Proc. Nat. Acad. Sci. USA
Vol. 72, No. 6, pp. 2284–2288, June 1975

NAD-Dependent Inhibition of Protein Synthesis by *Pseudomonas aeruginosa* Toxin

(inactivation of mammalian elongation factor 2/*Pseudomonas aeruginosa* exotoxin/ ADP-ribosyl transferases)

BARBARA H. IGLEWSKI AND DAVID KABAT

Departments of Microbiology and Biochemistry, University of Oregon Medical School, Portland, Oregon 97201

Communicated by Ray D. Owen, March 24, 1975

ABSTRACT **_Pseudomonas aeruginosa_ toxin (PA toxin) inhibits protein synthesis in a reticulocyte cell-free system. The inhibition requires NAD and results in a block at an elongation step of polypeptide assembly. PA toxin was found to act like diphtheria toxin fragment A. Both toxins catalyze the transfer of radioactivity from nicotinamide [*U*-^{14}C]adenine dinucleotide ([^{14}C]NAD) into covalent linkage with the 100,000 dalton elongation factor 2 (EF-2) protein. Furthermore, in the presence of a limiting amount of EF-2, excess toxin, and [^{14}C]NAD, the two toxins were non-additive in the amount of label transferred to EF-2. Unlike free fragment A of diphtheria toxin, the enzymatic activity of PA toxin is heat labile and neutralizable with antibody to PA toxin but not with antibody to fragment A. Although PA and diphtheria toxins have different cellular specificities and molecular properties and produce different clinical symptoms, their intracellular mechanisms of action appear to be identical.**

Pseudomonas aeruginosa produces a variety of extracellular substances (1–11). Although the role of each of these substances in the pathogenesis of pseudomonas infections in humans is poorly understood, the most likely virulence factor is the trypsin-sensitive, heat-labile protein exotoxin (PA toxin) originally described by Liu (3). PA toxin in very low concentrations produces hypotensive shock in dogs (6) and monkeys (12) and is lethal for mice (4). Furthermore, passive immunization with antibody produced against PA toxin prevents the lethal effects of infection with live cultures of *Pseudomonas aeruginosa* (13).

Previous data suggest that PA toxin inhibits RNA and protein synthesis in cultured mammalian cells (14) and protein synthesis in the liver, kidney, and spleen of intoxicated mice (15). Protein synthesis in cultured 3T3 mouse cells is inhibited by 2 hr incubation with as little as 0.02 μg/ml of PA toxin, whereas at this concentration RNA synthesis is not inhibited until 4–5 hr incubation (L. P. Elwell and B. H. Iglewski, unpublished observations). However, the sequence of events by which PA toxin inhibits protein synthesis has not been established and it is not known whether the inhibition is a primary or secondary effect of PA toxin action.

This report describes the NAD-dependent inhibitory effect of PA toxin on protein synthesis in a rabbit reticulocyte cell-free lysate and identifies an enzymatic activity of PA toxin which appears to be similar to the NAD-dependent ADP-ribosyl transferase activity which has been established for the A-fragment of diphtheria toxin (16–18). In both cases elongation factor 2 (EF-2) is covalently modified and thereby inactivated. However, the two toxins are dissimilar in cellular specificities, molecular and immunological properties, and in the diseases for which they are apparently responsible.

Abbreviations: PA toxin, *Pseudomonas aeruginosa* toxin; EF-2, elongation factor 2.

METHODS AND MATERIALS

Toxin and Antisera. A nonproteolytic strain of *Pseudomonas aeruginosa* (PA-103) kindly provided by P. V. Liu (19) was used throughout this study. PA toxin purified by precipitations with zinc acetate and $(NH_4)_2SO_4$ and chromatographed on columns of DEAE-cellulose and Sephadex G-200 (20) was obtained through the courtesy of L. P. Elwell (Dept. of Microbiology, Univ. of Washington, Seattle, Wash.). The PA toxin used had a mouse median lethal dose (LD_{50}) of 0.3 μg/20 g of Swiss Webster mouse and contained approximately 0.01 ng of endotoxin per μg of protein. Pony antiserum to PA toxin was kindly provided by P. V. Liu (13). Diphtheria toxin fragment A was obtained through the courtesy of R. J. Collier (17). Rabbit antiserum to fragment A was kindly provided by L. P. Elwell.

Cell-Free Protein Synthesis in a Lysate from Rabbit Reticulocytes. The methods used for preparing the rabbit reticulocyte lysate and for the incorporation of radioactive amino acids into hemoglobulin are described elsewhere (21, 22). Each lysate was stored frozen in aliquots in liquid N_2. We employed 0.4 ml of the lysate per ml of cell-free incorporation mixture. The mixture, which also contained an optimum concentration of hemin and 5 μCi/ml of L-[^{14}C]leucine (250 Ci/mol, New England Nuclear Corp.) was incubated at 29°. Aliquots (10 μl) were removed at intervals into 1 ml of H_2O containing 50 μg of unlabeled carrier bovine serum albumin at 0°. Proteins were then precipitated by adding 5% trichloroacetic acid and nucleic acids were hydrolyzed by incubating at 80° for 15 min. After chilling at 0° for 30 min, the protein precipitates were filtered on 0.45 μm Millipore membranes and were assayed for radioactivity in a Nuclear Chicago low-background gas-flow counter. In some experiments 0.2 ml portions of cell-free incorporation mixtures were analyzed by sucrose gradient sedimentation. In that case the samples were quickly chilled by dilution into 1.5 ml of 0.13 M NaCl, 5 mM KCl, 1.5 mM $MgCl_2$ that contained 0.1 mg/ml of cycloheximide at 0°. Cycloheximide was used in order to quickly block any further protein synthesis. The chilled samples were then layered onto 38 ml of 10–25% sucrose gradients in 0.25 M KCl, 0.01 M $MgCl_2$, 0.01 M Tris·HCl (pH 7.4) and were sedimented for 3 hr at 27,000 rpm in the SW27 Spinco rotor.

In these conditions the different sizes of polysomes out to pentasomes were well resolved from each other and the single ribosomes and subribosomal particles also separated. Following sedimentotion, the gradients were pumped through a Gilford spectrophotometer and the A_{260nm} was recorded. Fractions from the gradient were precipitated with 5% trichloroacetic acid and assayed for radioactivity as described above.

Polyacrylamide Gel Electrophoresis. Proteins were electrophoresed in 8 cm long × 0.6 cm diameter, 8% polyacrylamide gels in a buffer containing 0.6% sodium dodecyl sulfate as described in detail elsewhere (23). Following electrophoresis, gels containing radioactive protein were sectioned longitudinally into four slices (23, 24). The two internal slices were dried onto paper backing and exposed to x-ray film for autoradiography.

Enzymatic Activity. Aminoacyl-transferase-containing enzymes were prepared from crude extracts of rabbit reticulocyte as described by Allen and Schweet (25) and modified by Collier and Kandel (17). Nicotinamide adenine dinucleotide (NAD) transferase activity was measured according to the procedure of Collier and Kandel (17). The assay mixture in a total volume of 65 μl contained 50 mM Tris·HCl, pH 8.2, 0.1 mM EDTA, 40 mM dithiothreitol (Sigma), 25 μl of reticulocyte enzymes, 0.735 μM nicotinamide [*U*-^{14}C]adenine dinucleotide ([^{14}C]NAD) (136 Ci/mol, Amersham/Searle) and various amounts of diphtheria toxin fragment A, PA toxin, or toxin-antibody mixtures. After 1 hr incubation at 25°, 65 μl of 10% trichloroacetic acid was added and the precipitates were collected, washed as described previously (26), and analyzed in a low-background counter as described above.

Peptide Analysis. EF-2 preparations radioactively labeled by the toxins and [^{14}C]NAD were precipitated with 5% trichloroacetic acid, digested with TPCK trypsin (Worthington), and analyzed by CEL-300 thin-layer chromatography as described by Crawford and Gesteland (27). The solvents were *n*-butanol/acetic acid/water/pyridine (150:30:120:120) or *n*-butanol/acetic acid/5% ammonium hydroxide (55:30:15). Radioactive peptides were located on the thin-layers by autoradiography.

RESULTS

The effect of PA toxin on cell-free protein synthesis was studied in a lysate from rabbit reticulocytes. Fig. 1 shows that full inhibition by PA toxin occurs only in the presence of NAD. NAD alone had no effect, whereas PA toxin alone in the undialyzed lysate was slightly inhibitory. However, protein synthesis was completely blocked when both NAD and PA toxin were added. It is known that undialyzed reticulocyte lysates contain a small amount of NAD (28) and we, therefore, conclude that the apparent inhibition by PA toxin alone was very likely dependent upon the endogenous NAD. In fact, in some other lysates toxin alone caused no inhibition.

Protein synthesis in reticulocytes lysates is very active and occurs on polysomes (21). The ribosomes cycle repeatedly on mRNA and in optimally active lysates each ribosome is able to synthesize as many as 25 globins during a 60 min period of incubation (21). However, usually the system is less active. There is a rapid burst of [^{14}C]leucine incorporation into globin chains whose synthesis was initiated *in vivo*

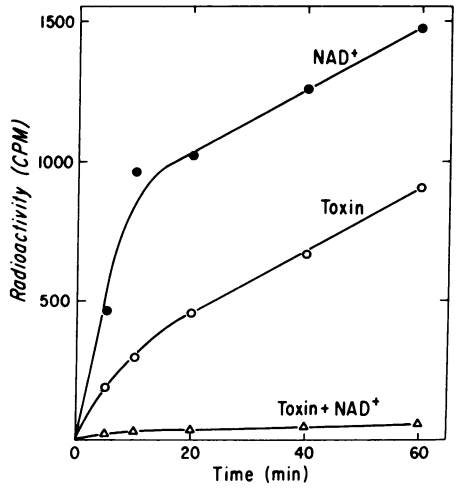

FIG. 1. Effect of *Pseudomonas aeruginosa* toxin and NAD on [^{14}C]leucine incorporation into protein in a reticulocyte lysate. Aliquots (0.2 ml) of the cell-free system were incubated either in the presence or absence of NAD (8 μg) and of PA toxin (3.6 μg). Aliquots (10 μl) were sampled at intervals.

before the lysate was made. During this early phase the rate of incorporation reflects the elongation of globin chains and their release from ribosomes. The slower secondary phase of incorporation (10–60 min) is rate-limited by initiation of new globin chains. Accordingly, polysomes are usually smaller during this secondary phase. As can be seen in Fig. 1, PA toxin plus NAD blocks the early phase of [^{14}C]leucine incorporation in the cell-free system. This suggests that elongation of chains is inhibited, because we would otherwise expect a burst of incorporation to precede the inhibition. This conclusion is also suggested by the data with PA toxin alone (Fig. 1), in which there is a partial inhibition of incorporation. The inhibition is more marked during the early phase when elongation is rate-limiting (67% inhibition at 5 min) than at later times when initiation is rate-limiting (only 21% inhibition at 60 min).

The conclusion that elongation of growing polypeptide chains is blocked by PA toxin plus NAD was substantiated by a study of [^{14}C]leucine incorporation into polysomes in the cell-free lysates. As seen in Fig. 2a, the radioactive leucine was extensively incorporated into polysomal nascent polypeptides during a 4 min incubation in the absence of toxin. However, no radioactivity was incorporated into polysomes in lysates that had been incubated in the presence of toxin (Fig. 2b). Fig. 2c shows a control experiment which demonstrates that PA toxin does not simply cause nascent chain release from polysomes. The lysates in that case were incubated for 4 min without toxin and then for an additional 4 min in the presence of toxin. Clearly, the radioactive chains that had been synthesized in the absence of toxin remained on the polysomes when toxin was subsequently added. As also seen in Fig. 2, polysomes remain intact when incubated with toxin, which shows that mRNA is not degraded.

Fragment A of diphtheria toxin inhibits protein synthesis elongation by catalyzing transfer of the ADP-ribosyl moiety of NAD onto EF-2 (16–18). ADP-ribosylated EF-2 is unable to translocate ribosomes along mRNA or to shift peptidyl-tRNA from acceptor to donor sites on eukaryotic ribosomes (16). Thereby, diphtheria toxin blocks the growth of nascent

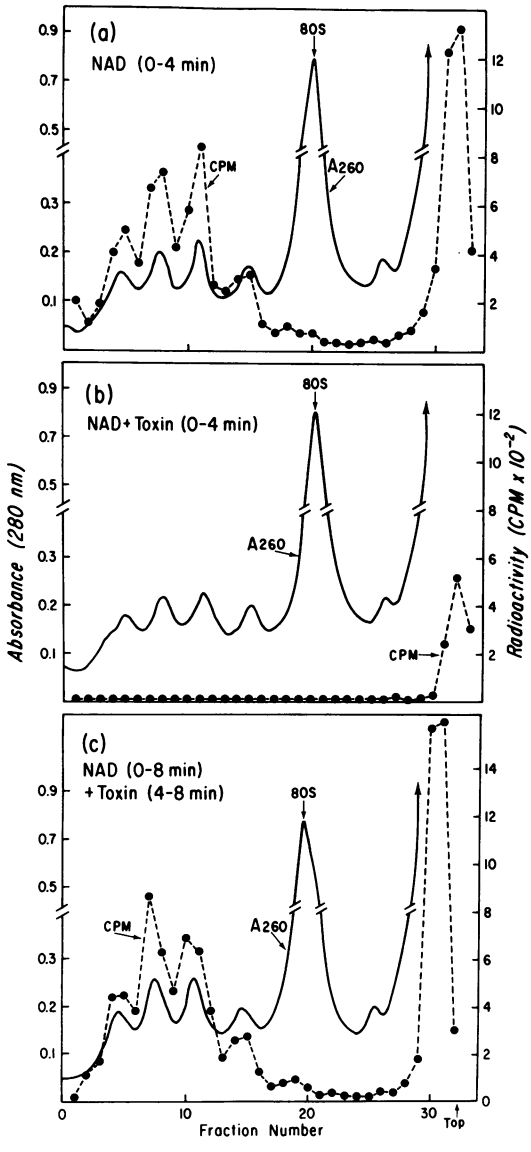

FIG. 2. Sucrose gradient sedimentation of polysomes from a reticulocyte lysate protein-synthesizing system labeled with [^{14}C]-leucine. The lysates (0.2 ml portions) each contained NAD and the following additional materials: (a) 4 min incubation, no additions, (b) 4 min incubation, plus 3.6 μg of PA toxin, (c) 4 min incubation with no additions followed by 4 min incubation with 3.6 μg of PA toxin.

polypeptide chains and causes ribosomes to become immobilized on the mRNA. Since PA toxin appeared also to require NAD and to inhibit protein synthesis at the level of chain elongation (Figs. 1 and 2), we determined whether it had enzymatic activity similar to fragment A of diphtheria toxin. As seen in Table 1, PA toxin was enzymatically active in transferring radioactivity from [^{14}C]NAD into acid-precipitable linkage with protein in a partially purified EF-2 preparation. The enzymatic activity of PA toxin was destroyed if it was preincubated with antibody to PA toxin but not with antibody to diphtheria toxin fragment A. Conversely, anti-fragment A neutralized the enzymatic activity of fragment A, whereas anti-PA did not.

The ADP-ribosyl transferase activity of diphtheria toxin fragment A is exceedingly stable to high temperature when

TABLE 1. *The effect of antisera on the NAD transferase activities of PA toxin and fragment A of diphtheria toxin**

Preincubation serum	Acid-Insoluble Radioactivity (cpm) H_2O	0.02 μg of fragment A	0.02 μg of PA Toxin
H_2O	164	1328	1651
Normal rabbit serum		1556	1080
Rabbit anti-fragment-A serum		284	1545
Normal horse serum, 1:10†		1684	1309
Pony anti-PA-toxin serum, 1:10†		1544	168

* The PA toxin or diphtheria toxin fragment A were preincubated with water, normal rabbit serum, diluted normal horse serum, or antisera at 37° for 15 min. The preincubation mixtures were then immediately chilled and added to the reaction mixture. Conditions for assay of NAD transferase activity were as described in *Methods and Materials*.

† A number of undiluted normal horse sera tested partially inactivated low concentrations of PA toxin. This could be minimized by diluting the normal horse sera 1:10 in H_2O. The pony anti-PA-toxin serum was therefore also diluted 1:10 in water prior to use.

not bound to fragment B (29, 30). As seen in Table 2, the enzymatic activity of PA toxin was destroyed by boiling for 30 min, whereas, the activity of diphtheria toxin fragment A was unaffected. These data and those in Table 1 suggest that PA toxin and diphtheria toxin fragment A are structurally different although they have similar enzymatic activities.

The EF-2 preparation used in the above studies was impure and contained many protein bands when analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In order to determine whether the substrate for PA toxin was EF-2 or some other protein, we incubated saturating amounts of PA toxin and diphtheria toxin fragment A either alone or together in the presence of excess [^{14}C]NAD and a constant, rate-limiting amount of reticulocyte enzyme preparation. As seen in Table 2, in these conditions the two toxins were non-additive in the amount of radioisotope they trans-

TABLE 2. *Comparison of the NAD transferase activities of PA toxin and diphtheria toxin fragment A*

Toxin added	Acid-insoluble radioactivity (cpm)
None	113
1 μg of diphtheria toxin fragment A	1485
1 μg of heated* diphtheria toxin fragment A	1509
1 μg of PA toxin	1381
1 μg of heated* PA toxin	208
1 μg of diphtheria toxin fragment A + 1 μg of PA toxin	1390

* The toxins were preincubated in boiling water for 30 min, rapidly cooled in an ice bath, and immediately added to the reaction mixture.

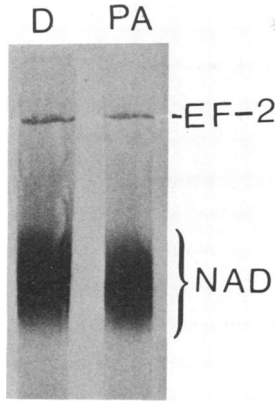

FIG. 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of proteins labeled by toxins with [^{14}C]NAD. The labeled proteins are visualized by autoradiography. A partially purified EF-2 preparation was incubated for 60 min with [^{14}C]-NAD and either with PA toxin or with diphtheria toxin fragment A as described in *Methods and Materials*. Following incorporation the solutions were adjusted to 0.6% sodium dodecyl sulfate, 5% sucrose, and 1% 2-mercaptoethanol and were incubated at 75° for 20 min before 50 μl aliquots were layered onto acrylamide gels. The unincorporated NAD electrophoresed rapidly as a diffuse band on the gels; it was removed if the gels were washed with 12.5% trichloroacetic acid before they were sectioned for autoradiography. D is the extract incubated with diphtheria toxin fragment A and PA is the extract incubated with *Pseudomonas aeruginosa* toxin. Both toxins label only one protein which migrates in the position expected for the 100,000 dalton EF-2 polypeptide.

ferred into acid-precipitable linkage with protein. Furthermore, when the [^{14}C]NAD-labeled reaction mixtures that had been incubated with either PA toxin or diphtheria toxin fragment A were electrophoresed on sodium dodecyl sulfate–polyacrylamide gels, the radioautographs of the gels were identical (Fig. 3). In both cases the only labeled protein had a molecular weight of about 100,000, the known size of EF-2 (31). These data suggest that PA toxin catalyzes the transfer of radioactivity from [^{14}C]NAD onto EF-2.

Evidence that the modifications of EF-2 by the two toxins are likely identical was shown by tryptic peptide analysis of the [^{14}C]NAD labeled EF-2 preparations. As is seen in Fig. 4, each toxin caused the labeling of only a single tryptic peptide. Furthermore, the tryptic peptides labeled by the action of the two toxins cochromatograph on CEL-300 thin-layer plates in two different solvent systems (see *Methods and Materials*). These data suggest that both toxins transfer the same portion of NAD onto the same amino-acid side chain of EF-2.

DISCUSSION

These results strongly suggest that PA toxin acts by the same intracellular mechanism as diphtheria toxin. Both catalyze the transfer of a portion of NAD onto EF-2, a protein required for ribosome translocation along mRNA during protein synthesis. Although we have not established by direct analysis that PA toxin transfers the ADP-ribosyl moiety of NAD rather than some other portion of NAD, it seems very likely that this will prove true. Thus when [*carbonyl*-^{14}C]nicotinamide adenine dinucleotide was substituted for the [^{14}C]NAD in the enzyme reaction (see *Methods and Materials*), no label was transferred to acid-precipitable

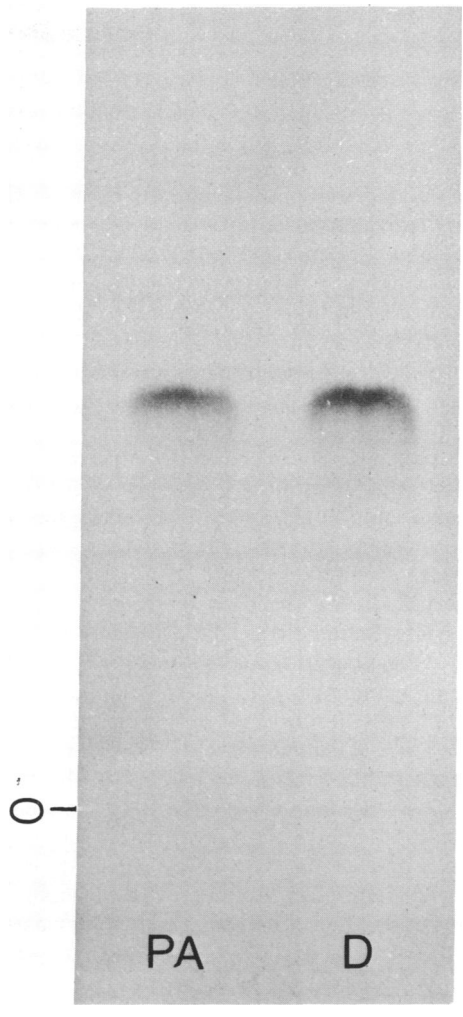

FIG. 4. Thin-layer chromatography of tryptic peptides from EF-2 labeled by toxins with [^{14}C]NAD. The radioactive tryptic peptides are visualized by autoradiography. A partially purified EF-2 preparation was incubated with [^{14}C]NAD and with either PA toxin (PA) or diphtheria toxin fragment A (D) as described in *Methods and Materials*. The tryptic peptides were chromatographed on a CEL-300 thin-layer plate. The solvent used was *n*-butanol/acetic acid/water/pyridine (150:30:120:120). O is the origin. Both toxins labeled the same tryptic peptide.

material in the presence of either 1 μg of PA toxin or 1 μg of diphtheria toxin fragment A. There are two known examples in bacteria of NAD-dependent covalent modification of proteins, both of which involve transfer of the ADP-ribosyl moiety (16, 32). Furthermore, our results suggest that the modifications by PA and diphtheria toxins occur at the same site on EF-2, since the modifications are non-additive (Table 2). Also, the toxins transfer a very similar amount of radioactivity from the [^{14}C]NAD onto EF-2 in conditions of toxin excess (Table 2). Finally, we have found that the two toxins modify the same tryptic peptide of EF-2, since these modified peptides cochromatograph on CEL-300 thin-layer plates in different solvent systems.

PA and diphtheria toxins are from unrelated bacteria, are not identical immunologically, are toxic to different cells and species (14, 33) and appear to differ in susceptibility to proteolytic attack. Intact diphtheria toxin has a molecular

weight of 64,000 and is cleaved by mild trypsin treatment and disulfide bond reduction into its A (24,000 dalton) and B (39,000 dalton) fragments. The A fragment is the active transferase, whereas the B fragment is required for toxicity and is believed responsible for binding to cell receptors and penetration through the plasma membrane (17, 18, 33). The intact diphtheria toxin is zymogen, since its transferase activity is only evident after proteolysis. The reported molecular weight of PA toxin is 50,000–54,000 (12, 13). Our preliminary data indicate that trypsin treatment of PA toxin under conditions causing fragmentation of diphtheria toxin (18) does not result in an increase in the enzymatic activity of PA toxin. This may simply mean our PA toxin is already fully nicked. However, a single comparison of PA toxin electrophoresed on sodium dodecyl sulfate–polyacrylamide gels in the presence or absence of 1% dithiothreitol showed identical patterns. Accordingly, it seems likely that the intact PA toxin may be the active transferase, but additional structural-functional studies are required.

It seems to us unlikely that PA and diphtheria toxins were conserved during evolution because of their ability to ADP-ribosylate mammalian EF-2. Rather, it seems more likely that ADP-ribosylation plays some role in bacteria and that a site on mammalian EF-2 merely happens to serve as a cross-reacting protein substrate. In this regard it is interesting that *Escherichia coli* infected with T4 phage synthesize a protein that catalyzes transfer of the ADP-ribosyl moiety of NAD onto the α-chain of *E. coli* RNA polymerase (32). Similarly, diphtheria toxin is synthesized only in *Corynebacterium diphtheriae* infected with β or related virus, and the toxin is encoded by the viral genome (34). Therefore, it would be important to know whether a virus plays a role in PA toxin synthesis.

Substantial information at the molecular level is known for only three bacterial toxins—diphtheria toxin (33), PA toxin, and cholera toxin (35). The latter causes activation or derepression of plasma membrane adenylate cyclase (35, 36) and may involve covalent modification of the membrane proteins. We were surprised to find that both diphtheria and PA toxins have the same intracellular mechanism of action. The probability of this happening by chance or by convergent evolution is obviously remote. Accordingly, we suggest that these two toxins may have had a common evolutionary origin, that some other bacterial toxins will be found to act similarly, and that NAD-dependent ADP-ribosylation of various target proteins will prove to be a widespread general mechanism of bacterial toxin action.

We thank Carol M. Jonasson for technical assistance, Dr. P. Dehlinger for initial advice and guidance concerning the reticulocyte lysate system, and Dr. M. B. Rittenberg for advice and encouragement. This work was supported by U.S. Public Health Service Grant IAI 11137, National Science Foundation Grant GB-39815 and grants from Phi Beta Psi sorority and the Oregon Heart Association.

1. Liu, P. V., Abe, Y. & Bates, J. L. (1961) *J. Infect. Dis.* **108,** 218–228.
2. Berk, R. S. (1964) *J. Bacteriol.* **88,** 559–565.
3. Liu, P. V. (1966) *J. Infect. Dis.* **116,** 112–116.
4. Liu, P. V. (1966) *J. Infect. Dis.* **116,** 481–489.
5. Bartell, P. F., Orr, T. E. & Garcia, M. (1968) *J. Infect. Dis.* **118,** 165–172.
6. Atik, M., Liu, P. V., Hanson, B. A., Amini, S. & Rosenberg, C. F. (1968) *J. Am. Med. Assoc.* **205,** 134–140.
7. Carney, S. A. & Jones, R. J. (1968) *Br. J. Exp. Pathol.* **49,** 395–410.
8. Coleman, R. G., Janssen, R. J. & Ludovici, P. P. (1969) *Proc. Soc. Exp. Biol. Med.* **131,** 311–315.
9. Meinke, G., Barum, J., Rosenberg, B. & Berk, R. (1970) *Infect. Immun.* **2,** 583–589.
10. Meinke, G. & Berk, R. S. (1970) *Proc. Soc. Exp. Biol. Med.* **135,** 360–363.
11. Kubota, Y. & Liu, P. V. (1971) *J. Infect. Dis.* **123,** 97–98.
12. Callahan, L. T. (1974) *Infect. Immun.* **9,** 113–118.
13. Liu, P. V. & Hsieh, H. (1973) *J. Infect. Dis.* **128,** 520–526.
14. Pavlovskis, O. R. & Gordon, F. B. (1972) *J. Infect. Dis.* **125,** 631–636.
15. Pavlovskis, O. R. & Shackelford, A. H. (1974) *Infect. Immun.* **9,** 540–546.
16. Honjo, T., Nishizuka, Y., Kato, I. & Hayaishi, O. (1971) *J. Biol. Chem.* **246,** 4251–4259.
17. Collier, R. J. & Kandel, J. (1971) *J. Biol. Chem.* **246,** 1496–1503.
18. Gill, D. M. & Dinius, L. L. (1971) *J. Biol. Chem.* **246,** 1485–1491.
19. Liu, P. V. (1973) *J. Infect. Dis.* **128,** 506–513.
20. Liu, P. V., Yoshii, S. & Hsieh, H. (1973) *J. Infect. Dis.* **128,** 514–519.
21. Adamson, S. D., Herbert, E. & Godchaux, W., III (1968) *Arch. Biochem. Biophys.* **125,** 671–683.
22. Baldy, M., Gaskill, P. & Kabat, D. (1972) *J. Biol. Chem.* **247,** 6665–6670.
23. Bitte, L. & Kabat, D. (1974) in *Methods in Enzymology,* eds. Moldave, K. & Grossman, L. (Academic Press, New York), Vol. 30, pp. 563–590.
24. Fairbanks, G., Jr., Levinthal, C. & Reeder, R. H. (1965) *Biochem. Biophys. Res. Commun.* **20,** 343–346.
25. Allen, E. S. & Schweet, R. S. (1962) *J. Biol. Chem.* **237,** 760–767.
26. Lightfoot, H. N. & Iglewski, B. H. (1974) *Biochem. Biophys. Res. Commun.* **56,** 351–357.
27. Crawford, L. V. & Gesteland, R. F. (1973) *J. Mol. Biol.* **74,** 627–634.
28. Collier, R. J. (1967) *J. Mol. Biol.* **25,** 83–98.
29. Drazen, R., Kandel, J. & Collier, R. J. (1971) *J. Biol. Chem.* **246,** 1504–1510.
30. Cukor, G., Solotorovsky, M. & Kuchler, R. J. (1973) *J. Bacteriol.* 277–283.
31. Collins, J. F., Raeburn, S. & Maxwell, E. S. (1971) *J. Biol. Chem.* **246,** 1049–1054.
32. Goff, C. G. (1974) in *Metabolic Interconversion of Enzymes 1973* (Springer-Verlag, New York), pp. 235–244.
33. Pappenheimer, A. M., Jr. & Gill, D. M. (1973) *Science* **182,** 353–358.
34. Uchida, T. D., Gill, D. M. & Pappenheimer, A. M., Jr. (1971) *Nature New Biol.* **233,** 8–11.
35. Finkelstein, R. A. (1973) *CRC Crit. Rev. Microbiol.* **2,** 553–623.
36. Cuatrecasas, P. (1973) *Biochemistry* **12,** 3547–3558, 3559–3566, 3567–3577, 3578–3581.