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Novel Macrolide Actin-inhibitors Isolated from Sea Sponges

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ABSTRTACT : *Several marine toxins with macrolide structure have been found to act on actin. One of these toxins is mycalolide B isolated from the genus Mycale. This compound belongs to macrolide antibiotics and consists of tris-oxazole with strong cytotoxic activity (IC_{50}: 10~50 nM for growth of L1210 murine leukemia cells). This compound was found to be an actin-depolymerizing agent with the mode of action distinct from that of the known actin inhibitor, cytochalasin D. Tolytoxin, a macrolide isolated from cyanobacteria with similar chemical structure to mycalolide B, seems to have similar effect. Another macrolide compound, aplyronine A, showed the effects similar to those of mycalolide B. Although bistheonellide A, a dimeric macrolide, did not show a severing effect, it depolymerized F-actin and sequestered G-actin by forming 1 : 2 complex with G-actins. Swinholide A has a structure and effects similar to those of bistheonellide A. In conclusion, mycalolide B, tolytoxin, aplyronine A, bistheonellide A and swinholide A are the members of "actin depolymerizing macrolide" the mechanism of which is different from that of cytochalasin D.*

Key Words : *Marine toxin, Actin depolymerizing toxin, Mycalolide B, Aplyronine A, Bistheonellide A, Swinholide A*

I. INHIBITORS OF ACTIN POLYMERIZATION

Actin is a component of cytoskeleton and regulates various cell functions including cell motility, cell division and muscle contraction. Actin is regulated by actin-binding proteins (Stossel, 1989). "Severing" proteins, including gelsolin and villin, block the barbed ends of actin filaments and enhance nucleation. Depactin, actophorin, destrin and actokinin are categorized as "nibbling" proteins, which sever F-actin and bind to G-actin in stoichiometry with no capping activity nor enhancement of nucleation. Profilin, which controls monomer polymerizability (Stossel, 1989), binds only to G-actin and decreases polymerization rate by sequestering G-actin (Pollard and Cooper, 1984).

Before the finding of macrolide actin inhibitors, cytochalasins, a group of fungal metabolites, are the only non-protein inhibitors of actin polymerization we had. These compounds serve as actin capping substances which bind to the barbed end of actin filaments and shift the polymerization-depolymerization equilibrium toward net depolymerization of F-actin (Cooper, 1987; Sampath and Pollard, 1991). Cytochalasin D decreases the lag phase by accelerating the nucleation (Howard and Lin, 1979). Therefore cytochalasin D acts on actin as "end blocking agent" like capping proteins such as alpha-actinin. Cytochalasin D also decreases the final level of polymerization. However, cytochalasin D is not able to completely inhibit the polymerization; actin partially remains in a polymerized state in the presence of cytochalasin D. The severing effect of cytochalasin is still controversial (Cooper, 1991).

II. ACTIN-DEPOLYMERIZING MACROLIDE

Mycalolide B was isolated by Fusetani *et al.* (1989) from the marine sponge Mycale sp. from the Bay of Gokasho, Kii Peninsula, Japan. This compound has the antifungal and cytotoxic effects (Fusetani *et al.*, 1989). Because this compound also inhibits Mg^{2+}-ATPase activity of native actomyosin prepared from chicken gizzard smooth muscle and inhibits smooth muscle contraction, Hori *et al.* (1993) suggested that mycalolide B acts directly on either actin or myosin molecules.

This possibility was examined by Saito *et al.* (1994)

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by monitoring the effect of mycalolide B on actin polymerization using fluorescent pyrenyl-actin. It has been shown that actin is polymerized in the presence of Mg^{2+} and K^{+}. Addition of Mg^{2+} polymerized G-actin after a lag phase. The lag phase is due to formation of actin dimers or trimers (nucleation), and this is the rate-limiting step for actin polymerization (Nishida and Sakai, 1983). Saito *et al.* (1994) found that mycalolide B inhibited actin polymerization without accelerating nucleation. Higher concentrations of mycalolide B completely inhibited polymerization.

Aplyronine A is a macrolide isolated from the sea hare Aplysia kurodai from the Pacific coast of Mie Prefecture, Japan with strong cytotoxicity against HeLa-S3 cells (Yamada *et al.*, 1993). Bistheonellide A is a dimeric macrolide isolated from marine sponge, *Theonella* sp., found in the Hachijo-jima Island, Japan (Kato *et al.*, 1987). This compound has a cytotoxicity against L1210 cells at nanomolar concentrations (Fusetani, 1990).

Aplyronine A (Saito *et al.*, 1996) and bistheonellide A (Saito *et al.*, 1998) inhibited the actin polymerization without accelerating nucleation, as Mycalolide B did.

III. DEPOLYMERIZING EFFECTS

Saito *et al.* (1994) reported that although both mycalolide B and cytochalasin D depolymerized F-actin, the initial rate of depolymerization caused by mycalolide B (half time <1 min) was much faster than that caused by cytochalasin D (half time >13 min). To know the reason for this difference, F-actin and G-actin were previously treated with mycalolide B and used as the nucleus for G-actin to polymerize. Measuring the initial rate of polymerization, it was found that the rate was fastest with the combination of non-treated F-actin and non-treated G-actin, faster with the combination of treated F-actin and non-treated G-action, and slowest with the combination of non-treated F-actin and treated G-action. On the other hand, the final polymerization levels were almost identical. This result indicates that mycalolide B has dual effects; to sever F-actin and increase the number of F-actin (or to increase nucleation), and to bind to G-actin and antagonize the polymerization.

The effect of mycalolide B to sever F-actin was confirmed using the dilution method as described by MacIver *et al.* (1991). When the pyrenyl-actin was diluted, fluorescence of action gradually decreased. This decrease is considered to be due to the net actin depolymerization from both ends of F-actin. Therefore, the rate of depolymerization should be proportional to the number of F-actin. When mycalolide B was applied to F-actin simultaneously with dilution, the initial depolymerization rate was greatly increased, suggesting that mycalolide B severed F-actin.

Aplyronine A showed similar effects to mycalolide B, suggesting that aplyronine A also severs F-actin (Saito *et al.*, 1996). In contrast, bistheonellide A did not increase the rate of depolymerization suggesting the absence of severing effect (Saito *et al.*, 1998).

Patterson *et al.* (1993) demonstrated that tolytoxin, a macrolide isolated from cyanobacteria with similar chemical structure to mycalolide B (Ishibashi *et al.*, 1986; Fusetani, 1987), inhibits actin polymerization and disrupts microfilaments but not microtubules of intermediate filaments conformation in A10 cells. However, the precise mechanism of its action on actin has not yet been revealed.

IV. STOICHIOMETRY

Since the intensity of pyrenyl-actin fluorescence correlated with the concentration of F-actin, there is a positive correlation between total concentration of actin and total concentration of F-action (or fluorescence). In the presence of 5 mM mycalolide B, the relationship between the total actin concentration and F-actin concentration (or fluorescence) was shifted in a parallel manner to the right by 5 mM of actin. Increase in the concentration of mycalolide B to 10 mM shifted the relationship to the right by another 5 mM of actin, suggesting the increase of the critical concentration (Saito *et al.*, 1994).

Aplyronine A showed similar effect to mycalolide B (Saito *et al.*, 1996). Similar results have been reported for Acanthamoeba profilin (Lal and Korn, 1985), which sequesters G-actin and inhibits polymerization.

These results indicate that mycalolide B and aplyronine A bind to G-actin in a 1 : 1 molar ratio and prevent the incorporation of G-actin into filaments.

On the other hand, the relationship between bistheonellide A concentration and the effects on polymer-

ization or depolymerization indicated that one molar of bistheonellide A binds with two molar of G-actins (Saito *et al.*, 1998). These results indicate that bistheonellide A is a "monomer sequestering agent" with no effect on nucleation or severing. However, supramaximal concentration of bistheonellide A (more than twice a concentration of actin) slightly increased the rate of depolymerization, suggesting that bistheonellide A may have weak severing activity.

Bubb *et al.* (1995) showed that swinholide A, a dimeric macrolide with a similar structure to that of bistheonellide A (Kato *et al.*, 1987; Carmeli and Kashman, 1985), severed F-actin and bond to G-actin by an 1 : 2 molar ratio.

V. EFFECTS ON ACTIN-ACTIVATED MYOSIN Mg^{2+}-ATPASE ACTIVITY

Myosin possesses a low level of Mg^{2+}-ATPase activity which is enhanced by F-actin (Offer *et al.*, 1972). Mycalolide B had no effect on the basal myosin Mg^{2+}-ATPase activity in the absence of F-actin. However, mycalolide B completely inhibited the myosin Mg^{2+}-ATPase activity activated by actin. Mycalolide B also inhibited the acto-S1 ATPase activity in a concentration-dependent manner and the maximum inhibition was obtained when its molar ratio to actin was higher than one. Saito *et al.* (1984) also confirmed that mycalolide B decreased viscosity of F-actin in the presence of S1, suggesting that mycalolide B depolymerizes actin in the presence of myosin and this may be the mechanism of inhibition of actin activation of myosin ATPase activity.

In contrast, cytochalasin D did not inhibit actin-activated myosin Mg^{2+}-ATPase activity (Saito *et al.* 1984). Lack of the inhibitory effect of cytochalasin D on actin-activated myosin-ATPase activity may be attributable to the inability of cytochalasin D to completely depolymerize F-actin. Short length of F-actin produced by cytochalasin D may still activate myosin Mg^{2+}-ATPase activity (Offer *et al.*, 1972).

VI. OTHER EFFECTS

Saito *et al.* (1984) confirmed that mycalolide B had no effect on the polymerization of tubulin isolated from swine brain, the Mg^{2+}-ATPase of skeletal muscle myosin in the absence of actin, the phosphorylation of smooth muscle myosin regulatory light chain, or agonist-induced increase in cytosolic Ca^{2+} concentrations in smooth muscle (Hori *et al.*, 1993). These results suggest that mycalolide B selectively acts to depolymerize F-action without changing other mechanisms including tubulin, Mg^{2+}-ATPase, myosin light chain kinase and Ca^{2+} channels.

VII. APPLICATION OF NOVEL ACTIN INHIBITORS TO ACTIN BIOLOGY

Mycalolide B is now commercially available and is widely used as a pharmacological tool. Sugidachi *et al.* (1998) demonstrated that mycalolide B and cytochalasin D inhibit platelet aggregation in a concentration dependent manner. Using DNase I inhibition assay, they confirmed that mycalolide B has a stronger effect than cytochalasin D to increase G-actin content in the activated platelet. They also found that cytochalasin D, but not mycalolide B, failed to increase G-actin at resting state, suggesting there are cytochalasin D-susceptible and unsusceptible actin assemblies in the platelet cells.

Kojima *et al.* (1999) recently reported that only mycalolide B disrupts circumferential actin filament in rat hepatocytes, and revealed the importance of association of actin with occuldin in the formation of tight junctions. Others (Fujii *et al.*, 1997; Hirasawa *et al.*, 1998) also used mycalolide B as a potent and alternative inhibitor of actin polymerization.

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