Xestoquinone Activates Skeletal Muscle Actomyosin ATPase by Modification of the Specific Sulfhydryl Group in the Myosin Head Probably Distinct from Sulfhydryl Groups SH_1 and SH_2[†]

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ABSTRACT: Xestoquinone isolated from a sea sponge *Xestospongia sapra* inhibited both Ca^{2+} and K^+-(EDTA) ATPase of skeletal muscle myosin. The inhibition was abolished in the presence of dithiothreitol. Xestoquinone reacted with 2-mercaptoethanol, a sulfhydryl (SH) compound. Unlike *N*-ethylmaleimide, a well-known SH reagent, modification of 2 mol of SH groups per myosin by xestoquinone caused a marked increase in the actomyosin ATPase activity. Kinetical analysis of stimulatory effects of xestoquinone indicates a decrease in the actin concentrations which gives half of the maximum velocity (V_{max}) of actomyosin ATPase reaction without affecting the V_{max}, suggesting an increase in the affinity of myosin for actin. *N*-Ethylmaleimide can still modify both the SH_1 and SH_2 groups after modification of 2 mol of SH groups by xestoquinone. Xestoquinone modified myosin SH groups which caused changes in the tryptophan fluorescence intensity and circular dichroism. These results suggest that xestoquinone modifies the specific SH groups in myosin distinct from SH_1 and SH_2, resulting in activation of actomyosin ATPase. It is also suggested that xestoquinone strengthens the interaction between actin and myosin through conformational change in the myosin molecule.

Muscle contraction is produced by the cyclic interaction between actin and myosin in which chemical energy in ATP molecules is converted into mechanical works (Tonomura, 1986). Recently, the three-dimensional structure of myosin subfragment-1 has been revealed by single crystal X-ray diffraction (Rayment *et al.*, 1993). However, the detailed profiles of actin−myosin interaction still remain to be elucidated. The chemical modification is one of the most useful techniques to clarify the structure and functions of proteins. The myosin molecules contains over 40 thiol residues, 12 or 13 of which are on each of the two myosin heads (Lowey *et al.*, 1969). Chemical modification of the sulfhydryl (SH)[1] groups of myosin has been investigated intensively (Inoue *et al.*, 1979). K^+(EDTA) ATPase was abolished by incorporating 2 mol of *N*-ethylmaleimide (NEM) into the SH groups (SH_1) of myosin, whereas Ca^{2+} ATPase was enhanced by the modification (Sekine *et al.*, 1962, 1964). Further incorporation of 2 mol of NEM into the other SH groups (SH_2) of SH_1-modified myosin inhibited the Ca^{2+} ATPase activity (Sekine & Yamaguchi, 1963). Balint *et al.* (1978) suggested that the SH groups were located near the junction between the myosin head and rod regions. Recently, however, Rayment *et al.* (1993) have demonstrated that α helices containing these two SH groups are lying under the nucleotide binding site which locates on the thick portion of the myosin head. A significant change in distance between the two SH groups during the ATPase reaction is induced when nucleotide binds (Burke & Reisler, 1977; Wells & Yount, 1982; Huston *et al.*, 1988). The functions of the SH groups other than SH_1 and SH_2 were unknown because specific SH reagents for such SH groups have not been found. *p*-Chloromercuriobenzoate (PCMB) modified not only other types of SH groups but also the SH_1 and SH_2 groups (Kitagawa *et al.*, 1961).

In the survey of biochemical probes for clarifying the relationship of the structure and function of a contractile system, we have found several useful substances and clarified their mechanism of action (Kobayashi *et al.*, 1984; Takito *et al.*, 1986; Nakamura *et al.*, 1987; Seino *et al.*, 1991; Furukawa *et al.*, 1993). Xestoquinone (Figure 1a) was isolated from a sea sponge *Xestospongia sapra* as an activator of actomyosin ATPase as well as muscle contraction (Nakamura *et al.*, 1985; Kobayashi *et al.*, 1991a,b). Xestoquinone directly modifies a device in the contractile machinery, resulting in an enhanced contractility of striated muscle filaments. It was also reported that xestoquinone is a novel leading compound for valuable cardiotonic agents, because of its unique mechanism of a positive inotropic effect on cardiac muscle (Kobayashi *et al.*, 1991a). In this report, we have shown for the first time that xestoquinone modifies specific SH groups in myosin distinct from SH_1 and SH_2 to strengthen the interaction between actin and myosin probably

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[1] Abbreviations: SH, sulfhydryl group; S-1, myosin subfragment-1; NEM, *N*-ethylmaleimide; IAA, iodoacetoamide; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); MOPS, 3-(*N*-morpholino)propanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol; CD, circular dichroism; PCMB, *p*-chloromercuriobenzoate.

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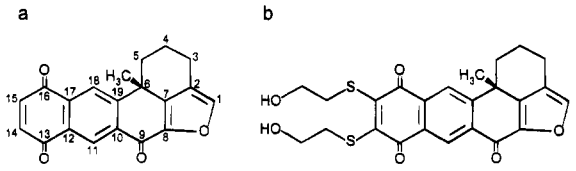

FIGURE 1: Chemical structure of xestoquinone (a) and the product (b) of the reaction with 2-mercaptoethanol.

through conformational change, resulting in enhancement of actomyosin ATPase.

MATERIALS AND METHODS

Materials. Xestoquinone was isolated from an Okinawan sea sponge *X. sapra* as described previously (Nakamura *et al.*, 1985). Briefly, the fresh sea sponge was extracted with methanol, and the methanol extract was chromatographed on silica gel columns to yield pure xestoquinone. Xestoquinone was dissolved in dimethyl sulfoxide. Myosin was prepared from rabbit skeletal white muscle according to the method of Margossian and Lowey (1992). Myosin subfragment-1 (S-1) was prepared by chymotryptic digestion of myosin as described by Weeds and Taylor (1975). Actin was prepared from acetone powder by the method of Spudich and Watt (1971). Chemicals used in this study were all analytical grade.

SH Modifications. SH modification of myosin or its active fragment, S-1, was carried out as described previously (Reisler, 1982). Myosin (2.4 mg/mL) or S-1 (0.8 mg/mL) was incubated with various concentrations of xestoquinone at 0 °C in the presence of 0.03 M NaCl and 25 mM Tris-HCl (pH 8.0). For the specific modification of the SH_1 and SH_2 groups, myosin was incubated with NEM (20 μM) at 0 °C in the presence of 0.03 M NaCl, 25 mM Tris-HCl (pH 8.0), and 1 mM MgADP. For modification of the SH_1 groups, myosin was incubated with iodoacetoamide (IAA) (10 μM) at 0 °C in the presence of 0.03 M NaCl and 25 mM Tris-HCl (pH 8.0). The mixture was diluted 10 times with ice-cold water and then dialyzed against the medium containing 0.5 M NaCl and 25 mM Tris-HCl (pH 8.0). The amount of SH groups labeled by 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) was determined (Ellman, 1959). The amount of the SH groups modified by xestoquinone, NEM, IAA, or a combination of them was calculated from the difference between the amount of DTNB-modified SH groups in control myosin and that in myosin treated with SH reagents.

ATPase Assays. Myosin ATPase assay solutions were as follows: 0.1 mg/mL myosin, 500 mM KCl, 10 mM $CaCl_2$, 1 mM ATP, and 20 mM MOPS−KOH buffer (pH 7.0) for Ca^{2+} ATPase; 0.1 mg/mL myosin, 500 mM KCl, 5 mM EDTA, 1 mM ATP, and 50 mM Tris-HCl (pH 7.5) for K^+-(EDTA) ATPase; and 0.1 mg/mL myosin, 0−0.4 mg/mL actin, 50 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 2 mM ATP, and 20 mM HEPES/Tris buffer (pH 6.8) for actomyosin ATPase. All ATPase reactions were carried out at 37 °C. The ATPase activity was determined from the amount of phosphate liberated, measured by the malachite green method described by Chan *et al.* (1986) which is highly sensitive to inorganic phosphate. Furthermore, the initial rate of actomyosin ATPase reaction was measured to avoid any effect of the superprecipitation of actomyosin on the rate.

Fluorescence Measurements of Intrinsic Tryptophan in Myosin. The excitation (emission wavelength, 330 nm) and emission (excitation wavelength, 280 nm) spectra of intrinsic tryptophan fluorescence were recorded with a Hitachi F-2000 fluorescence spectrophotometer. Xestoquinone has no autofluorescence at the excitation and emission wavelengths.

Optical Activity Measurements. Far UV circular dichroism (CD) spectra of myosin were measured in the 200−300 nm region in the presence of 0.5 M NaCl and 25 mM Tris-HCl (pH 7.6) with a spectropolarimeter (J-720, Japan Spectroscopic Corporation, Inc.).

Reaction of Xestoquinone with SH Compounds. NMR spectra were recorded on a JEOL α 400 spectrometer using TMS as an internal standard. To the solution of 0.8 mg of xestoquinone in 0.4 mL of CD_3OD was added 20 μL of 0.1 M 2-mercaptoethanol solution in CD_3OD at room temperature. The ^1H NMR spectrum of this mixed solution was measured immediately. From the result of the ^1H NMR spectrum, the production of the xestoquinone−2-mercaptoethanol adduct was confirmed. Then the product was purified by HPLC (Senshu pak ODS, 90% methanol). The detailed analysis of the structure was performed through the interpretation of the DQF-COSY spectra of the xestoquinone−2-mercaptoethanol adduct and its acetate derivative.

Table 1: Effect of XQN on the Myosin ATPase Activities[a]

ATPase	ATPase activity (% of control)	
	(−)-dithiothreitol	(+)-dithiothreitol (1 mM)
K^+(EDTA) ATPase	21 ± 5[b]	98 ± 7
Ca^{2+} ATPase	60 ± 15[b]	101 ± 3
Mg^{2+} ATPase	110 ± 18	99 ± 11
actomyosin ATPase	142 ± 8[b]	105 ± 15

[a] Myosin (2.4 mg/mL) was incubated with xestoquinone (20 μM) in the presence or absence of 1 mM dithiothreitol for 30 min, and then the myosin ATPase and actomyosin ATPase activities were determined as described in Materials and Methods. Data are mean ± standard error (n = 4). The control values of each ATPase activity were as follows: K^+(EDTA) ATPase, 2.51 ± 0.36 μmol (mg of myosin^{-1} min^{-1}); Ca^{2+} ATPase, 0.43 ± 0.065 μmol (mg of myosin^{-1} min^{-1}); Mg^{2+} ATPase, 0.0124 ± 0.0018 μmol (mg of myosin^{-1} min^{-1}); actomyosin ATPase, 0.512 ± 0.114 μmol (mg of myosin^{-1} min^{-1}). [b] Significantly (P < 0.05) different from the control value.

RESULTS

Modification of ATPase Activities and SH Groups in Myosin by Xestoquinone. Effects of xestoquinone on myosin ATPase activities of rabbit skeletal muscle were investigated (Table 1). Xestoquinone (20 μM) markedly inhibited K^+-(EDTA) ATPase and Ca^{2+} ATPase but did not affect Mg^{2+} ATPase. On the other hand, the actomyosin ATPase activity was clearly increased by xestoquinone. Actomyosin ATPase of active myosin subfragment (S-1) was also stimulated by xestoquinone (Table 2). Pretreatment of myosin with dithiothreitol (1 mM) abolished these effects of xestoquinone, suggesting the modification of myosin SH groups by xestoquinone. This modification seems to be irreversible because DTT failed to reverse established inhibition by xestoquinone. Furthermore, incubation of xestoquinone with myosin decreased the amount of free xestoquinone which was able to be extracted with ethyl ether (Figure 2), suggesting that xestoquinone is incorporated into myosin molecules and activate actomyosin ATPase through modification of myosin SH groups.

Myosin was incubated with various concentrations of xestoquinone, and then unmodified myosin SH groups were

Table 2: Effects of Xestoquinone on SH Groups and Actomyosin ATPase of Myosin Subfragment-1[a]

xestoquinone (μM)	ATPase activity (% of control)	modified SH (mol/mol of subfragment-1)
0	100 ± 1.2	0
12	127 ± 1.5[b]	1.2

[a] Myosin subfragment-1 (0.8 mg/mL) was incubated with xestoquinone for 30 min, and then the amounts of modified SH groups of subfragment-1 and the actomyosin ATPase activities were determined. Data are mean ± standard error (n = 4). The control value of actomyosin ATPase activity was 0.62 ± 0.11 μmol (mg of myosin^{-1} min^{-1}). [b] Significantly (P < 0.05) different from the control value.

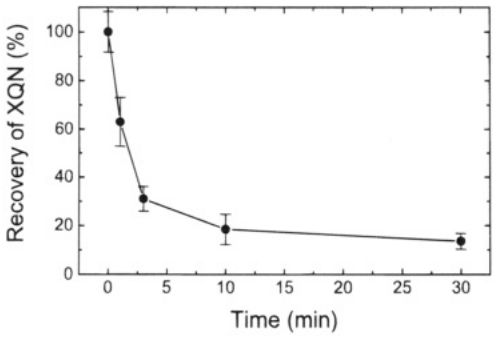

FIGURE 2: Incorporation of xestoquinone into myosin molecules. Myosin (9.6 mg/mL) was incubated with xestoquinone (40 μM) in the medium containing 0.03 M NaCl and 25 mM Tris-HCl (pH 8.0) at 0 °C. The reaction was terminated by adding ethyl acetate and extracting free xestoquinone. The amount of xestoquinone in ethyl acetate was estimated by its optical density at 400 nm. Data were expressed as percentages of the control value obtained in the absence of myosin (mean ± standard error, n = 3).

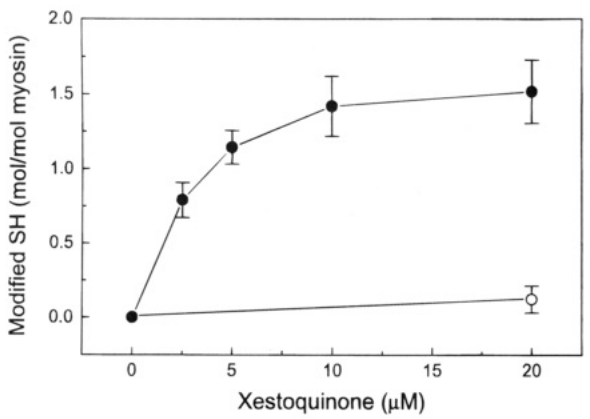

FIGURE 3: Concentration-dependent modification of SH groups in myosin by xestoquinone. Myosin (2.4 mg/mL) was incubated with various concentrations of xestoquinone (0−20 μM) in the medium containing 0.03 M NaCl and 25 mM Tris-HCl (pH 8.0) at 0 °C for 30 min. The reaction was terminated by diluting 10 times with ice-cold water, and then myosin was dialyzed into a buffer (0.5 M NaCl, 25 mM Tris-HCl, pH 7.6) at 4 °C to remove free xestoquinone and to dissociate the myosin filaments. The amount of modified SH groups was determined by using the DTNB method as described under Materials and Methods. ●, in the absence of dithiothreitol; ○, in the presence of 1 mM DTT.

titrated with DTNB. As shown in Figure 3, the amount of modified SH groups in myosin increased with an increase in the concentrations (0−20 μM) of xestoquinone and reached a plateau at about 20 μM. Twenty micromolar xestoquinone caused the modification of 1.6 mol of SH groups per myosin, which was abolished in the presence of 1 mM DTT. Similar results were obtained by using S-1.

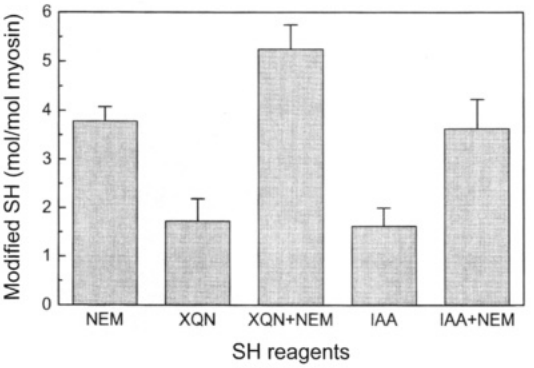

FIGURE 4: Modification of skeletal muscle myosin by SH reagents. Myosin (2.4 mg/mL) was modified by *N*-ethylmaleimide (20 μM), iodoacetoamide (10 μM), xestoquinone (20 μM), or a combination of them as described in Figure 3, and then the amount of modified SH groups was determined: NEM, *N*-ethylmaleimide; XQN, xestoquinone; IAA, iodoacetoamide.

Xestoquinone modified 1.2 mol of SH groups of S-1 (Table 2).

Myosin has two fast reacting SH groups, *i.e.*, SH_1 and SH_2. *N*-Ethylmaleimide (NEM) can modify one or both of them, depending on the conditions, while iodoacetoamide (IAA) only modifies SH_1. As shown in Figure 4, a 4-fold molar excess of NEM per myosin modified 3.8 mol of SH groups per myosin, suggesting the modification of both the SH_1 and SH_2 groups. The modification was confirmed by the complete loss of both the activities of K^+(EDTA) ATPase and Ca^{2+} ATPase (data not shown). A 2-fold molar excess of xestoquinone modified 1.7 mol of SH groups per myosin. Incubation of xestoquinone-treated myosin with NEM (20 μM) increased the amount of modified SH groups from 1.7 to 5.3 mo/mol of myosin. The further increase in the amount of SH groups modified by NEM (3.6 mol/mol of myosin) was nearly equal to that modified by NEM alone (3.8 mol/mol of myosin). IAA (10 μM) modified 1.6 mol of SH groups per myosin. Incubation of IAA-treated myosin with NEM increased the value from 1.6 to 3.6 mol/mol of myosin. However, the amount of SH groups modified by IAA + NEM (3.6 mol/mol of myosin) was nearly equal to that modified by NEM alone (3.8 mol/mol of myosin).

Demonstration of the Reaction of Xestoquinone with SH Groups. In order to demonstrate that xestoquinone can react with SH groups, we followed the reaction between xestoquinone and 2-mercaptoethanol as a simple SH compound. From the result of the ^{1}H NMR spectrum, the production of the xestoquinone−2-mercaptoethanol adduct was confirmed. 2-Mercaptoethanol (2 mol) reacted with 1 mol of xestoquinone. ^{1}H NMR (400 MHz, $CDCl_3$) of xestoquinone−2-mercaptoethanol: δ 9.01 (1H, s), 8.22 (1H, s), 7.54 (1H, br s), 3.83 (2H, t, J = 4.8 Hz), 3.81 (2H, t, J = 4.8 Hz), 3.49 (2H, t, J = 4.8 Hz), 3.41 (2H, t, J = 4.8 Hz), 2.89 (1H, ddd, J = 16.4, 7.6, 2.4 Hz), 2.62 (1H, ddd, J = 16.4, 9.5, 8.7 Hz), 2.55 (1H, ddd, J = 13.8, 4.2, 4.2 Hz), 2.29 (1H, m), 2.19 (1H, m), 1.75 (1H, ddd, J = 13.8, 13.8, 5.2 Hz), 1.52 (3H, s). The product was purified by HPLC and then acetylated (2 mol/mol of the product). ^{1}H NMR (400 MHz, $CDCl_3$) of the acetate derivative: δ 9.01 (1H, s), 8.21 (1H, s), 7.55 (, br s), 4.33 (2H, t, J = 6.4 Hz), 4.32 (2H, t, J = 6.4 Hz), 3.69 (2H, t, J = 6.4 Hz), 3.53 (2h, t, J = 6.4 Hz), 2.90 (1H, dd, J = 17.2, 8.0 Hz), 2.63 (1H, ddd, J = 17.2, 10.1, 8.9 Hz), 2.57 (1H, dt, J = 14.4, 3.8 Hz), 2.1−2.4 (2H, m), 2.00 (3H, s), 1.98 (3H, s), 1.76 (1H,

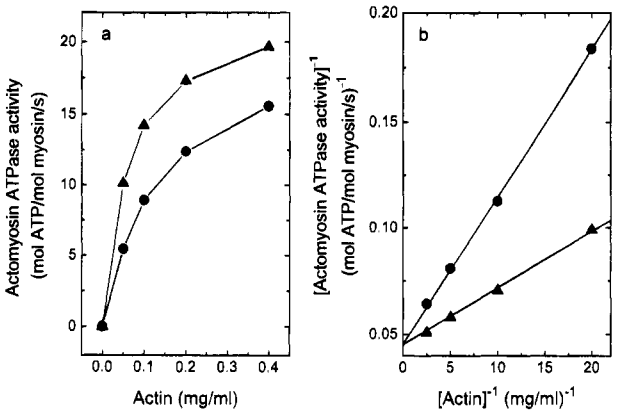

FIGURE 5: Effect of modification of myosin by xestoquinone on the actomyosin ATPase activity in the presence of various concentrations of actin. (a) Myosin (2.4 mg/mL) was treated with xestoquinone (20 μM) as described in Figure 3, and then the actomyosin ATPase activity was measured in the presence of actin at various concentrations (0−0.4 mg/mL). The amount of modified SH groups of myosin was 1.78 mol/mol of myosin. (b) Double reciprocal plots of the data in a. ●, control myosin; ▲, myosin treated with xestoquinone.

td, J = 14.4, 5.6 Hz), 1.54 (3H, s). H-1 (δ 7.54) was assigned by detailed analysis of DQF-COSY spectrum, indicating the correlation between an aromatic proton at δ 7.54 (H-1) and aliphatic protons at δ 2.89 and 2.62 (H-3) (see Figure 1a). These results suggest that xestoquinone can react with the SH group of 2-mercaptoethanol. Figure 1b indicates the structure of the product.

The Kinetics of Xestoquinone Activation with Respect to Actin. The actomyosin ATPase activity was measured in the presence of actin at various concentrations (Figure 5a). The amount of modified SH groups of myosin used was 1.78 mol/mol of myosin. Xestoquinone activated actomyosin ATPase at any concentrations of actin used. The actomyosin ATPase activities increased with increasing actin concentrations in both intact and xestoquinone-modified myosin. Figure 5b shows the double reciprocal plots of the data in Figure 5a. The reciprocal of the activities increased linearly with an increase in the reciprocal of actin concentrations. The ATPase activity at an infinite concentration of actin, V_{max} [21.6 mol of ATP (mol of myosin^{-1} s^{-1})] was not affected by the modification of myosin by xestoquinone. However, the value of K_{actin}, *i.e.*, the actin concentration which gives half of the V_{max} value, decreased from 0.149 to 0.057 mg/mL by xestoquinone, suggesting that xestoquinone strengthens the interaction between actin and myosin.

Effects of Xestoquinone on Fluorescence Intensity of Tryptophan and Circular Dichroism in Myosin. Conformational changes of contractile proteins are often accompanied by a change in fluorescence intensity of intrinsic tryptophan (Inoue *et al.*, 1979; Cooke, 1982). Figure 6a,b shows the effect of SH modification of myosin by xestoquinone on the fluorescence intensity of intrinsic tryptophan at varied excitation and emission wavelengths, respectively. The amount of modified SH groups of myosin used was 1.6 mol per myosin. The spectra clearly show that xestoquinone markedly decreased the tryptophane fluorescence intensity by 25%. NEM modified both the SH_1 and SH_2 groups (3.9 mol of SH groups per myosin) but did not change significantly the tryptophan fluorescence intensity as shown in Figure 6c,d. There was a close correlation between the decrease in fluorescence intensity of tryptophan and the

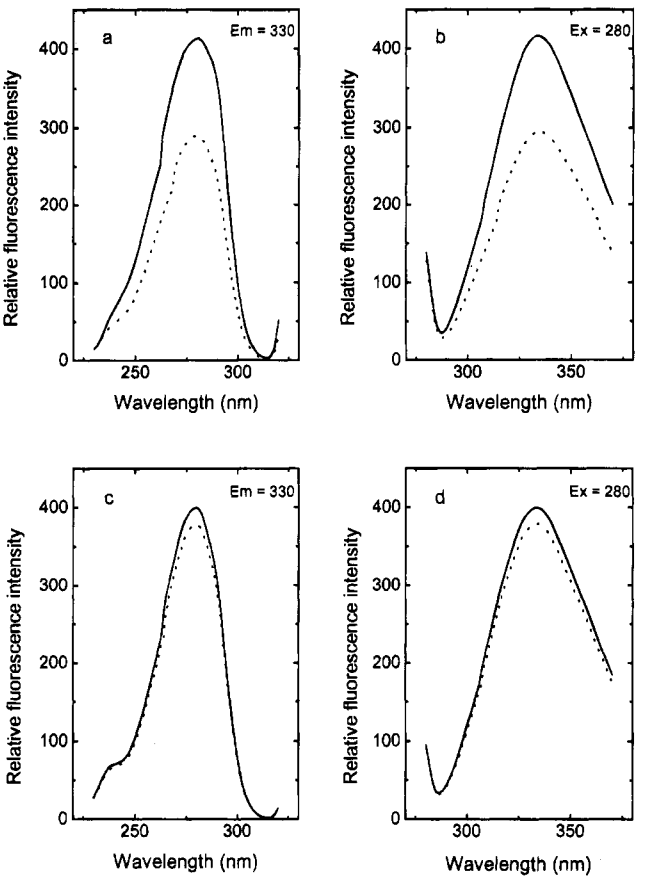

FIGURE 6: Fluorescence change in tryptophan residues of myosin induced by xestoquinone (a and b) and *N*-ethylmaleimide (c and d). Myosin (2.4 mg/mL) was modified by xestoquinone (20 μM) or *N*-ethylmaleimide (20 μM) as described in Figure 4. Excitation (a and c) and emission (b and d) spectra were recorded. Excitation and emission wavelengths were 280 and 330 nm, respectively. Solid lines, control; dashed lines, myosin treated with SH reagents. The amount of modified SH groups were 1.60 (a and b) and 3.9 (c and d) mol/mol of myosin.

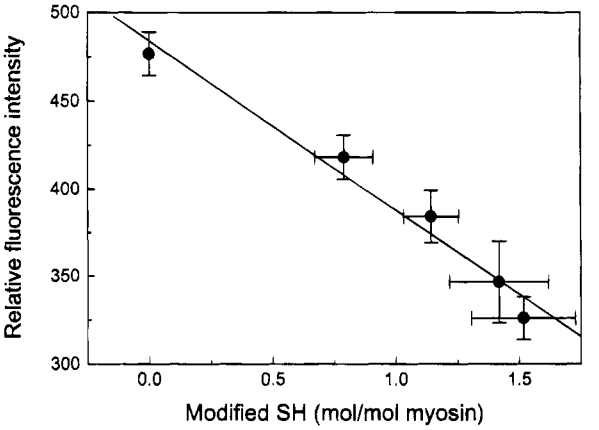

FIGURE 7: Relationship between the amount of modified SH groups and the change in fluorescence intensity of tryptophan residues in myosin. Myosin (2.4 mg/mL) was modified with various concentrations (0−20 μM) of xestoquinone as described in Figure 3. The amount of modified SH groups and the fluorescence intensity were measured as described in Figures 3 and 6, respectively.

amount of SH groups modified by xestoquinone (Figure 7).

Circular dichroism (CD) has been extensively applied in the study of conformational changes of protein (William *et al.*, 1982). Figure 8a shows the far-UV CD spectra of myosin treated by various concentrations of xestoquinone. Negative optical rotation around 220 nm indicates the

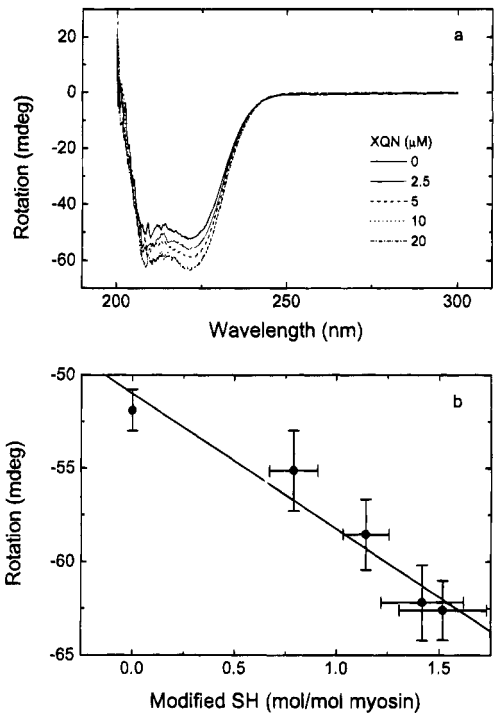

FIGURE 8: Circular dichroism of myosin modified by various concentrations of xestoquinone. Myosin (2.4 mg/mL) was modified by xestoquinone at various concentrations (0−20 μM) as described in Figure 3, and then circular dichroism measurements were carried out. (a) Typical circular dichroic spectra of myosin. (b) Relationship between the amount of modified SH groups and the change in optical rotation at 220 nm.

α-helical content. Negative optical rotation measured at 220 nm increased with increasing concentrations of xestoquinone from 0 to 20 μM. The optical rotation was closely correlated with the modification of the SH groups in myosin (Figure 8b).

DISCUSSION

Actomyosin ATPase of rabbit skeletal muscle was activated by xestoquinone, whereas K^+(EDTA) ATPase and Ca^{2+} ATPase were markedly inhibited by it. The actomyosin ATPase activities of both intact and xestoquinone-modified myosin increased with increasing actin concentrations. Kinetical analysis indicates that the K_{actin} value of actomyosin ATPase, *i.e.*, the actin concentration which gives half of the V_{max} value, decreases without a change in the V_{max} value. These results suggest that xestoquinone increases the actomyosin ATPase activity through reinforcement of the interaction between actin and myosin.

These modifications of the ATPase activities by xestoquinone were abolished in the presence of DTT. Titration of SH groups of myosin and its active subfragment S-1 with DTNB indicated a decrease in the amount of unmodified SH groups after the treatment of them with xestoquinone. Furthermore, xestoquinone can react with a SH group in 2-mercaptoethanol. 2-Mercaptoethanol (2 mol) reacted with 1 mol of xestoquinone, but only one SH group per myosin head did. This discrepancy may be due to the difference between the free SH compound (2-mercaptoethanol) and SH groups included in myosin molecules. These results support our idea that xestoquinone modifies myosin SH groups. Two well-known SH groups, SH_1 and SH_2, locate on the heavy chain of S-1 (Sekine & Kielley, 1964). NEM has been intensively used for the specific modification of the SH_1 and SH_2 groups and causes dramatic changes in the myosin ATPase activities. Selective modification of the myosin SH_1 groups by NEM or IAA causes the inhibition of K^+(EDTA) ATPase and activation of both the Ca^{2+} ATPase and Mg^{2+} ATPase (Sekine *et al.*, 1962; Sekine & Yamaguchi, 1963; Sekine & Kielley, 1964; Reisler, 1982). Subsequent modification of the SH_2 groups by NEM results in complete loss of all myosin ATPase activities. In contrast, actomyosin ATPase was activated by xestoquinone, although Ca^{2+} ATPase and K^+(EDTA) ATPase were markedly inhibited without affecting Mg^{2+} ATPase. Titration of SH groups with DTNB indicated that xestoquinone modified myosin SH groups stoichiometrically, and about 2 mol of SH groups per myosin (probably 1 mol/mol of myosin head) was modified by xestoquinone at 20 μM. NEM further modified 4 mol of SH groups in xestoquinone-modified myosin, and the amount of SH groups modified by NEM was nearly equal to that of the SH_1 + SH_2 groups, indicating that xestoquinone does not interfere with the modification of the SH_1 and SH_2 groups by NEM. There may be something about its size or structure that prevents xestoquinone from labeling at these fast reacting SH groups, because this compound can react with 2-mercaptoethanol, a simple SH compound. Further investigation is required to clarify the specificity of SH modification by xestoquinone. In addition, PCMB, a nonspecific SH reagent, activates actomyosin ATPase with loss of the Ca^{2+} control of the ATPase reaction even in the presence of troponin and tropomyosin (Kitagawa *et al.*, 1961; Tonomura *et al.*, 1961; Daniel & Hartshorne, 1972), whereas xestoquinone elevated its activity with Ca^{2+} control (unpublished observation). These observations suggest that xestoquinone stimulates the actomyosin ATPase activity through modification of 1 mol of the specific SH groups per myosin head distinct from the SH_1, SH_2, and PCMB-modified SH groups. However, the possibility that PCMB modified the same site that xestoquinone modifies and that the loss of Ca^{2+} control is due to further nonspecific modification could not be completely excluded.

Various physicochemical techniques including electron spin resonance, fluorometry, absorption, spectrophotometry, and CD have provided useful information about conformational changes of physiologically important proteins. The relationship between the conformation of the myosin molecule and its function has been intensively studied by analysis of fluorescence spectra of intrinsic tryptophan (Cooke, 1982). The intensity of tryptophan fluorescence of myosin was markedly decreased by xestoquinone, accompanied by the activation of actomyosin ATPase. There was a close correlation between the change in the fluorescence intensity and the modification of the SH groups in myosin (Figure 5). The CD signal at 220 nm increased with an increase is the amount of SH groups modified by xestoquinone, suggesting the increase in the α-helical content of myosin (William *et al.*, 1982). These results suggest that xestoquinone induces a conformational change through modification of the myosin SH groups, which may be related to the activation of actomyosin ATPase. Furthermore, the tryptophan fluorescence intensity was decreased by xestoquinone, whereas that was not affected by NEM. These results also support our idea that xestoquinone modifies the specific SH groups distinct from the SH_1 and SH_2 groups.

In summary, it is suggested that the actomyosin ATPase is activated by xestoquinone through the conformational change of the myosin molecule due to modification of the specific SH groups in myosin heads distinct from SH_1 and SH_2. Xestoquinone becomes an excellent tool for studies on not only muscle contraction but also other cell motilities in which myosin is involved.

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