Goniodomin A Induces Modulation of Actomyosin ATPase Activity Mediated through Conformational Change of Actin*

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Goniodomin A, one of the polyether macrolides, isolated from the dinoflagellate Goniodoma pseudogonialax stimulated skeletal actomyosin ATPase activity 2–3 times, with a peak at 3 × 10⁻⁶ to 3 × 10⁻⁷ M. The effect of goniodomin A was dependent on the concentration of actin, but not of myosin. The actomyosin ATPase activity was increased by pretreatment of actin (but not of myosin) with goniodomin A. Goniodomin A induced a remarkable but transient increase in fluorescence intensity (excitation wavelength, 277 nm; emission wavelength, 329 nm) of actin. The maximum response was obtained with concentrations of goniodomin A in the 10⁻⁵ to 10⁻⁴ M range in the presence of 5 μM F-actin. However, the ATPase activity and fluorescence intensity of myosin were not changed by goniodomin A at concentrations from 10⁻⁶ to 10⁻³ M. Interestingly, goniodomin A induced a remarkable but transient increase in the fluorescence intensity of actomyosin in a concretion-dependent manner, with a peak at 3 × 10⁻⁷ M. This profile was quite similar to that found in the stimulation of the actomyosin ATPase activity induced by goniodomin A. To investigate further the effect of goniodomin A, actin was labeled with N-(1-pyrenyl)iodoacetamide. Goniodomin A at 10⁻³ M had no effect on the fluorescence intensity of pyrenyl-actin (excitation wavelength, 385 nm; emission wavelength, 407 nm), but increasing concentrations of goniodomin A to 3 × 10⁻⁶ M remarkably decreased its intensity. This effect was potentiated by heavy meromyosin. Actin molecules treated with goniodomin A were completely sedimented by mild centrifugation (for 15 min at 12,000 × g). Electron microscopic observations suggest that actin filaments associate with each other to form a gel in the presence of 3 × 10⁻⁶ M goniodomin A. The conformational change of actin molecules, resulting from stoichiometric binding of goniodomin A to actin monomers in filaments, may modify the interaction between actin and myosin.

The force of muscle contraction is produced by the interaction between actin and myosin molecules in a process that involves cross-bridge cycling coupled with the hydrolysis of ATP (1). Actomyosin is a precise machinery for transduction of chemical energy in ATP molecules into mechanical works. From the viewpoint of enzymology, myosin is an ATPase whose activity is stimulated by the interaction with actin. There is much evidence suggesting that the conformational changes of actin and myosin are tightly linked to cross-bridge cycling (1). Although the binding sites involved in the interaction between myosin and actin molecules have been determined (2–4), the role of these conformational changes and the interactions in muscle contraction remain to be elucidated. Therefore, novel tools that provide information on conformational changes and interactions of contractile proteins will be useful.

In a survey of marine natural products that affect muscle contraction, we have found several interesting substances that modulate the actomyosin system (5–9). Goniodomin A, a polyether macrolide, was first obtained from the dinoflagellate Goniodoma pseudogonialax (see Fig. 1 for its chemical structure) as an antibiotic substance by Murakami et al. (5). There have been no reports on the pharmacological and biochemical properties of goniodomin A. In this study, we have shown for the first time that goniodomin A induces modulation of actomyosin ATPase activity mediated through conformational change of actin. Goniodomin A might become a useful tool for the investigation of the relationship between the structure and function of actin.

**EXPERIMENTAL PROCEDURES**

**Materials**—Goniodomin A was purified from the dinoflagellate G. pseudogonialax (5). Briefly, the dinoflagellate was cultivated, collected, and extracted with ethanol/dichloromethane (1:1). The extract was partitioned between water and ether. The organic layer was subjected to column chromatography on silica gel, followed by reversed-phase high pressure liquid chromatography to yield goniodomin A. Myosin was prepared from rabbit skeletal white muscle according to the method of Margossian and Lowey (10). Actin was prepared from acetone powder by the method of Spudich and Watt (11). All other reagents were of analytical grade.

**ATPase Assays**—Actomyosin ATPase reactions were carried out at 37 °C in a medium of 50 mM KCl, 2 mM MgCl₂, 2 mM EGTA, 1 mM ATP, and 20 mM HEPES/Tris buffer (pH 6.8) in the presence or absence of goniodomin A. The ATPase activity was determined from the amount of phosphate liberated, measured by the malachite green method described by Chan et al. (12).

The dilution method was employed to investigate the irreversibility of the effect of goniodomin A. Myosin (0.1 mg/ml), actin (0.1 mg/ml), or actomyosin (0.1 mg of each of actin and myosin/ml) was incubated with goniodomin A for 5 min and then diluted 10 times. The dilution medium contained fresh actin or myosin (or both) at final concentrations of 0.1 and 0.01 mg/ml, respectively. Then, the reaction of actomyosin ATPase was carried out under the same conditions as described above. Goniodomin A increased the activity of actomyosin ATPase to near-maximum levels at 3 × 10⁻⁷ M, but did not produce any effect on the activity at 3 × 10⁻⁸ M. Then, we used 3 × 10⁻⁸ M goniodomin A for preincubation.

**Fluorescence Enhancement of Actin and Myosin Induced by Goniodomin A**—The fluorescence intensity of intrinsic tryptophan residues of actin or myosin was recorded at 329 nm (excitation wavelength, 277 nm).
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RESULTS

Effect of Goniodomin A on Actomyosin ATPase Activity—The effect of goniodomin A on the skeletal actomyosin ATPase was investigated at various concentrations of actin and myosin. As shown in Fig. 2a, at concentrations $>10^{-5}$ M, the actomyosin ATPase activity increased with increasing concentrations of goniodomin A. The peak value of the response was increased by -10 times (Fig. 2b). Activities are expressed as percentages of the activity of actomyosin ATPase without the treatments described above (0.183 μmol/min/mg of myosin). Values are means ± S.D. (n = 3). ***, significantly different from vehicle, p < 0.01; *; p < 0.05.

ATPase activity increased with an increase in goniodomin A concentration and reached a peak at $10^{-7}$ M. The peak value was 2.8–3.3 times higher than that of the control. Further increasing the goniodomin A concentration decreased the activity by 20% of the control (Fig. 2a). Most of the same values were obtained with various concentrations of myosin (0.01–0.1 mg/ml). On the other hand, this effect of goniodomin A was dependent on actin concentration. When the actin concentration was increased by 4 times, the concentration of goniodomin A that induced the maximum response increased by ~10 times (Fig. 2b). Goniodomin A did not affect the Mg$^{2+}$-ATPase activity of

**Fig. 1. Chemical structure of goniodomin A.**

**Fig. 2. Concentration-dependent effects of goniodomin A on actomyosin ATPase activity.** a, the actomyosin ATPase activity with three different concentrations of myosin (0.01 (a), 0.05 (b), and 0.1 (c)) mg/ml and 0.1 mg/ml actin; b, the actomyosin ATPase activity with various concentrations of actin (0.05 (I), 0.1 (M), and 0.2 (A) mg/ml) and 0.05 mg/ml myosin. Actomyosin was preincubated with various concentrations of goniodomin A for 5 min. ATPase activities were measured by the malachite green method as described under "Experimental Procedures." ATPase activities are presented as values relative to control values obtained in the absence of goniodomin A under each condition. Control values are as follows: a, 0.485 μmol/min/mg of myosin for 0.1 mg/ml myosin, 0.381 μmol/min/mg of myosin for 0.05 mg/ml myosin, and 0.236 μmol/min/mg of myosin for 0.01 mg/ml myosin; b, 0.299 μmol/min/mg of myosin for 0.05 mg/ml actin, 0.390 μmol/min/mg of myosin for 0.1 mg/ml actin, and 0.411 μmol/min/mg of myosin for 0.2 mg/ml actin. Values are means of triplicate measurements.

**Fig. 3. Effects on actomyosin ATPase activities of preincubation of its components with goniodomin A.** Actomyosin consisting of 0.1 mg/ml actin and 0.1 mg/ml myosin alone (a), or 0.1 mg/ml actin alone (b), or 0.1 mg/ml actin alone (c) was incubated with $3 \times 10^{-5}$ M goniodomin A (shaded columns) or vehicle (hatched columns) for 5 min at room temperature. These solutions were diluted 10 times with solutions containing 0.1 mg/ml actin (a), 0.11 mg/ml actin (b), or 0.011 mg/ml myosin plus 0.1 mg/ml actin (c), respectively. The actomyosin ATPase activity was measured by the malachite green method as described under "Experimental Procedures." Activities are expressed as percentages of the activity of actomyosin ATPase without the treatments described above (0.183 μmol/min/mg of myosin). Values are means ± S.D. (n = 3). ***, significantly different from vehicle, p < 0.01; *; p < 0.05.

**Fig. 4. Fluorescence change in tryptophan residues of F-actin induced by goniodomin A.** a, fluorescence change in tryptophan residues of F-actin induced by adding $3 \times 10^{-5}$ M goniodomin A. Goniodomin A was added at the time indicated by the arrow. b, dose-dependent effect of goniodomin A on the fluorescence intensity of tryptophan of F-actin. The reaction mixture contained 0.2 mg/ml F-actin, 50 mM KCl, 2 mM MgCl$_2$, 2 mM EGTA, and 20 mM HEPES/Tris (pH 6.8). Fluorescence intensities are presented as values relative to that before the addition of goniodomin A.
The reaction mixture contained 0.1 mg/ml myosin, 0.5 mg/ml actin, 0.5 mM MgCl₂, 2 mM EGTA, and 20 mM HEPES-Tris (pH 6.8). Fluorescence intensities are presented as values relative to that before the addition of goniodomin A. These results suggest that the effect of goniodomin A on the fluorescence intensity of tryptophan of myosin induced by goniodomin A was transient (Fig. 6a). The fluorescence intensity increased, reached a peak level at ~25 s, and decreased toward the control level. As shown in Fig. 6b, the peak level increased with increasing concentrations of goniodomin A. The fluorescence intensity decreased with further increases in concentration. This profile was similar to that found in the activation of the actomyosin ATPase activity by goniodomin A (cf. Fig. 2a and b). These results suggest that the site of action of goniodomin A is actin, but this effect is further modified in the presence of myosin.

Effects of Goniodomin A on Fluorescence Intensity of Pyrenyl-F-Actin—To analyze further the effect of goniodomin A, actin was labeled with N-(1-pyrenyl)iodoacetamide as a reporter of conformational changes of actin molecules. As seen in Fig. 7, goniodomin A at concentrations >10⁻⁶ M greatly decreased the fluorescence intensity of pyrenyl-F-actin. At 0.1 mg/ml F-actin, the 50 and 100% inhibitory concentrations of goniodomin A were 2.5 × 10⁻⁶ and 3 × 10⁻⁶ M, respectively, whereas at 0.2 mg/ml F-actin, the values were 5 × 10⁻⁶ and 6 × 10⁻⁶ M, respectively. In the presence of 10⁻⁶ M goniodomin A, the fluorescence intensity did not change at 0.2 mg/ml F-actin, whereas it slightly but significantly dropped at 0.1 mg/ml F-actin. These data suggest that the effect of goniodomin A on F-actin is caused by its stoichiometric binding to actin molecules.

Effects of Goniodomin A on Fluorescence Intensity of Tryptophan in Contractile Proteins—The fluorescence of intrinsic tryptophan has provided useful information on conformational changes of contractile protein molecules (16). Addition of goniodomin A to an actin solution caused some increase in the intensity of its fluorescence (Fig. 4a). The increase reached a plateau within 50 s and was maintained for at least several minutes. The fluorescence of actin increased with an increase in goniodomin A concentrations from 10⁻⁷ to 3 × 10⁻⁵ M (Fig. 4b). The increase in the fluorescence reached a plateau at ~10 μM goniodomin A. The concentration of actin was 0.2 mg/ml (4.76 μM). Therefore, it was suggested that goniodomin A stoichiometrically binds to actin. The fluorescence of myosin, however, was not changed by adding goniodomin A at concentrations between 3 × 10⁻⁸ and 3 × 10⁻⁶ M (Fig. 5, a and b). In the case of actomyosin, the aspect of its fluorescence change was quite different. The increase in the fluorescence intensity induced by goniodomin A was transient (Fig. 6a). The fluorescence intensity increased, reached a peak level in ~25 s, and decreased toward the control level. This profile was similar to that found in the activation of the actomyosin ATPase activity by goniodomin A (cf. Fig. 2a and b). These results suggest that the site of action of goniodomin A is actin, but this effect is further modified in the presence of myosin.
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**Effect of Goniodomin A on Polymerization of Pyrenyl-Actin**

Polymerization of pyrenyl-actin in the presence of goniodomin A was monitored by fluorescence intensity measurement. The polymerization was slightly but definitely accelerated by goniodomin A at $10^{-6} \text{ M}$ during the initial period, but the level at full polymerization was somewhat lower (Fig. 9, spectrum b) than that of the control (spectrum a). This decrease was observed in the same concentration range of goniodomin A as that observed in polymerized F-actin (Fig. 7). Not only the rate, but also the level of polymerization decreased in the presence of $2 \times 10^{-6} \text{ M}$ goniodomin A (Fig. 9, spectrum c), and further increasing the concentration of goniodomin A to $3-5 \times 10^{-6} \text{ M}$ completely inhibited the polymerization of actin (Fig. 9, spectra d and e).

Electron Microscopy of Actin Filaments under Influence of Goniodomin A—The measurements of the fluorescence intensity of pyrenyl-actin suggest that F-actin is not formed under the influence of a sufficient concentration of goniodomin A (see Figs. 7 and 9). Unexpectedly, an F-actin network was observed under an electron microscope (Fig. 10A). Higher magnifications clearly demonstrated an association of actin filaments to form a meshwork (Fig. 10B). Although a fine structure was not clear, the network was formed by entanglement of side-by-side associated actin filaments consisting of two to five actin filaments in the presence of $3 \times 10^{-6} \text{ M}$ goniodomin A. This means that goniodomin A did not depolymerize pyrenyl-actin filaments, although the fluorescence intensity was almost reduced to the level of actin monomers.

A sedimentation experiment was carried out to prove the above observations. Any F-actin is not sedimentable by centrifugation for 15 min at $12,000 \times g$ (Fig. 11a). This was true even in the presence of $10^{-5} \text{ M}$ goniodomin A (Fig. 11b). On the other hand, ~60% of F-actin was precipitated by this mild centrifugation at $10^{-6} \text{ M}$ (Fig. 11c), and >95% of F-actin was sedimented to confirm network formation at $3 \times 10^{-6} \text{ M}$ (Fig. 11d). These observations confirm that actin was polymerized into filaments even in the presence of $3 \times 10^{-6} \text{ M}$ goniodomin A.

Fluorescence Intensity of Acto-H-meromyosin under Influence of Goniodomin A—Pyrenyl-actin (0.1 mg/ml) was polymerized together with H-meromyosin (0.2 mg/ml) for 12 h at 25 °C in the presence of 50 mM KCl, 2 mM MgCl$_2$, and 2 mM Tris-HCl (pH 8.0), and then goniodomin A was added to the solution. The fluorescence intensity spectra shown in Fig. 12 (a and b) revealed that there were significant decreases in acto-H-meromyosin as compared to pyrenyl-F-actin alone (Fig. 8, a and b, spectrum 1). Addition of $5 \times 10^{-7}$ or $7 \times 10^{-7} \text{ M}$ goniodomin A resulted in a marked drop (Fig. 12, a and b, spectra 3 and 4). Goniodomin A, even at $10^{-6} \text{ M}$, hardly affected the fluorescence intensity of pyrenyl-F-actin alone (see Figs. 7 and 8); however, the same concentration of goniodomin A markedly decreased the fluorescence intensity of acto-H-meromyosin (Fig. 12, a and b, spectrum 5). In addition, goniodomin A ($10^{-6} \text{ M}$) did not dissociate myosin from F-actin under both low (50 mM) and high (0.6 M) ionic strength conditions in the sedimentation experiment, suggesting a stable goniodomin A-actin-myosin complex.

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**Fig. 7. Effects of varied concentration of goniodomin A on fluorescence intensity of pyrenyl-F-actin.** G-actin was polymerized for 3 h at 25 °C in the presence of 50 mM KCl, 2 mM MgCl$_2$, and 2 mM Tris-HCl (pH 8.0), and then, various concentrations of goniodomin A were added. Excitation wavelength was at 365 nm, and emission wavelength was at 407 nm. The polymerization was slightly but definitely accelerated by goniodomin A at $10^{-6} \text{ M}$.

**Fig. 8. Effects of goniodomin A on fluorescence intensity of pyrenyl-actin at varied excitation and emission wavelengths.** Conditions were as described for Fig. 7, except that F-actin concentration was 0.1 mg/ml, a, excitation wavelength, 265 nm; b, emission wavelength, 407 nm. The arrow indicates intensity excitation wavelength at 365 nm. Spectrum 1, F-actin alone; spectrum 2, $10^{-6} \text{ M}$ goniodomin A; spectrum 3, $2 \times 10^{-6} \text{ M}$; spectrum 4, $3 \times 10^{-6} \text{ M}$; spectrum 5, $5 \times 10^{-6} \text{ M}$; spectrum 6, G-actin alone.

**Fig. 9. Effects of goniodomin A on polymerization process of G-actin.** Polymerization of actin was started by the addition of 50 mM KCl and 2 mM MgCl$_2$ at 25 °C and pH 8.0. Excitation wavelength was at 365 nm, and emission wavelength was at 407 nm. Spectrum a, G-actin alone; spectrum b, $10^{-6} \text{ M}$ goniodomin A; spectrum c, $2 \times 10^{-6} \text{ M}$; spectrum d, $3 \times 10^{-6} \text{ M}$; spectrum e, $5 \times 10^{-6} \text{ M}$.
Goniodomin A has two opposite effects on the actomyosin ATPase activity. One is the stimulatory effect that appears at concentrations lower than $\sim 10^{-8} \text{ M}$, and the other is the inhibitory one at higher concentrations. The stimulatory effect of goniodomin A was dependent on the concentration of actin, but not of myosin (Fig. 2). Pretreatment of actomyosin or actin with goniodomin A produced an enhancement of the actomyosin ATPase activity, whereas pretreatment of myosin did not affect it (Fig. 3). The intensity of tryptophan fluorescence of actin increased with increasing goniodomin A concentrations and reached a plateau at $10^{-5} \text{ M}$, but this was not true for myosin (Figs. 4 and 5). Goniodomin A modified the tryptophan fluorescence of actomyosin in the range of goniodomin A concentrations from $10^{-8}$ to $10^{-6} \text{ M}$, which was quite similar to the profile found for the actomyosin ATPase activity (Figs. 2 and 6). The fluorescence intensity of pyrenyl-F-actin at $2.4$ and $4.8 \times 10^{-6} \text{ M}$ was reduced to the level of G-actin in the presence of $3 \times 10^{-6}$ and $6 \times 10^{-6} \text{ M}$ goniodomin A, respectively. It is consistent with our view that goniodomin A stoichiometrically binds to actin molecules, resulting in a conformational change of actin monomers in filaments (Figs. 4b and 7). These results suggest that the stimulation of actomyosin ATPase activity by goniodomin A at lower concentrations is mediated through the conformational changes of actin molecules. The spectra of pyrenyl-F-actin in the presence of goniodomin A are quite similar to those of pyrenyl-G-actin. However, according to electron microscopic observations, goniodomin A does not depolymerize F-actin at all even in the presence of an excess amount of goniodomin A ($3 \times 10^{-6} \text{ M}$), but forms a meshwork of actin filaments (Fig. 10).
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Goniodomin A is an essential tool for the investigation of the detailed nature of actomyosin and its conformational changes. Although the detailed nature of goniodomin A-bound actin molecules still remains unclear, further information about the nature of goniodomin A-bound actin may be obtained by detailed investigation of the effects of goniodomin A on native actomyosin ATPase. In addition, the technique of fluorescence measurement using pyrenyl-actin has been routinely used for polymerization assay (13). However, our results with goniodomin A indicate that this technique is not always valid for the measurement of polymerizability of actin.

In summary, goniodomin A stoichiometrically binds to actin monomers in filaments, causing conformational changes, and this may change the actomyosin ATPase activity of skeletal muscle. These effects of goniodomin A were markedly potentiated in the presence of myosin.

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