Structure–Activity Analysis of the Activation of Pertussis Toxin†

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ABSTRACT: *Bordetella pertussis*, the causative agent of whooping cough, releases pertussis toxin in an inactive form. The toxin consists of an A protomer containing one S1 peptide subunit and a B oligomer containing several other peptide subunits. The toxin binds to cells via the B oligomer, and the S1 subunit is activated and expresses ADP-ribosyltransferase and NAD glycohydrolase activities. Treatment of purified toxin with dithiothreitol (DTT) in vitro increases both activities. ATP and the detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) synergistically reduce the $A_{0.5}$ (activation constant) for DTT from >100 mM to 200 μM. We studied the structure–activity relationships of activators of the toxin. In the presence of CHAPS (1%) and DTT (10 mM) the following compounds increased the NAD glycohydrolase activity of the toxin with the following $A_{0.5}$'s in μM and fraction of the ATP effect in parentheses: ATP, 0.2 (1.0); ADP, 6 (0.8); UTP, 15 (0.7); GTP, 35 (0.6); pyrophosphate, 45 (0.7); triphosphate, 60 (0.6); tetraphosphate, ≥170 (≥0.4). Thus, the polyphosphate moiety is sufficient to stimulate the toxin, and the adenosine moiety confers upon ATP its extraordinary affinity for the toxin. Phospholipid and detergents could substitute for CHAPS in the activation of the toxin. Glutathione substituted for DTT with an $A_{0.5}$ of 2 mM, a concentration within the range found in eucaryotic cells. Thus, membrane lipids and cellular concentrations of glutathione and ATP are sufficient to activate pertussis toxin without the need for a eucaryotic enzymatic process.

The bacterium *Bordetella pertussis* causes the disease "whooping cough". The bacterium releases several toxins involved in the symptoms and pathogenesis of the disease. One of these toxins, termed pertussis toxin, is particularly important for two reasons. First, vaccination with this toxin can confer considerable protection against infection by *B. pertussis*. Second, the toxin is proving to be a powerful tool in investigations of regulation of transmembrane signaling of animal cells [for a review see Weiss and Hewlett (1986)].

Pertussis toxin is organized as an A protomer, containing one S1 subunit, and a B oligomer, containing one S2, one S3, two S4, and one S5 subunits (Tamura et al., 1982; Sekura et al., 1983). The A protomer catalyzes the transfer of ADP-ribose from NAD either to acceptor proteins, i.e., ADP-ribosyltransferase activity, or to water, i.e., NAD glycohydrolase (NADase)[1] activity (Katada et al., 1983; Moss et al., 1983). The ADP-ribosylation of specific guanine nucleotide binding proteins produces intoxication (Katada & Ui, 1982a,b). The B oligomer binds the toxin to target cells.

B. pertussis releases pertussis toxin in a stable but inactive form (Tamura et al., 1982; Katada et al., 1983; Moss et al., 1983). This observation prompts this question: What mechanism activates the toxin in vivo? Recent studies have shed light on how activation may take place. In early studies of the pure toxin, expression of NADase activity was achieved by treating the toxin or the purified S1 subunit with high concentrations of DTT, in some cases for several hours (Katada et al., 1983; Moss et al., 1983). Subsequently we found that, in the presence of DTT, ATP promotes both the NADase and ADP-ribosyltransferase activities by interacting directly with the toxin (Lim et al., 1985). The activation did not appear to require hydrolysis of the ATP (Lim et al., 1985). Subsequently, Moss et al. (1986) established that the detergent CHAPS, in the presence of ATP, lowers the $A_{0.5}$ for DTT from >100 mM to 200 μM and accelerates the activation of the toxin so that it occurs within minutes. Moss et al. (1986) also established that CHAPS similarly activated the isolated S1 subunit, in both the absence and presence of ATP. Recently, Burns and Manclark (1986) demonstrated that the combination of ATP and CHAPS causes the release of the S1 subunit from the B oligomer.

In this paper, we describe structure–activity relationships of activators of the toxin. These relationships indicate that membrane lipids and cellular concentrations of glutathione and ATP are sufficient to activate pertussis toxin without the need for a eucaryotic enzymatic process.

Experimental Procedures

Materials. Pertussis toxin was from List Biological Laboratories, Campbell, CA. A volume of 500 μL of water was added to a vial containing 50 μg of toxin, and the toxin was used without further treatment unless otherwise stated. According to the manufacturer, this addition suspends the toxin in 10 mM sodium phosphate, pH 7, and 50 mM NaCl. DTT

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[1] Abbreviations: NADase, NAD glycohydrolase; DTT, dithiothreitol; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CHAPSO, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate; Zwittergent-*n*, alkyl sulfobetaines where *n* represents the number of carbons in the alkyl group; $C_{12}E_8$, octaethylene glycol dodecyl ether; BSA, bovine serum albumin; LPC, lysophosphatidylcholine (palmitoyl); Triton X-100 and NP-40, trade names for poly(oxyethylene) *p-tert*-octylphenols; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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was from Boehringer Mannheim. NP-40 was from Almega. CHAPS, CHAPSO, Zwittergents, and $C_{12}E_8$ were from Calbiochem. Triton X-100, sodium dodecyl sulfate, and electrophoresis reagents were from Bio-Rad. Sodium thiophosphate was from Alfa. [*nicotinamide*-4-^{3}H]NAD was from Amersham; [*adenylate*-^{32}P]NAD was from New England Nuclear. X-ray film was from Kodak. Other reagents were from Sigma.

NADase Assay. The assay involves the hydrolysis of NAD labeled in the nicotinamide moiety and ion-exchange column chromatography to resolve product from substrate. Unless otherwise noted, the assay was initiated by adding 0.5–2.5 μg toxin to a reaction mixture for a final volume of 100 μL. Final concentrations in the 100 μL were 25 μM [^{3}H]NAD (25 000–75 000 cpm/tube), 50 mM Tris-HCl, pH 8.0, 1% w/w CHAPS, 10 mM DTT, and 50 mM NaCl. Unless otherwise noted, incubations were for 90 min at 37 °C. The NADase reaction was terminated by adding 0.85 mL of ice-cold water. The entire 0.95 mL was then pipetted onto a QAE-Sephadex column (chloride form) and the [^{3}H]nicotinamide eluted with 3 mL of H_2O directly into a scintillation vial and counted. NADase activity was linear with time up to 3 h at 37 °C if ≤10% of the NAD was hydrolyzed. One unit of NADase activity is defined as 1 pmol of NAD hydrolyzed/h.

ADP-ribosyltransferase Assay. The transfer of ^{32}P from [^{32}P]NAD to acceptor proteins was detected by incubating proteins with pertussis toxin under the same conditions as the NADase assay, except that CHAPS was usually omitted (see Results and Discussion). The 37 °C incubation was terminated after 60 min by adding 10 μL of 0.15% w/v sodium deoxycholate; the contents of the tube were mixed and the tubes transferred to an ice bath. After 10 min, 10 μL of ice-cold 72% w/v trichloroacetic acid was added, the contents of the tube were mixed, 900 μL of water was added, and the precipitate was collected by centrifugation (1800*g*, 25 min). The supernatant was discarded, and the pellet was resuspended in a buffer containing sodium dodecyl sulfate and β-mercaptoethanol, heated for 1 min at 95 °C, and electrophoresed by using 10% polyacrylamide gels as described (Laemmli, 1970). The gels were dried and used with enhancing screens to expose X-ray film.

Results and Discussion

At least three classes of compounds can synergistically combine to activate purified pertussis toxin. In this paper we will use the following nomenclature to refer to sites binding these substances: *A site* (for *adenosine site*) and *P site* (for *polyphosphate site*) for the two subsites of the *ATP site*; *L site* (for *lipid site*) for sites binding activators with hydrophobic character; *S site* (for *sulfhydryl site*) for sites that lead to activation when reduced by DTT.

ATP-Site Structure–Activity Relationships. In our original report we found that adenine nucleotides, but not other nucleotides, stimulated the ADP-ribosyltransferase activity of pertussis toxin (Lim et al., 1985). Subsequently it was found that GTP could stimulate the NADase activity of the toxin, albeit not to the same extent as ATP (Moss et al., 1986). Apart from the different reactions monitored (ADP-ribosyltransferase vs. NADase), a key difference distinguishes these two studies: in the first detergents were absent; in the second CHAPS was present. These results prompted us to screen other compounds in the presence of CHAPS, using the NADase assay (see Table I). Under the conditions of this experiment, every triphosphonucleotide and inorganic polyphosphate examined strongly stimulated the toxin, as did ADP. Adenosine, inorganic phosphate, glucose 6-phosphate, and amino acid monophosphates were ineffective. This study clearly demonstrates that only a polyphosphate moiety, and not a nucleoside moiety, is required for stimulation of pertussis toxin.

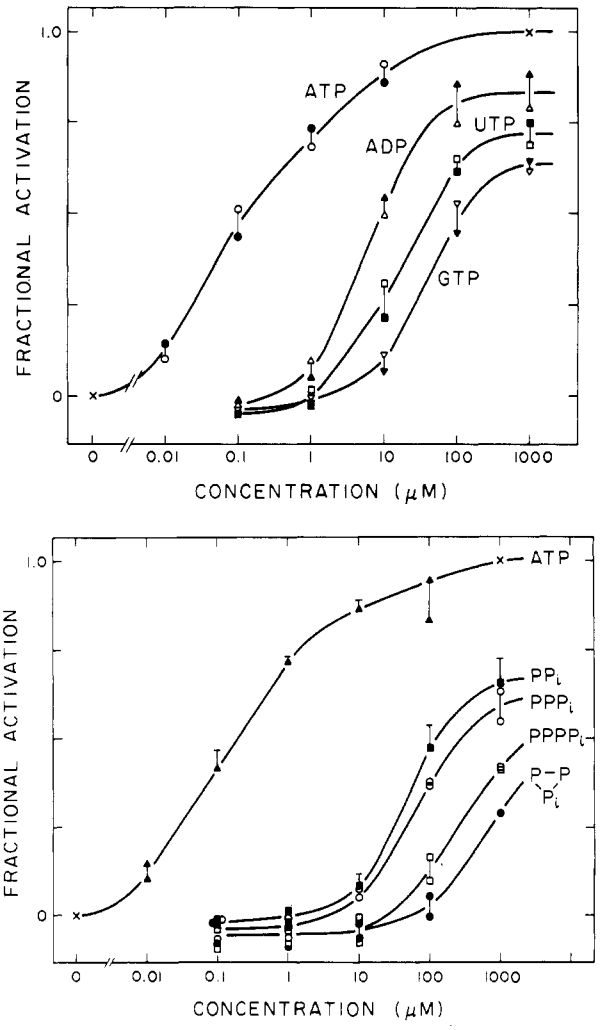

FIGURE 1: Activation of pertussis toxin NADase activity by nucleotides and polyphosphates. Pertussis toxin (1 μg/assay tube) was assayed for NADase activity in the presence of various activators at several concentrations. For each activator, at least two experiments were performed, with at least duplicates at each point. For activator concentrations covered by two experiments, each symbol represents the average of a duplicate from one experiment. For concentrations covered by more than two experiments, the average is shown with its standard error bar. Fractional activation is defined as (activity − activity in the absence of activator)/(activity in the presence of 1 mM ATP − activity in the absence of activator). The NADase activities (units/μg of toxin) for the four experiments, without ATP-site activator and with 1 mM ATP, were 71 and 250, 56 and 234, 40 and 192, and 37 and 164, respectively. In the top panel, the open symbols represent data from one experiment, and the closed symbols represent data from another. In the bottom panel, the compounds listed from top to bottom are ATP, pyrophosphate, triphosphate, tetraphosphate, and trimetaphosphate.

We next determined the $A_{0.5}$'s for several of these substances in the presence of CHAPS, using the NADase assay (Figure 1). Of all the tested compounds, ATP showed by far the lowest $A_{0.5}$, approximately 200 nM. The $A_{0.5}$'s of the other substances were 30–1000 times greater. Examination of the data shown in Figure 1 indicates the following $A_{0.5}$'s in μM and fraction of the ATP effect in parentheses: ATP, 0.2 (1.0); ADP, 6 (0.8); UTP, 15 (0.7); GTP, 35 (0.6); pyrophosphate, 45 (0.7); triphosphate, 60 (0.6); tetraphosphate, ≥170 (≥0.4). It is interesting to note that the $A_{0.5}$'s of GTP and triphosphate are similar—suggesting that, under these assay conditions, the

Table I: Activation of Pertussis Toxin by Compounds Containing Phosphate[a]

compd	fractional activation	compd	fractional activation
GTP	0.87 ± 0.01	trimetaphosphate	0.69 ± 0.22
CTP	1.03 ± 0.09	tetraphosphate	0.92 ± 0.12
UTP	0.92 ± 0.03	thiophosphate	0.12 ± 0.06
ITP	0.85 ± 0.01	pyridoxal-P	0.53 ± 0.06
ADP	1.07 ± 0.06	glucose-6-P	−0.03 ± 0.02
AMP	0.26 ● 0.09	glycerol-2-P	−0.01 ± 0.03
Ado	0.02 ± 0.02	serine-P	−0.04 ± 0.02
phosphate	0.01 ± 0.01	threonine-P	0.01 ● 0.01
pyrophosphate	0.85 ± 0.11	tyrosine-P	0.02 ± 0.02
triphosphate	0.98 ± 0.14		

[a] Various compounds at 1 mM were screened for the ability to stimulate the NADase activity of the toxin. Before assay the toxin (1 μg/assay tube) was incubated at 37 °C for 10 min with 20 mM DTT and 2% CHAPS. Assays were for 3 h at 37 °C. Fractional activation is defined as (activity − activity in the absence of activator)/(activity in the presence of 1 mM ATP − activity in the absence of activator). The value given is the average ± the range for two separate experiments; duplicates were measured in each experiments. The NADase activity in units/μg of toxin in the absence of any ATP-site activator and in the presence of 1 mM ATP, respectively, was 44 and 111 for the first experiment and 18 and 97 for the second experiments. "P" denotes phosphate.

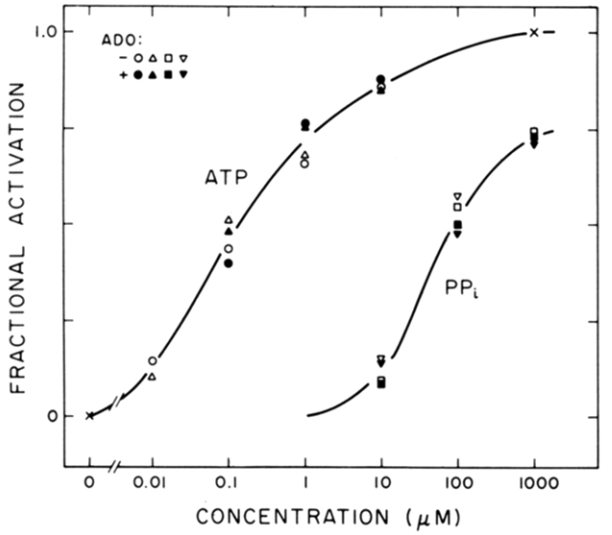

FIGURE 2: Effect of adenosine (Ado) on pertussis toxin NADase activity. Pertussis toxin NADase activity was assayed as described in Figure 1. Two identical experiments were performed, with at least duplicate tubes at each concentration of activator. Each symbol represents the average of the duplicates from one experiment, and different symbols are used to represent data from the first (○, ●, □, ■) and second (▽, ▼, △, ▲) experiments. The NADase activities (units/μg of toxin), without ATP-site activator and with 1 mM ATP, respectively, were 40 and 192 for the first experiment and 37 and 164 for the second experiment.

guanosine portion of GTP does little to either aid or interfere with binding or activation. These results further suggest that the adenosine portion of ATP is crucial for its low $A_{0.5}$.

With this conclusion in mind, we next sought to determine if adenosine interacted with the toxin, even though it was not an activator. Adenosine, at 1 mM, failed to significantly alter the dose–response relationship of either ATP or pyrophosphate (Figure 2). Thus, although this nucleoside is important in contributing to the high affinity of ATP for the toxin, there is no evidence to suggest that alone it interacts with binding sites that stimulate the toxin.

We next compared these NADase results to results obtained from the ADP-ribosyltransferase assay (Figure 3). In the NADase assays described above (Table I) CHAPS is present;

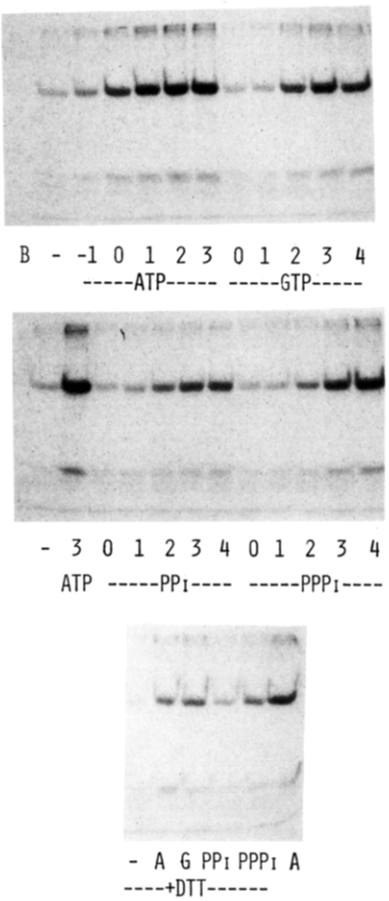

FIGURE 3: Activation of pertussis toxin ADP-ribosyltransferase activity by nucleotides and polyphosphates. Pertussis toxin ADP-ribosyltransferase activity was assayed by using BSA as an acceptor protein in the presence of various concentrations of ATP-site activators; CHAPS was omitted. For lanes 1–5 in the bottom panel, pertussis toxin was treated with 20 mM DTT at 37 °C for 30 min prior to the addition of [^{32}P]NAD (4.6 μCi) and BSA (5 μg). In the other lanes the toxin was used without prior treatment. The numbers refer the log of the concentration of the activator in μM, and "−" means no activator was added. All tubes contained 0.5 μg of pertussis toxin except for the one labeled B: no toxin was present in this sample. PP_i refers to pyrophosphate and PPP_i to triphosphate. In the bottom panel activators were present at 1 mM, and A refers to ATP and G to GTP. The main band in the middle of the panels is ADP-ribosylated BSA. The faint bands at the top are other proteins contained in the BSA preparation. The bands near the bottom of the panels are due to ADP-ribose incorporated into pertussis toxin itself.

in its absence NADase activity is so reduced that it is difficult to assay. In contrast, in the ADP-ribosyltransferase assay we found that CHAPS obliterated the ability of the toxin to ADP-ribosylate BSA (data not shown). This result is similar to the earlier observation that CHAPS reduces the ADP-ribosylation of transducin by pertussis toxin (Moss et al., 1986). Whether this effect reflects an interaction of the detergent with the toxin or the protein substrate is unknown. In the absence of CHAPS, we found that triphosphates stimulated ADP-ribosyltransferase activity in a fashion similar to their stimulation of NADase activity except that the $A_{0.5}$'s were increased 2–10-fold. The results shown in Figure 3 are typical of several experiments. Visual inspection of this type of data indicated that ATP stimulated the ADP-ribosyltransferase activity with an $A_{0.5}$ of approximately 1 μM; GTP, pyrophosphate, and inorganic triphosphate all had $A_{0.5}$'s of approximately 100 μM. All of the triphosphates appeared to have the same maximal effectiveness. In contrast, pyrophosphate was only weakly effective.[2] This result is similar

to our previous finding that, in the absence of CHAPS, ADP is a much weaker stimulator of ADP-ribosyltransferase activity than ATP (Lim et al., 1985). Taken together, these results suggest that CHAPS decreases the $A_{0.5}$ for these compounds and increases the effectiveness of diphosphates such as ADP and pyrophosphate in the activation process. The results also indicate that the concentration of ATP is sufficient to activate pertussis toxin in vivo.

We also considered the possibility that the interaction of ATP with pertussis toxin might resemble its interaction with diphtheria toxin. In the case of diphtheria toxin, inorganic polyphosphates also interact with the toxin, leading to a nomenclature that refers to the site interacting with the phosphate portion of ATP as the P site (Proia et al., 1980; Lory et al., 1980). However, in contrast to pertussis toxin, ATP inhibits diphtheria toxin. In particular, a dinucleotide, adenylyl(3′–5′)uridine 3′-monophosphate, inhibits diphtheria toxin with extraordinary affinity (K_i = 2 nM) (Collins & Collier, 1984). We found that this nucleotide, at 1 mM, had no effect on pertussis toxin NADase activity in the presence of CHAPS, either in the presence of ATP or in the absence of ATP (data not shown). Thus, although these two toxins share some characteristics, these data indicate that ATP binds to and regulates them in two distinct ways.

L-Site Structure–Activity Relationships. Moss et al. (1986) found that CHAPS, a zwitterion analogue of cholic acid, activates pertussis toxin. They also found that Triton X-100, another detergent, and a variety of phospholipids also activate the toxin, although to a lesser extent than CHAPS. We sought to better define the structural characteristics of lipophilic substances that activate the toxin. First, we considered the possibility that phospholipids might exert their effect not only by hydrophobic interactions analogous to those of CHAPS but also by interacting with the P site that mediates ATP activation. This possibility was raised by the observation that in the presence of CHAPS some monophosphates (e.g., AMP, thiophosphate, and pyridoxal phosphate) activated the toxin, albeit weakly (Table I). We examined the ability of palmitoyllysophosphatidylcholine (LPC), at a concentration well above its critical micellar concentration, to substitute for ATP in an NADase assay containing CHAPS. Unlike ATP, LPC failed to activate the toxin in the presence of CHAPS. However, in the absence of CHAPS, LPC did act synergistically with ATP to activate the toxin (data not shown). These results indicate that phospholipids can interact with L sites and promote activation but do not productively interact with P sites.

We next determined the ability of other detergents to activate the toxin (Table II). The detergents studied fall into four classes: cholic acid analogues (Hjelmeland et al., 1983), alkyl sulfobetaines (Gonenne & Ernst, 1978), and poly(ethylene glycol) monionic and alkyl anionic detergents (Helenius & Simons, 1975). Of all the detergents studied, the cholic acid analogues CHAPS [as originally described by Moss et al. (1986)] and CHAPSO allowed for the greatest expression of NADase activity. Cholic acid itself supported half the activity seen with CHAPS and did not inhibit the ability of CHAPS to activate the toxin. In contrast, deoxycholic acid inhibited activity in the presence or absence of CHAPS. Poly(ethylene glycol) nonionic surfactants also stimulated the toxin half as well as CHAPS and did not inhibit CHAPS stimulation of the toxin. Of this type of surfactant studied, two (Triton X-100 and NP-40) are *p*-octylphenol derivatives and the other ($C_{12}E_8$) is a dodecyl alkyl derivative. Despite this substantial difference in the hydrophobic portion of the molecule, the detergents behaved in similar fashion. Other alkyl detergents, such as the alkyl sulfobetaines and anionic detergents, inhibited the activity of the toxin to varying extents. Thus the possibility is raised that it is the poly(ethylene glycol) portion of the nonionic detergents that activates pertussis toxin. These results (Table II) and those of Moss et al. (1986) show that a variety of lipophilic substances can activate the toxin in vitro and suggest that no specific lipid is involved in vivo.

Table II: Effect of Detergents on the NADase Activity of Pertussis Toxin[a]

detergent	fractional activity without CHAPS	fractional activity with CHAPS
cholic acid analogues		
none	0.19	1
CHAPSO	1.05	1.04
cholic acid	0.43	1.02
deoxycholic acid	0.05	0.29
poly(ethylene glycol) nonionic detergents		
NP-40	0.54	0.97
Triton X-100	0.58	1.05
$C_{12}E_8$	0.59	1.03
alkyl sulfobetaines		
Zwittergent-8	0.15	0.41
Zwittergent-10	0.05	0.18
Zwittergent-12	0.02	0.39
Zwittergent-14	0.03	0.68
alkyl anionic detergents		
sodium dodecyl sulfate	0.02	0.03
sodium lauroylsarcosinate	0.01	0.01

[a] Activity was measured in the presence of various detergents at a 1% (w/w) concentration in either the absence or presence of CHAPS (1% w/w). In all assay tubes, 1 mM ATP was present. Fractional activity is defined as activity divided by activity in the presence of 1% CHAPS. The value given is the average for two separate experiments; in each experiment duplicates were measured for each condition. The results of the two experiments differed by less than 4% of the activity in the presence of CHAPS alone, which in the two experiments was 153 and 158 units/μg.

S-Site Structure–Activity Relationships. DTT effectively activates pertussis toxin in vitro, but this question arises: What is the mechanism in intact cells? Two possibilities are likely. Either the amount and character of intracellular reduced substances are sufficient (in the presence of ATP and lipid) to reduce the disulfide bonds in pertussis toxin or an enzymatic process is involved. An enzymatic mechanism has been suggested for either disulfide reduction or proteolytic cleavage—either of which can activate the toxin (Katada et al., 1983). As shown in Figure 4, we examined the ability of glutathione to substitute for DTT in the in vitro activation of the purified toxin: In the presence of ATP and CHAPS, glutathione activated pertussis toxin with an $A_{0.5}$ of 2 mM, a concentration within the range found in eucaryotic cells (Meister & Anderson, 1983). Thus, cellular ATP and membrane lipids could allow intracellular glutathione to activate pertussis toxin in vivo, without the need for an enzymatic process.

[2] The relative ineffectiveness of pyrophosphate is best seen in the bottom panel of Figure 3. In the upper two panels it is evident that some ADP-ribose is incorporated into BSA in the absence of any added activator. In the bottom panel, it is evident that the treatment of pertussis toxin with DTT decreased incorporation of ADP-ribose in both the presence and absence of activators. Under these latter conditions, it is clear that pyrophosphate stimulates the toxin to a lesser extent than do the triphosphates. Although DTT is required for activity, these results question the desirability of treating pertussis toxin with DTT prior to ADP-ribosylation reactions.

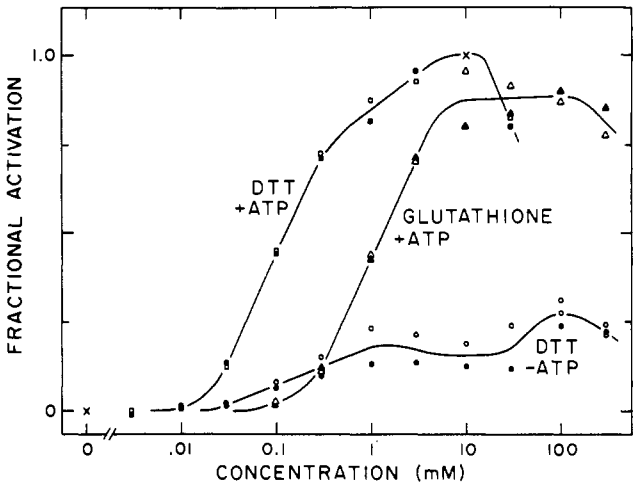

FIGURE 4: Activation of pertussis toxin NADase activity by sulfhydryl reducing reagents. Pertussis toxin NADase activity was assayed in the presence of various sulfhydryl reducing reagents at several concentrations. The assay buffer contained 1.0% CHAPS and 1 mM ATP. The data from two experiments are shown. Fractional activation is defined as [activity – activity in the absence of reducing reagent (which was essentially 0)]/[activity in the presence of 10 mM DTT – activity in the absence of reducing reagent]. The NADase activities (units/μg of toxin) for the two experiments, at 10 mM DTT without and with 1 mM ATP, respectively, were 18 and 147 for the first experiment and 36 and 195 for the second experiment. Examination of the ability of cysteine to activate pertussis toxin yielded essentially the same curve as did glutathione. For clarity, the cysteine results are not shown.

ACKNOWLEDGMENTS

We thank Eleanor Leung for technical assistance.

Registry No. ATP, 56-65-5; ADP, 58-64-0; UTP, 63-39-8; GTP, 86-01-1; CTP, 65-47-4; ITP, 132-06-9; AMP, 61-19-8; CHAPSO, 82473-24-3; NP-40, 39454-98-3; NAD-glycohydrolase, 9032-65-9; pyrophosphate, 14000-31-8; triphosphate, 14127-68-5; tetraphosphate, 16132-64-2; glutathione, 70-18-8; adenosine, 58-61-7; phosphate, 14265-44-2; trimetaphosphate, 15705-55-2; pyridoxal-P, 54-47-7; glucose-6-P, 56-73-5; glycerol-2-P, 17181-54-3; serine-P, 407-41-0; threonine-P, 1114-81-4; tyrosine-P, 21820-51-9; cholic acid, 81-25-4; deoxycholic acid, 83-44-3; Triton X-100, 9002-93-1; octaethylene glycol dodecyl ether, 3055-98-9; Zwittergent-8, 15178-76-4; Zwittergent-10, 15163-36-7; Zwittergent-12, 14933-08-5; Zwittergent-14, 14933-09-6; sodium dodecyl sulfate, 151-21-3; sodium lauroylsarcosinate, 137-16-6.

REFERENCES

Burns, D. L., & Manclark, C. R. (1986) *J. Biol. Chem. 261*, 4324–4327.

Collins, C. M., & Collier, R. J. (1984) *J. Biol. Chem. 259*, 15159–15162.

Gonenne, A., & Ernst, R. (1978) *Anal. Biochem. 87*, 28–38.

Helenius, A., & Simons, K. (1975) *Biochim. Biophys. Acta 415*, 29–79.

Hjelmeland, L. M., Nebert, D. W., & Osborne, J. C. (1983) *Anal. Biochem. 130*, 72–82.

Katada, T., & Ui, M. (1982a) *J. Biol. Chem. 257*, 7210–7216.

Katada, T., & Ui, M. (1982b) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3129–3133.

Katada, T., Tamura, M. L., & Ui, M. (1983) *Arch. Biochem. Biophys. 224*, 290–298.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lim, L.-K., Sekura, R. D., & Kaslow, H. R. (1985) *J. Biol. Chem. 260*, 2585–2588.

Lory, S., Carroll, S. F., & Collier, R. J. (1980) *J. Biol. Chem. 255*, 12016–12019.

Meister, A., & Anderson, M. E. (1983) *Annu. Rev. Biochem. 52*, 711–760.

Moss, J., Stanley, S. J., Burns, D. L., Hsia, J. A., Yost, D. A., Myers, G. A., & Hewlett, E. L. (1983) *J. Biol. Chem. 258*, 11879–11882.

Moss, J., Stanley, S. J., Watkins, P. A., Burns, D. L., Manclark, C. R., Kaslow, H. R., & Hewlett, E. L. (1986) *Biochemistry 25*, 2720–2725.

Proia, R. L., Wray, S. K., Hart, D. A., & Eidels, L. (1980) *J. Biol. Chem. 255*, 12025–12033.

Sekura, R. D., Fish, F., Manclark, C. R., Meade, B., & Zhang, Y.-L. (1983) *J. Biol. Chem. 258*, 14647–14651.

Tamura, M., Nogimori, K., Murai, S., Yajima, M., Ito, K., Katada, T., Ui, M., & Ishii, S. (1982) *Biochemistry 21*, 5516–5522.

Weiss, A. A., & Hewlett, E. L. (1986) *Annu. Rev. Microbiol. 40*, 661–686.