Powerful Activation of Skeletal Muscle Actomyosin ATPase by Goniodomin A Is Highly Sensitive to Troponin/Tropomyosin Complex

KIMIHIRO MATSUNAGA, KEIGO NAKATANI, MASAHIRO MURAKAMI, KATSUMI YAMAGUCHI, and YASUSHI OHIZUMI

Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Sendai, Japan (K.M., K.N., Y.O.); Laboratory of Marine Biochemistry, Graduate School of Agricultural Life Sciences, The University of Tokyo, Yayoi, Tokyo, Japan (M.M.); and Faculty of Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan (K.Y.)

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ABSTRACT

Goniodomin A has been shown to cause the conformational change of actin to modify actomyosin ATPase activity. Goniodomin A induced a potent stimulation of the actomyosin ATPase activities of the actin-myosin reconstituted system and natural actomyosin in the range of 10^{-8} to 10^{-7} M. When the concentration was increased above 10^{-7} M, actomyosin ATPase activity was decreased. Interestingly, the troponin/tropomyosin complex caused a concentration-dependent inhibition of the goniodomin A-induced stimulation of actomyosin ATPase activity. In the presence of a high concentration of the troponin/tropomyosin complex, goniodomin A decreased actomyosin ATPase activity in a concentration-dependent manner. The enhancement of the ATPase activity of troponin/tropomyosin-free natural actomyosin by goniodomin A was larger than that obtained with natural actomyosin. Goniodomin A at lower concentrations enhanced the superprecipitation of natural actomyosin but decreased it at higher concentrations. The ATPase activity of skeletal muscle myofibrils and the contractile response of skinned fibers to Ca^{2+} were never activated and were decreased by this compound, suggesting an inhibition by the troponin/tropomyosin complex. In the far ultraviolet circular dichroism, goniodomin A above 10^{-8} M increased the negative ellipticity at 220 nm, suggesting an increase in the $alpha$-helical content of actin. These results suggest that goniodomin A increases and decreases actomyosin ATPase activity, probably through the stimulatory and inhibitory sites on actin, respectively. It is also suggested that the troponin/tropomyosin complex binds to actin to inhibit the goniodomin A-induced enhancement of actomyosin ATPase activity, probably by affecting the stimulatory site on the molecule.

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 (dos Remedios and Moens, 1995; Holmes, 1996; Cooke, 1997). Actomyosin is a precise machine for the transduction of chemical energy in ATPase molecules into mechanical work (Sugi, 1993). Myosin is an ATPase whose activity is stimulated by the interaction with actin. Although the binding sites involved in the interaction between myosin and actin molecules have been determined (Rayment et al., 1993a,b), the role of conformational changes and the interactions of these proteins in muscle contraction remain to be elucidated. Furthermore, the regulatory protein system, the troponin/tropomyosin complex, plays an important role in the regulatory process of the skeletal muscle contraction (Holmes, 1995; Cooke, 1997; Squire and Morris, 1998). Therefore, novel tools that provide information of interactions of contractile proteins will be useful (Ohizumi, 1997). The superprecipitation of actomyosin is generally accepted to be basically the same phenomenon in vitro as a contraction in skeletal muscle cells (Szent-Györgyi, 1951). Circular dichroism (CD) has been extensively applied in the study of conformational changes of protein (William et al., 1982).

In the course of our survey on biologically active substances from marine resources, much attention has been given to compounds affecting the contractile apparatus, because these compounds are very useful as tools for elucidating the relationship between structure and function in the contractile and regulatory proteins (Ohizumi, 1997). Recently, we isolated several natural products that affect myosin and actin functions, such as purealin, which modulates myosin ATPase activity (Takito et al., 1986; Nakamura et al., 1987), and xestoquinone, which modulates the specific sulf-hydrl groups of myosin (Kobayashi et al., 1991a,b; Sakamoto et al., 1995). In further research, goniodomin A (Fig. 1)

ABBREVIATION: CD, circular dichroism.
myosin. The mixture was preincubated in the absence of goniodomin A and ATP at 30°C for 5 min, followed by the addition of goniodomin A and further preincubation. The reaction was started by the addition of ATP and stopped by the addition of an equal volume of cold 10% trichloroacetic acid. The amount of inorganic phosphate liberated during the 5-min incubation was determined according to the method of Martin and Doty (1949).

Measurement of Superprecipitation Activity. The superprecipitation activity was examined as described previously (Ohizumi et al., 1998). The superprecipitation was induced by the addition of 0.4 mM ATP in 0.3 mg/ml natural actomyosin, 0.8 mM CaCl₂, 2 mM MgCl₂, 50 mM KCl, 1 mM EGTA, and 20 mM Tris·HCl at pH 6.8 and 25°C, and the change in the absorbance at 660 nm was followed.

Measurement of Contractile Response of Skinned Fibers. Psoas muscles of male guinea pig were excised and washed rapidly with a Ringer’s solution containing 150 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 5.5 mM glucose, and 5 mM HEPES (pH 7.4) and were immediately transferred into relaxing solution containing 74.7 mM K-methanesulfonic acid, 5.4 mM Mg-methanesulfonic acid, 4 mM ATP, 10 mM EGTA, and 20 mM piperazine-N,N’-bis(2-ethanesulfonic acid)-KOH (pH 7.0). A small muscle bundle of four or five fibers (~0.1 mm in diameter and ~3 mm in length) was dissected from the psoas muscle. One end of the fiber was secured to the tissue holder by a ligature and the other end was connected to a force-displacement transducer (Acers AE801; Horten, Norway); the compliance of tension measurement system was ~0.5 mm/g for measurement of isometric contraction of the fiber at 20–23°C. Fibers were treated with the relaxing solution containing 50 µg/ml saponin for 30 min and then with a 0.5% Triton X-100 solution for 15 min (Endo and Lino, 1980; Horiiuti, 1986). Various solutions for skinned fiber experiments were prepared as described elsewhere (Kobayashi et al., 1991a). A criterion for discarding skinned preparations was the total decline in a maximum contractile response to Ca²⁺, and the survival time of the preparation was at least 5 h.

CD Measurements. CD spectrum was measured as previously reported (Sakamoto et al., 1995) with a slight modification. F-actin (0.1 mg/ml) was incubated with various concentrations of goniodomin A in the medium containing 50 mM KCl, 1 mM MgCl₂, and 25 mM Tris·HCl (pH 6.8) at 37°C for 5 min. The reaction mixture was dialyzed into a buffer (50 mM KCl, 1 mM MgCl₂, 25 mM Tris·HCl, pH 6.8) at 4°C for 2 h to remove dimethyl sulfoxide used as a dissolving solvent of goniodomin A. Far-UV CD spectra of F-actin were measured in the 200- to 300-nm region in the medium consisting of 50 mM KCl, 1 mM MgCl₂, and 25 mM Tris·HCl (pH 6.8) with a spectropolarimeter (J-720, Japan Spectroscopic Corporation, Inc.) at 25°C.

Statistical Analysis. The data are expressed as mean ± S.E. Statistical comparisons were made with the use of Student’s t test. P < .05 was considered significant.

Results

Effects of Goniodomin A on Reconstituted Actomyosin ATPase Activity. Goniodomin A enhanced the ATPase activity of actomyosin reconstituted from actin and myosin in the range of 10⁻⁸ to 10⁻⁷ M in a concentration-dependent manner, but the ATPase activity was decreased by further increasing goniodomin A concentration (Fig. 2). The tropinin-tropomyosin complex decreased goniodomin A-induced elevation of the actomyosin ATPase activity of the actin-myosin reconstituted system in a concentration-dependent manner (Fig. 2). Goniodomin A (10⁻⁸ to 3 × 10⁻⁶ M) caused a concentration-dependent inhibition of the ATPase activity of actomyosin reconstituted from 0.1 mg/ml actin and 0.1 mg/ml myosin in the presence of 0.8 mg/ml tropinin and 0.8 mg/ml tropomyosin (Fig. 2).
Effects of Goniodomin A on Natural Actomyosin ATPase Activity. Natural actomyosin was used for investigation of the effect of goniodomin A because it contained more principal protein components for the contractile system than actomyosin reconstituted from actin and myosin. As shown in Fig. 3A, goniodomin A caused a concentration-dependent increase in the natural actomyosin ATPase activity in the range of $3 \times 10^{-2}$ to $3 \times 10^{-7}$ M but its ATPase activity was decreased by further increasing the goniodomin A concentration. This effect was not affected by the SH group protecting reagent dithiothreitol ($10^{-2}$ M, data not shown). The tropo-nin-tropomyosin complex decreased the goniodomin A-induced elevation of the natural actomyosin ATPase activity in a concentration-dependent manner (Fig. 3A). Goniodomin A caused a concentration-dependent decrease in the ATPase activity of $0.3 \text{ mg/ml}$ natural actomyosin in the presence of $0.2 \text{ mg/ml}$ troponin and $0.2 \text{ mg/ml}$ tropomyosin (Fig. 3A). The goniodomin A-induced modulations of the ATPase activities of the actin-myosin reconstituted system (Fig. 2) and natural actomyosin (Fig. 3A) are closely correlated. The concentration-response curve of the ATPase activity for $\text{Ca}^{2+}$ was shifted to the upper direction by goniodomin A ($10^{-2}$ M; Fig. 3B).

Effects of Goniodomin A on Troponin/Tropomyosin-Free Natural Actomyosin ATPase Activity. The ATPase activity was enhanced with an increase in goniodomin A concentration and reached a peak at $10^{-7}$ M (Fig. 3A). The peak value was 240% higher than that of the natural actomyosin ATPase activity. Further increase in the goniodomin A concentration up to $3 \times 10^{-7}$ to $3 \times 10^{-6}$ M decreased the ATPase activity.

Effects of Goniodomin A on Myofibril ATPase Activity. Goniodomin A decreased the ATPase activity of myofibrils in a concentration-dependent manner ($>10^{-8}$ M; Fig. 4A). This profile was similar to that found for the goniodomin A-induced inhibition of the ATPase activity of natural actomyosin (Fig. 3A) and actomyosin reconstituted from actin and myosin (Fig. 2) in the presence of a high concentration of the troponin/tropomyosin complex. The concentration-response curve of the myofibril ATPase activity for $\text{Ca}^{2+}$ was decreased by goniodomin A at $10^{-6}$ M to $10^{-5}$ M $\text{Ca}^{2+}$ (Fig. 4B).
Effects of goniodomin A on Superprecipitation Activity of Natural Actomyosin. The effect of goniodomin A was examined on the superprecipitaion of skeletal muscle natural actomyosin, an in vitro model reaction of muscle protein contraction, by monitoring the turbidity change. After the addition of ATP, the turbidity increased for 3 min. Figure 5A shows the typical recording traces of the superprecipitation activity of natural actomyosin in the presence of various concentrations of goniodomin A. As shown in Fig. 5B, goniodomin A (10^-8 to 3 x 10^-5 M) enhanced the superprecipitation activity in a concentration-dependent manner, but further increase in the goniodomin A concentration decreased it. This profile was similar to that found in the goniodomin A-induced modulation of the ATPase activity of natural actomyosin (Fig. 3A). The concentration-response curve of the superprecipitation activity of natural actomyosin for Ca^{2+} was shifted to the upper direction by goniodomin A (2 x 10^-8 M; Fig. 5C).

Effects of Goniodomin A on Contractile Response of Chemically Skinned Fibers. To measure the contractile force of chemically skinned fibers under the direct influence of Ca^{2+}, concentration, the fibers were prepared from guinea pig skeletal muscles by sufficient treatment with detergents to destroy the function of both the cell membrane and sarcoplasmic reticulum membrane. As shown in Fig. 6A, goniodomin A (>10^-6 M) inhibited the contraction of skinned fibers in a concentration-dependent manner. The concentration-response curve of the contractile response of skinned fibers for Ca^{2+} was shifted to the lower direction by goniodomin A (10^-5 M; Fig. 6B).

Effects of Goniodomin A on CD of Actin. Figure 7A shows the far-UV CD spectra of actin treated with various concentrations of goniodomin A. Goniodomin A increased the negative ellipticity at 220 nm in a concentration-dependent manner (Fig. 7B).

Discussion
Tropinin and troponyosin, the muscle regulatory proteins, in concert play a physiologically significant role in the regulatory process of the actomyosin ATPase activity and the striated muscle contraction (Holmes, 1995; Cooke, 1997; Squire and Morris, 1998). Goniodomin A (>10^-8 M, <10^-7
M) induced a profound enhancement of the ATPase activities of actomyosin reconstituted from actin and myosin, troponin/tropomyosin-free natural actomyosin, and natural actomyosin. At higher concentrations (>3 × 10⁻⁷ M), goniodomin A caused an inhibition of the ATPase activity. It is well known that superprecipitation of skeletal natural actomyosin is an in vitro model reaction of muscle protein contraction (Szent-Györgyi, 1951). The superprecipitation activity of natural actomyosin was increased by goniodomin A at lower concentrations but was inhibited by it at higher concentrations. It has been previously reported that the conformational change of actin molecules, resulting from stoichiometric binding of goniodomin A to actin monomers in filaments, modifies the interaction between myosin and actin (Furukawa et al., 1993). Ca²⁺-, Mg²⁺-, or K⁺-EDTA-ATPase activity of myosin was not affected by goniodomin A, suggesting an elimination of the possible involvement of direct stimulation of myosin ATPase in the mechanism of actomyosin ATPase modulation. These results suggest that at lower concentrations, goniodomin A directly enhances the interaction of actin and myosin to activate the ATPase activity and thus increases the superprecipitation of natural actomyosin, but at higher concentrations, goniodomin A inhibits it, resulting in the decrease in the ATPase activity, and thus reduces the superprecipitation activity.

An interesting observation is that in the actin-myosin reconstituted system and natural actomyosin, the troponin/tropomyosin complex (regulatory proteins of muscle contraction) significantly reduced the goniodomin A-induced enhancement of the ATPase activities. In the presence of a sufficient amount of the troponin/tropomyosin complex, goniodomin A at any concentration used did not activate the actomyosin ATPase activity but rather inhibited it. The enhancement of the ATPase activity of troponin/tropomyosin-free natural actomyosin was greater than that obtained from natural actomyosin. Goniodomin A at any concentration used did not activate myofibril ATPase activity and the tension development of skinned fibers but rather decreased both the functions. A probable explanation for these findings is that goniodomin A fails to potentiate the actomyosin ATPase activity through the stimulatory site on actin. It is also suggested that the troponin/tropomyosin complex causes a marked inhibition of the goniodomin A-induced activation of actomyosin ATPase activity through the stimulatory site on actin.

It is generally accepted that in skeletal muscles, Ca²⁺ first interacts with troponin-C, resulting in a shift of the position of tropomyosin on skeletal thin filament and leading to the physiological contraction of muscle fibers. Goniodomin A at a low concentration enhances the response to Ca²⁺ in the superprecipitation and ATPase activity of natural actomyosin, but at a high concentration, goniodomin A decreases it in the ATPase activity of myofibrils and tension development of skinned fibers. These results suggest that goniodomin A can modify the function of actomyosin ATPase and contractile response at a wide concentration range of Ca²⁺.

Various physiological techniques, including electron spin resonance, fluorometry, and absorption spectrophotometry,
have provided useful information about the conformational changes of physiologically important proteins. We previously reported that at higher concentrations (>10⁻⁷ M), goniodomin A caused a concentration-dependent increase in the fluorescence intensity in tryptophan residues of actin (Furukawa et al., 1993). In the present study, the ATPase activity of actin-myosin reconstituted system and natural actomyosin increased with an increase in the goniodomin A concentration and reached a peak at 10⁻⁷ M. Further increase in the goniodomin A concentration caused a concentration-dependent decrease in the ATPase activity. The profile of a concentration-response curve of the goniodomin A-induced decrease in the ATPase activity was similar to that of the goniodomin A-induced increase in the fluorescence intensity. It is possible that there is a close correlation between the decrease in the ATPase activity and the increase in the fluorescence intensity induced by goniodomin A. (A detailed study on both the relationship is under way.) On the other hand, the relationship between the conformation of the actin molecule and its function has been extensively studied by the analysis of the CD spectra (Murphy, 1971; De La Cruz and Pollard, 1995). The far-UV CD spectra of actin shows two negative ellipticities at 208 and 220 nm, and negative optical rotation around 220 nm indicates the α-helical content (Feinberg et al., 1996). Goniodomin A increased the negative ellipticity at 220 nm in the CD spectrum of actin in a concentration-dependent manner, suggesting binding to a site that changes the conformation of actin into α-helical. These results suggest that goniodomin A shifts the dynamic equilibrium of actin between a helical conformation and a more lipticity at 220 nm in the CD spectrum of actin in a concentration-dependent manner, suggesting binding to a site that changes the conformation of actin into α-helical. These results suggest that goniodomin A shifts the dynamic equilibrium of actin between a helical conformation and a more random structure towards a helical. It is also suggested that the increase in α-helical content of actin by goniodomin A at a concentration of ≤10⁻⁷ M enhances actomyosin ATPase activity but at a concentration of ≥3 × 10⁻⁷ M inhibits it.

Goniodomin A may provide a selective modulator of actin for the study of not only the relationship between structure and function of actin but also the molecular mechanism of the activation of actomyosin ATPase that is regulated by the tropinin/tropomyosin complex.

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Send reprint requests to: Yasushi Ohizumi, Ph.D., Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tokohu University, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan. E-mail: ohizumi@mail.pharm.tohoku.ac.jp

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