Stimulation of the Thiol-Dependent ADP-Ribosyltransferase and NAD Glycohydrolase Activities of *Bordetella pertussis* Toxin by Adenine Nucleotides, Phospholipids, and Detergents†

Joel Moss,* Sally J. Stanley, and Paul A. Watkins‡

Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

Drusilla L. Burns and Charles R. Manclark

Division of Bacterial Products, National Center for Drugs and Biologics, Food and Drug Administration, Bethesda, Maryland 20892

Harvey R. Kaslow

Department of Physiology, University of Southern California, Los Angeles, California 90033

Erik L. Hewlett

Departments of Medicine and Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908

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ABSTRACT: Pertussis toxin catalyzed ADP-ribosylation of the guanyl nucleotide binding protein transducin was stimulated by adenine nucleotide and either phospholipids or detergents. To determine the sites of action of these agents, their effects were examined on the transducin-independent NAD glycohydrolase activity. Toxin-catalyzed NAD hydrolysis was increased synergistically by ATP and detergents or phospholipids; the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was more effective than the nonionic detergent Triton X-100 > lysophosphatidylcholine > phosphatidylcholine. The $A_{0.5}$ for ATP in the presence of CHAPS was 2.6 μM; significantly higher concentrations of ATP were required for maximal activation in the presence of cholate or lysophosphatidylcholine. In CHAPS, NAD hydrolysis was enhanced by ATP > ADP > AMP > adenosine; ATP was more effective than MgATP or the nonhydrolyzable analogue adenyl-5′-yl imidodiphosphate. GTP and guanyl-5′-yl imidodiphosphate were less active than the corresponding adenine nucleotides. Activity in the presence of CHAPS and ATP was almost completely dependent on dithiothreitol; the $A_{0.5}$ for dithiothreitol was significantly decreased by CHAPS alone and, to a greater extent, by CHAPS and ATP. To determine the site of action of ATP, CHAPS, and dithiothreitol, the enzymatic (S_1) and binding components (B oligomer) were resolved by chromatography. The purified S_1 subunit catalyzed the dithiothreitol-dependent hydrolysis of NAD; activity was enhanced by CHAPS but not ATP. The studies are consistent with the conclusion that adenine nucleotides, dithiothreitol, and CHAPS act on the toxin itself rather than on the substrate; adenine nucleotides appear to be involved in the activation of toxin but not the isolated catalytic unit.

Hormone-sensitive adenylate cyclase synthesizes cAMP from ATP in animal tissues (Rodbell, 1980; Cooper & Londos, 1982; Gilman, 1984; Lefkowitz et al., 1984). The membrane-associated complex consists, at a minimum, of inhibitory and stimulatory hormone receptors linked through different guanyl nucloetide binding regulatory proteins, termed G_i[1] and G_s for those involved in inhibition and stimulation, respectively, to a catalytic unit (Gilman, 1984; Lefkowitz et al., 1984). G_i and G_s are heterotrimers, composed of α, β, and γ subunits (Gilman, 1984; Hildebrandt et al., 1984). the β and γ subunits are identical in both regulatory proteins (Gilman, 1984; Hildebrandt et al., 1984); the α subunits of G_i and G_s bind guanine nucleotides and are believed to be in the active state following dissociation of $G_{\beta\gamma}$ and when the nucleotide site is occupied with GTP or a GTP analogue (Gilman, 1984; Lefkowitz et al., 1984; Northup et al., 1983a,b; Bokoch et al., 1984; Codina et al., 1984; Katada et al., 1984). Both $G_{i\alpha}$ and $G_{s\alpha}$ have intrinsic GTPase activity; hydrolysis of bound GTP to GDP results in inactivation (Cassel & Selinger, 1976; Koski & Klee, 1981; Brandt et al., 1983; Cerione et al., 1984; Kanaho et al., 1984; Sunyer et al., 1984).

At least three bacterial toxins exert their effects on animal cells through the adenylate cyclase system (Moss et al., 1984). The toxins disrupt control of the cyclase by catalyzing the transfer of ADP-ribose from NAD to the α subunit of the guanyl nucleotide binding proteins (Gilman, 1984; Moss et al., 1984; Cassel & Pfeuffer, 1978; Gill & Meren, 1978; Johnson et al., 1978; Northup et al., 1980; Katada & Ui, 1982a,b; Bokoch et al., 1983; Codina et al., 1983). Choleragen

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* Address correspondence to this author.

‡ Present address: The Kennedy Institute for Handicapped Children, Baltimore, MD 21205.

[1] Abbreviations: App(NH)p, adenyl-5′-yl imidodiphosphate; Gpp(NH)p, guanyl-5′-yl imidodiphosphate; G_i, inhibitory guanine nucleotide binding protein of adenylate cyclase; G_s, stimulatory guanine nucleotide binding protein of adenylate cyclase; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; SDS, sodium dodecyl sulfate; T_α, transducin α subunit; $T_{\beta\gamma}$, transducin $\beta\gamma$ subunits; Tris, tris(hydroxymethyl)aminomethane.

(cholera toxin) and *Escherichia coli* heat-labile toxin, agents involved, in part, in the pathogenesis of cholera and "traveler's diarrhea", respectively, activate cyclase by ADP-ribosylating G_s (Gilman, 1984; Moss et al., 1984). In membrane systems containing stimulatory receptors and catalytic subunit, ADP-ribosylated G_s exhibits increased sensitivity to GTP due apparently to a decreased GTPase activity (Cassel & Selinger, 1977); basal GTPase activity was not, however, affected by ADP-ribosylation (Kahn & Gilman, 1984). *Bordetella pertussis* toxin ("islet-activating protein"), an etiologic agent in pertussis or whooping cough, catalyzes the ADP-ribosylation of $G_{i\alpha}$ (Katada & Ui, 1982a,b; Bokoch et al., 1983; Codina et al., 1983). Modification of $G_{i\alpha}$ results in the "uncoupling" of G_i from inhibitory hormone receptors (Kurose et al., 1983; Hsia et al., 1984a); the effect of toxin-catalyzed ADP-ribosylation of G_i is a loss of inhibitory agonist action, permitting unopposed activity of the stimulatory arm of the cyclase system (Katada & Ui, 1982a,b; Moreno et al., 1983; Hsia et al., 1984b).

Pertussis toxin, an oligomeric protein consisting of an enzymatically active subunit (S_1) linked to a binding oligomer (S_2S_4)(S_5)(S_3S_4) (Tamura et al., 1982), in addition to ADP-ribosylating G_i also catalyzes the hydrolysis of NAD (Moss et al., 1983; Katada et al., 1983); NAD glycohydrolase activity is dependent on the reduction of intrachain disulfide bonds in S_1 (Katada et al., 1983). Studies of the ADP-ribosylation of $G_{i\alpha}$ and transducin, a related protein, revealed in some systems in addition to a thiol dependency a stimulation by phospholipid and/or ATP (Bokoch et al., 1984; Katada & Ui, 1982b; Tsai et al., 1984); since NAD hydrolysis was stimulated by ATP, the effect of the nucleotide appeared to be directly on the toxin (Lim et al., 1985). To determine the loci of action of these effectors, we investigated the requirements for toxin-catalyzed ADP-ribosyltransferase and NAD glycohydrolase activities with both the holotoxin and its S_1 subunit.

Materials and Methods

Materials. ATP, ADP, AMP, adenosine, GTP, thymidine, cholate, phosphatidylinositol, lysolecithin, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and NAD were purchased from Sigma Chemical Co. (St. Louis, MO); App(NH)p and Gpp(NH)p were from Boehringer Mannheim (Indianapolis, IN); $MgCl_2$ and potassium phosphate were from Fisher (Springfield, NJ); Triton X-100 was from Research Product International (Elk Grove Village, IL); [*carbonyl*-^{14}C]NAD (52 mCi/mmol) was from Amersham (Arlington Heights, IL); [^{32}P]NAD (1 mCi/0.039 μmol) was from New England Nuclear (Boston, MA); CHAPS was from Calbiochem-Behring (San Diego, CA). CM-Sepharose (C-L-6B) was from Pharmacia (Piscataway, NJ).

Assays. Unless otherwise noted, NAD glycohydrolase activity was assayed in a total volume of 0.1 mL containing 50 mM potassium phosphate (pH 7.5), 22.4 μM [*carbonyl*-^{14}C]NAD (40 000 cpm), and other additions as indicated. Reaction was initiated with pertussis toxin (~1 μg). After 3 h at 30 °C, two 30-μL samples were transferred to columns (0.5 × 4 cm) of AG 1-X2 that had been equilibrated with water. Columns were washed 4 times with 1.2 mL of water to separate [*carbonyl*-^{14}C]nicotinamide, released during the reaction, from [*carbonyl*-^{14}C]NAD (Moss et al., 1976; Moss & Vaughan, 1977). All data are means of values from duplicate assays in representative experiments, each of which was replicated at least twice.

[^{32}P]ADP-ribosylation reactions were carried out for 30 min at 30 °C in a total volume of 0.1 mL. Mixtures contained 50 mM potassium phosphate (pH 7.5), 20 mM thymidine, 20 μM [α-^{32}P]NAD (1–2 μCi/assay), activated pertussis toxin (1 μg/assay), and other additions as indicated. After termination of the reaction with cold 10% trichloroacetic acid, precipitated proteins were subjected to electrophoresis on 10% polyacrylamide gels containing 0.1% SDS (Laemmli, 1970; Burns et al., 1983). Dried gels exposed Kodak X-Omat XAR film for 4–24 h. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

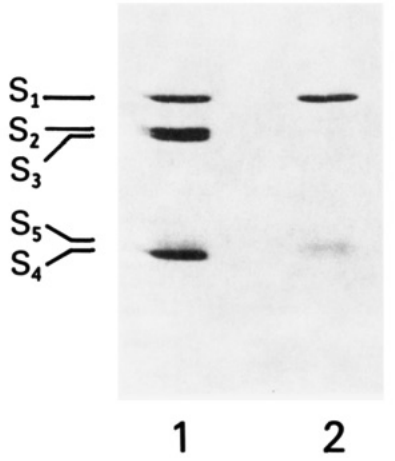

FIGURE 1: Electrophoresis of pertussis toxin and subunits. Pertussis toxin, 20 μg (lane 1), and a sample containing S_1 and S_5 subunits, 6 μg (lane 2), were reduced with 25 mM dithiothreitol in the presence of 1% SDS and subjected to electrophoresis on 15% polyacrylamide gels containing SDS as described under Materials and Methods.

Isolation and Activation of Pertussis Toxin. Pertussis toxin, purified as described by Burns et al. (1983), except for the use of fetuin–Sepharose in place of haptoglobin–Sepharose, was activated by incubation in 50 mM glycine (pH 8.0) containing ovalbumin, 1 mg/mL, with 20 mM dithiothreitol and other indications as indicated for 10 min at 30 °C.

Preparation of Pertussis Toxin Subunits. The subunits of pertussis toxin were isolated by two procedures. Preparation I was similar to that followed by Tamura et al. (1982). Purified pertussis toxin was dialyzed against 5 M urea in 10 mM potassium phosphate, pH 8.3, for 48 h at 4 °C and then applied to a fetuin–Sepharose affinity column that had been equilibrated with the same buffer. The nonadherent fraction containing the S_1 subunit was collected; the adherent protein containing the B oligomer was eluted with 0.1 M Tris, pH 10–0.5 M NaCl. Each fraction was concentrated on an Amicon YM-10 membrane and dialyzed against 2 M urea in 100 mM potassium phosphate, pH 7–0.5 M NaCl for 18 h at 4 °C.

In the second preparation, pertussis toxin (0.3 mg) was dialyzed against 4 M urea in 10 mM sodium phosphate, pH 7.0, for 18 h at 4 °C. An equal volume of 10 mM sodium phosphate, pH 7.0, was added, and this solution (500 μL) was immediately added to a microcentrifuge tube that contained 160 μL of packed CM Sepharose that had been washed with 10 mM sodium phosphate, pH 7.0, containing 2 M urea. After centrifugation at 12800*g* for 1 min, the supernatant that contained the S_1 and S_5 subunits was collected. The purity of the subunits is given in Figure 1.

Results

Pertussis toxin catalyzed ADP-ribosylation of transducin α subunit was enhanced by ATP and phosphatidylcholine vesicles, lysophosphatidylcholine, and ionic and nonionic detergents (Figure 2). With T_α or $T_\alpha T_{\beta\gamma}$ as substrate, lysophosphatidylcholine, phosphatidylcholine, and cholate were clearly more effective than CHAPS (Figure 2). With $T_\alpha T_{\beta\gamma}$

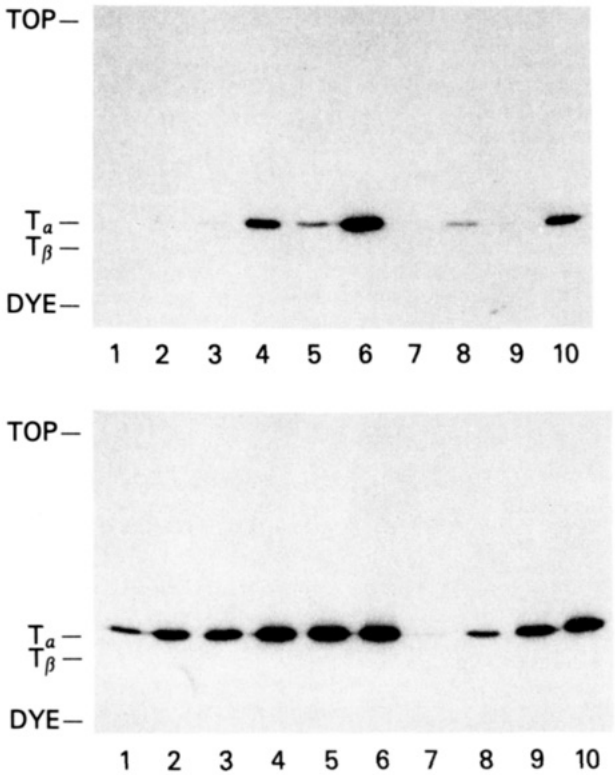

FIGURE 2: Effect of ATP, phospholipids, and detergents on the [^{32}P]ADP-ribosylation of transducin by pertussis toxin. T_α (2.5 μg) (upper panel) or $T_\alpha T_{\beta\gamma}$ (3 μg) (lower panel) was incubated for 30 min at 30 °C in 50 mM potassium phosphate, pH 7.5, 20 mM thymidine, 20 μM [α-^{32}P]NAD, and activated pertussis toxin (1 μg) with (lanes 2, 4, 6, 8 and 10) or without (lanes 1, 3, 5, 7, and 9) 1 mM ATP and other additions as indicated (final volume = 0.1 mL). Final concentrations were as follows: (lanes 3 and 4) phosphatidylcholine (PC), 0.15 mg/mL; (lanes 5 and 6) lysophosphatidylcholine (lyso-PC), 0.1 mg/mL; (lanes 7 and 8) CHAPS, 1.0%; (lanes 9 and 10) cholate, 0.1%. Electrophoresis and autoradiography were carried out as described under Materials and Methods. Markers at left indicate the relative positions of T_α and T_β.

Table I: Effect of Phospholipids and Detergents on NAD Glycohydrolase Activity of Pertussis Toxin[a]

prepn	additions	NAD glycohydrolase act. (pmol·min^{-1}) no ATP	100 μM ATP
I	none	0	0.018
	phosphatidylcholine (30 μg/mL)	0.012	0.130
	phosphatidylserine (30 μg/mL)	0	0.100
	phosphatidylethanolamine (30 μg/mL)	0	0.180
	phosphatidylinositol (30 μg/mL)	0	0.094
	lysophosphatidylcholine (30 μg/mL)	0	0.284
II	none	0	0.083
	CHAPS (1%)	0.250	5.70
	Triton X-100 (1%)	0.041	1.4
	lysophosphatidylcholine (30 μg/mL)	0.026	0.43

[a] Pertussis toxin (129 μg/mL) was incubated in 50 mM glycine, pH 8.0, 50 mM potassium phosphate, 20 mM dithiothreitol, and ovalbumin (1 mg/mL) for 10 min at 30 °C. Assays containing in addition 50 mM potassium phosphate, 20 mM thymidine with or without 100 μM ATP, and the indicated concentrations of phospholipids or detergent were initiated with 0.02 mL of toxin. Two 0.03-mL samples were transferred to columns of AG 1-X2 columns to isolate [*carbonyl*-^{14}C]-nicotinamide. Data are values from duplicate assays.

as substrate, the lysolecithin-enhanced ADP-ribosylation exhibited a smaller ATP effect (Figure 2). Prior studies demonstrated increased ADP-ribosylation of G_i by toxin in the presence of ATP. To determine whether ATP, phospholipids,

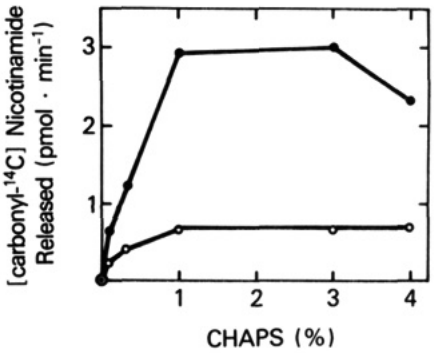

FIGURE 3: Effect of CHAPS and ATP on the NAD glycohydrolase activity of pertussis toxin. Pertussis toxin (110 μg) was incubated in 67 mM glycine hydrochloride, pH 8.0, 87 mM dithiothreitol, 1.25 mg/mL ovalbumin, 333 mM NaCl, and 66.7 mM potassium phosphate in a total volume of 1.2 mL for 10 min at 30 °C. Assays containing 20 mM thymidine without (○) or with 1 mM ATP (●) and the indicated concentration of CHAPS were initiated with 0.02 mL of the toxin solution.

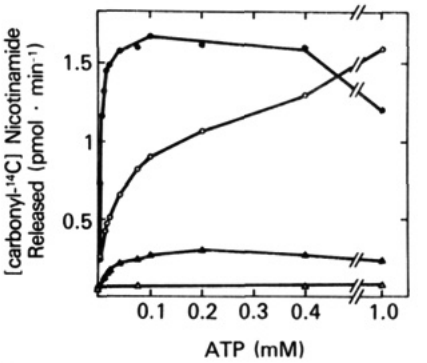

FIGURE 4: Effect of ATP on lysolecithin- and detergent-stimulated NAD glycohydrolase activity. Pertussis toxin, 51 μg/mL (mix A) or 154 μg/mL (mix B), in 50 mM glycine hydrochloride, pH 8.0, containing 50 mM potassium phosphate, 250 mM NaCl, 1 mg/mL ovalbumin, and 20 mM dithiothreitol was incubated for 10 min at 30 ° C. Assays containing 1% CHAPS (●), 0.1 mg/mL lysolecithin (○), 0.1% cholate (▲), or no additions (△) and the indicated concentration of ATP were initiated with 0.2 mL of mix A (●) or mix B (△, ▲, ○).

and detergents act through the toxin or the ADP-ribose acceptor, their effects on the G_i- and transducin-independent NAD glycohydrolase activity of toxin were examined. NAD hydrolysis by thiol-activated toxin was stimulated synergistically by ATP and either phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, or lysophosphatidylcholine (Table I); lysophosphatidylcholine was effective at levels above the critical micellar concentration. The zwitterionic detergent CHAPS and the nonionic detergent Triton X-100 also stimulated glycohydrolase activity synergistically with ATP (Table I). CHAPS was clearly more effective in enhancing ATP-stimulated NAD glycohydrolase than were the phospholipids (Table I); these data contrast with the findings noted in Figure 2 on ADP-ribosylation of transducin. In the presence of ATP, CHAPS exhibited optimal activity at concentrations above the critical micelle concentration (Figure 3); synergistic effects of lysolecithin and cholate with ATP differed from those of CHAPS (Figure 4). The concentrations of ATP at which significant activation was observed varied with the phospholipid detergent (Figure 4). In CHAPS, the $A_{0.5}$ for ATP was 2.6 μM (Figure 5). In lysophosphatidylcholine, a Lineweaver–Burk plot was curvilinear. In the presence of maximally effective concentrations of CHAPS and ATP, a constant reaction rate for the NAD hydrolysis activity was not observed for ~10 min (Figure 6).

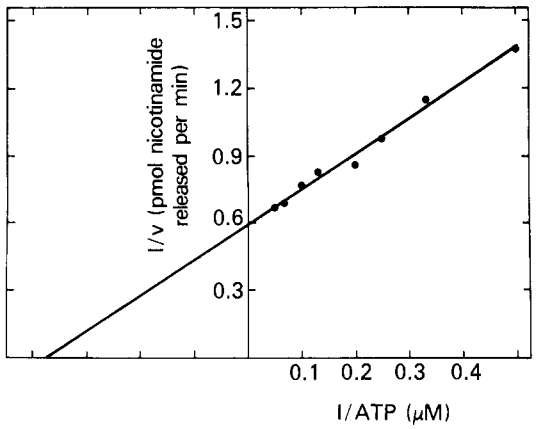

FIGURE 5: Effect of ATP on the NAD glycohydrolase activity of pertussis toxin. Data are from assays with CHAPS shown in Figure 3.

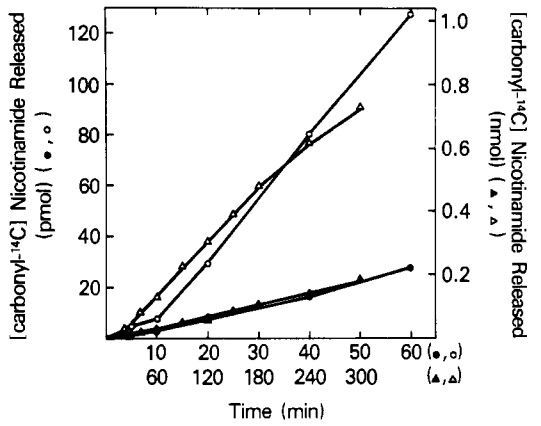

FIGURE 6: Time course of NAD hydrolysis in the presence of CHAPS and ATP. Pertussis toxin (50 μg/mL) in 100 mM glycine (pH 8.0) containing 36.3 mM potassium phosphate, 181 mM NaCl, 20 mM dithiothreitol, and 1 mg/mL ovalbumin was incubated for 10 min at 30 °C. Assays (total volume 0.9 mL) containing 57.3 mM potassium phosphate (pH 7.5), 1% CHAPS, 22.4 μM [*carbonyl*-^{14}C]-NAD (~10^6 cpm), 20 mM glycine, 36.2 mM NaCl, 4 mM dithiothreitol, and 0.2 mg/mL ovalbumin without (●, ▲) or with 100 μM ATP (○, △) were initiated with 0.18 mL of toxin solution. At the indicated times, two 0.03-mL samples were run over AG 1-X2 columns to isolate [*carbony*-^{14}C]nicotinamide. Data are means of values from duplicate assays.

Table II: Effect of Adenine and Guanine Nucleotides and Mg^{2+} on NAD Glycohydrolase Activity of Pertussis Toxin[a]

additions	NAD glycohydrolase act. (pmol·min^{-1})	additions	NAD glycohydrolase act. (pmol·min^{-1})
none	0.64	adenosine (1 mM)	0.59
ATP (0.1 mM)	2.87	GTP (0.1 mM)	1.33
ATP (1 mM)	2.90	GTP (1 mM)	2.29
App(NH)p (0.1 mM)	1.51	Gpp(NH)p (1 mM)	0.56
App(NH)p (1 mM)	2.23	$MgCl_2$ (5 mM)	0.59
ADP (1 mM)	2.06	$MgCl_2$ (5 mM) plus ATP (1 mM)	0.88
AMP (1 mM)	0.56		

[a] Assays containing 20 mM thymidine with or without 1% CHAPS and the indicated additions were initiated for 3 h at 30 °C with 0.02 mL of toxin activated as described in Figure 2. Data from assays with CHAPS are reported. No activity was observed without CHAPS.

ATP was considerably more effective in CHAPS than were the nonhydrolyzable derivatives App(NH)p, ADP, AMP, or adenosine (Table II); both ATP and App(NH)p were more active than the corresponding guanosine derivatives (Table II).

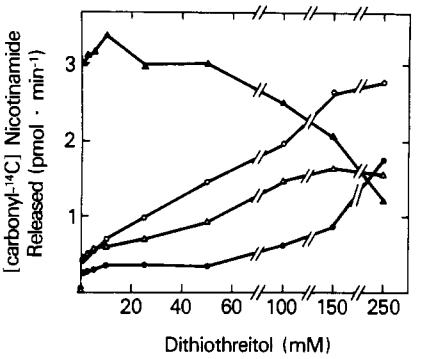

FIGURE 7: Effect of dithiothreitol, ATP, and CHAPS on the NAD glycohydrolase activity of pertussis toxin. Assays containing 60 mM potassium phosphate (pH 7.5), 50 mM NaCl, and the indicated concentration of dithiothreitol, with 3 mg/mL ovalbumin (●), ovalbumin plus 100 μM ATP (○), 1% CHAPS (△), or CHAPS plus ATP (▲), were initiated with 3.1 μg (●, ○) or 0.46 μg (△, ▲) of toxin (not activated). The activity in the absence of dithiothreitol was 0.0 (●), 0.036 (○), 0.030 (△), and 0.022 (▲) pmol·min^{-1}.

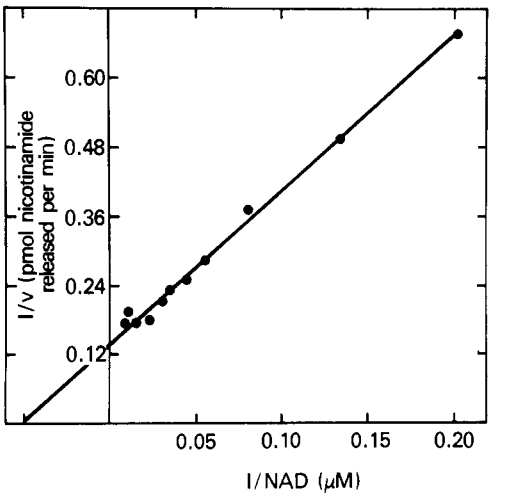

FIGURE 8: Effect of NAD on the glycohydrolase activity of pertussis toxin. Pertussis toxin (50 μg/mL) was incubated in 50 mM glycine, pH 8.0, containing 36.5 mM potassium phosphate, 183 mM NaCl, 1 mg/mL ovalbumin, and 20 mM dithiothreitol for 10 min at 30 °C (final volume 2.0 mL). Reaction was initiated with 20 μL in an assay mix containing 50 mM potassium phosphate, pH 7.5, 1% CHAPS, 1 mM ATP, and the indicated concentration of [*carbonyl*-^{14}C]NAD (59 600 cpm).

Table III: Activation of NAD Glycohydrolase Activity of S_1 Subunit by CHAPS and ATP[a]

additions	[*carbonyl*-^{14}C]nicotinamide release (pmol·min^{-1})
none	0.32
CHAPS (1%)	2.21
ATP (100 μM)	0.27
CHAPS plus ATP	2.30

[a] Assays containing 1 mg/mL ovalbumin, 1 mM dithiothreitol, 0.2 M urea, and the indicated concentrations of CHAPS and ATP were initiated with toxin S_1S_5 subunit (0.08 μg) (preparation II) not previously activated. Similar results were obtained with preparation I.

Mg^{2+} had little effect on the glycohydrolase activity and inhibited the effect of ATP (Table II). The CHAPS- and ATP-dependent activation of toxin was dependent on dithiothreitol (Figure 7); the concentration of thiol at which maximal activity was observed was reduced from ~150 to <5 mM by the inclusion of CHAPS and ATP in the assay mix (Figure 7); synergistic effects of CHAPS and ATP were observed at suboptimal concentrations of dithiothreitol. The K_m for NAD

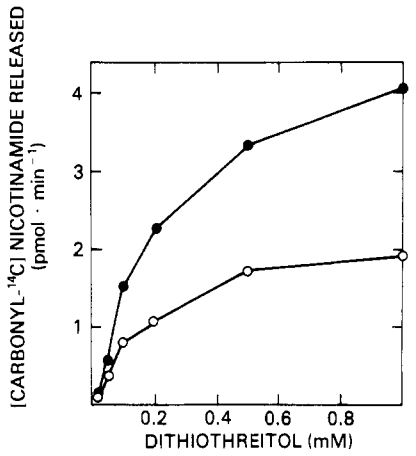

FIGURE 9: Effect of dithiothreitol on the NAD glycohydrolase activity of pertussis toxin and its S_1 protomer. Assays containing 1 mg/mL ovalbumin, 100 μM ATP, 1% CHAPS, 0.2 M urea, and the indicated concentration of dithiothreitol were initiated with toxin (2.2 μg) (○) or S_1 (0.08 μg) (●) (preparation II) not previously activated. After 3 h at 30 °C, two 0.04-mL samples were run over AG 1-X2 columns to isolate [*carbonyl*-^{14}C]nicotinamide. Similar results were obtained with preparation I.

with CHAPS- and ATP-stimulated toxin ($K_m \simeq 20$ μM) was similar to that obtained with toxin activated in the presence of high concentrations of thiol (Moss et al., 1983) (Figure 8).

To identify the sites of action for CHAPS and ATP, the enzymatically active S_1 component of toxin containing S_5 was separated from the S_2, S_3, and S_4 subunits. The activity of S_1 was increased by CHAPS but not by ATP (Table III); as expected, the B oligomer was inactive (data not shown). The CHAPS-stimulated S_1 activity was dependent on dithiothreitol (Figure 9). The $A_{0.5}$ values for dithiothreitol with S_1 or holotoxin were similar (Figure 9).

Discussion

This paper demonstrates that pertussis toxin catalyzed ADP-ribosylation of transducin was enhanced synergistically by adenine nucleotides, phospholipids, and detergents; as noted previously, thiol was necessary for the demonstration of activity (Moss et al., 1983; Katada et al., 1983). Initial reports by Ui and co-workers on ADP-ribosylation of G_i in membranes prepared from C6 cells demonstrated a requirement for ATP (Katada & Ui, 1982a). ADP-ribosylation of purified G_i incubated with receptor incorporated into phospholipid vesicles was stimulated by adenine nucleotide (Tsai et al., 1984). Since Lim et al. (1985) demonstrated that ATP stimulated NAD hydrolysis, it appeared that the nucleotide interacted directly with the toxin. This paper, showing that ATP and detergents directly affect the catalytic activity of the holotoxin, would explain why modification of a guanine nucleotide binding protein was enhanced by adenine nucleotides. Since ATP is not absolutely required with S_1, and may contaminate membrane and NAD preparations, it is evident why in some systems exogenous ATP was not necessary to observe toxin-catalyzed ADP-ribosylation of G_i (Bokoch et al., 1984).

Prior studies had noted a dithiothreitol requirement for expression of the NAD glycohydrolase and ADP-ribosyltransferase activities of pertussis toxin (Moss et al., 1983; Katada et al., 1983). Maximal activation was only achieved with holotoxin at high dithiothreitol concentrations of >100 mM. The concentration of dithiothreitol necessary for activation was reduced by disruption of toxin structure with detergents, phospholipids, or urea or by use of the purified S_1 subunit (Katada et al., 1983). The fact that the $A_{0.5}$ for thiol is extremely sensitive to ATP in the presence of agents that presumably promote disruption of toxin, such as CHAPS, might explain in part why ADP-ribosylation of G_i in extensively washed membrane preparations from NG108-15 cells was stimulated by, but not dependent on, the presence of dithiothreitol (Moss et al., 1983). Conceivably, the presence of adenine nucleotides or their generation from the degradation of NAD would potentiate the action of endogenous thiol. Similarly, it appears that membrane phospholipids act synergistically with ATP to promote activation of toxin.

In order to determine the sites of action of CHAPS and ATP, the enzymatically active S_1 subunit of toxin was resolved from the surface-binding components by chromatography. CHAPS stimulated the NAD glycohydrolase activity of S_1, suggesting that CHAPS is directly involved in activation of S_1. In contrast, ATP activates only the holotoxin, perhaps by releasing S_1 from the B oligomer. The activity of the S_1 is ATP-independent. Collier and co-workers have shown that diphtheria toxin has a tightly bound nucleotide, ApUp (Lory & Collier, 1980; Barbieri et al., 1981; Collier et al., 1982). Conceivably, in the case of pertussis toxin, ATP may be mimicking a similar nucleotide invovled in the stimulation of enzymatic activity. Currently, studies are directed at identification of that nucleotide.

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Registry No. ADP, 58-64-0; AMP, 61-19-8; ATP, 56-65-5; CHAPS, 75621-03-3; GTP, 86-01-1; ADP-ribosyltransferase, 58319-92-9; NAD glycohydrolase, 9032-65-9; Mg, 7439-95-4; Triton X-100, 9002-93-1; cholate, 81-25-4; adenosine, 58-61-7; adenyl-5′-yl imidodiphosphate, 25612-73-1; guanyl-5′-yl imidodiphosphate, 34273-04-6; dithiothreitol, 3483-12-3.

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Role of Tropomyosin in Smooth Muscle Contraction: Effect of Tropomyosin Binding to Actin on Actin Activation of Myosin ATPase†

Hidetake Miyata and Samuel Chacko*
Department of Pathobiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104
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ABSTRACT: The binding of gizzard tropomyosin to gizzard F-actin is highly dependent on free Mg^{2+} concentration. At 2 mM free Mg^{2+}, a concentration at which actin-activated ATPase activity was shown to be Ca^{2+} sensitive, a molar ratio of 1:3 (tropomyosin:actin monomer) is required to saturate the F-actin with tropomyosin to the stoichiometric ratio of 1 mol of tropomyosin to 7 mol of actin monomer. Increasing the Mg^{2+} could decrease the amount of tropomyosin required for saturating the F-actin filament to the stoichiometric level. Analysis of the binding of smooth muscle tropomyosin to smooth muscle actin by the use of Scatchard plots indicates that the binding exhibits strong positive cooperativity at all Mg^{2+} concentrations. Calcium has no effect on the binding of tropomyosin to actin, irrespective of the free Mg^{2+} concentration. However, maximal activation of the smooth muscle actomyosin ATPase in low free Mg^{2+} requires the presence of Ca^{2+} and stoichiometric binding of tropomyosin to actin. The lack of effect of Ca^{2+} on the binding of tropomyosin to actin shows that the activation of actomyosin ATPase by Ca^{2+} in the presence of tropomyosin is not due to a calcium-mediated binding of tropomyosin to actin.

Contraction in striated muscle is regulated by the interaction of Ca^{2+} and troponin–tropomyosin complex which lies along actin filaments (Ebashi & Endo, 1968). While calcium sensitivity of the actomyosin system in striated muscle resides in the complete complement of the troponin–tropomyosin complex, tropomyosin alone can either inhibit (Eaton et al., 1975) or potentiate (Bremel et al., 1972) the actin-activated adenosine-5′-triphosphatase (ATPase)[1] activity depending on the conditions of the assays.

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[1] Abbreviations: EGTA, ethylene glycol bis(aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; DTT, dithiothreitol; EDTA, (ethylenedinitrilo)tetraacetic acid; ATP, adenosine 5′-triphosphate; ATPase, adenosine-5′-triphosphatase; P_i, inorganic phosphate.

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