Pseudomonas toxin binds Triton X-114 at low pH

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Pseudomonas toxin was found to bind Triton X-114 at pH values below pH 5.0, whereas much less binding was observed at higher pH values. The toxin bound Triton X-114 at a higher pH value in the presence of 0.14 M-NaCl, -KCl or -NaNO₃ than at low salt concentrations. The results suggest that low pH in an intracellular compartment facilitates the transport of *Pseudomonas* toxin across the membrane and into the cytosol by inducing a conformational change in the molecule.

INTRODUCTION

Pseudomonas exotoxin A, which is produced by *Pseudomonas aeruginosa*, inhibits protein synthesis in cells by ADP-ribosylation of elongation factor 2 (Iglewski & Kabat, 1975) in the same way as diphtheria toxin (Pappenheimer, 1977). The entry of *Pseudomonas* toxin into cells involves binding to surface receptors (Manhart *et al.*, 1984), endocytosis from coated pits and possibly transfer to the Golgi apparatus (Morris & Saelinger, 1986). It is well documented that low pH in intracellular vesicles is required for efficient intoxication (Fitzgerald *et al.*, 1983; Sundan *et al.*, 1984). However, it is not clear if the low pH is required for correct routing of the toxin to a compartment suitable for entry into the cytosol, for activity of enzymes that may be involved in processing of the toxin, or for a necessary conformational change of the toxin molecule. In the case of diphtheria toxin low pH induces a conformational change of the molecule required for transport across the membrane (Draper & Simon, 1980; Sandvig & Olsnes, 1980, 1981). As a consequence of the conformational change, diphtheria toxin becomes able to bind Triton X-100 (Sandvig & Olsnes, 1981). We therefore decided to study whether *Pseudomonas* toxin binds Triton at low pH in a similar manner to diphtheria toxin.

MATERIALS AND METHODS

Pseudomonas aeruginosa exotoxin A was obtained from the Swiss Serum and Vaccine Institute, Berne, Switzerland. Diphtheria toxin was obtained from Connaught Laboratories (Willowdale, Ont., Canada) and purified as previously described (Sandvig & Olsnes, 1981).

¹²⁵I-labelled toxins were prepared by the Iodogen method (Fraker & Speck, 1978). This procedure gave a specific radioactivity of 10 000–20 000 c.p.m./ng of toxin. Control experiments showed that the labelling had no effect on the biological activity of the toxins. The radioactively labelled toxins were used to study the distribution of toxin between water and Triton X-114 as described in the legend to Fig. 1.

Triton X-114 was obtained from Fluka, Buchs, Switzerland, and pretreated as previously described (Madshus *et al.*, 1984).

RESULTS AND DISCUSSION

To test if *Pseudomonas* toxin binds a non-ionic detergent at low pH, we studied the interaction of the toxin with Triton X-114. This detergent is miscible with water at 0 °C, whereas two phases are formed at temperatures above 20 °C. When proteins are added to the detergent/water mixture at 0 °C, and the solution is subsequently incubated at 37 °C, hydrophilic proteins are present in the detergent-poor phase whereas amphiphilic proteins are found in the detergent-rich phase (Bordier, 1981). In the following the two phases are referred to as the water phase and the Triton phase respectively.

As shown in Fig. 1(a), ¹²⁵I-labelled *Pseudomonas* toxin entered the Triton phase at pH values below pH 5, whereas at higher pH values most of the toxin was found in the water phase. The ability of the toxin to enter the Triton phase was influenced by the presence of salt. Thus at low salt concentration only 70% of the toxin was found in the Triton phase at pH 3, whereas in the presence of 0.14 M-NaCl, 0.14 M-NaNO₃ or 0.14 M-KCl 80–90% of the toxin was already in the Triton phase at pH 4. There was no further shift in the curve by addition of 0.28 M-NaCl or by addition of 2 mM-CaCl₂ in addition to 0.14 M-NaCl. *Pseudomonas* toxin was found to retain full biological activity after incubation at 37 °C for 30 min at pH values between 4 and 11 (results not shown). The ability to enter the Triton phase at low pH is therefore not due to denaturation of the toxin. Also, 70% of the toxin present in the Triton phase at pH 4.0 entered the water phase when the pH was increased to pH 7.0. Furthermore, unlabelled *Pseudomonas* toxin showed the same pH-dependence for entry into the Triton phase as ¹²⁵I-labelled toxin. This was demonstrated by estimating the amount of toxin in the water phase by the ability to intoxicate L-cells (results not shown).

In contrast with the results shown here with *Pseudomonas* toxin, the protein toxins abrin (Sandvig & Brown, 1987), ricin and viscumin (results not shown), which do not require low pH to intoxicate cells (for review see Olsnes & Sandvig, 1985), do not bind Triton X-114 at low pH.

Analogous to our present finding with *Pseudomonas* toxin, it was previously reported that the ionic strength in the buffer affected the pH required to induce a

Fig. 1. Effect of pH on the ability of *Pseudomonas* toxin to bind Triton X-114

A mixture of buffer (300 μ l) containing 5 mM-Mes/5 mM-gluconic acid adjusted to different pH values with Tris or gluconic acid and Triton X-114 (100 μ l) was incubated for 15 min at 4 °C with (a) 125 I-labelled *Pseudomonas* toxin or (b) 125 I-labelled diphtheria toxin (approx. 20000 c.p.m. per sample). The samples were then heated to 37 °C, they were centrifuged for 2 min in an Eppendorf centrifuge, and the radioactivity in the two phases was measured. The amount of radioactivity in the Triton X-114 phase, as a percentage of the total radioactivity in each sample, is given at the ordinate.

conformational change of diphtheria toxin (Blewitt *et al.* 1984, 1985). The data in Fig. 1(b) show that the salt concentration also influenced the ability of 125 I-labelled diphtheria toxin to enter the Triton phase.

The increased ability of *Pseudomonas* toxin to bind Triton at low pH could be due to neutralization of charged residues. However, in spite of the apparent lack of hydrophobic stretches in the molecule (Allured *et al.*, 1986), it is possible that a conformational change could confer hydrophobic properties on the molecule. As discussed by Blewitt *et al.* (1984), neutralization of carboxy groups should occur at a lower pH value in the presence than in the absence of salt (Perrin & Demsey, 1974).

This, together with our findings, suggests that in the case of *Pseudomonas* toxin also there is a conformational change at low pH. Our data are consistent with the findings by Farahbakhsh *et al.* (1986) and Zalman & Wisniewski (1985), who showed that *Pseudomonas* toxin enters artificial lipid membranes at pH 4, as measured by the ability of photoreactive probes to label the toxin.

The pH required to induce binding of Triton X-114 to *Pseudomonas* toxin seems to be slightly lower than the pH in endosomes (Tycko & Maxfield, 1982), and clearly lower than that required for entry of diphtheria toxin into the detergent phase. However, a number of factors, such as the lipid composition of the membrane (Chung & London, 1987), binding to cell-surface receptors (Madhus *et al.*, 1984) and temperature (Blewitt *et al.*, 1985), can affect the pH-dependence.

Pretreatment of *Pseudomonas* toxin with urea and 2-mercaptoethanol, a method commonly used to increase the enzymic activity of the toxin (Vasil *et al.*, 1977), did not alter the ability of the toxin to enter the Triton phase. Also, incubation of the toxin at 80 °C did not alter the

pH-dependence of the entry of *Pseudomonas* toxin into the Triton phase (results not shown). This is in contrast with results obtained with diphtheria toxin, which changes conformation at pH 7.0 when incubated at 60 °C (Zhao & London, 1986).

When cells with surface-bound diphtheria toxin are exposed to low pH, toxin entry is induced, apparently directly through the surface membrane. In contrast, entry of surface-bound *Pseudomonas* toxin could not be induced even when the cells were exposed to pH 3. The normal entry of diphtheria toxin appears to occur in early endosomes. Several observations suggest that *Pseudomonas* toxin does not enter from this organelle. The fact that low temperature (19 °C) strongly inhibits the action of *Pseudomonas* toxin, but not that of diphtheria toxin, suggests that fusion with a different intracellular compartment is required for entry of *Pseudomonas* toxin. In fact, this toxin was observed in the Golgi region (Morris & Saelinger, 1986). Furthermore, cycloheximide sensitizes cells to *Pseudomonas* toxin (Sandvig *et al.*, 1986), possibly by preventing newly synthesized proteins from entering the Golgi apparatus, where they may compete with *Pseudomonas* toxin for transport or processing. Also, low concentrations of monensin (0.1 μ M) that do not affect the pH in endosomes (Marsh *et al.*, 1982), but are known to inhibit processes in trans Golgi apparatus (Tartakoff, 1983), efficiently protect against *Pseudomonas* toxin (Sundan *et al.*, 1984). It is conceivable that structural alterations of *Pseudomonas* toxin that may occur during the intracellular transport could change the pH requirement for the acquisition of amphiphilic properties.

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