One-dimensional Peptide Mapping of the Subunits of Pertussis Toxin

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Pertussis toxin (pertussigen) purified from the cytoplasmic fraction of Bordetella pertussis strain 18334, phase 1, consisted of five subunits which included an additional subunit (S1a) not previously reported. Subunits S1, S1a and S2 showed extensive structural homology when analysed by one-dimensional peptide mapping, indicating that the latter two were probably derived from proteolytic cleavage of the largest subunit, S1. Subunits S3 and S4,5 generated only a limited number of peptides following chemical and enzymic degradation, but these subunits differed structurally from each other and from those showing structural homology.

INTRODUCTION

Toxigenic strains of Bordetella pertussis phase 1 consistently produce three distinct toxins: the heat-labile toxin, the lipopolysaccharide endotoxin and pertussis toxin (PT) (Wardlaw & Parton, 1983). Pertussis toxin has many biological activities, which include sensitization to histamine (Munoz & Bergman, 1968), promotion of lymphocytosis and leucocytosis (Arai & Sato, 1976; Morse & Morse, 1976), activation of pancreatic islets (Katada & Ui, 1977) and haemagglutinating activity (Irons & MacLennan, 1979). Recently, toxin-mediated ADP-ribosylation of mammalian cell membrane proteins was demonstrated by Katada & Ui (1982).

Many of the subunit-toxins with ADP-ribosyl transferase activity produced by other organisms consist of two components: the A (active) moiety with ADP-ribosyl transferase activity, and the B (binding) moiety (Gill, 1978). The B moiety generally consists of either a single peptide, as in diphtheria toxin (Collier, 1975), or five identical subunits, as with cholera toxin (Gill, 1976) and heat-labile enterotoxin of Escherichia coli (Gill et al., 1981). Recently, Tamura et al. (1982) reported that PT was an oligomeric protein consisting of an A protomer (S1) with ADP-ribosyl transferase activity and a B oligomer which promoted the binding of the native toxin to receptor molecules. However, unlike other subunit-toxins, the B oligomer of PT was reported to consist of four dissimilar subunits, S2, S3, S4 and S5.

The object of this study was to investigate the possible structural relationship of the various subunits of PT by means of one-dimensional peptide mapping and to determine whether the structure of this toxin conformed with the A–B model proposed for other bacterial toxins.

METHODS

Organism and growth conditions. B. pertussis strain 18334, phase 1, was grown in shaken (150 cycles min⁻¹) cyclodextrin liquid medium (Imaizumi et al., 1983) for 48 h at 37 °C. Bacteria from 10 l medium were harvested by centrifugation at 10000 g for 30 min and used for purification of cell-associated PT.

Partial purification of PT. Bacteria (150 g wet wt) were disrupted by a single passage through a French press at 2000 bar and envelope fragments were removed by centrifugation at 30000 g for 1 h at 4 °C. PT in the cytoplasmic fraction was partially purified by affinity chromatography on a Fetuin-Sepharose 4B column based on the method

Abbreviations: NCS, N-chlorosuccinimide; PT, pertussis toxin; TLCK, N-tosyl-L-phenylalanine chloromethyl ketone.
described by Sekura et al. (1983). Bound toxin was eluted with 0-1 M-Tris, 0-5 M-NaCl, pH 10-0, containing 3 M-KSCN (Cowell et al., 1979), the eluate immediately brought to pH 7-5 with 1-0 M-HCl and the toxin concentrated by ultrafiltration using a YM30 membrane (Amicon). The retentate was then dialysed for 16 h against 100 vols 0-01 M-phosphate, 0-15 M-NaCl, pH 7-4. The yield of toxin was approximately 10 mg.

IsoHation of subunits by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Affinity-purified PT (approximately 500 µg) was solubilized in buffer containing 1-5% (w/v) SDS and 2-5% (v/v) 2-mercaptoethanol and the subunits were separated by SDS-PAGE (Laemmli, 1970) at a constant current of 40 mA using a 15% (w/v) acrylamide resolving gel. The subunits were visualized by staining briefly with Coomassie brilliant blue R250 (Cleveland et al., 1977) and each of the stained bands was excised and stored at −20 °C.

One-dimensional peptide mapping. Gel slices (each of 7–10 µg protein) were treated with N-chlorosuccinimide (NCS) in urea (Lischwe & Ochs, 1982) and inserted into the wells of a 4% (w/v) acrylamide stacking gel, and the peptides were separated on a 17% (w/v) acrylamide resolving gel as above. Untreated slices were also electrophoresed in parallel for comparison.

Enzymic digestion of subunits was carried out according to Cleveland et al. (1977) with either staphylococcal V8 proteinase (EC 3.4.21.19; Miles Laboratories) or TLCK-treated a-chymotrypsin (EC 3.4.21.1; Sigma). Each enzyme (1-0 µg) was added to the well containing the gel slice and limited proteolysis was allowed to take place in the stacking gel by electrophoresing at 10 mA until the dye marker reached the bottom of the stacking gel. The peptide fragments generated subsequently separated in a 17% (w/v) acrylamide gel at a constant current of 40 mA.

Molecular mass markers (Sigma) used were bovine serum albumin (68 kDal), ovalbumin (45 kDal), glyceraldehyde-3-phosphate dehydrogenase (36 kDal), carbonic anhydrase (29 kDal), trypsinogen (24 kDal), trypsin inhibitor (20-1 kDal) and b-lactalbumin (14-2 kDal). The gels were stained with silver according to the method described by Oakley et al. (1980).

RESULTS AND DISCUSSION

The SDS-PAGE patterns of reduced PT and isolated subunits are shown in Fig. 1 (a, b) and the apparent molecular masses of the subunits are summarized in Table 1. Under the conditions used, subunits S4 and S5 were not separable and instead a single peptide band corresponding to an apparent molecular mass of 14 kDal was detected. This peptide (henceforth referred to as S4,5) was also reported by Sekura et al. (1983). This effect was not peculiar to the toxin preparation used in this study since examination under identical conditions of purified PT kindly provided by M. Ui (Dept of Physiological Chemistry, Hokkaido University, Japan) and by A. Robinson (PHLS CAMR, Porton Down, Salisbury, UK) failed to show any separation of the two subunits (data not shown). The other major subunits detected were S1, S2 and S3 with apparent molecular masses of 28, 24-5 and 23-5 kDal, respectively, values similar to those reported by Tamura et al. (1982) and Sekura et al. (1983). A fifth subunit referred to as S1a and having an apparent molecular mass of 26 kDal was also detectable between S1 and S2. This subunit has not been reported by other workers.

Treatment of S1 and S1a with NCS in urea generated fragments a, b, c, d and e and c, d, e, g and j respectively (Fig. 2, lanes 1, 2) of which three peptides (c, d and e) were common to both subunits. Degradation of S2 with this reagent produced three major fragments, g, i and j (lane 3). Two of these fragments (g and j) were detected from S1a. Enzymic degradation of S1, S1a and S2 with a-chymotrypsin produced the following fragments: a, b, d, e and f; b, d, e and f; and b, d and e respectively (Fig. 3, lanes 1, 2 and 3) of which three fragments (b, d and e) were common to all three subunits. In addition, fragment f was common to S1 and S1a. Fragments common to all three subunits were also generated by digestion with staphylococcal V8 proteinase (data not shown). Such homology shown by the peptide maps of S1, S1a and S2 subunits was indicative of the structural relationship of the latter two polypeptides with the catalytic subunit S1 (the A moiety) of PT. Subunits S1a and S2 may be degradation products of S1 produced by the action of B. pertussis proteolytic enzymes which can be detected in both cytoplasmic fractions and culture supernates (unpublished observations). The observation that subunit S1a was markedly diminished in PT purified from culture supernates (unpublished observations) supports the suggestion that this subunit was a partially degraded form of S1 and that full conversion to S2 possibly occurred either during or after release of the toxin into the culture medium. Cleavage of S3 with NCS reagent (Fig. 2, lane 4) generated only two fragments (f and h), which differed from those of S1, S1a and S2. Low molecular mass degradation products of S4,5 were not detected.
Peptide mapping of pertussis toxin subunits

Fig. 1. SDS-PAGE of (a) partially purified PT, and (b) gel slices containing toxin subunits. Lane 1 shows molecular mass marker proteins and lanes 2–6 show S1, S1a, S2, S3 and S4,5 respectively.

Table 1. Comparison of the apparent molecular mass of the subunits of PT when examined by SDS-PAGE

<table>
<thead>
<tr>
<th>Subunit . . .</th>
<th>S1</th>
<th>S1a</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S4,5</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-0</td>
<td>26-0</td>
<td>24-5</td>
<td>23-5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>14-0</td>
<td>This study</td>
</tr>
<tr>
<td>28-0</td>
<td>–</td>
<td>23-0</td>
<td>22-0</td>
<td>11-7</td>
<td>9-3</td>
<td>–</td>
<td>–</td>
<td>Tamura et al. (1982)</td>
</tr>
<tr>
<td>30-0</td>
<td>–</td>
<td>26-1</td>
<td>25-4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>13-9</td>
<td>Sekura et al. (1983)</td>
</tr>
</tbody>
</table>

(Fig. 2, lane 5). The susceptibility of all the subunits to degradation by NCS in urea was indicative of the presence of tryptophan residues (Lischwe & Ochs, 1982). Degradation of S3 with α-chymotrypsin produced only one major fragment (c) (Fig. 3, lane 4). This, as well as the fragments generated with staphylococcal V8 proteinase (data not shown) were unique to this particular subunit. Much of the native S4,5 remained undegraded following treatment with α-chymotrypsin (Fig. 3, lane 5) and staphylococcal V8 proteinase (data not shown). The absence of homology between the peptide maps of S1, S3 and S4,5 suggests that these subunits share no homologous amino acid sequences and were therefore the products of different precursors. The B moiety of PT thus consisted of at least two or possibly three dissimilar subunits.

In conclusion, one-dimensional peptide mapping of the subunits of cell-associated PT revealed three structurally related subunits but the subunit structure of the toxin appears more complex than that of other bacterial toxins with similar enzymic activity.

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Fig. 2. Cleavage of the subunits of PT with NCS reagent. Lanes 1–5 show cleaved S1, S1a, S2, S3 and S4,5 respectively and lane 6 shows molecular mass markers as for Fig. 1(b). Fragments a to j were generated by subunits S1, S1a, S2 and S3. See text for details. Fragment g in lane 2 (faint band) is arrowed.

Fig. 3. SDS-PAGE of the subunits digested with α-chymotrypsin. Lanes 1–5 show digests of S1, S1a, S2, S3 and S4,5 respectively and lane 6 shows enzyme alone. Fragments a to f were generated by subunits S1, S1a, S2 and S3. See text for details.

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