ADP-Ribosylation of Rainbow Trout (Onchorhynchus mykiss) Actin by Botulinum C2 Toxin

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Intracellular actin of rainbow trout macrophages was ADP-ribosylated by botulinum C2 toxin, which is composed of two nonlinked protein components, component I and trypsinized component II. The actin in the supernatants of various tissue homogenates of the trout was also directly ADP-ribosylated by component I of C2 toxin, indicating that fish actin other than those of land vertebrates is susceptible to enzymatic modification by component I of C2 toxin.

We have reported that component I of botulinum C2 toxin (C2T) ADP-ribosylates nonmuscle (β/γ-actins), but not muscle (α-actin), actin monomers, inactivates the actin to interact with unmodified actin, and subsequently inhibits the polymerization of actin within the cells (10). This enables the toxin to cause cellular damage to both cultured cells and whole animals (6, 7, 9), because the cytoplasmic actin is widely distributed in most cells. However, these findings have been obtained with actin from mammalian cells. The modification of only nonmuscle actin by C2T indicates that component I of the toxin recognizes the structural difference between α- and β-isoforms in mammalian cells, although the difference in primary structure of these actins is only 6.4% and the amino acid residue at Arg-177, which is the target site for modification of β-actin, is common to both isoforms (13). These facts indicate that component I of C2T recognizes a difference in the configuration around the target site between mammalian and fish actins. In the present study, we investigated fish actin, the β-actin of which differs from mammalian β-actin by 1% in amino acid sequence (5), and attempted to see whether actin other than that of land vertebrates is ADP-ribosylated by component I of C2T.

Components I and II were purified from the culture fluid of Clostridium botulinum type C strain 92-13 as described previously (9). Trypsinized component II was prepared as described previously (8). α-32P-NAD (29.6 TBq/mmol), purchased from NEN Research Products, was diluted with 1 M imidazole-HCl buffer containing 0.1 mM CaCl2, 0.5 mM ATP, 0.5 mM dithiothreitol, and 0.8 mM sodium azide (pH 7.5) (buffer A). Rainbow trout (Onchorhynchus mykiss) was obtained from a commercial farm. To determine whether C2T ADP-ribosylates intracellular actin in trout macrophages, cells of trout head kidneys were cultured in Eagle’s minimum essential medium containing 10% fetal calf serum and 5% rainbow trout serum, as described previously by Kodama and coworkers (2,3). Protein was determined by the method of Bradford (1), with bovine serum albumin as the standard.

To see whether the actin in trout tissues is ADP-ribosylated by component I of C2T, supernatant fractions were prepared by homogenization (10%, wt/vol) in buffer A and then centrifuged at 13,000 × g for 15 min. Fractions containing 100 μg of protein were incubated for 30 min at 37°C with 1 μg of component I and 100 nmol of α-32P-NAD in a total volume of 60 μl of buffer A. The reaction mixtures were electrophoresed on a 10% acrylamide slab gel by the method of Laemmli (4), and then the dried gel was exposed to Fuji RX film for 2 days at −80°C. The molecular size of the radiolabeled band was estimated by using molecular weight markers (Daichi Chemical Co., Tokyo, Japan). The autoradiogram shows a radiolabeled protein band of 46 kDa with the supernatant fractions of various trout tissues, except muscle tissue (Fig. 1A). The results indicate that the trout actin in these tissues is modified by component I, whereas that in muscle tissue is not.

The ADP-ribosylation of intracellular actin of trout macrophages, which are rich in actin, was examined by exposing the cells at 20°C to 300 ng of C2T, a mixture of 150 ng of component I plus 150 ng of trypsinned component II, in 150 μl of minimum essential medium-bovine serum albumin per well of a 96-well plastic plate. After the first incubation, cell lysate prepared in buffer A was again incubated at 37°C for 30 min with 1 μg of component I in the presence of 100 nmol of α-32P-NAD in a total volume of 100 μl of buffer A (11). Figure 1B shows that a 46-kDa protein in the lysate was ADP-ribosylated, although ADP-ribosylation of the protein in the cells that had been exposed first to the toxin decreased depending on the incubation period. This indicates that the intracellular actin of the cells is ADP-ribosylated by component I of the toxin, which enters the cells through the binding site induced by trypsinned component II on the cell membrane (12).

To examine the direct ADP-ribosylation of actin in trout macrophages, cells cultured in plastic dishes were suspended in buffer A, and lysate containing 100 μg of protein was incubated at 37°C for 30 min with 1 μg of component I and 100 nmol of α-32P-NAD in 60 μl of buffer A. The actin band in trout macrophage lysate, as well as that of porcine brain, was ADP-ribosylated by component I of C2T (Fig. 1C).

Actin is highly conserved protein in evolution. In land vertebrates, actin is classified into at least six isoforms. Four muscle-type actins participate in muscle contraction, whereas cytoplasmic actins participate in a variety of cellular functions. Recently, Lie et al. reported that the fish actin gene is 99% conserved at amino acid levels with mammalian β-actin and that the amino acid residue of the actin at position 177 is arginine (5). The fact that trout cytoplasmic actin is susceptible to enzymatic modification by component

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FIG. 1. (A) ADP-ribosylation of actin in the supernatant fraction of trout tissue homogenates by component I; lanes: 1, brain; 2, muscle; 3, kidney; 4, head kidney; 5, heart; 6, spleen. Arrowheads indicate (a) top of the gel, (b) β-galactosidase (116 kDa), (c) bovine serum albumin (66 kDa), (d) aldolase (42 kDa), and (e) carbonic anhydrase (30 kDa). (B) Intracellular ADP-ribosylation of trout macrophages by C2T. The cells were first exposed to the toxin for 60 (lane 1), 30 (lane 2), 15 (lane 3), or 60 (with no toxin; lane 4) min. (C) ADP-ribosylation of trout actin in the macrophage lysate by component I. Lanes: 1 and 3, lysate and purified porcine brain actin were incubated in the presence of component I and α-32P-NAD, respectively; 2 and 4, lysate and porcine brain actin were incubated in the presence of NAD and the absence of component I, respectively.

I of C2T suggests that the molecular configuration around the target site for modification differs between fish actins in muscle and nonmuscle cells, whereas it is very similar between cytoplasmic actins in fish and mammalian cells.

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