Oligomerization of the Hemolytic Lectin CEL-III from the Marine Invertebrate Cucumaria echinata Induced by the Binding of Carbohydrate Ligands*

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The hemolytic lectin CEL-III is a Ca\(^{2+}\)-dependent, galactose/GalNAc-specific lectin purified from the marine invertebrate Cucumaria echinata (Holothuroidea). We found that this lectin forms ion-permeable pores in erythrocyte and artificial lipid membranes that have specific carbohydrate ligands on the surface. The hemolytic activity of CEL-III exhibited characteristic pH dependence; activity increased remarkably with pH in the alkaline region, especially above pH 9. When rabbit erythrocyte membrane was examined by immunoblotting using anti-CEL-III antiserum after treatment with CEL-III, the irreversible binding of the CEL-III oligomer increased with pH, indicating that the increase in hemolytic activity at higher pH is associated closely with the amount of oligomer irreversibly bound to the membrane. Surface hydrophobicity of CEL-III, as measured by the fluorescent probe 8-anilino-1-naphthalenesulfonate, increased markedly with the binding of specific ligands such as lactose, lactulose, and N-acetyllactosamine at pH 9–10 in the presence of 1 M NaCl. The enhancement of surface hydrophobicity induced by the binding of carbohydrates was also accompanied by the formation of a CEL-III oligomer, which was found to be the same size on sodium dodecyl sulfate-polyacrylamide gel electrophoresis as the oligomer that formed in CEL-III-treated erythrocyte membranes. Far-UV circular dichroism spectra of CEL-III and the oligomer revealed a definite difference in secondary structure. These data suggest that the binding of CEL-III to specific carbohydrate ligands on the erythrocyte surface induces a conformational change in the protein, leading to the exposure of a hydrophobic region which triggers oligomerization and the irreversible binding of the protein to the membrane.

We purified four Ca\(^{2+}\)-dependent, galactose/N-acetylgalactosamine (GalNAc)-specific lectins from the Holothuroidea Cucumaria echinata (1). Interestingly, these lectins exhibited hemolytic activity, especially toward rabbit and human erythrocytes. Unlike typical enzymatic reactions, hemolysis by CEL-III was more efficient at lower temperatures (around 10°C) and higher pH (up to pH 10), whereas little hemolysis was observed in the acidic region. In addition, since CEL-III induced a release of carboxyfluorescein from liposomes containing a glycolipid with GalNAc at the end, we assumed that hemolysis by CEL-III was not due to enzymatic activity but mediated by the formation of ion-permeable pores in the erythrocyte membrane, leading to osmotic rupture of the cells. The hemolytic activity is also Ca\(^{2+}\)-dependent and is inhibited by galactose or GalNAc-containing carbohydrates such as lactose, suggesting that the Ca\(^{2+}\)-dependent binding of CEL-III to specific carbohydrate receptors on the cell surface is an essential step for hemolytic action (2).

Many of the pore-forming proteins reported so far are of bacterial origin. Among them, Staphylococcus aureus \(\alpha\)-toxin is one of those most extensively studied (3–8). This protein is known to form oligomers upon binding to the lipid bilayer of target cells so that ion-permeable pores with a diameter of 1–3 nm are formed in the membrane. Recently, its oligomer has been shown to be a heptamer (9). The formation of the \(\alpha\)-toxin oligomer is also induced by interaction with deoxycholate, suggesting that hydrophobic interaction of the protein with the membrane or detergent micelles is important for oligomerization (10). Although the detailed mechanism is still uncertain, some data suggested that the \(\alpha\)-toxin oligomers after insertion into the membrane (11, 12). The three-dimensional orientation of an ion-permeable pore formed by aerolysin, another pore-forming toxin from Aeromonas hydrophila, has been illustrated recently (13). This protein forms heptameric cylindrical structures on the target cell surface following activation by partial digestion by some proteases (14–16). This exposes the hydrophobic region of the protein. As these cases show, it seems very important to investigate the hydrophobic nature of pore-forming proteins for the elucidation of their mechanism of action.

In the case of CEL-III, the formation of heterogeneous high molecular mass species was seen on SDS-PAGE, when the stacking gel was used (2). This suggests that CEL-III molecules tend to interact with each other to form oligomers under certain conditions. Although CEL-III is entirely soluble in aqueous solution, some hydrophobic interaction with the membrane also seems to be important for its hemolytic action. Therefore there is probably an additional step that triggers the exposure of the protein’s hydrophobic region, as with the proteolytic activation of aerolysin. In this study, we found that the binding of specific carbohydrates induced an enhancement of surface hydrophobicity as well as oligomerization of CEL-III in aqueous solution, which suggests that binding to carbohydrate receptors on the cell surface may also lead to a conformational

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1 The abbreviations used are PAGE, polyacrylamide gel electrophoresis; ANS, 8-anilino-1-naphthalenesulfonate; Bis-Tris, 2-(2-hydroxyethyl)aminot-2-(hydroxymethyl)-propane-1,3-diol.
change in CEL-III, thereby facilitating interaction with the membrane.

**EXPERIMENTAL PROCEDURES**

**Materials**—The C. echinata samples were generously provided by N. Ikeda (Fukuoka Fisheries and Marine Technology Research Center). The samples were stored at −30 °C until use. The rabbit blood was obtained from Nippon Bio-Test Laboratories (Tokyo). Cellulofine GCL-2000 K was obtained from Seikagaku Kogyo (Tokyo). The peroxidase-conjugated goat anti-mouse IgG was from Organon Teknika (West Chester, PA). The colloidal gold (5 nm)-protein A was from Wako (Osaka), and 8-anilino-1-naphthalenesulfonate (ANS) was from Tokyo Kasei.

**Purification of CEL-III**—CEL-III was purified from the body fluid of C. echinata using column chromatography on lactosyl-Cellulofine, GalNAC-Cellulofine, and Sepharcl S-200 as described previously (2). Chromatographic changes in absorbance at 280 nm were monitored by using a Beckman Optima XL-A analytical ultracentrifuge. Changes in absorbance at 280 nm were analyzed using a Beckman An-60 Ti rotor using a Beckman Optima XL-A analytical ultracentrifuge.

**Immunoblotting**—Immunoblotting was performed using mouse anti-CEL-III antisera as described previously (2).

**Measurement of Surface Hydrophobicity with ANS**—The surface hydrophobicity of CEL-III was measured with a fluorescent probe ANS (18, 19). Five microliters of 2.4 mM ANS were added to 0.3 ml of CEL-III solution (5.5 mg/ml) in buffers containing 1 mM NaCl and 30 mM CaCl2 with and without 10 mM carbohydrates (final ANS concentration 40 μM). After 30 min at room temperature, the fluorescence at 490 nm was recorded with excitation at 380 nm.

**Analytical Ultracentrifugation**—Sedimentation velocity analysis was performed at 60,000 or 40,000 rpm, at 20 °C using 0.19 mg/ml protein in an An-60 Ti rotor using a Beckman Optima XL-A analytical ultracentrifuge. Changes in absorbance at 260 nm were monitored using a Beckman XLAVEL program based on the method of Goldberg (20).

**Circular Dichroism (CD) Spectroscopy**—CD spectra of the CEL-III monomer and oligomer were recorded using a JASCO J-720 spectropolarimeter. The spectra were measured in a 1-mm path length cell at 20 °C. The molecular ellipticity (θ) was calculated using the mean residue weight of 115.

**Electron Microscopy**—Rabbit erythrocytes (5% (v/v) suspension) were treated with CEL-III (0.11 mg/ml) in 0.4 ml of 5 mM glycine/NaOH buffer, pH 10, containing 0.15 M NaCl and 10 mM CaCl2, at 20 °C. After 5 min, membranes were collected by centrifugation at 13,000 × g for 2 min and washed with 20 mM EDTA in 10 mM Tris-HCl buffer, pH 8, to remove CEL-III bound to carbohydrate chains on the membrane by Ca2+-dependent lectin activity. The membranes were then incubated with mouse anti-CEL-III antisera (1,000-fold dilution) (2), then with colloidal gold-protein A (20-fold dilution) in 0.1 ml of Tris-buffered saline. Each incubation was for 30 min at room temperature. After suspension in Tris-buffered saline, the membranes were stained with 3% (w/v) uranyl acetate and examined using a Hitachi H-600A electron microscope (75 kV).

**RESULTS**

The pH Dependence of the Irreversible Binding of CEL-III to the Erythrocyte Membrane—Our previous results indicated that hemolysis by CEL-III depends largely on pH; although little hemolytic activity was observed in the acidic region, hemolysis by CEL-III increased remarkably with pH, especially above pH 9 (2). However, it is obvious that this pH effect is not due to any decrease in the carbohydrate binding capacity of CEL-III in the acidic region, since a hemagglutination assay in the presence of an osmotic protectant, such as dextran 4, showed a somewhat higher hemaggulination activity in the acidic region (data not shown). This suggests that the higher hemolytic activity at higher pH is due to some factor other than the capacity of CEL-III to bind to the carbohydrate ligands on the erythrocyte surface. To clarify the mechanism for such a pH-dependent hemolysis, the irreversible binding of CEL-III to the erythrocyte membrane was examined. In this experiment, rabbit erythrocytes treated with CEL-III at various pH values were washed with lactose and EDTA to remove CEL-III bound to the carbohydrate chains on the erythrocyte surface, and the proteins irreversibly bound to the membrane were detected by immunoblotting analysis using anti-CEL-III antiserum. As shown in Fig. 1A, the immunoblot gave stronger bands of CEL-III oligomer with a molecular mass of about 270 kDa at higher pH, whereas only very weak bands were seen in the acidic region. This result indicates that the strong hemolytic activity at higher pH is closely related to the effective irreversible binding of CEL-III to the erythrocyte membrane and its oligomerization in this pH region, which probably damages the membrane with the formation of ion-permeable pores. Since the molecular mass of CEL-III was estimated at 45 kDa, the oligomer seen on the immunoblot may comprise six molecules of CEL-III monomer. Treatment of the samples with 2-mercaptoethanol did not affect the migration of the oligomer in the gel as shown in Fig. 1B, suggesting that the oligomer was not assembled by disulfide bond between CEL-III monomers.

**Increased Surface Hydrophobicity of CEL-III and Oligomerization Induced by the Binding of Specific Carbohydrates**—The change in surface hydrophobicity of CEL-III was examined with a fluorescent probe ANS (18, 19) at different pH values, as one of the possible factors affecting the increased irreversible binding of CEL-III to the membrane. When added to the protein solution, ANS exhibited little change in fluorescence intensity in the pH range 5–10, regardless of the presence of lactose. In contrast, in the presence of 1 mM NaCl a marked enhancement of fluorescence at higher pH, especially at pH 9–10, was observed only in the presence of lactose (Fig. 2). The fluorescence profile of ANS resembles the hemolytic activity.

![Fig. 1](image1.png) Immunoblotting of erythrocyte membranes treated with CEL-III at different pH values. Rabbit erythrocytes were treated with CEL-III (0.13 mg/ml) for 30 min at 20 °C in buffers at various pH values. The following buffers containing 0.15 M NaCl and 10 mM CaCl2 were used: pH 5, 10 mM sodium acetate; pH 6 and 7, 10 mM Bis-Tris; pH 8, 10 mM Tris-HCl; pH 9 and 10, 10 mM glycine/NaOH. After washing with 0.1 mM lactose and 10 mM EDTA, the resulting membranes were solubilized with the sample buffer containing 2% SDS without (panel A) or with (panel B) 2.5% (v/v) 2-mercaptoethanol and subjected to SDS-PAGE (5% gel) and immunoblotting. Detection was done using mouse anti-CEL-III antiserum.
profile in the same pH range (2), strongly suggesting a close correlation between the degree of surface hydrophobicity of CEL-III and its hemolytic activity. Fig. 3 shows the effects of several carbohydrates on the surface hydrophobicity of CEL-III. Although lactose, lactulose, and N-acetyllactosamine induced relatively high enhancement of hydrophobicity, other carbohydrates showed less effect. When the effect of NaCl concentration was examined (Fig. 4), it was found that in the presence of lactose, the hydrophobicity of CEL-III increased remarkably with increasing NaCl concentration, up to 1.15 M. The effect of NaCl was much less in the absence of lactose, although a slight increase in hydrophobicity was observed.

When CEL-III was subjected to SDS-PAGE after treatment with different carbohydrates at pH 10 in the presence of 1 M NaCl, formation of the oligomer was observed at 270 kDa (Fig. 5A). This is the same position as the CEL-III oligomer bound irreversibly to the erythrocyte membrane (Fig. 5B). In this case, the effects of the different carbohydrates were similar to the effects on hydrophobicity increase, as measured by ANS fluorescence. These results indicate a close relationship between the hydrophobicity enhancement and the oligomerization of CEL-III upon the binding of carbohydrates. Therefore, it seems reasonable to suppose that under these conditions, the hydrophobicity enhancement and the oligomerization of CEL-III reflect structural changes to CEL-III which occur during interaction with the erythrocyte membrane.

Molecular Size of the CEL-III Oligomer—Fig. 6A shows the separation of the CEL-III monomer and oligomer induced by the binding of lactose using a Sephacryl S-500HR column. When CEL-III was applied to the column after incubation in 10 mM glycine/NaOH buffer, pH 10, containing 1 M NaCl and 1 M CaCl₂, two major peaks appeared at fractions 54 and 62 (peak 1 and peak 2, respectively). These fractions were found by SDS-PAGE (Fig. 6B) to correspond to the CEL-III oligomer and monomer, respectively. On the other hand, CEL-III incubated in the buffer containing 10 mM EDTA instead of lactose and CaCl₂ showed only a single major peak corresponding to the CEL-III monomer with some shoulders at higher molecular mass positions, indicating that the oligomer could not be induced effectively without the binding of lactose. Although the oligomer in peak 1 showed a band at 270 kDa on SDS-PAGE (Fig. 6B), ultracentrifugal analysis revealed that the sedimentation coefficients of these oligomer and monomer as 37 and 4.1 S, respectively, suggesting that the oligomer formed much larger molecular species in aqueous solution. As shown in Fig. 6C, lane 1, nondenaturing PAGE of peak 1 showed a band at the top of the gel, but its size was reduced by the addition of 0.1% Triton X-100 prior to application to the gel (lane 2). These results indicate that the CEL-III oligomer induced by the binding of lactose forms larger aggregates of the oligomers held together by relatively weak interactions that can be disrupted by treatment with detergent. The CEL-III oligomer is obviously active in terms of its carbohydrate binding activity, since it still possesses hemagglutinating activity to at least the same degree as the CEL-III monomer when examined in the presence of an osmotic protectant (data not shown). However, no hemolytic activity of the oligomer was detected.

CD Spectra of the CEL-III Monomer and Oligomer—Far-UV CD spectra of CEL-III and its oligomer were measured to evaluate the conformational changes accompanying exposure of the hydrophobic region and the oligomerization induced by the binding of lactose. As shown in Fig. 7, the CEL-III monomer gave a spectra with negative and positive peaks at 206 and 229 nm, respectively. The α-helix, β-sheet, β-turn, and unordered structure content were calculated to be 0, 46, 24, and 30%, respectively, based on the method of Yang et al. (21). After oligomerization, the negative band of the spectrum shifted to longer wavelengths, and the positive peak at around 229 nm

Fig. 2. Changes in the surface hydrophobicity of CEL-III at different pH values as measured by ANS fluorescence. Fluorescence intensity at 490 nm was recorded with excitation at 380 nm. Measurements were carried out in the presence of 10 mM CaCl₂ and 1 mM NaCl, with (●) or without (○) 10 mM lactose. The following buffers were used: pH 5.4–7.1, 10 mM bis-Tris; pH 7.6–8.7, 10 mM Tris-HCl; pH 8.8–9.9, 10 mM glycine/NaOH.

Fig. 3. Effects of various carbohydrates on the hydrophobicity of CEL-III upon the binding of lactose. The surface hydrophobicity of CEL-III was measured in 10 mM glycine/NaOH, pH 10, containing 10 mM CaCl₂, 1 mM NaCl, and indicated carbohydrates at 1 mM. ANS fluorescence was measured at 490 nm with excitation at 380 nm. Bars represent the means ± S.D. of four measurements. LacNac, N-acetyllactosamine.

Fig. 4. Effects of NaCl concentration on the hydrophobicity of CEL-III upon the binding of lactose. Measurements were performed in 10 mM glycine/NaOH, pH 10, containing 10 mM CaCl₂, 1 mM lactose, and indicated concentrations of NaCl. ANS fluorescence was measured at 490 nm with excitation at 380 nm. Bars represent the means ± S.D. of three measurements.
The α-helix, β-sheet, β-turn, and unordered structure were calculated to be 0, 51, 22, and 27%, respectively. This indicates that the CEL-III oligomerization process is accompanied by a change in secondary structure, which may be closely related to the enhancement of the surface hydrophobicity of the protein.

**Electron Microscopy—**Fig. 8 shows the electron micrographs of negatively stained rabbit erythrocyte membrane treated with CEL-III. After incubation with CEL-III at pH 10 for 5 min, the CEL-III-treated erythrocyte membranes (panel B) were uneven in appearance compared with the control membrane (panel A), with several clusters of colloidal gold suggesting the presence of CEL-III oligomers irreversibly bound to the membrane. Treating the erythrocytes with CEL-III for 30 min damaged the membrane more severely (panel C). Ring- or arc-like structures observed on the membrane treated with some bacterial pore-forming toxins (3, 22–27) were not seen in this case.

**DISCUSSION**

Our previous data indicated that hemolysis is caused by ion-permeable pores formed by oligomerized CEL-III being inserted into the erythrocyte membrane. Such a mechanism, damaging the membrane through pore formation, is also common to many cytolytic protein toxins of microbial origin. One of the remarkable features of CEL-III is that the hemolytic activity increases markedly at higher pH, especially above pH 9, whereas it causes almost no lysis in the acidic region. This is in contrast to some other toxins, such as staphylococcal α-toxin (7), diphtheria toxin (28), and anthrax toxin (29), which require acidic pH to interact with the cell membrane. An investigation into the pH-dependent changes in the activity and structure of CEL-III was therefore expected to provide important clues for the elucidation of the mechanism of the hemolytic action of CEL-III.

Measurement of the irreversible binding of CEL-III to the erythrocyte membrane revealed that CEL-III was bound to the membrane as an oligomer probably consisting of six monomers. The binding of the CEL-III oligomer to the membrane increased with pH in the alkaline region, suggesting that irreversible binding accounts for the remarkable increase in hemolytic activity at high pH. In contrast, CEL-III appears to be active in terms of its carbohydrate binding ability even at low pH, since no remarkable decrease in hemagglutinating activity was observed in the pH range 5–10 when measured in the presence of an osmotic protectant (data not shown). The difference in pH dependence between hemolytic and carbohydrate binding activity strongly suggests that hemolysis is governed not only by the binding efficiency of the protein to carbohydrate receptors on the cell surface, but also by another step involving the pH dependence of the interaction between CEL-III and the membrane. Fluorescence measurements using ANS indicated that the surface hydrophobicity of CEL-III increased markedly in the alkaline region, especially above pH 9, only when complexed with lactose in the presence of a high concentration of salt. In addition, formation of the CEL-III oligomer was also observed after such treatment. These results suggest that the binding of specific carbohydrates, especially lactose, induced a conformational change in CEL-III which exposed the hydrophobic region and led to oligomerization of the protein through hydrophobic interaction. However, the reason why a high salt...
concentration (e.g. 1 M NaCl) is required remains unknown. It seems possible that a high concentration of NaCl may promote the oligomerization of CEL-III by weakening hydrogen bonds between water and polar groups of the protein. Alternatively, the salt may directly affect the interaction between amino acid residues within the protein monomer and facilitate conformational change upon the binding of specific carbohydrates, leading to hydrophobic interaction between the proteins.

Although the oligomer induced by the binding of lactose showed a band at 270 kDa on SDS-PAGE, the actual size of the oligomer in aqueous solution was found to be much larger, as shown by the ultracentrifugal analysis. The band of oligomer seen on SDS-PAGE may represent a structural core unit held by strong interactions, which is further assembled in aqueous solution relatively weakly. The oligomer of 270 kDa appears quite stable once formed, since it does not dissociate after boiling for several minutes in the SDS-PAGE sample buffer. Immunoblotting of CEL-III-treated erythrocyte membrane indicated that the oligomer formed in the membrane is of the same size as the oligomer formed in aqueous solution upon the binding of lactose. This suggests that both oligomers are similarly assembled, and therefore an investigation into the structural changes to CEL-III induced by the binding of carbohydrate in solution can also be expected to provide useful information concerning oligomer formation processes in the membrane.

Electron microscopy of rabbit erythrocyte membrane treated with CEL-III suggested that the protein bound to the membrane in clusters within 5 min, whereas the CEL-III oligomer bound to the membrane was not directly visible. Nevertheless, severe lesions were seen in the membrane after treatment for 30 min. Such a drastic change in the membrane may be partly due to the relatively high concentration of CEL-III (0.11 mg/ml) used in this experiment to facilitate the detection of bound CEL-III. Since, after incubation for several hours, hemolysis occurred with a much lower CEL-III concentration (e.g. 5 µg/ml) even in the presence of an osmotic protectant (data not shown), the size of the pores formed by CEL-III might increase with time and cause severe damage to the membrane, as observed by electron microscopy.

Enhancement of hydrophobicity and oligomerization of CEL-III in solution was effectively induced by disaccharides containing a β-1,4-glycosidic bond, such as lactose, lactulose, and N-acetyllactosamine, whereas α-1,6-linked galactosides and monosaccharides induced less change. In our previous study, however, GalNAc was most effective at inhibiting hemolysis by
CEL-III (1), indicating that it can bind strongly to CEL-III. The monosaccharide structure is probably too small to induce conformational change and subsequent oligomerization.

Some toxins have been shown to increase their hydrophobicity upon the binding of specific carbohydrate ligands. Pertussis toxin is composed of a catalytic A subunit and receptor-binding B oligomer. The B oligomer binds to carbohydrate receptors on target cells by lectin activity (30, 31). The binding of the A subunit to some glycoproteins or oligosaccharides was found to enhance the hydrophobicity of the toxin, which may contribute to the functional binding of the toxin to the cell membrane and the delivery of the A subunit into the cytosol (32). Binding of the carbohydrate moiety of the receptor to the Shiga-like toxin B subunit also induces exposure of the hydrophobic region on the surface of the protein, resulting in aggregation in vitro due to hydrophobic interaction between protein molecules (33). Such an exposure of the hydrophobic region is expected to promote insertion of the protein into the lipid membrane after specific binding to the cell surface carbohydrate receptors. Although conformational change to the Shiga-like toxin B subunit upon binding with the carbohydrate is thought to be limited to a local site comprising a tryptophan residue (33), the conformational change to CEL-III appears more extensive and includes the backbone structure as revealed by the far-UV CD spectra (Fig. 7). As described above, oligomerization and the irreversible binding of CEL-III to the erythrocyte membrane may also be triggered by conformational change upon binding with specific carbohydrate receptors on the erythrocyte membrane. Carbohydrate receptors on the cell surface may contribute not only by increasing the local concentration of these proteins at the membrane, but also by inducing the conformational change essential for strong interaction with the membrane.

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REFERENCES

1. Hatakeyama, T., Kohzaki, H., Nagatomo, H., and Yamasaki, N. (1994) J. Biochem. (Tokyo) 116, 209–214
2. Hatakeyama, T., Nagatomo, H., and Yamasaki, N. (1995) J. Biol. Chem. 270, 3560–3564
3. Füssle, R., Bhakdi, S., Szegedi, A., Tranum-Jensen, J., Kranz, T., and Wellems, T. (1981) J. Biol. Chem. 256, 83–94
4. Hildebrand, A., Pöhl, M., and Bhakdi, S. (1991) J. Biol. Chem. 266, 17195–17200
5. Tobkes, N., Wallace, B. A., and Bayley, H. (1985) Biochemistry 24, 1915–1920
6. Ikigai, H., and Nakae, T. (1985) Biochem. Biophys. Res. Commun. 130, 175–181
7. Forti, S., and Menestrina, G. (1989) Eur. J. Biochem. 181, 767–773
8. Jursch, R., Hildebrand, A., Hobom, G., Tranum-Jensen, J., Ward, R., Kehoe, M., and Bhakdi, S. (1994) Infect. Immun. 62, 2249–2256
9. Gouaux, J. E., Braha, O., Hobaugh, M. R., Song, L., Cheley, S., Shustak, C., and Bayley, H. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 12828–12831
10. Bhakdi, S., Füssle, R., and Tranum-Jensen, J. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5475–5479
11. Ikigai, H., and Nakae, T. (1987) J. Biol. Chem. 262, 2156–2160
12. Walker, B., Krishnasastry, M., Zorn, L., and Bayley, H. (1992) J. Biol. Chem. 267, 21782–21786
13. Parker, M. W., Buckley, J. T., Postma, J. P. M., Tucker, A. D., Leonard, K., Pattus, F., and Tsernoglou, D. (1994) Nature 367, 292–295
14. van der Goot, F. G., Lakey, J., Pattus, F., Kay, C. M., Sorokine, O., Dorselaer, A. V., and Buckley, J. T. (1992) Biochemistry 31, 8566–8570
15. Garlant, W. J., and Buckley, J. T. (1988) Infect. Immun. 56, 1249–1253
16. Wilsmsen, H. U., Leonard, K. R., Tichelaar, W., Buckley, J. T., and Pattus, F. (1992) EMBO J. 11, 2457–2463
17. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenke, D. C. (1985) Anal. Biochem. 150, 76–85
18. Weber, G., and Young, L. B. (1964) J. Biol. Chem. 239, 1415–1423
19. Stryer, L. (1975) J. Biol. Chem. 250, 482–495
20. Goldberg, R. J. (1953) J. Phys. Chem. 57, 194–202
21. Yang, J. T., Wu, C.-S. T., and Martinez, H. M. (1986) Methods Enzymol. 130, 200–209
22. Duncan, J. L., and Schlegel, R. (1975) J. Cell Biol. 67, 160–173
23. Smyth, C. J., Freer, J. H., and Arbuthnot, J. P. (1975) Biochim. Biophys. Acta 382, 479–493
24. Cowell, J. L., Kim, K. S., and Bernheimer, A. W. (1978) Biochim. Biophys. Acta 507, 230–241
25. Freer, J. H., Arbuthnot, J. P., and Bernheimer, A. W. (1968) J. Bacteriol. 95, 1153–1168
26. Bhakdi, S., Weller, U., Walev, I., Martin, E., Jonas, D., and Palmer, M. (1993) Mol. Microbiol. 8, 167–175
27. Morgan, P. J., Hyman, S. C., Byron, O., Andrew, P. W., Mitchell, T. J., and Rowe, A. J. (1994) J. Biol. Chem. 269, 25315–25320
28. Donovan, J. J., Simon, M. I., and Montal, M. (1985) J. Biol. Chem. 260, 8817–8823
29. Singh, Y., Klimpel, K. R., Arora, N., Sharma, M., and Leppa, S. H. (1994) J. Biol. Chem. 269, 29039–29046
30. Brennan, M. J., David, L. J., Kimmer, J. G., and Mandark, C. R. (1988) J. Biol. Chem. 263, 4899–4904
31. Saukkonen, K., Burnette, W. N., Mar, V. L., Masure, H. R., and Tuomanen, E. I. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 118–122
32. Spangler, B. D., Heerze, L. D., Clark, C. G., and Armstrong, G. D. (1993) Arch. Biochem. Biophys. 305, 153–158
33. Hilaire, P. M. S., Boyd, M. K., and Toone, E. J. (1994) Biochemistry 33, 14652–14663