Clostridium novyi α-Toxin-catalyzed Incorporation of GlcNAc into Rho Subfamily Proteins

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The lethal and edema-inducing α-toxin from Clostridium novyi causes rounding up of cultured cell lines by redistribution of the actin cytoskeleton. α-Toxin belongs to the family of large clostridial cytotoxins that encompasses Clostridium difficile toxin A and B and the lethal toxin from Clostridium sordellii. Toxin A and toxin B have been recently identified as monogliescosyltransferases to modify the low molecular mass GTPases of the Rho subfamily (Just, I., Selzer, J., Wilm, M., Von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) Nature 375, 500–503 and Just, I., Wilm, M., Selzer, J., Rex, G., Von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) J. Biol. Chem. 270, 13932–13936). We report here the identification of the α-toxin-catalyzed modification of Rho. Using electrospray mass spectrometry, the mass of the modification was determined as 203 Da, consistent with a N-acetyl-hexosamine moiety. UDP-N-acetylglucosamine selectively served as cosubstrate for α-toxin-catalyzed modification into the Rho subfamily proteins Rho, Rac, Cdc42, and Rhog. The acceptor amino acid of N-acetylglucosaminylation was identified by mutagenesis as Thr-37 in Rho (equivalent to Thr-35 in Rac/Cdc42), which is located in the effector domain of the GTPases. C. novyi α-toxin seems to mediate its cytoxic effects on cells by mimicking endogenous post-translational modification of cellular proteins.

Clostridium novyi type A strains have been identified as the causative organisms of gas gangrene infections of humans and animals (1). Type A strains produce an exotoxin, termed α-toxin, that exhibits in vivo both lethal and edematizing activity (1). In tissue culture, α-toxin is cytotoxic, causing cell shape changes that are accompanied by disruption of the microfilament cytoskeleton and by minor effects at the vimentin and tubulin system (2–4). Recently, α-toxin has been cloned and sequenced (5). As deduced from these data, α-toxin has a molecular mass of 250,166 Da and shows 48% homology with Clostridium difficile toxin A (ToxA)† and toxin B (ToxB). ToxA and ToxB are the major virulence factors of pathogenic C. difficile strains and have been identified as the causative agents of the antibiotic-associated diarrhea and the fatal form, the pseudomembranous colitis (6, 7). Furthermore, α-toxin shows 34% homology to the lethal toxin from Clostridium sor- dellii (5, 8), which is causally involved in diarrhea and enterotoxaemia in domestic animals and gas gangrene in man (1, 9). These clostralid toxins share common structural features. The C-terminal part of the single-chained toxins covers repetitive peptides that are most likely involved in cell receptor binding, followed by a small hydrophobic intermediate region which probably participates in the translocation of the toxins into the cytoplasm of the target cell (5, 10). The N-terminal part carries the biological activity (11). The common property of these intracellularly acting protein toxins is their cytotoxic activity, which leads to preferential destruction of the microfilament system of cell monolayers.

Recently, C. difficile ToxA and ToxB have been identified as monogliescosyltransferases that selectively modify the low molecular mass GTP-binding proteins of the Rho subfamily (12, 13). The target proteins Rho, Rac, and Cdc42 are involved in the regulation of the actin cytoskeleton. Whereas Rho controls the formation of focal adhesions and stress fibers (14), Rac participates in membrane ruffling (15) and Cdc42 in formation of filopodia (16, 17). Furthermore, the Rho subfamily proteins have been identified as being involved in the activation of transcription factors via the Ras-regulated pathway (18) and via a Ras-independent signal cascade (19–21).

ToxC and ToxB-catalyzed glucosylation, which occurs at Thr-37 in RhoA, causes functional inactivation leading to depolymerization of the actin filament system (12). Glucosylation of Rho in Thr-37 blocks subsequent ADP-riboseylation by Clostridium botulinum C3 exoenzyme in Asn-41 (22, 23).

Here we report the identification of C. novyi α-toxin as a N-acetyl-glucosaminytransferase that modifies the Rho subtype proteins.2

EXPERIMENTAL PROCEDURES

Materials—[α-32P]NAD, UDP-[14C]Glc, UDP-[14C]GalNAc, UDP-[14C]Gal, and UDP-[14C]GlcNAc were obtained from DuPont NEN. All other reagents were of analytical grade and purchased from commercial sources. C. botulinum C3 exoenzyme (24) and C. difficile toxin B (25) were purified as described.

Purification of C. novyi α-Toxin—α-Toxin was purified by a modified procedure according to Ref. 26. C. novyi type A (strain 19402, kindly donated by Dr. G. Schallehn, Bonn, Germany) was cultured anaerobically for 4 days at 37 °C in a medium containing 3% tryptone, 2% yeast extract, 0.4% glucose, and 0.1% cystine- HCl, pH 7.2. After centrifugation, the proteins of the culture supernatant were precipitated with 290 g/liter ammonium sulfate (60% of saturation) for 2 h at 4 °C. Precipitated proteins were collected by centrifugation, extracted twice with 25 mM sodium phosphate, pH 7.2, and dialyzed for 24 h against 25 mM sodium phosphate, pH 7.2. The dialyzed solution was applied to anion
exchange chromatography (DE-Sephacel column; 2.5 × 15 cm). Separation was carried out using a linear gradient of 0 to 2 M NaCl in 25 mM sodium phosphate, pH 7.2. Bound α-toxin eluted at about 80 mM NaCl. Eluted toxin was concentrated by ultrafiltration (Amicon; cut-off, 10,000 kDa) followed by gel permeation chromatography (Superose 12 column, 20 × 1 cm; elution buffer 200 mM NaCl in 25 mM sodium phosphate, pH 7.2). Final yield was 0.5 mg of α-toxin/liter of culture.

Cell Culture—NIH 3T3 cells were grown in Dulbecco’s medium supplemented with 10% fetal calf serum, 4 mM glutamine/penicillin/streptomycin. After 24 h the medium was changed, and the cells were incubated with the toxins for the indicated times. Before cell lysis, the cells were rinsed with ice-cold phosphate-buffered saline, pH 7.2, and were then disrupted mechanically by sonication (five times on ice) in the presence of lysis buffer (2 mM MgCl₂, 40 mg/ml aprotinin, 0.3 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 50 mM HEPES, pH 7.4) followed by centrifugation for 10 min at 2,000 × g. The supernatant was used for glycosylation reactions.

ADP-ribosylation of Rho in Lysates from Toxin-treated NIH 3T3 Cells—NIH 3T3 cells (2 × 10⁶ cells/well) were incubated with α-toxin (400 ng/ml) for the indicated periods of time. Thereafter, the cells were lysed as described above, and the cell lysates were centrifuged at 600 × g for 10 min at 100,000 × g. The pellets were dissolved in 2 mM MgCl₂/1 mM dithiothreitol/0.3 mM phenylmethylsulfonyl fluoride/50 mM HEPES, pH 7.4, and used as membrane fraction. [³²P]ADP-ribosylation of the membrane fraction was carried out as described. The SDS-PAGE was evaluated with PhosphorImager SF (Molecular Dynamics). The amount of ADP-ribosylation was calculated as the percentage of control (nontreated cells).

ADP-ribosylation Reaction—Cell lysates or membrane fractions were incubated in buffer (10 mM thymidine/2 mM MgCl₂/50 mM HEPES, pH 7.4) with [³²P]NAD (0.3 μCi/50 mM HEPES, pH 7.4) and with 1 μg/ml C3 exoenzyme for 15 min at 37°C. The reaction was terminated by the addition of Laemmli sample buffer.

Preparation of Recombinant GTP-binding Proteins—Rhoa, RhoAAT₃⁷⁷, RhoA, and Rac1, Cdc42, RhoG, and H-Ras were prepared from their respective E. coli. The supernatant (cytosolic fraction) was incubated for 15 min at 37°C. Thereafter, this fraction was added to each reaction mixture, and the mixture was analyzed with the PhosphorImager. Thereafter, this fraction was added to each reaction mixture, and the mixture was analyzed with the PhosphorImager.

Glycosylation of Rho Subfamily Proteins

Identification of the Modification—To test whether α-toxin also exhibits glucosyltransferase activity as do C. difficile ToxA and ToxB, lysates from NIH 3T3 cells and recombinant Rho subfamily proteins, respectively, were incubated with α-toxin in the presence of UDP-[¹⁴C]glucose. Surprisingly, under conditions where ToxA/B elicited full glucosyltransferase activity, α-toxin did not catalyze incorporation of glucose from UDP-glucose into cellular or recombinant proteins (data not shown). Therefore, we first tested whether the Rho protein is actually the target of α-toxin. To this end, ADP-ribosyltransferase C3 was used to detect alterations in the Rho protein. C3 selectively ADP-ribosylates the Rho subtype proteins RhoA, B, and C in Asn-41, thereby inactivating Rho (34–37). In lysates prepared from α-toxin-treated NIH 3T3 cells, C3-catalyzed [³²P]ADP-ribosylation of Rho was decreased in a time-dependent manner comparable with the effects of ToxA and ToxB on cellular Rho (Fig. 1A). Heat inactivation of α-toxin abolished both cytotoxic effects on cells and inhibition of Rho-ADP-ribosylation (not shown), indicating a specific effect of α-toxin on the ADP-ribosylation of Rho. As observed with ToxA/B, α-toxin affected ADP-ribosylation of recombinant Rho protein exclusively in the presence of a cytosolic fraction indicating a cytosolic cofactor is essential for α-toxin activity (Fig. 1B). This cytosolic cofactor was partially characterized as a heat-stable, nonproteinaceous agent with a molecular mass between 500 and 3000 Da. Partial purification of the cofactor was essential to produce sufficient amounts of α-toxin-modified recombinant Rho, which was subsequently subjected to electrospray mass spectrometry to determine the molecular mass of the modification. The mass of the complete Rho protein modified by α-toxin was 203 Da higher than the unmodified protein (not shown), suggesting that modified Rho contains a covalently bound N-acetyl-hexosamine moiety (221 Da of N-acetyl-hexosamine minus 18 Da of glucose). These data are consistent with monoglycosylation of Rho.

The identification of the type of hexosamine was performed with biochemical methods by testing several sugars to inhibit α-toxin-induced decrease in Rho ADP-ribosylation. Because the molecular mass of the cosubstrate was >500 Da, mers N-acetyl-hexosamines were excluded, and the activated forms (UDP-N-acetyl-hexosamines) were tested. As shown in Fig. 2, α-toxin induced inhibition of C3-catalyzed ADP-ribosylation of Rho exclusively in the presence of UDP-GlcNAc. Other UDP-hexoses did not mediate this effect. To corroborate the incorporation of GlcNAc directly, [¹⁴C]labeled nucleotide sugars were used. As shown in Fig. 3A, α-toxin catalyzed incorporation of [¹⁴C]GlcNAc but not of [¹⁴C]Glc or [¹⁴C]GalNAc into Rho. Denaturing either Rho or C3-catalyzed reaction complete inhibition of modification consistent with the notion that the native protein structure is essential for this type of glycosylation. Taken together, these data indicate that C. novyi α-toxin is a...
ADP-ribosylation by C3 exoenzyme. PhosphorImager data of the F500) for 60 min at 37°C. Thereafter, the samples were subjected to the presence of either rat brain cytosol or cytosolic subfractions (F3000 or F5000) as described under "Experimental Procedures." The amount of ADP-ribosylation was calculated as the percentage of control cells.

Desaminylated, whereas other GTPases of the Ras superfamily were not target for ADP-ribosylation. Recombinant H-Ras, Arf1, Rab5, and Ran were not target for α-toxin.

Glycosylation of Rho subfamily proteins

N-Acetylgalcosaminyltransferase that utilizes the cosubstrate UDP-GlcNAc to transfer the GlcNAc moiety to the Rho protein.

Substrate Specificity—To identify the substrate proteins of α-toxin, several recombinant low molecular mass GTPases were tested. As illustrated in Fig. 3B, Rho, Rac, Cdc42, and RhoG, all members of the Rho subfamily, were N-acetylgalcosaminylated, whereas other GTPases of the Ras superfamily namely H-Ras, Arf1, Rab5, and Ran were not target for α-toxin.

N-Acetylgalcosaminylation is significantly stimulated in the presence of KCl with maximal effects at 150 mM, whereas NaCl had no stimulatory effect. Thus, α-toxin modifies the same recombinant substrate proteins as do ToxA and ToxB from C. difficile.

Acceptor Amino Acid—To test whether α-toxin uses the same acceptor site as ToxA and ToxB, sequential glycosylation was performed. Modification of RhoA with α-toxin in the presence of unlabeled UDP-GlcNAc, followed by a second glycosylation in the presence of UDP-[14C]Glc and ToxB resulted in blocked incorporation of [14C]Glc (Fig. 4). The same was true when the first glycosylation was performed with ToxB and UDP-Glc. These results indicate that α-toxin shares the same acceptor amino acid in RhoA, namely Thr-37. To prove the acceptor amino acid Thr-37 by a different approach, we tested whether exchange of Thr in position 37 for Ala abolishes incorporation of GlcNAc (Fig. 4). RhoAThr37Ala was still substrate for ADP-ribosyltransferase C3, indicating no gross changes in the overall protein structure. Incorporation of GlcNAc by α-toxin, however, was completely blocked. Thus, Thr-37 in Rho is the acceptor amino acid of ToxA and ToxB (12, 13).

Applying the crystal structure of H-Ras to Cdc42, Thr-35 of Thr-37 to RhoA and ToxA and ToxB (12, 13). The hydroxyl group of the preceding amino acid Pro to Ala (RhoA Pro36Rho) decreased but did not completely blocked modification. As can be deduced from the primary structure of the Rho subfamily proteins, Thr-37 in RhoA corresponds to Thr-35 in Rac and Cdc42. α-Toxin shares the acceptor amino acid of the Rho subfamily proteins with ToxA and ToxB (12, 13).

FIG. 1. Influence of α-toxin on C3-catalyzed ADP-ribosylation. A, time-dependent effect of C. novyi α-toxin on ADP-ribosylation of Rho. NIH 3T3 cells were treated with α-toxin (400 ng/ml) for increasing periods of time. Thereafter, the cells were lysed, and the membrane fractions were used for [32P]ADP-ribosylation with C3 (1 μg/ml) as described under "Experimental Procedures." The amount of ADP-ribosylation was calculated as the percentage of control cells. B, α-toxin-induced decrease in ADP-ribosylation of recombinant Rho. Recombinant RhoA (50 μg/ml) was incubated with α-toxin (40 μg/ml) in the presence of either rat brain cytosol or cytosolic subfractions (F3000 or F5000) for 60 min at 37°C. Thereafter, the samples were subjected to [32P]ADP-ribosylation by C3 exoenzyme. PhosphorImager data of the SDS-PAGE are shown.

FIG. 2. Influence of various nucleotide-hexoses on α-toxin-induced decrease in C3-catalyzed ADP-ribosylation. Recombinant RhoA (50 μg/ml) was incubated with α-toxin (40 μg/ml) in the presence of the indicated UDP-hexoses (100 μM) for 60 min at 37°C. Thereafter, the samples were subjected to [32P]ADP-ribosylation with C3 (1 μg/ml) as described under "Experimental Procedures." The amount of ADP-ribosylation was calculated as the percentage of control cells. B, α-toxin-induced decrease in ADP-ribosylation of recombinant Rho. Recombinant RhoA (50 μg/ml) was incubated with α-toxin (40 μg/ml) in the presence of either rat brain cytosol or cytosolic subfractions (F3000 or F5000) for 60 min at 37°C. Thereafter, the samples were subjected to [32P]ADP-ribosylation by C3 exoenzyme. PhosphorImager data of the SDS-PAGE are shown.

FIG. 3. Cosubstrate and protein substrates of α-toxin. A, α-toxin-induced incorporation of GlcNAc into RhoA. RhoA (50 μg/ml) was incubated in the presence of the indicated UDP-[14C]hexoses (100 μM) for 30 min at 37°C with α-toxin (40 μg/ml). B, protein substrate specificity of α-toxin. The indicated recombinant low molecular mass GTPases (50 μg/ml) (dissolved in a buffer containing 2 mM MgCl2, 0.3 mM GDP, 150 mM KCl, 50 mM HEPES, pH 7.5) were incubated with α-toxin (40 μg/ml) and UDP-[14C]GlcNAc (30 μM) for 30 min at 37°C. PhosphorImager data of the SDS-PAGE are shown.
Glycosylation of Rho Subfamily Proteins

Cdc42 is a better substrate for α-toxin in the GDP-bound form than in the GTP-bound form (Fig. 5). Binding of the nonhydrolyzable GTPγS almost completely blocked modification of Cdc42, suggesting that incorporation of GlcNAc into GTP-bound Cdc42 is due to GTP hydrolysis. Coordination of the Cdc42, suggesting that incorporation of GlcNAc into GTP-bound Cdc42 is due to GTP hydrolysis. Coordination of the γ-phosphate results in a conformation of Thr-35, which is incompatible with attachment of GlcNAc. In the GDP-bound form incorporation did not exceed 1 mol of GlcNAc/mol of Cdc42, consistent with monoglycosylation of Cdc42. These data are in agreement with the data on ToxA and ToxB, which catalyze monoglycosylation (12, 13).

Thr-37 in Rho (Thr-35 in Cdc42 and Rac) is located in the effector domain of these GTPases. In case of Rac and Cdc42, the p65PAK kinase has been identified as an effector, whereas the effector of the Rho subtype protein appears to be a p150 serine/threonine kinase (39). In Rac two different effector sites have been identified to interact with the serine/threonine p65PAK kinase and p67PAK (40). The N-terminal site covers residues 22–45 embracing the acceptor of GlcNAc, Thr-35. It is conceivable that the hydrophilic GlcNAc moiety in this crucial domain impedes with effector coupling resulting in blocked signal cascade.

In Vivo Substrate Proteins—Incubation of cell lysates with α-toxin and UDP-[14]C[GlcNac resulted in labeling of two protein bands (Fig. 6). Immunoblot analysis of gel electrophoretically separated cellular proteins with anti-RhoA and anti-Cdc42, respectively, showed that the lower band correponds to Cdc42 and the upper one to RhoA (data not shown). In contrast to ToxA/B, α-toxin catalyzed only a faint labeling of Rho (upper band). It seems that cellular Rho is a poor substrate for α-toxin. However, this observation is not unique to NIH 3T3 cells. Lysates from various cultured cell lines showed a low extent in N-acetyl-glucosaminylation of Rho compared with Cdc42/Rac. To test whether isoprenylation is the basis for this observation NIH 3T3 cells were treated with Lovastatin (30 μM for 24 h) to block isoprenylation. However, α-toxin-catalyzed incorporation of GlcNac into Rho from this lysates did not increase (data not shown). Furthermore, proteolytical degradation of Rho was excluded to cause low incorporation of GlcNac into Rho. Thus, for unknown reasons recombinant Rho is a superior substrate to cellular Rho.

To test whether Cdc42 and Rac were modified by α-toxin in the intact cell, the method of [14]C-galactosylation of proteins bearing a GlcNAc moiety was employed (32). Lysates from α-toxin-treated NIH 3T3 cells were denatured by incubation at 95 °C in the presence of detergents followed by incubation with UDP-[14]C[Gal and galactosyltransferase from bovine milk. As demonstrated in Fig. 7A, [14]C-galactosylated proteins in the molecular mass range of 20–22 kDa were only detected in lysates from α-toxin-treated cells. Identification of the [14]C-galactosylated protein was performed in a second approach. Cell lysates were electrotransferred to nitrocellulose and the blot slice corresponding to the molecular mass range of 15–25 kDa was cut out and was subjected to the [14]C-galactosylation reaction (Fig. 7B). Thereafter, the same blot slice was probed with anti-Cdc42 identifying the labeled protein as Cdc42 (Fig. 7B). These data suggest that α-toxin modifies Cdc42 in intact cells.

O-linked N-acetylgalactosaminylation is not unique to C. novyi α-toxin, but it is a common post-translational modification in eukaryotic cells. Characteristic for O-GlcNAc-bearing proteins is their intracellular localization in the cytoplasm and in the nuclear space, respectively (41). This kind of modification is as highly dynamic as phosphorylation (41). The function of monosaccharide attachment is not fully understood, but there is evidence that O-glycosylation with GlcNAc is involved in nuclear targeting and formation of multimeric protein structures (41, 42). It is conceivable that bacterial toxins mimic regulatory mechanisms of eukaryotic cells to interfere effectively with the signal cascade of their target cells. C. novyi α-toxin seems to belong to those bacterial toxins that mediate their cytotoxic effects by mimicking endogenous post-translational modification of cellular proteins as has been reported for diphtheria toxin/pseudomonas exotoxin A (43) and for cholera toxin (44).
that mimic endogenous ADP-ribosylation.

In conclusion, α-toxin from C. novyi has been identified as a monoglycosyltransferase that catalyzes incorporation of GlcNAc into cellular Rho, Cdc42, and Rac. Modification of Thr-35 in Cdc42/Rac causes inactivation of these GTPases resulting in redistribution of the actin cytoskeleton. In common with in Cdc42/Rac causes inactivation of these GTPases resulting in redistribution of the actin cytoskeleton. In common with

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REFERENCES

1. Hatheway, C. L. (1990) Clin. Microbiol. Rev. 3, 66–98
2. Pette, P., Oksche, A., Mauger, F., Eichel-Streiber, C., Popoff, M. R., and Habermann, E. (1991) Toxicon 29, 877–887
3. Müller, H., Von Eichel-Streiber, C., and Habermann, E. (1992) Infect. Immun. 60, 3002–3006
4. Oksche, A., Nakov, R., and Habermann, E. (1992) Infect. Immun. 60, 67–70
5. Hofmann, F., Herrmann, A., Habermann, E., and Von Eichel-Streiber, C. (1995) Mol. Gen. Genet. 247, 670–679
6. Lyerly, D. M., Krivan, H. C., and Wilkins, T. D. (1988) Clin. Microbiol. Rev. 1, 1–18
7. Kelly, C. P., Poonthaklis, C., and LaMont, J. T. (1994) N. Engl. J. Med. 330, No. 4, 257–262
8. Green, G. A., Schü, V., and Montel, H. (1995) Gene (Amst.) 161, 57–61
9. McGregor, J. A., Soper, D. E., Lowell, G., and Todd, J. K. (1989) Am. J. Obstet. Gynecol. 161, 987–995
10. Eichel-Streiber, C., and Sauerborn, M. (1990) Gene (Amst.) 96, 107–113
11. Eichel-Streiber, C. (1995) in Genetics and Molecular Biology of Anamorphic Bacteria (Sebold, M., ed) pp. 264–289, Springer-Verlag, New York
12. Just, I., Selzer, J., Wilm, M., Von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) Nature 375, 500–503
13. Just, I., Wilm, M., Selzer, J., Rex, G., Von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) J. Biol. Chem. 270, 13892–13936
14. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
15. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1992) Cell 70, 491–410
16. Nobes, C. D., and Hall, A. (1995) Cell 81, 53–62
17. Kosma, B., Ahmed, S., Best, A., and Lim, L. (1995) Mol. Cell. Biol. 15, 1842–1952
18. Minden, A., Liu, A., Clarlet, F.-X., Abo, A., and Karin, M. (1995) Cell 81, 1147–1157
19. Hill, C. S., Wynn, J., and Treisman, R. (1995) Cell 81, 1159–1170
20. Coso, O. A., Chiarriello, M., Yu, J.-C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. S. (1995) Cell 113, 1137–1146
21. Olson, M. F., Ashworth, A., and Hall, A. (1995) Science 269, 1270–1272
22. Just, I., Fritz, G., Aktories, K., Grey, M., Popoff, M. R., Bougot, P., Hegenbarth, S., and Von Eichel-Streiber, C. (1994) J. Biol. Chem. 269, 10706–10712
23. Just, I., Selzer, J., Von Eichel-Streiber, C., and Aktories, K. (1995) J. Clin. Invest. 95, 1026–1031
24. Aktories, K., Rosener, S., Blaschke, U., and Chhatwal, G. S. (1988) Eur. J. Biochem. 172, 445–450
25. Eichel-Streiber, C., Harperath, U., Bosse, D., and Hadding, U. (1987) Microb. Pathog. 2, 307–318
26. Bette, P., Frevert, J., Maurer, F., Suttrop, N., and Habermann, E. (1989) Infect. Immun. 57, 2507–2513
27. Laemmli, U. K. (1970) Nature 227, 680–685
28. Fenn, J. B., Mann, M., Meng, C. K., Wong, S. P., and Whitehouse, C. M. (1989) Science 246, 64–69
29. Mann, M., and Wilm, M. (1995) Trends Biochem. Science. 20, 219–224
30. Wilm, M., and Mann, M. (1994) Int. J. Mass Spectrom Ion Processes 136, 167–180
31. Wilm, M., and Mann, M. (1996) Anal. Chem. 68, 1–8
32. Roquemore, E. P., Chou, T.-Y., and Hart, G. W. (1994) Methods Enzymol. 230, 443–460
33. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
34. Sekine, A., Fujimura, M., and Narumiya, S. (1989) J. Biol. Chem. 264, 8602–8605
35. Chardin, P., Boquet, P., Madaule, P., Popoff, M. R., Ruin, E. J., and Gill, D. M. (1989) EMBO J. 8, 3197–3202
36. Braun, U., Habermann, B., Just, I., Aktories, K., and Vandekerckhove, J. (1989) FEBS Lett. 243, 70–76
37. Just, I., Mohr, C., Schallehn, G., Menard, L., Didie, J., Vandekerckhove, J., van Damme, J., and Aktories, K. (1992) J. Biol. Chem. 267, 10274–10280
38. Pai, E. F., Kabsch, W., Krengel, U., Holmes, K. C., John, J., and Wittinghofer, A. (1995) Nature 374, 299–314
39. Bairoch, A., and Claverie, J.-M. (1988) Nature 323, 219–224
40. Diekmann, D., Nobes, C. D., Berbelo, P. D., Abo, A., and Hall, A. (1995) EMBO J. 14, 5297–5305
41. Haitaniwa, R. S., Kelly, W. G., Roquemore, E. P., Blomberg, M. A., Dong, L.-Y. D., Kreppel, L., Chou, T.-Y., and Hart, G. W. (1992) Biochem. Soc. Trans. 20, 264–269
42. Hart, G. W., Haitaniwa, R. S., Holt, G. D., and Kelly, W. G. (1989) Annu. Rev. Biochem. 58, 841–874
43. Iglewski, W. J., and Fendrick, J. L. (1990) in ADP-ribosylating Toxins and G Proteins (Moss, J., and Vaughan, M., eds) pp. 511–524, American Society for Microbiology, Washington, D.C.
44. Jacque, C., Thibault, H., Lambert, B., and Correze, C. (1986) Nature 323, 182–184