaud, and B. H. Holland.** 1986. *Escherichia coli* hemolysin may damage target cell membranes by generating transmembrane pores. Infect. Immun. **52**: 63–69.
6. **Bernheimer, A. W., and L. S. Avigad.** 1974. Partial characterization of aerolysin, a lytic exotoxin from *Aeromonas hydrophila*. Infect. Immun. **9**:1016–1021.
7. **Bernheimer, A. W., and L. S. Avigad.** 1980. Inhibition of bacterial and other cytolysins by glycophorin. FEMS Lett. **9**:15–17.
8. **Bernheimer, A. W., and B. Rudy.** 1986. Interactions between membranes and cytolytic peptides. Biochim. Biophys. Acta **864**:123–141.
9. **Calton, G. J.** 1984. Immunosorbent separations. Methods Enzymol. **104**:381–387.
10. **Dahr, W., K. Beyreuther, and J. J. Moulds.** 1987. Structural analysis of the major human erythrocyte membrane sialoglycoprotein from Miltenberger class VII cells. Eur. J. Biochem. **166**:27–30.
11. **Dahr, W., P. Issitt, J. Moulds, and B. Pavone.** 1978. Further studies on the membrane glycoprotein defects of S-s- and En(a−) erythrocytes. Hoppe-Seyler's Z. Physiol. Chem. **359**: 1217–1224.
12. **Dodge, J. T., C. Mitchell, and D. J. Hanahan.** 1963. The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch. Biochem. Biophys. **100**: 119–131.
13. **Dufourc, E. J., I. C. P. Smith, and J. Dufourc.** 1986. Molecular details of melittin-induced lysis of phospholipid membranes as revealed by deuterium and phosphorus NMR. Biochemistry **25**:6448–6455.
14. **Fowler, V., and D. Branton.** 1977. Lateral mobility of human erythrocyte membrane proteins. Nature (London) **268**:23–26.
15. **Freer, J. H., and T. H. Birbeck.** 1982. Possible conformation of delta-lysin, a membrane-damaging peptide of Staphyloccocus aureus. J. Theoret. Biol. **94**:535–540.
16. **Freer, J. H., T. H. Birbeck, and M. Bakoo.** 1984. Interaction of staphylococcal δ-lysin with phospholipid monolayers and bilayers—a short review, p. 181–189. *In* J. E. Alouf, F. J. Fehrenbach, J. H. Freer, and J. Jeljaszewicz (ed.), Bacterial toxins. Academic Press, Inc. (London), Ltd., London.
17. **Furthmayr, H.** 1978. Structural comparison of glycophorins and immunochemical analysis of genetic variants. Nature (London) **271**:519–524.
18. **Harshman, S., and M. Bondurant.** 1985. Susceptibility to staphylococcal alpha toxin of Friend virus-infected erythroblasts during differentiation. Infect. Immun. **48**:114–118.
19. **Howard, S. P., and J. T. Buckley.** 1982. Membrane glycoprotein receptor and hole-forming properties of a cytolytic protein toxin. Biochemistry **21**:1662–1667.
20. **Howard, S. P., and J. T. Buckley.** 1985. Activation of the hole-forming toxin aerolysin by extracellular processing. J. Bacteriol. **163**:336–340.
21. **Howard, S. P., and J. T. Buckley.** 1986. Molecular cloning and expression in Escherichia coli of the gene for the hemolytic toxin aerolysin of Aeromonas hydrophila. Mol. Gen. Genet. **204**:289–295.
22. **Howard, S. P., W. J. Garland, M. J. Green, and J. T. Buckley.** 1987. Nucleotide sequence of the gene for the hole-forming toxin aerolysin of *Aeromonas hydrophila*. J. Bacteriol. **169**: 2869–2871.
23. **Hugo, F., J. Reichwein, M. Arvand, S. Krämer, and S. Bhakdi.** 1986. Use of a monoclonal antibody to determine the mode of transmembrane pore formation by streptolysin O. Infect. Immun. **54**:641–645.
24. **Lundblad, R. L., and C. M. Noyes.** 1984. Chemical reagents for protein modification, p. 62–66 and 108–111. CRC Press, Boca Raton, Fla.
25. **Maharaj, I., and H. B. Fackrell.** 1980. Rabbit erythrocyte band 3: a receptor for staphylococcal alpha toxin. Can. J. Microbiol. **26**:524–531.
26. **Marchesi, V. T., and E. P. Andrews.** 1971. Glycoproteins: isolation from cell membranes with lithium diiodosalicylate. Science **174**:1247–1248.
27. **Markwell, M. K., and C. F. Fox.** 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3a,6a-diphenylglycouril. Biochemistry **17**: 4807–4817.
28. **Markwell, M. A. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert.** 1978. A modification of the Lowry procedure to simplify protein detection in membrane and lipoprotein samples. Anal. Biochem. **87**:206–210.
29. **Neville, D. M.** 1971. Molecular weight determination of protein-dodecyl sulphate complexes by gel electrophoresis in a discontinuous buffer system. J. Biol. Chem. **246**:6328–6334.
30. **Silversmith, R. E., and G. L. Nelsesteren.** 1986. Assembly of the membrane attack complex of complement on small unilamellar phospholipid vesicles. Biochemistry **25**:852–857.