Mini-Review

ADP-ribosylation of Actin by Clostridial Toxins

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A number of clostridial bacteria produce toxins which ADP-ribosylate monomeric actin. These include botulinum C2 toxin (2), *Clostridium perfringens* iota toxin (23, 26), *Clostridium spiroforme* toxin (20, 27), and an ADP-ribosyltransferase produced by *Clostridium difficile* (21). The toxins have turned out to be valuable tools for investigating the actin cytoskeleton since ADP-ribosylation of actin blocks its polymerization. Furthermore, ADP-ribosylated actin acts as a capping protein that binds to the fast-growing barbed end of polymerized F-actin to further inhibit polymerization of nonmodified actin. This class of newly isolated toxins can be used to investigate the dynamic turnover of G- and F-actin in living cells. The best studied example of these new toxins is botulinum C2 toxin, which is described in this review in more detail.

Botulinum C2 Toxin

Botulinum C2 toxin, which is produced by various strains of *Clostridium botulinum* types C and D, has been purified to apparent homogeneity (16). The binary toxin comprises two nonlinked subunits, component I and II, whose pharmacological actions depend on their functional communication (8, 10–12, 14, 15, 17, 22).

Component II of botulinum C2 toxin has a molecular mass of ∼100 kD on SDS-PAGE (16) and is responsible for the binding of the toxin to the eukaryotic cell surface (14). This component has to be activated by trypsin treatment to produce a binding site for component I (13), an ∼72-kD proteolytic fragment. It has been reported that this activated component II forms an oligomeric structure with a molecular mass of 365 kD having both hemagglutinating and hemolytic activities. The monomer possesses only hemagglutinating activity (13). So far, the nature of the cell surface receptor for component II and the mechanism of cell entry of component I is not known.

The 50-kD component I of botulinum C2 toxin possesses ADP-ribosylating activity (25). The protein substrate is nonmuscle actin (1, 2). The modification on actin is due to mono-ADP-ribosylation since no increase in the molecular mass of labeled protein is observed during ADP-ribosylation and phosphodiesterase treatment of [^{32}P]ADP-ribosylated actin releases labeled 5'-AMP. The K_m of the ADP-ribosylation reaction for NAD is 1–5 μM (1) and the pH optimum is between pH 7 and 8 using homopolyarginine as the substrate (28). As found for other bacterial ADP-ribosyltransferases, botulinum C2 toxin possesses glycohydrolase activity and splits NAD into ADP-ribose and nicotinamide.

Other Actin–ADP-ribosylating Toxins

In addition to botulinum C2 toxin, *C. perfringens* iota toxin (23, 26), *C. spiroforme* toxin (20, 27), and an ADP-ribosyltransferase from *C. difficile* (21) (distinct from *C. difficile* toxins A and B) also ADP-ribosylate actin. These "iota-like" toxins are binary in structure and are immunologically closely related to each other representing a subfamily of actin–ADP-ribosylating toxins (27, 30). Botulinum C2 toxin is not recognized by antibodies raised against these toxins. Moreover, the binding components of the iota-like toxins can substitute for each other in their transport ability, whereas a botulinum C2 toxin binding component cannot be interchanged with the other toxins (27). Recently, a cell line has been described which is apparently sensitive to botulinum C2 toxin but not to iota-like toxins, suggesting that different cell surface receptors are involved (37).

ADP-ribosylation of Actin by Botulinum C2 Toxin

Both isoforms of nonmuscle actin (β and γ) are good substrates for C2 toxin (33). Skeletal muscle actin (α) is modified by the toxin only to a limited extent (2). Furthermore, the substrate of botulinum C2 toxin and of the other toxins is monomeric G-actin, not polymerized F-actin. For unknown reasons, the ability of purified actin to accept ADP-ribosylation declines during storage; and whereas 0.9 mol ADP-ribose per mole of protein can be incorporated into freshly prepared actin, this rapidly decreases with time. 2-wk-old actin is usually not more than 15–30% ADP-ribosylated.

Botulinum C2 toxin ADP-ribosylates nonmuscle actin on Arg-177 (33). Since skeletal muscle actin also has Arg at residue 177 (31), it has been suggested that the preceding amino acid (methionine in skeletal muscle actin; leucine in nonmuscle actins) is important for ADP-ribosylation. However, *C. perfringens* iota toxin modifies Arg-177 in skeletal muscle actin also (32). Thus, the specificity of ADP-ribosylation depends not only on the type of actin isoform but also on the toxin used.

ADP-ribosylated Actin Behaves Like a Capping Protein

Several findings indicate that ADP-ribosylation of actin blocks its ability to polymerize. Botulinum C2 toxin reduces the viscosity of polymerized liver actin as measured by the falling ball device (2). Electron microscopy supports the view that the toxin drastically decreases actin polymerization and formation of filaments (1). More recent investigations in-
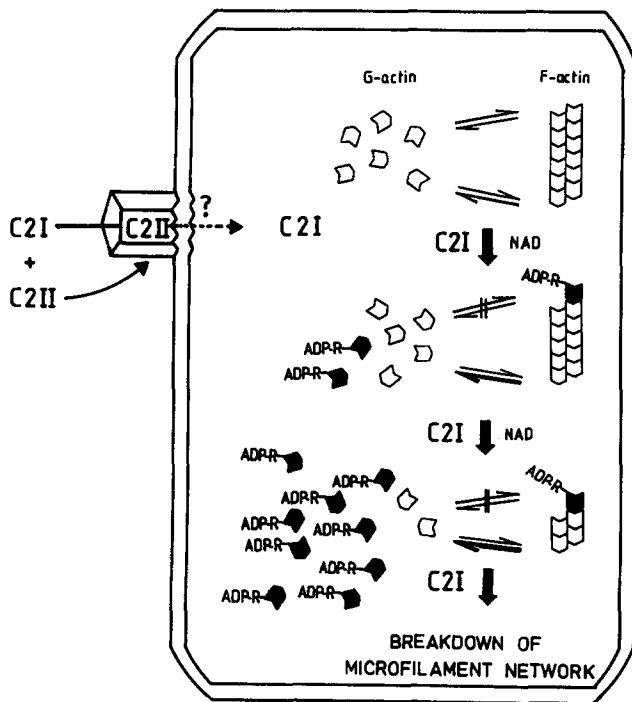
Figure 1. Model of the action of botulinum C2 toxin on the microfilament network of cells. By an unknown mechanism, component II (*C2II*) of the binary botulinum C2 toxin transfers the ADP-ribosylating component I (*C2I*) into the cell. ADP-ribosylation by the toxin turns actin into a capping protein, which binds to the barbed end of filaments to inhibit polymerization. The actin concentration is increased by depolymerization at the pointed ends. G-actin monomers released are substrates of the toxin. As ADP-ribosylated actin loses its ability to polymerize, it will accumulate, which finally causes breakdown of the microfilament network.

dicate that ADP-ribosylated actin inhibits polymerization of unmodified actin like a capping protein (34, 36). However, ADP-ribosylated actin does not influence elongation at the pointed ends of actin filaments that are capped at the barbed ends by gelsolin (34). These findings indicate that ADP-ribosylated actin acts like a barbed end-capping protein. Accordingly, ADP-ribosylated actin increases the critical concentration for actin polymerization from the low value characteristic of the barbed end to the high critical concentration characteristic of the pointed ends (18, 35). In contrast to gelsolin, ADP-ribosylated actin did not act as a nucleus for formation of new actin filaments. The equilibrium constant K for the binding of ADP-ribosylated actin to the barbed end of actin filaments is $\sim 10^8$ M^{-1} (34). Monomeric actin ADP-ribosylated by botulinum C2 toxin or iota toxin also has reduced ATPase activity (6).

Botulinum C2 Toxin Is a Cytotoxin

Injection of both components of botulinum C2 toxin (12 μg) into the intestinal loop of mice causes fluid accumulation after a delay of 1–2 h (11). The toxic effect is increased by trypsin treatment of component II. Morphological changes after toxin application indicate induction of an acute inflammation (15). Cytoplasmic vacuolation, desquamation, and finally necrosis of epithelial cells and of the lamina propria occurs. Botulinum C2 toxin increases the vascular permeability (12,

24), an effect observed even when each component of the toxin was applied at different times and at separate locations.

Botulinum C2 toxin causes rounding-up of a variety of types of cells, although cells vary in their sensitivity. Vero cells round-up at 0.5 ng/ml botulinum C2 toxin while FL (human amnion) cells required concentrations 200 times higher (17). As in studies with ligated intestinal loops, rounding begins in ~ 1 h, followed by lysis after 24–48 h (17). Y-1 adrenal cells were shown to withstand the toxin for at least 24 h, although morphological changes occurred (37).

A Model for the Cytotoxic Effects of Botulinum C2 Toxin

For the pathophysiological relevance of actin ADP-ribosylation, it was particularly important to establish that modification of actin occurred in intact cells. Treatment of intact cells with botulinum C2 toxin or with other actin-modifying toxins reduced or blocked subsequent [^{32}P]ADP-ribosylation of actin in cell lysates (2, 22). Rounding-up of chicken cells correlated with toxin-induced ADP-ribosylation of actin in a time- and concentration-dependent manner. In ^{32}P-loaded chicken cells botulinum C2 toxin labeled a 43-kD protein, which was identified as actin by binding to a DNase I column and subsequent protease mapping (22). ADP-ribosylated actin was exclusively found in Triton-soluble but not in Triton-insoluble fractions, suggesting that G-actin but not F-actin is the substrate of the toxin in intact cells (22).

Rounding-up induced by botulinum C2 toxin is associated with destruction of the microfilament network of intact cells (22). After treatment of chicken embryo cells for 3 h with botulinum C2 toxin, stress fibers became condensed, shortened, and broken. After 6 h of toxin treatment, stress fibers mostly vanished and heaps of material amorphously stained by fluorescein–phalloidin appeared. At this stage at least 95% of cells were alive as determined by trypan blue exclusion. The G-actin content of toxin-treated fibroblasts increased concomitantly with ADP-ribosylation of actin (3, 22). Together these findings indicate that botulinum C2 toxin-induced treatment of intact cells causes depolymerization of actin filaments.

From the data available, the following model for the cytotoxic effects of actin ADP-ribosylating toxins has been proposed (Fig. 1). It is generally accepted that in nonmuscle cells monomeric and polymeric actin are in dynamic equilibrium and their levels are regulated by the transition between G- and F-actin and by actin-binding proteins (7, 19). Botulinum C2 toxin and other actin-modifying toxins apparently interfere with this equilibrium by ADP-ribosylation of actin. The modified actin caps the barbed end of actin filaments thereby blocking further polymerization at this end of the filaments, while the pointed ends continue depolymerizing. The cellular pool of G-actin, which serves as substrate for botulinum C2 toxin, increases. As ADP-ribosylated actin is not capable of polymerization, the toxin traps actin in its monomeric form, which is no longer available for formation of microfilament networks. All these events finally cause destruction of the microfilament network.

ADP-ribosylating Toxins Are Tools to Study the Function of Actin

Treatment of neutrophils with the toxin blocked random and

chemotactic agent-induced migration (10), in line with the view that polymerization of actin is involved in cell movement (29). Like cytochalasin treatment, toxin treatment increased stimulated, but not basal, superoxide anion production (4, 10), secretion of N-acetylglucosamine and of vitamin B_{12}-binding protein from human neutrophils (10). Treatment of PC12 cells at 20°C with botulinum C2 toxin increased the [^{3}H]noradrenaline release stimulated by carbachol, K$^+$ depolarization, and by ionophore A 23187 1.5–3-fold (8). Concomitantly, actin was ADP-ribosylated. Toxin treatment at 37°C showed a biphasic effect on noradrenaline release from PC12 cells which was interpreted as an involvement of actin in secretion at more than one site. In Y-1 adrenal cells, botulinum C2 toxin increased steroid secretion about twofold (37). Böttinger et al. (5) reported the inhibition of histamine release from mast cells after treatment with botulinum C2 toxin.

Recently, it has been found that botulinum C2 toxin inhibits electrically stimulated contractions of guinea pig ileum longitudinal smooth muscle preparations (9). This effect was most likely caused by a direct toxin action on smooth muscle cells, because Ba^{2+}-induced contractions were also inhibited by botulinum C2 toxin. As botulinum C2 toxin ADP-ribosylates G-actin but not F-actin, the latter studies indicate that monomeric actin is relevant for smooth muscle function and indicate a dynamic transition between G- and F-actin, also in smooth muscle cells. The increasing number of studies suggest that actin–ADP-ribosylating toxins are powerful tools for investigating the cellular functions of actin.

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