Activation by Thiol of the Latent NAD Glycohydrolase and ADP-ribosyltransferase Activities of Bordetella pertussis Toxin (Islet-activating Protein)*

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Pertussis toxin (islet-activating protein) activates adenylate cyclase in susceptible cells by ADP-ribosylating an inhibitory component of the cyclase system. This toxin, assayed in a cell-free system in the presence of high concentrations of thiols, catalyzed the hydrolysis of NAD to ADP-ribose and nicotinamide. This NAD glycohydrolase activity co-chromatographed on Sephacryl S-200 in 6.5 M urea, pH 3.2, 0.1 M glycine with the ADP-ribosyltransferase activity of the toxin, as monitored by the transfer of \( ^{32}P \)ADP-ribose from \( [^{32}P] \)NAD to a 41,000-Da protein in NG108-15 neuroblastoma x glioma hybrid cells. In the absence of thiol, the native holotoxin was enzymatically inactive. Following addition of 250 mM dithiothreitol to the assay, maximal enzymatic activity was evident after a delay of \( \approx 1 \) h, with 20 mM thiol, the delay was longer. The \( K_a \) for NAD with the fully activated enzyme was 25 \( \mu \)M; the \( K_a \) did not appear to vary with the extent of activation. Thiols were necessary in a cell-free system to demonstrate NAD glycohydrolase activity. When extensively washed membranes were used as a source of 41,000-Da substrate, thiol was necessary to observe ADP-ribosylation in some cases (human erythrocytes) and significantly stimulated activity in others (NG108-15 cells). In contrast to the bacterial toxins choleragen and Escherichia coli heat-labile enterotoxin that ADP-ribosylate stimulatory components of the cyclase system, pertussis toxin did not transfer ADP-ribose to low molecular weight guanidino compounds, such as arginine or agmatine.

The adenylate cyclase system is critical to the control of certain metabolic pathways by hormones and bacterial toxins. Hormones acting through specific cell surface receptors appear to have either stimulatory or inhibitory effects on cyclase (1–3). With the stimulatory receptors, it appears that activation of the catalytic unit depends on a coupling protein that requires GTP for activity and is known as Go, or G/P factor (1–2). With the inhibitory hormones, a similar case has been made for the presence of a GTP-dependent inhibitory coupling factor (G) (3). At least three bacterial toxins appear to exert their effects on cells through activation of the adenylate cyclase system (4–7). Choleragen (cholera toxin) and Escherichia coli heat-labile enterotoxin, the agents responsible in part for cholera and "traveler's diarrhea," respectively, activate cyclase by catalyzing the transfer of the ADP-ribose moiety of NAD to G, protein (4, 8–10). ADP-ribosylation of G, appears to promote the association of the protein with GTP and maintenance of an active G, GTP complex (11, 12). A toxin from Bordetella pertussis appears to increase the responsiveness of cyclase to stimulatory hormones and decreases the effect of inhibitory agonists (6, 7). The effects of this toxin result from ADP-ribosylation of a 41,000-Da membrane protein (13). Based on the effects of the modification of cyclase and the effects of GTP on action of inhibitory hormones, this protein has been termed G,, the inhibitory GTP-binding protein. The ADP-ribosylation of G, appears to be catalyzed by both holotoxin and a specific subunit, termed S, (14).

In the present report, we note that the enzymatic activity of the toxin is latent; activation of the toxin was achieved in the presence of high concentrations of thiol. Some membrane preparations were capable of toxin activation independent of exogenous thiol, whereas others were not. Pertussis toxin showed significant differences in enzymatic properties from choleragen and E. coli heat-labile enterotoxin.

EXPERIMENTAL PROCEDURES

Methods

NAD Glycohydrolase and ADP-Ribosyltransferase Assays—The NAD glycohydrolase activity of pertussis toxin was determined in a standard assay mixture that contained 50 mM potassium phosphate, pH 7.5, 0.24 mM [carbonyl-\( ^{14} \)C]NAD (\( \approx 40,000 \) cpm), 1 \( \mu \)g/ml of ovalbumin, and the indicated additions in a total volume of 0.3 ml. After incubation for 18 h at 30 °C, or as indicated, two 0.1-ml samples were run over AG 1-X2 columns as described previously (15, 16); this procedure resolves [carbonyl-\( ^{14} \)C]nicotinamide from [carbonyl-\( ^{14} \)C]NAD. All assays were run in duplicate.

The radioactivity of products of the reaction in the presence of [carbonyl-\( ^{14} \)C]NAD and [adenine-\( ^{14} \)C]NAD were [carbonyl-\( ^{14} \)C]nicotinamide and [adenine-\( ^{14} \)C]ADP-ribose, respectively, as determined by high pressure liquid chromatography (Fig. 1). The ratio of [carbonyl-\( ^{14} \)C]nicotinamide released to [adenine-\( ^{14} \)C]ADP-ribose formed was \( \approx 0.9 \). The addition of agmatine to the assay containing [adenine-\( ^{14} \)C]NAD did not lead to the formation of [adenine-\( ^{14} \)C]ADP-ribose-agmatine; the products of the reaction were analyzed by high performance liquid chromatography using [adenine-\( ^{14} \)C]ADP-ribose-agmatine synthesized by an erythrocyte NAD:arginine ADP-ribosyltransferase as a standard (17).

Pertussis Toxin—The toxin from B. pertussis was purified as described by Burns et al. (18).

Preparation of Cell Membranes—NG108-15 neuroblastoma \( \times \) glioma hybrid cells as described (19) were suspended in 20 volumes of 50 mM Tris-HCl, pH 7.5, containing 5 mM MgCl\(_2\) and homogenized with 25 strokes on a Dounce tissue grinder. The homogenate was
Activation of Pertussis Toxin by Thiol

**Materials**

Agmatine, mercaptoethanol, cysteine, glutathione, NAD, and ATP were purchased from Sigma; AG 1-X2 from Bio-Rad; \([\alpha^{-32P}]\)NAD from New England Nuclear; and dithiothreitol from Schwarz/Mann. Other agents were purchased as previously described (20). \([\text{carbonyl-}^{14}\text{C}]\)NAD (specific activity 57 mCi/mmol) and \([\text{adenine-U-}^{14}\text{C}]\)NAD (specific activity 287 mCi/mmol) were from Amersham Corp. NG108-15 cells were a gift of Dr. Werner A. Klee, National Institute of Mental Health, Bethesda, MD.

**RESULTS**

In the presence of dithiothreitol, pertussis toxin catalyzed the release of \([\text{carbonyl-}^{14}\text{C}]\)nicotinamide from \([\text{carbonyl-}^{14}\text{C}]\)NAD (Fig. 2). Maximal nicotinamide release was observed with \(-250\) mM dithiothreitol. The rate of nicotinamide formation was less in \(20\) mM than in \(250\) mM dithiothreitol; a delay in the rate of reaction was observed at both thiol...

**Fig. 1.** Analysis by high pressure liquid chromatography of the products of the reaction of pertussis toxin and \([\text{carbonyl-}^{14}\text{C}]\)NAD (A) or \([\text{adenine-U-}^{14}\text{C}]\)NAD (B). Pertussis toxin (14 \(\mu\)g) was incubated for 6 \(h\) at 30 °C in 50 \(\mu\)l of 90 mM potassium phosphate, pH 7.5, containing 20 mM dithiothreitol, 100 mM NaCl, and 1 mg/ml of ovalbumin. To initiate the assay, 0.25 ml of a mixture was added to give a final concentration in 0.3 ml of 65 mM potassium phosphate, pH 7.5, 1.2 mg/ml of ovalbumin, and either 20 mM or 250 mM dithiothreitol, and either 32.4 \(\mu\)M \([\text{carbonyl-}^{14}\text{C}]\)NAD (38,700 cpm) or 30.5 \(\mu\)M \([\text{adenine-U-}^{14}\text{C}]\)NAD (34,400 cpm). After 18 \(h\) at 30 °C, the samples containing \([\text{carbonyl-}^{14}\text{C}]\)NAD were assayed by chromatography on AG 1-X2 and those containing \([\text{adenine-U-}^{14}\text{C}]\)NAD were analyzed by high pressure liquid chromatography (A); samples containing \([\text{adenine-U-}^{14}\text{C}]\)NAD were analyzed by high pressure liquid chromatography (B). In both cases, 100 \(\mu\)l were injected. All assays were run in duplicate. Control samples were run in the presence of buffer to determine the basal rate (~5%) of \([\text{carbonyl-}^{14}\text{C}]\)NAD or \([\text{adenine-U-}^{14}\text{C}]\)NAD breakdown. The ratio of \([\text{carbonyl-}^{14}\text{C}]\)nicotinamide release to \([\text{adenine-U-}^{14}\text{C}]\)ADP-ribose formed was 0.96. In the presence of 75 mM agmatine, no peak was observed that had the mobility of \([\text{adenine-U-}^{14}\text{C}]\)ADP-ribose-agmatine; the ratio of \([\text{carbonyl-}^{14}\text{C}]\)nicotinamide release to \([\text{adenine-U-}^{14}\text{C}]\)ADP-ribose formed was 0.92.

**Fig. 2 (left).** Effect of dithiothreitol on the release of \([\text{carbonyl-}^{14}\text{C}]\)nicotinamide from \([\text{carbonyl-}^{14}\text{C}]\)NAD catalyzed by pertussis toxin. Pertussis toxin (14 \(\mu\)g) initiated reaction in a standard assay mixture containing the indicated concentration of thiol. Reaction was run as described under "Experimental Procedures." Two 0.05-ml samples were run over AG 1-X2 columns to determine \([\text{carbonyl-}^{14}\text{C}]\)nicotinamide release.

**Fig. 3 (center).** Effect of dithiothreitol on the rate of release of \([\text{carbonyl-}^{14}\text{C}]\)nicotinamide from \([\text{carbonyl-}^{14}\text{C}]\)NAD catalyzed by pertussis toxin. Pertussis toxin (15.9 \(\mu\)g) initiated the reaction in an assay mixture containing 50 mM potassium phosphate, pH 7.5, 1 mg/ml of ovalbumin, 32.4 \(\mu\)M \([\text{carbonyl-}^{14}\text{C}]\)NAD, and either 20 mM (A, ○) or 250 mM (C, □) dithiothreitol in a total volume of 0.6 ml; in two instances, pertussis toxin (15.9 \(\mu\)g) was incubated at 30 °C for 18 \(h\) in 0.2 ml of 50 mM potassium phosphate, pH 7.5, 1 mg/ml of ovalbumin, and either 20 mM (A) or 250 mM (A) dithiothreitol prior to addition to the assay mixture. At the indicated times, two 0.05-ml samples were run over AG 1-X2 columns to determine \([\text{carbonyl-}^{14}\text{C}]\)nicotinamide release.

**Fig. 4 (right).** Effect of NAD concentration on activity of the pertussis toxin. Pertussis toxin (159 \(\mu\)g/ml) was incubated for 18 \(h\) at 30 °C in a mixture containing 50 mM potassium phosphate, pH 7.5, 1 mg/ml of ovalbumin, 250 mM dithiothreitol; 50 \(\mu\)l (7.95 \(\mu\)g) of the activated toxin initiated reaction in a mixture containing 5.83 mM potassium phosphate, pH 7.5, 1.2 mg/ml of ovalbumin, 292 mM dithiothreitol, and the indicated concentrations of \([\text{carbonyl-}^{14}\text{C}]\)NAD (33,800 cpm). Reaction was run for 6 \(h\) at 30 °C.
concentrations and could be reduced by incubations with thiol prior to assay (Fig. 3). In addition to dithiothreitol, other thiols were also capable of activating the toxin (Table I); dithiothreitol and \( \beta \)-mercaptoethanol appeared to be more effective than glutathione.

The \( K_m \) for NAD in the thiol-dependent reaction was \( \approx 20 \mu M \) (Fig. 4); the \( K_m \) was similar with toxin preparations that varied over a 10-fold range in the extent of activation (data not shown).

The effects of various thiols on the release of \( [\text{carbonyl-}^{14}\text{C}] \) nicotinamide from \( [\text{carbonyl-}^{14}\text{C}] \) NAD catalyzed by pertussis toxin are shown in Table I. Dithiothreitol and \( 8 \)-mercaptoethanol appeared to be more activating than glutathione or cysteine.

### Table I

| Additions | \([\text{carbonyl-}^{14}\text{C}]\) Nicotinamide released (pmol) |
|-----------|---------------------------------------------------------------|
| None      | 0                                                             |
| Dithiothreitol | 1140                                                          |
| 20 mM     | 5870                                                          |
| 250 mM    | 393                                                           |
| Glutathione | 240                                                          |
| 20 mM     | 359                                                           |
| 250 mM    | 649                                                           |
| Cysteine  | 401                                                           |
| 20 mM     | 4375                                                          |
| 500 mM    |                                                               |

FIG. 5. Co-chromatography of the ADP-ribosyltransferase and NAD glycohydrolase activities of pertussis toxin on gel permeation columns. Pertussis toxin was dialyzed extensively against 6.5 M urea, pH 3.2, 0.1 M glycine, as described previously (15, 22); this procedure was adapted from that used to resolve the enzymatically active A subunit of choleragen from the B or binding subunit. The sample (34 \( \mu \)g) was then chromatographed on a Sephacryl G-200 column (1.2 \( \times \) 94.7 cm); 1.0-ml fractions were collected. A, identification of column fractions containing ADP-ribosyltransferase activity. Samples of NG108-15 membranes (150 \( \mu \)g of protein) were incubated with 10 \( \mu l \) of column fraction in 50 mM potassium phosphate buffer, pH 7.5, containing 20 \( \mu M \) [\( ^{32} \text{P} \)] NAD (\( \approx 20 \mu \text{Ci/ml} \), 0.5 mM ATP, 0.5 mM GTP in 50 mM potassium phosphate, pH 7.5. Samples were incubated at 37 \( ^\circ \)C for 30 min. Trichloroacetic acid precipitation, electrophoresis, and autoradiography were performed as described in the legend to Fig. 5. A. \([^{32}P]\)ADP-ribosylation of human erythrocyte membranes: Lane 1, minus toxin, minus dithiothreitol; Lane 2, plus toxin, minus dithiothreitol; Lane 3, minus toxin, plus dithiothreitol; Lane 4, plus toxin, plus dithiothreitol. B. \([^{32}P]\)ADP-ribosylation of NG108-15 membranes: Lane 1, minus toxin, minus dithiothreitol; Lane 2, plus toxin, minus dithiothreitol; Lane 3, minus toxin, plus dithiothreitol; Lane 4, plus toxin, plus dithiothreitol. Arrows point to pertussis toxin substrates.

FIG. 6. Effect of dithiothreitol on the \([^{32}P]\)ADP-ribosylation of erythrocyte and NG108-15 membranes. Pertussis toxin (60 \( \mu \)g/ml) was incubated for 8 min at 37 \( ^\circ \)C in buffer containing 50 mM glycine, pH 8.0, 0.25 mg/ml of ovalbumin, with or without 20 mM dithiothreitol. 25 \( \mu l \) of one of the toxin mixtures or an identical mixture not containing toxin were then added to samples of erythrocyte membranes (50 \( \mu g \) of protein) or NG108-15 membranes (50 \( \mu g \) of protein) to give a final volume of 100 \( \mu l \) which also contained 20 \( \mu M \) [\( ^{32} \text{P} \)] NAD (\( \approx 20 \mu \text{Ci/ml} \), 20 mM thymidine, 0.5 mM ATP, 0.5 mM GTP in 50 mM potassium phosphate, pH 7.5. Samples were incubated at 37 \( ^\circ \)C for 30 min. Trichloroacetic acid precipitation, electrophoresis, and autoradiography were performed as described in the legend to Fig. 5. A. \([^{32}P]\)ADP-ribosylation of human erythrocyte membranes: Lane 1, minus toxin, minus dithiothreitol; Lane 2, plus toxin, minus dithiothreitol; Lane 3, minus toxin, plus dithiothreitol; Lane 4, plus toxin, plus dithiothreitol. B. \([^{32}P]\)ADP-ribosylation of NG108-15 membranes: Lane 1, minus toxin, minus dithiothreitol; Lane 2, plus toxin, minus dithiothreitol; Lane 3, minus toxin, plus dithiothreitol; Lane 4, plus toxin, plus dithiothreitol. Arrows point to pertussis toxin substrates.
not shown). NAD glycohydrolases are fairly ubiquitous; to establish that the thiol-dependent hydrolytic reaction noted in pertussis toxin preparations was catalyzed by the toxin, the preparation was dialyzed against 6.5 M urea in 0.1 M glycine, pH 3.2, and chromatographed on a Sephaeryl G-200 column. The ADP-ribosyltransferase activity of the toxin was monitored by the transfer of radioactivity from [32P]NAD to a 41,000-Da membrane protein in NG108-15 cells (Fig. 5A), and co-chromatographed with NAD glycohydrolase activity (Fig. 5B).

Although thiol was clearly necessary to demonstrate the NAD glycohydrolase activity of pertussis toxin, it did not appear to be necessary in all systems to demonstrate [32P] ADP-ribosylation of membrane proteins (Figs. 6, A and B). A dithiothreitol requirement was observed for erythrocyte membranes (Fig. 6A); it was not observed for membranes from NG108-15 cells, although the thiol enhanced [32P]ADP-ribosyltransferase (Fig. 6B).

**DISCUSSION**

The enzymatic activity of bacterial toxins dependent on ADP-ribosylation for their action (e.g. diphtheria toxin, *Pseudomonas exotoxin A*, cholera, *E. coli* heat-labile enterotoxin) appears to be latent; upon mild denaturation, proteolytic digestion, or reduction of the native protein, there is a large increase in enzymatic activity (15, 26–29).

It appears that activation of cholera by thiol results from reduction of a single disulfide bond linking two peptides in the A subunit (30). With pertussis toxin, there is no evidence that a similar critical bond linking two proteins exists (14). Pertussis toxin is an oligomeric protein; one subunit, termed S1, appears to possess ADP-ribosyltransferase activity (14). Conceivably, however, thiol may act to release the S1 subunit from the holotoxin complex or to cleave a disulfide in S1.

The *Km* for NAD with thiol-activated pertussis toxin was ~25 μM; this figure is considerably lower than that observed with cholera and *E. coli* heat-labile enterotoxin, where the *Km* values were ~4–8 mM (15, 27). It is similar, however, to the *Km* values obtained with diphtheria toxin and *Pseudomonas exotoxin A* (31, 32). Katada and Ui (33), investigating the ADP-ribosylation reaction catalyzed by pertussis toxin, report a low *Km* in the micromolar range; the apparent *Km* observed in the ADP-ribosylation reaction, however, may be dependent on the effect of the ADP-ribose acceptor G, on the toxin’s affinity for NAD. Thus, one might not expect a priori that the apparent *Km* values in the ADP-ribosyltransferase and NAD glycohydrolase reactions would be similar. In addition, the NAD concentration is affected by endogenous NAD glycohydrolase present in membrane preparations.

Although thiol was clearly necessary to demonstrate NAD glycohydrolase activity, a requirement for thiol was not demonstrated in studies by Katada and Ui (33). Indeed, the present studies demonstrate that thiol is necessary only in some membrane systems. These findings suggest that membrane contain a factor(s) capable of activating pertussis toxin. For cholera, it has been shown that disulfide exchange catalyzed by an oxidoreductase resulted in significant activation of the toxin at low thiol concentrations (34). Perhaps a similar factor may potentiate activation of pertussis toxin by endogenous thiols.

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**REFERENCES**

1. Ross, E. M., and Gilman, A. G. (1980) *Annu. Rev. Biochem.* 49, 533–564
2. Rodbell, M. (1980) *Nature (Lond.*) 284, 17–22
3. Cooper, D. M. F., and Londo, C. (1982) in *Horizons in Biochemistry and Biophysics* (Kohn, L. D., ed) Vol. 6, pp. 309–335, John Wiley and Sons, New York
4. Moss, J., and Vaughan, M. (1981) *Mol. Cell. Biochem.* 37, 75–90
5. Gill, D. M. (1977) in *Advances in Cyclic Nucleotide Research* (Greengard, P., and Robison, G. A., eds) Vol. 8, pp. 85–118, Raven Press, New York
6. Katada, T., and Ui, M. (1981) *J. Biol. Chem.* 256, 8310–8317
7. Katada, T., Amano, T., and Ui, M. (1982) *J. Biol. Chem.* 257, 3739–3746
8. Cassel, D., and Pfeuffer, T. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 75, 2669–2673
9. Gill, D. M., and Meren, R. (1978) *Proc. Natl. Acad. Sci. U. S. A.* 76, 2050–2054
10. Johnson, G. L., Kaslow, H. R., and Bourne, H. R. (1978) *J. Biol. Chem.* 253, 7120–7123
11. Cassel, D., and Seilinger, Z. (1977) *Proc. Natl. Acad. Sci. U. S. A.* 74, 3307–3311
12. Nakaya, S., Moss, J., and Vaughan, M. (1980) *Biochemistry* 19, 4871–4874
13. Katada, T., and Ui, M. (1982) *Proc. Natl. Acad. Sci. U. S. A.* 79, 3129–3133
14. Tamura, M., Nogimori, K., Murai, S., Yajima, M., Ito, K., Katada, T., Ui, M., and Ishii, S. (1982) *Biochemistry* 21, 5516–5522
15. Moss, J., Mangnelli, V. C., and Vaughan, M. (1976) *Proc. Natl. Acad. Sci. U. S. A.* 73, 4424–4427
16. Moss, J., and Vaughan, M. (1977) *J. Biol. Chem.* 252, 2455–2457
17. Yosh, D. A., and Moss, J. (1983) *J. Biol. Chem.* 258, 4926–4929
18. Burns, D. L., Hewlett, E. L., Moss, J., and Vaughan, M. (1983) *J. Biol. Chem.* 258, 1435–1438
19. Koski, G., and Klee, W. A. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 4185–4189
20. Watkins, P. A., Moss, J., and Vaughan, M. (1981) *J. Biol. Chem.* 256, 4985–4989
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
22. Finkelstein, R. A., Boesman, M., Neoh, S. H., LaRue, M. K., and Delaney, R. (1974) *J. Immunol.* 113, 145–150
23. Moss, J., Stanley, S. J., and Watkins, P. A. (1980) *J. Biol. Chem.* 255, 5838–5840
24. Krustensen, T., and Hoitlund, J. (1976) *Eur. J. Biochem.* 70, 441–446
25. Priess, J., Schlaeger, R., and Hitz, H. (1971) *FEBS Lett.* 19, 244–246
26. Peppenheimer, A. M., Jr. (1977) *Annu. Rev. Biochem.* 46, 69–94
27. Moss, J., and Richardson, S. H. (1978) *J. Clin. Invest.* 62, 281–285
28. Moss, J., Osborne, J. C., Jr., Fishman, P. H., Nakaya, S., and Robertson, D. C. (1981) *J. Biol. Chem.* 256, 12861–12865
29. Vasil, M. L., Kabat, D., and Iglewski, B. H. (1977) *Infect. Immun.* 16, 353–361
30. Mekalanos, J. J., Collier, R. J., and Romig, W. R. (1979) *J. Biol. Chem.* 254, 5855–5861
31. Chung, D. W., and Collier, R. J. (1977) *Biochim. Biophys. Acta* 483, 248–257
32. Chung, D. W., and Collier, R. J. (1977) *Infect. Immun.* 16, 832–841
33. Katada, T., and Ui, M. (1982) *J. Biol. Chem.* 257, 7210–7216
34. Moss, J., Stanley, S. J., Morin, J. E., and Dixon, J. E. (1980) *J. Biol. Chem.* 255, 11085–11087
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