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Structure-Activity Relationships of an Exotoxin of *Pseudomonas aeruginosa*

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The relation of the structure of *Pseudomonas aeruginosa* exotoxin A (PA toxin) to its enzymatic activity (adenosine 5'-diphosphate-ribosyl transferase) in vitro and to its toxicity in vivo was examined. PA toxin is produced as a single polypeptide chain with a molecular weight of about 71,500. PA toxin is produced by *Pseudomonas* as a toxic proenzyme that lacks enzymatic activity. Adenosine 5'-diphosphate-ribosyl transferase activity is expressed when the molecule is denatured and reduced or when its is cleaved by *Pseudomonas* proteases to yield an enzymatically active 27,000-dalton fragment (fragment a). A 45,000-dalton protein is tentatively identified as the enzymatically inactive fragment b of PA toxin. Enzymatically active forms of the toxin lack toxicity for mouse L-cells or mouse lethality. Thus, it is concluded that the native toxin proenzyme is required for toxicity and that a structural rearrangement must precede its intracellular activity.

Pseudomonas aeruginosa exotoxin A (PA toxin) originally described by Liu (13), is a potentially important virulence factor (3, 16, 17, 20, 21). PA toxin inhibits mammalian protein synthesis by the same mechanism as diphtherial toxin (10). Both toxins catalyze the transfer of the adenosine 5'-diphosphate-ribosyl moiety of nicotinamide adenine dinucleotide onto the same amino acid of elongation factor 2 in a stereochemically identical fashion (9, 10, 11). However, the structure-function relationships of PA toxin have not been determined.

Although PA toxin and diphtherial toxin have identical enzymatic activities, they do not cross-react immunologically, and some additional differences in structural properties have been reported (9, 11). Diphtherial toxin must be cleaved and reduced to produce an enzymatically active fragment (fragment A) (4). In contrast, it has been reported that the intact PA toxin is an active adenosine 5'-diphosphate-ribosyl transferase (ADPR-transferase) (9, 11). There is, however, a smaller-molecular-weight protein (30,000) found in PA toxin preparations which also has ADPR-transferase activity (9, 11). The relationship of this smaller protein to PA toxin has not been established.

Recent data suggest that the ADPR-transferase activity of PA toxin is not solely responsible for toxicity to mouse L-cells or for mouse lethality (24). A similar relationship of toxicity and enzyme activity is seen with diphtherial toxin where another portion of the toxin molecule (fragment B), separate from the enzymatically active fragment A, is required for toxicity to mammalian cells and intact animals (4).

In the present study, additional structure-function relationships are described, and data are presented suggesting that the PA toxin is a single polypeptide produced as a toxic proenzyme (71,500 daltons). Upon activation, this protein is an active ADPR-transferase enzyme and nontoxic to mammalian cells or intact animals. In addition, data are presented indicating that the PA toxin may be cleaved and, without the need for concomitant reduction, may yield a nontoxic 27,000-dalton, enzymatically active fragment. Based on cumulative data, a molecular model of *P. aeruginosa* PA toxin is presented.

MATERIALS AND METHODS

Preparation of PA toxin and its enzymatically active fragment. *P. aeruginosa* strain PA103 (kindly provided by P. V. Liu, Louisville, Ky.) was used throughout this study for toxin production. PA toxin was produced and purified as previously described (15, 18, 24) with the following modifications. The $(NH_4)_2SO_4$-precipitated toxin was chromatographed on a column of Sephadex G-200 and then on a diethylaminoethyl-cellulose column. The fractions from the columns were monitored by the ADPR-transferase assay described below and by mouse lethality. All columns were kept at 4°C, and the toxin preparations were stored at −70°C between each step of purification.

The purification of the enzymatically active, non-

toxic fragment of PA toxin was carried out as follows. The crude cultural supernatant of PA103 was allowed to stand at 4°C for 1 to 2 weeks before it was precipitated with zinc acetate and $(NH_4)_2SO_4$. This preparation was then chromatographed on Sephadex G-200 and diethylaminoethyl-cellulose columns. Preparations obtained in this manner were nonlethal for mice but had high ADPR-transferase activity. The enzymatically active nontoxic fragment was further purified as follows. Urea (10 M) was deionized by passage through a mixed-bed, ion-exchange resin, no. AG 501-XE(D) (Bio-Rad, Richmond, Calif.). The toxin was dialyzed against 8 M urea, 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 1 mM 2-mercaptoethanol, pH 7.4, and was fractionated by gel filtration on a column of Bio-Gel A-5m (30 by 1 cm; Bio-Rad) at 22°C with dialysis buffer at a flow rate of 6 ml/h.

Conventional and SDS-PAGE. Conventional polyacrylamide discontinuous gel electrophoresis (PAGE) and elution of active toxin from gels were performed as previously described (24). A modification of the Weber and Osborn method (25) was used for sodium dodecyl sulfate (SDS)-PAGE. Polyacrylamide gels containing 7.5% recrystallized acrylamide (Eastman) in 0.1 M sodium phosphate buffer, pH 7.2, 0.1% SDS were prepared according to the method of Weber and Osborn (25). The gel dimensions were 9 by 0.6 cm. Protein samples, 20 to 100 μg, were dissolved in 10 μl of 0.1 M sodium phosphate buffer containing 1% SDS, 10% sucrose, and, when indicated, 1% (wt/vol) dithiothreitol (DTT). Samples were unheated or heated at 100°C for 5 min prior to loading. The gels were subjected to electrophoresis at room temperature until the tracking dye (0.002% bromophenol blue) reached the distal end of the gels or for 7 to 8 h at a constant current of 5 mA/gel in the buffer system described by Weber and Osborn (25). Gels were stained in 0.1% Coomassie brilliant blue in 40% methanol and 7.5% acetic acid for 2 to 4 h and destained by diffusion in 40% methanol. Determination of molecular weights by SDS-PAGE has been described by Weber and Osborn (25). Cholera toxin was kindly provided by Richard A. Finkelstein (University of Texas, Southwestern Medical School); bovine serum albumin, ovalbumin; and chymotrypsinogen were commercially obtained (Schwarz/Mann), and diphtherial toxin was purified in this laboratory by Lynn P. Elwell (University of Washington) (9).

Production of rabbit anti-PA toxin and Ouchterlony immunodiffusion assay. Production of a highly specific rabbit anti-PA toxin was as follows. Quantities (25 or 50 μg) of PA toxin purified as described above were subjected to electrophoresis on conventional discontinuous gels. The gels were fractionated, the proteins were eluted as previously described (24), and the eluate from each fraction was assayed for ADPR-transferase activity as described below. Only those fractions showing maximum enzymatic activity were pooled and used for immunization of rabbits. Rabbits were immunized with approximately 50 μg of PA toxin in complete Freund adjuvant. Injections were given by the subcutaneous, intramuscular, and intraperitoneal routes. The rabbits were boosted with 50 μg of PA toxin in the same manner 2 to 3 weeks after the primary immunization. The rabbits were exsanguinated 2 to 3 weeks after the second immunization.

Ouchterlony immunodiffusion analysis of PA toxin, toxin fragments, and antitoxin is a modification of the procedure of Ouchterlony (19) as described by Finkelstein and Lo Spalluto (6).

Assay for ADPR-transferase activity and activation of toxin. Aminoacyl transferase-containing enzymes were prepared from crude extracts of rabbit reticulocytes as described by Allen and Schweet and modified by Collier and Kandel (1, 5). ADPR-transferase activity was measured according to the procedure of Collier and Kandel (5) as previously described (11, 24).

Crude or purified preparations of PA toxin were "activated" by one of two methods: (i) dialysis against 0.01 M Tris (pH 8.0) for 24 h and freezing at −20°C for 12 h (unactivated controls were identical preparations stored at −70°C) or (ii) treatment of toxin preparations for 15 min at room temperature with a final concentration of 4 M urea and 1% DTT (unactivated controls were identical toxin preparations without urea and DTT, which were diluted in the same manner as the treated toxin preparations). After various combinations of treatments with urea or DTT, the toxin preparations were dialyzed for 18 to 24 h against 0.01 M Tris buffer, pH 8.0. Dialyzed toxin preparations were then assayed for ADPR-transferase activity, inhibition of protein synthesis in mouse L-cells, and/or mouse lethality (24).

Assay of growth of and toxin production by PA103. A 200-ml portion of media described by Liu (15) in a 2-liter flask was inoculated with approximately 5×10^8 viable cells of PA103 and incubated at 32°C on a reciprocating shaker (200 linear excursions per min). Growth of PA103 was monitored at hourly intervals by determining the number of colony-forming units on nutrient agar (Difco). Toxin production during growth was detected as follows. Samples (5 ml) of the PA103 culture were removed at hourly intervals. The cultural supernatant was obtained by immediate centrifugation at $10,000 \times g$ for 30 min. Each supernatant was immediately stored at −70°C until assayed for ADPR-transferase activity by the method previously described (24).

RESULTS

Potentiation of ADPR-transferase activity in PA toxin preparations. During the course of the present study it has been observed many times that the ADPR-transferase activity of PA toxin preparations stored at −20°C appeared to increase when compared with preparations that were stored at −70°C. There was approximately a sixfold increase in enzyme activity when a crude cultural supernatant was stored at −20°C instead of −70°C (Table 1). Furthermore, it has been reported (13) that the ADPR-transferase activity of PA toxin can be increased by treatment with a denaturant (urea) and a reducing agent (DTT); however, nothing is known about the structure-activity relation-

TABLE 1. *Activation of the ADPR-transferase in crude cultural supernatants*

Treatment	cpm[a]
None	156
−70°C	203
−20°C	1,200
4 M urea	208
1% DTT	217
4 M urea + 1% DTT	2,306

[a] Acid-insoluble radioactive counts per minute from the ADPR-transferase assay.

ships of PA toxin after such treatment. Table 1 shows the ADPR-transferase activity of crude cultural supernatants activated with 4 M urea and 1% DTT. There is greater than a 10-fold increase in enzyme activity of the activated sample over an identical untreated sample or one stored at −70°C (Table 1).

The enzyme activity of purified toxin preparations was also activatable. However, the increase in enzyme activity of the treated purified preparations was not as large as the increase in activity obtained when crude culture supernatants were used (Tables 1 and 2). It is possible that the purification methods activate some of the PA toxin, thereby reducing the proportion of activatable molecules. Urea or DTT alone did not appear to increase significantly the ADPR-transferase activity of crude or purified preparations of PA toxin (Tables 1 and 2).

ADPR-transferase production during growth of *P. aeruginosa*. Results presented in Fig. 1 demonstrate that significant ADPR-transferase activity is not seen in untreated culture supernatants until 19 h, well into the stationary phase of growth. In contrast, when activatable ADPR-transferase is measured, activity is seen as early as 10 h, while the cells are still in the logarithmic phase. When activated samples are measured at 20 and 21 h, ADPR-transferase activity has reached a peak level. Enzymatic activity in untreated samples, on the other hand, appears to be still increasing as late as 22 to 23 h, well into the stationary phase of growth. This increase in enzyme activity may be a reflection of spontaneous activation and/or fragmentation of the toxin, resulting in an increase of ADPR-transferase activity.

These data (Fig. 1) and the results of the activation experiments presented in Table 1 indicate that the PA toxin is produced as a proenzyme, which may be spontaneously activated (Fig. 1), activated by treatment with a combination of a denaturing and a reducing agent, or by freezing (−20°C) and thawing (Tables 1 and 2).

Effect of activated toxin on inhibition of protein synthesis in mouse L-cells and/or mouse lethality. It has been recently reported that the mouse L-cell toxicity and the mouse

TABLE 2. *Effect of enzyme activation on the mouse lethality of purified PA toxin preparations*

Toxin prepn	Enzyme activity (units)[a]	Mouse LD_{50} (μg)[b]
Untreated	1.0	0.25
Toxin + urea	1.3	ND
Toxin + DTT	1.1	0.25
Toxin + urea, DTT	3.2	>5.0

[a] Enzyme activities are based on the acid-insoluble radioactivity counts per minute from the ADPR-transferase assay where counts per minute from the assay of the untreated toxin preparation are equivalent to 1.0 activity unit.

[b] LD_{50}, 50% lethal dose; ND, not determined.

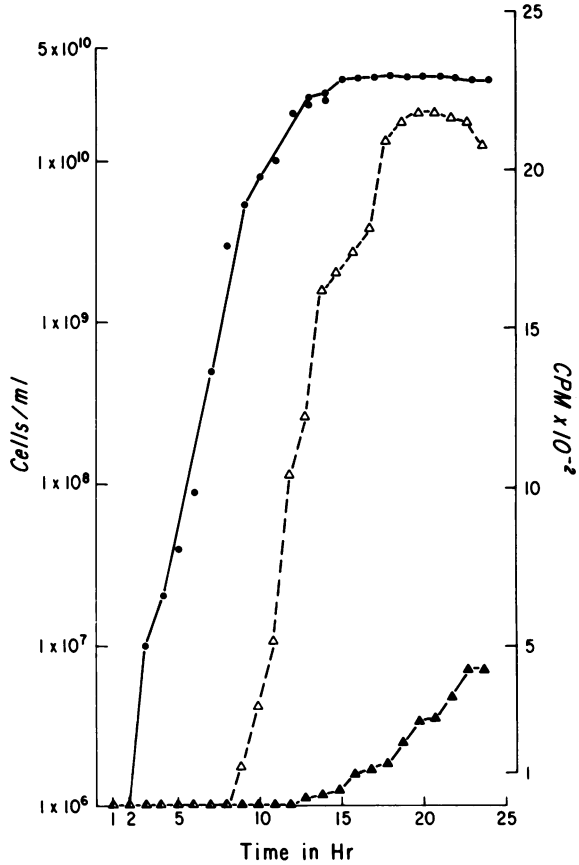

FIG. 1. *PA toxin (ADPR-transferase) production during growth. Cells per milliliter (●) are based on colony-forming units of PA103 on nutrient agar. Counts per minute is the acid-insoluble radioactivity counts per minute from the ADPR-transferase assay of unactivated (▲) and activated (urea + DTT treated) (△) samples.*

toxicity of PA toxin may be reduced or destroyed by thermal denaturation without significantly reducing ADPR-transferase activity (23). Thus, it was of interest to examine the mouse lethality and cell toxicity of activated PA toxin. A purified toxin preparation treated with urea and DTT showed a greater than threefold increase in enzyme activity (Table 2). In contrast, the same toxin preparation had a greater than 10-fold reduction in mouse lethality (Table 2) and a 25-fold reduction in toxicity to mouse L-cells (Fig. 2). Other combinations, such as toxin treated with urea or toxin treated with DTT, demonstrated either no change or a slight increase in enzymatic activity (Table 2). In each instance where there was an increase in enzymatic activity, there was a decrease in mouse lethality or L-cell toxicity (Fig. 2).

SDS-PAGE of PA toxin. When unactivated and activated (urea- and DTT-treated) samples of PA toxin were compared by SDS-PAGE there were no apparent major differences seen in the gels stained for protein (Fig. 3, inset). In addition, duplicates of the above gels run at the same time were sliced and the fractions were assayed for ADPR-transferase activity. The enzyme activity profiles of the gel with the unactivated sample (Fig. 3A) and the gel with the activated sample (Fig. 3B) were essentially the same.

Iglewski et al. (9) have reported the presence of an approximately 30,000-dalton protein with ADPR-transferase protein in PA toxin preparations. Figure 4 demonstrates the presence of such a protein in a partially purified preparation of PA toxin that was repeatedly frozen at −20°C and thawed during each stage of purification. It was observed during the course of the present study that repeated freezing and thawing of crude or partially purified PA toxin preparations resulted in an increase in the smaller ADPR-transferase, with a concomitant decrease in the larger-molecule-weight species. Figure 4 (inset) shows the gel of a toxin preparation treated with 1% SDS at room temperature (Fig. 4A) and a gel of a toxin preparation treated with 1% SDS and 1% DTT and heated at 100°C for 10 min (Fig. 4B). The smaller-molecular-weight protein with ADPR-transferase activity was seen regardless of whether a reducing agent (DTT) was used, and the relative ADPR-transferase activities associated with the larger and smaller proteins was not changed by reduction and heating under the conditions described (Fig. 4). These and additional data described below indicate that the 27,000-dalton, enzymatically active protein is a fragment rather than a subunit of the PA toxin.

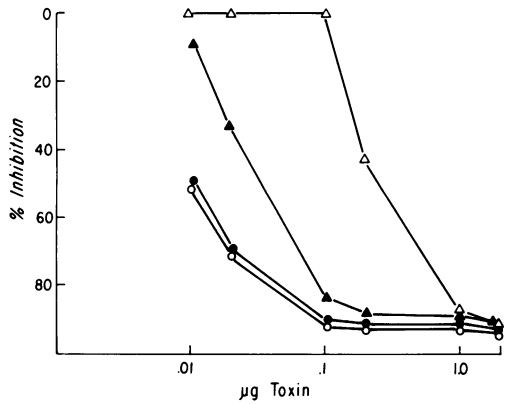

FIG. 2. *Effect of activated toxin preparations on inhibition of protein synthesis in mouse L-cells. Percent inhibition is based on 7,200 cpm of ^{3}H-labeled amino acid incorporated for controls without toxin. Symbols: Untreated toxin* (○) *toxin + DTT* (●); *toxin + urea* (▲); *and toxin + urea, DTT* (△).

Characterization of an enzymatically active fragment (fragment a) of a PA toxin. The smaller-molecular-weight ADPR-transferase has been purified from preparations of PA toxin as described above. This enzymatically active protein was compared to PA toxin by Ouchterlony immunodiffusion. Figure 5 shows a reaction of partial identity between smaller ADPR-transferase and the purified PA toxin.

The enzymatically active fragment was nontoxic for mice or mouse L-cells. Doses of 50 μg/mouse were nonlethal, and 1,000-fold greater concentrations than are normally required of intact PA toxin to inhibit protein synthesis in mouse L-cells had no effect (unpublished observations). These results and those presented in Fig. 3, 4, and 5 strongly suggest that the small-molecular-weight (~27,000) protein found in some PA toxin preparations is a nontoxic fragment of PA toxin with ADPR-transferase activity. This protein will be referred to hereafter as fragment a of PA toxin.

Molecular weights of toxin and fragments. Estimation of molecular weights by SDS-PAGE (Fig. 6, Table 3) indicates that that of the intact PA toxin is about 71,500. This value is in reasonably close agreement with previously reported molecular weights of PA toxin as determined by various methods (3, 9, 11, 13). The enzymatically active fragment a of PA toxin is about 27,000 molecular weight, which is very similar to the molecular weight of diphtherial toxin fragment A (24,000). There is an additional protein consistently seen in gels of PA toxin preparations (Fig. 4) that contain the fragment a protein. This protein is only seen when fragment a is present and could be the putative fragment b of PA toxin. This protein is

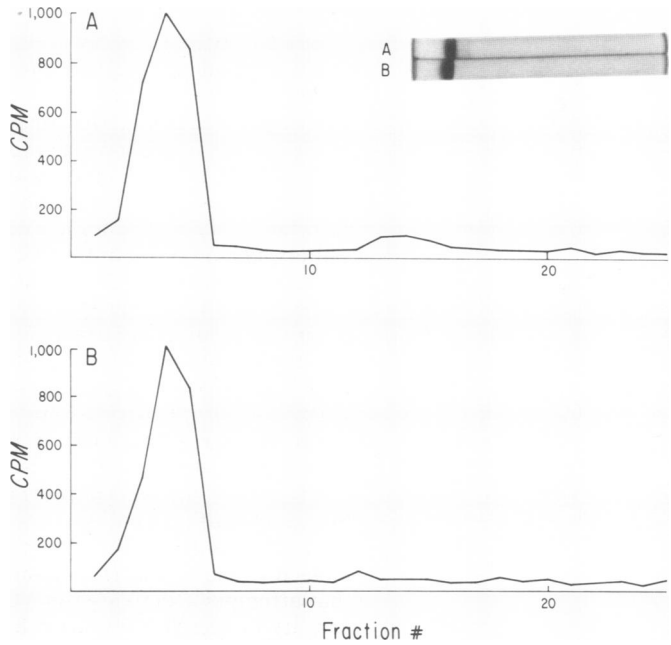

FIG. 3. *SDS-PAGE of PA toxin; effect of activation. Inset, Stained gels; graph, ADPR-transferase activity profile of fractionated gels. PA toxin untreated prior to application on gel (A) and activated (urea + DTT treated) PA toxin (B).*

approximately 45,000 daltons in size and is the only protein observed in PA toxin preparations or fragmented preparations that is the appropriate size to be the "b" portion of the PA toxin molecule. Unsuccessful attempts to isolate this putative b fragment of PA toxin may be a result of its relative insolubility, as is the case with the fragment of B of diphtherial toxin (4).

DISCUSSION

In recent years it has become clear that although there are obvious major differences in the properties of bacterial and other protein toxins, there are some striking similarities in their structure-function relationships. Diphtherial toxin and *Vibrio cholerae* and *Escherichia coli* enterotoxins, as well as two plant toxins, abrin and ricin, each consist of two basic components, both of which are necessary for the expression of toxicity (4, 7, 8, 22, 23). There is a carrier component termed fragment B (B for binding) that is responsible for making initial contact with the cell surface receptors, and there is an effector portion of the toxin molecule usually designated fragment A (A for active) that catalyzes a specific intracellular reaction. For example, the effector or fragment A moiety of diphtherial toxin is an enzyme (ADPR-transferase) that catalyzes a specific intracellular reaction, leading to inhibition of protein synthesis. More recently, data have been reported suggesting that the A fragments of cholera and *E. coli* enterotoxins are also enzymes (7, 8). Furthermore, it is also of interest that the toxins described above are apparently produced as proenzymes, and in this form they are virtually, if not entirely, devoid of enzyme activity (5, 7, 8, 22, 23).

It is now clear that PA toxin can inhibit protein synthesis in vitro by the same mechanism as diphtherial toxin fragment A (9, 10, 11). Both proteins act as an ADPR-transferase enzyme and inactivate elongation factor 2, thereby blocking peptide chain elongation (4, 9, 11). However, as described above, the structure-activity relationships of diphtherial toxin are reasonably well understood (see review by R. J. Collier [4]), whereas much less information is available concerning PA toxin.

Based on the cumulative data presented in this report and data recently reported (13, 24), a proposed molecular model of PA toxin is presented in Fig. 7. Some notable similarities in structure-activity relationships between PA toxin and other bacterial toxins, and, most specifically, diphtherial toxin, are seen. There are also some important differences.

The PA toxin is produced as a single and intact polypeptide (molecular weight, 71,500)

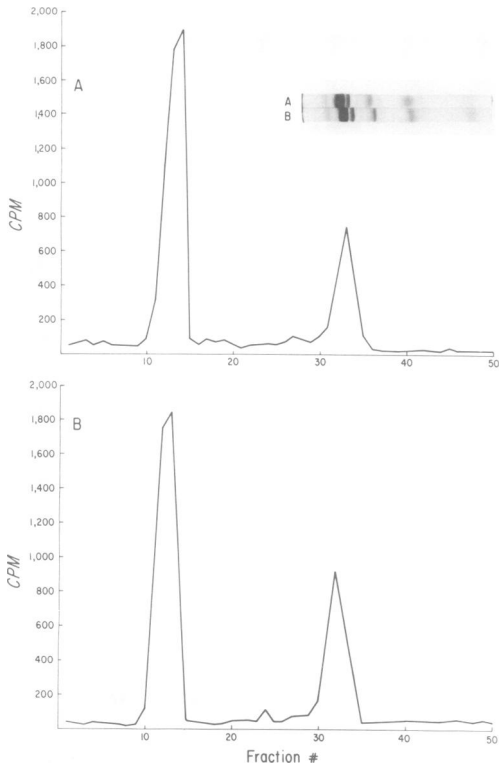

FIG. 4. *SDS-PAGE of partially purified PA toxin. Inset, Stained gels; graph, ADPR-transferase activity profile of fractionated gels. PA toxin preparation treated with 1% SDS at 25°C for 10 min (A) and PA toxin preparation treated with 1% SDS and 1% DTT at 100°C for 10 min (B).*

and contains four intrachain disulfide bonds (Fig. 3, 6 and 7; 13). The amino acid analysis of PA toxin has revealed that there are approximately eight one-half-cystine residues in the molecule (13), with no half-cystines in the enzymically active fragment a (D. Chung and R. J. Collier, personal communication). Furthermore, data presented in Fig 4 indicate that fragment a is not held to fragment b by a disulfide bond. Thus, four disulfide bonds have been placed within the b fragment of the PA toxin model in Fig. 7. The data presented in Fig. 1 and Table 1 demonstrate that the PA toxin, like diphtherial toxin, is produced as a toxic proenzyme, which has little if any ADPR-transferase activity. This specific enzymatic activity can be expressed or potentiated by (i) treatment of toxin with the combination of a denaturing and a reducing agent or (ii) cleavage of the polypeptide chain to produce a fragment (fragment a) of about 27,000 daltons (Fig. 4–7). This fragment is an active ADPR-transferase, and its activity cannot be increased by denaturation and reduction as with intact toxin. Ouchterlony immunodiffusion analysis of a purified preparation of this enzymatically active fragment indicates that this protein (fragment a) is immunologically related to purified intact PA toxin (Fig. 5). In addition, the amount of PA toxin fragment a is increased when the cell-free crude culture supernatant is incubated at 4°C for several days (see Materials and Methods) or at 37°C for 18 to 24 h (Dominic Chung and R. John Collier, personal communication). Therefore, it is unlikely that the 27,000-dalton ADPR-transferase is a distinct product of *P. aeruginosa*; rather it is an enzymatically active fragment produced by post-translational cleavage of the intact PA toxin. A similar situation is seen with diphtherial toxin where a portion of the isolated toxin is commonly in the nicked form, which is due to the action of bacteria proteases (4). However, unlike diphtherial toxin, a reduction of a disulfide bond is not necessary to dissociate the PA fragment a from the rest of the molecule (Fig. 4 and 7). It should be noted here that attempts to activate the ADPR-transferase activity of purified PA toxin by producing the enzymatically active fragment a or similar fragments by controlled treatment with specific proteases such as trypsin or a partially purified *Pseudomonas* protease (12) have not yet been successful.

Data presented in Table 1 and Fig. 2 indicate

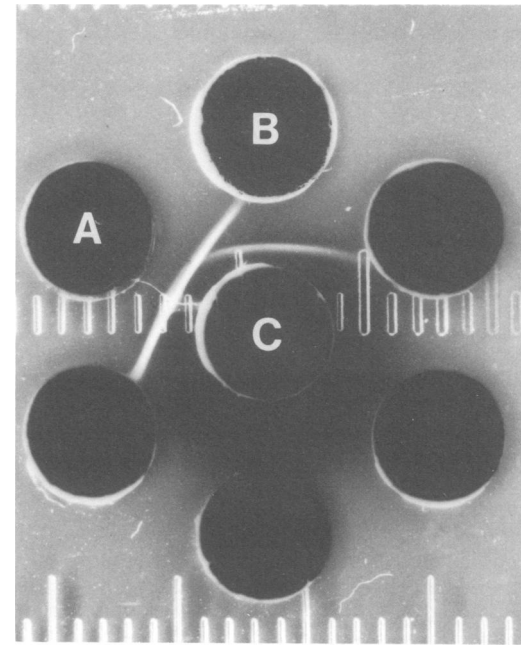

FIG. 5. *Ouchterlony immunodiffusion analysis of PA toxin and PA toxin fragment a. Well A, Purified PA toxin, 100 μg/ml; well B, purified PA toxin fragment a, 100 μg/ml; well C, rabbit anti-PA toxin.*

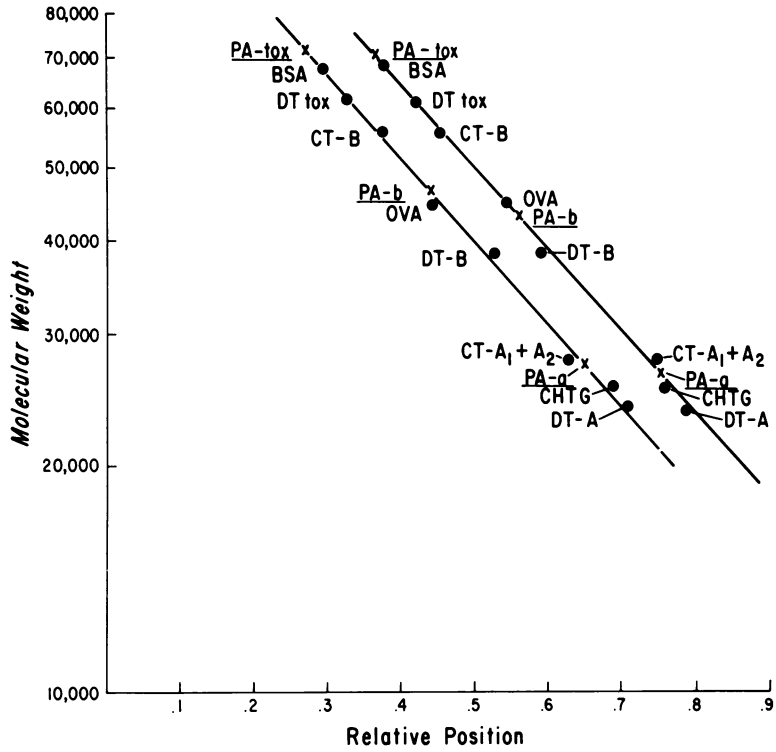

FIG. 6. *Estimation of molecular weights of toxins and fragments by SDS-PAGE. The results of two separate experiments are shown. For the lower line the usual 0.26% cross-linker (bis-acrylamide) was used, but half this amount was used for the upper line. The molecular weights of the marker proteins have been previously reported (4, 7, 22), BSA, bovine serum albumin (between 67,000 and 69,000); DT tox, intact diphtherial toxin (63,000); CT-B, fragment B of cholera enterotoxin (56,000); OVA, ovalbumin (44,000 to 46,000); DT-B, fragment B of diphtherial toxin (39,000); CT-A_1, fragments A_1 plus A_2 of cholera enterotoxin; CHTG, chymotrypsinogen (25,700; DT-A, fragment A of diphtherial toxin (24,000).*

TABLE 3. *Molecular weights of toxin and fragments*

Material tested	Mol wt	
	Range	Mean[a] ± SD[b]
PA toxin	70,000–72,500	71,500 ± 1,000
PA fragment b	43,500–46,500	45,000 ± 1,350
PA fragment a	26,500–27,500	27,000 ± 350

[a] Average of six separate experiments.
[b] SD, Standard deviation.

that as ADPR-transferase activity increases the toxicity of the preparation to mouse L-cells and to mice is significantly reduced. Also, as previously mentioned, the PA fragment a is nontoxic to cells or intact animals even at high doses. These data and previously reported results (24) strongly suggest that ADPR-transferase alone is not sufficient for toxicity of PA toxin and that there is an additional portion of the molecule (e.g., fragment b), distinct from the enzyme-active site, which is required for expression of toxicity. The 45,000-dalton protein seen in PA toxin preparations only when fragment a is present appears to be a likely candidate for the putative fragment b portion of the PA toxin molecule. Attempts to isolate this 45,000-dalton protein from polyacrylamide gels and to determine its immunological relationship to intact toxin have been unsuccessful as yet. Thus, the fragment b of PA toxin may be similar to diphtherial toxin fragment B, which denatures and precipitates rapidly after dissociation from fragment A and can only be maintained in solution with a high concentration of urea or guanidine or with detergents (4). An alternative possibility is that the b portion of PA toxin is completely destroyed by *Pseudomonas* proteases. Studies are in progress to attempt to produce a "b" fragment under controlled conditions with purified proteases isolated from *P. aeruginosa*. Although data have been presented demonstrating that the PA toxin can be cleaved to produce an enzymatically active fragment (fragment a) and a putative fragment b, it is not clear whether cleavage is actually required for expression of toxicity. ADPR-transferase activity is also expressed by

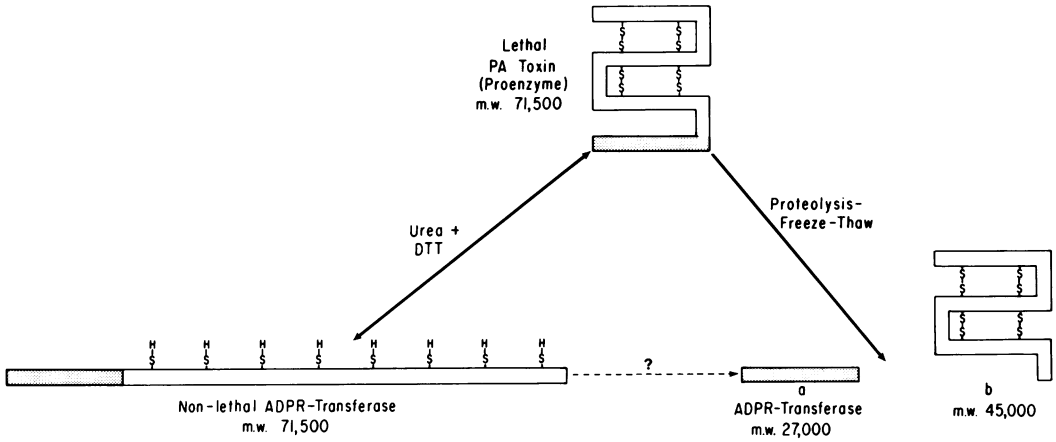

FIG. 7. *Proposed molecular model of PA toxin: structure-activity relationships.*

treating PA toxin with a denaturant and a reducing agent, which does not lead to the production of fragments (Tables 1 and 2; Fig. 3 and 7). Thus, the mechanisms by which the ADPR-transferase activity of PA toxin becomes activated in vivo remains to be clarified.

In summary, PA toxin resembles the structure-activity relationship of other bacterial toxins. That is, this toxin consists of an enzymatically active effector moiety (fragment a) and a carrier moiety (fragment b), which is most likely responsible for binding the toxin to receptors on the cell surface.

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