The Mode of Inhibitory Action of \( \alpha \)-Mangostin, a Novel Inhibitor, on the Sarcoplasmic Reticulum Ca\(^{2+} \)-Pumping ATPase from Rabbit Skeletal Muscle

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ABSTRACT—\( \alpha \)-Mangostin, the principal ingredient of the fruit hull of *Garcinia mangostana*, caused a concentration-dependent decrease in the activities of both Ca\(^{2+} \)-ATPase and Ca\(^{2+} \)-transport of the sarcoplasmic reticulum from rabbit skeletal muscle with an IC\(_{50}\) value of 5 \( \mu \)M. Neither Ca\(^{2+} \) release nor other enzyme activities were affected by \( \alpha \)-mangostin. Kinetic analysis of the inhibitory effects of \( \alpha \)-mangostin on Ca\(^{2+} \)-ATPase suggests that the inhibition of the ATPase is a noncompetitive-type with respect to ATP or Ca\(^{2+} \). \( \alpha \)-Mangostin may become a useful pharmacological tool for clarifying the physiological functions of Ca\(^{2+} \)-pumping ATPase and sarcoplasmic reticulum.

Keywords: \( \alpha \)-Mangostin, Ca\(^{2+} \)-pumping ATPase, Sarcoplasmic reticulum

Many physiological processes such as muscle contraction, secretion, cell growth and differentiation are regulated through the level of cytosolic Ca\(^{2+} \) concentrations ([Ca\(^{2+} \)]). For this Ca\(^{2+} \) signaling mechanism to work, living cells keep the resting [Ca\(^{2+} \)] below 10\(^{-7} \) M by means of the Ca\(^{2+} \) transport system and maintain a high gradient of Ca\(^{2+} \) ions across both the plasma membrane and endoplasmic/sarcoplasmic reticulum membrane. The best understood system of Ca\(^{2+} \) transport is the Ca\(^{2+} \)-ATPase in sarcoplasmic reticulum (SR). Although the molecular structure of the ATPase and its regulatory mechanisms are well-studied (1, 2), functions of the ATPase domains except for the catalytic site and Ca\(^{2+} \) binding domain remain to be elucidated.

In the course of our survey of natural products that affect Ca\(^{2+} \)-pumping ATPase, we found various types of modulators (3-5). Mangosteen, *Garcinia mangostana*, is well-known for its use as a folk medicine in South-East Asia. \( \alpha \)-Mangostin, an anti-inflammatory and anti-ulcer active substance, was isolated as a major compound from the fruit hull (6, 7). However, the detailed pharmacological properties of this compound have not been studied yet.

Here we present data indicating that \( \alpha \)-mangostin is a noncompetitive inhibitor of the Ca\(^{2+} \)-pumping ATPase from skeletal muscle SR. This is the first report of a non competitive inhibitor, although many competitive inhibitors have been reported. \( \alpha \)-Mangostin might provide a useful pharmacological tool for clarifying the physiological functions of SR Ca\(^{2+} \)-pumping ATPase.

\( \alpha \)-Mangostin was isolated from the fruit hull of *Garcinia mangostana* as previously reported (8). \( \alpha \)-Mangostin was dissolved in dimethyl sulfoxide of which the final concentration was kept at less than 0.1010 in all experiments. SR was prepared from rabbit skeletal muscle by the method of Meissner et al. (9). Protein concentrations were determined by the Bradford method (10).

Unless otherwise stated, the reaction mixture for Ca\(^{2+} \)-ATPase contained 0.02 mg/ml SR, 0.2 mM ATP, 1 mM CaCl\(_2\), 5 mM MgCl\(_2\), 90 mM KCl, 1 mM EGTA and 50 mM MOPS/KOH buffer (pH 7.2 at 30°C). The mixture was preincubated in the absence of ATP for 5 min, followed by the addition of \( \alpha \)-mangostin and further preincubation for 5 min. The reaction was started by the addition of ATP. The amount of inorganic phosphate liberated during 5 min was determined by the method of Chan et al. (11). Enzyme activities of actomyosin-ATPase (rabbit skeletal), Na\(^+\),K\(^+\)-ATPase (porcine cerebral cortex) and plasma membrane Ca\(^{2+} \)-ATPase (red blood cell ghost) were determined as described by Seino et al. (12).
Fig. 1. Inhibitory effect of α-mangostin on the activity of Ca\textsuperscript{2+}-ATPase from skeletal muscle sarcoplasmic reticulum (SR). A: chemical structure of α-mangostin. B: the concentration-response curve for α-mangostin in the activity of Ca\textsuperscript{2+}-ATPase from skeletal muscle SR. Ca\textsuperscript{2+}-ATPase was preincubated with various concentrations of α-mangostin for 5 min. The Ca\textsuperscript{2+}-ATPase activities were calculated by the quantity of phosphate liberated during a 5-min incubation. Vertical lines indicate S.E.M. (n=3). *P<0.05 vs control.

Fig. 2. The double-reciprocal plot for the ATP (A) and Ca\textsuperscript{2+} (B) concentrations in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+}-ATPase in the presence of α-mangostin. A: the Ca\textsuperscript{2+}-ATPase reaction was carried out in the presence of various concentrations of ATP. B: the Ca\textsuperscript{2+}-ATPase reaction was carried out in the presence of various concentrations of CaCl\textsubscript{2}. α-Mangostin was added 5 min before the start of the ATPase reaction. The amount of inorganic phosphate liberated during the 5-min ATPase reaction was assayed. Vertical lines indicate S.E.M. (n=3). The concentrations of α-mangostin are as follows: ○, 0; ●, 4 \times 10\textsuperscript{-6} M; ▲, 5 \times 10\textsuperscript{-6} M; ▼, 6 \times 10\textsuperscript{-6} M.
The Ca$^{2+}$ concentration of SR suspension was measured at 30°C with a Ca$^{2+}$ electrode as described elsewhere (12).

The significant difference between means of data was evaluated by Student's t-test, and a P value less than 0.05 was considered statistically significant.

As shown in Fig. 1, α-mangostin caused a concentration-dependent decrease in the activity of Ca$^{2+}$-pumping ATPase with an IC$_{50}$ value of 5 μM. The kinetics of inhibition by α-mangostin were examined with respect to ATP and Ca$^{2+}$ for SR Ca$^{2+}$-ATPase. Figure 2A shows the double-reciprocal plot for the ATP concentration in the kinetics of Ca$^{2+}$-ATPase. The slopes and intercepts were increased by α-mangostin and three lines crossed at a point on the horizontal axis. The value of $V_{\text{max}}$ decreased from 905 to 95 μmol/min/mg protein, while that of $K_m$ was constant (0.43 mM). This indicates that α-mangostin shows a noncompetitive inhibition pattern with respect to the ATP. In a reciprocal plot for the free Ca$^{2+}$ concentration, the slopes and intercepts were increased by α-mangostin and three lines crossed at a point on the horizontal axis (Fig. 2B). The $V_{\text{max}}$ value decreased from 470 to 77 μmol/min/mg protein, while the $K_m$ value was constant (0.25 μM), indicating the noncompetitive inhibition pattern of α-mangostin with respect to Ca$^{2+}$. These results suggest that α-mangostin inhibits SR Ca$^{2+}$-pumping ATPase activity by binding to a site that is distinct from the ATP- or Ca$^{2+}$-binding site.

The Ca$^{2+}$-pumping activity of skeletal SR can be visualized by monitoring directly the extravesicular Ca$^{2+}$ concentration of SR with a Ca$^{2+}$ electrode (12). Figure 3A demonstrates that upon the addition of 0.2 mM ATP, free Ca$^{2+}$ concentrations decreased rapidly due to the formation of Ca-ATP complexes and further decreased gradually due to Ca$^{2+}$ uptake by SR. The earlier part of the Ca$^{2+}$ uptake was almost linear in an antilogarithmic plot (data not shown). The effect of α-mangostin on the Ca$^{2+}$-pumping activity of SR was examined by using this system. After pretreatment with α-mangostin, the slopes of time-course curves of Ca$^{2+}$ uptake by SR was gentler than that of the control in a concentration-dependent manner. The IC$_{50}$ value was estimated to be 4.6 μM (Fig. 3B). This value is in close agreement with the concentration that inhibits the Ca$^{2+}$-ATPase activity. α-Mangostin did not induce Ca$^{2+}$ release and affected neither the
caffeine-induced Ca\(^{2+}\) release from SR nor other ATPases such as Na\(^+,K^+\)-ATPase, plasma membrane Ca\(^{2+}\)-ATPase and actomyosin-ATPase (data not shown). These results suggest a specific inhibitory effect of \(\alpha\)-mangostin on Ca\(^{2+}\) uptake by SR Ca\(^{2+}\)-ATPase.

In conclusion, \(\alpha\)-mangostin inhibits the SR Ca\(^{2+}\)-pumping ATPase without affecting the ATP- or Ca\(^{2+}\)-binding site. It has been reported that numerous Ca\(^{2+}\)-ATPase inhibitors compete with ATP and/or Ca\(^{2+}\) at each binding site (2). \(\alpha\)-Mangostin, a noncompetitive SR Ca\(^{2+}\)-pumping ATPase inhibitor, may become a useful tool for investigating the physiological functions of the SR and Ca\(^{2+}\)-pumping ATPase.

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REFERENCES

1 MacLennan DH, Brandl CJ, Korczak B and Green NM: Amino-acid sequence of a Ca\(^{2+}\)+Mg\(^{2+}\)-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence. Nature 316, 696–700 (1990)

2 Inesi G, Cantilina T, Yu X, Nikic D, Sagara Y and Kirtley ME: Long-range intramolecular linked functions in activation and inhibition of SERCA ATPases. Ann NY Acad Sci 672, 32–48 (1992)

3 Kobayashi M, Ishida Y, Shoji N and Ohizumi Y: Cardiotonic action of [8]-gingerol, an activator of the Ca\(^{2+}\)-pumping adenosine triphosphatase of sarcoplasmic reticulum, in guinea pig atrial muscle. J Pharmacol Exp Ther 246, 667–673 (1988)

4 Sasaki S, Kusumi T, Ohtani I and Ohizumi Y: Ptilomycalin A, a novel ATPase inhibitor competitively interacts with ATP at its binding site in Na\(^+,K^+\)-ATPase and Ca\(^{2+}\)-ATPase. Eur J Pharmacol (in press)

5 Kobayashi J, Ishibashi M, Nakamura H, Hirata Y, Yamasa T, Sasaki T and Ohizumi Y: Symbioramide, a novel Ca\(^{2+}\)-ATPase activator from the cultured dinoflagellate Symbiodinium sp. Experientia 44, 800–802 (1988)

6 Shankaranarayanan D, Gopalakrishnan C and Kameswaran L: Pharmacological profile of mangostin and its derivative. Int Pharmacodyn Ther 239, 257–269 (1979)

7 Pai BR, Natarajan S, Suguna N, Kameswaran L, Shankaranarayanan D and Gopalakrishnan C: Synthesis and pharmacology of mangostin-3,6-di-O-glucoside. J Nat Prod 42, 361–365 (1979)

8 Yates P and Stout GH: The structure of mangostin. J Am Chem Soc 80, 1691–1699 (1958)

9 Meissner G, Coner GE and Fleischer S: Isolation of SR by zonal centrifugation and purification of Ca\(^{2+}\)-pump and Ca\(^{2+}\)-binding proteins. Biochim Biophys Acta 298, 246 (1976)

10 Bradford MM: A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72, 248–254 (1976)

11 Chan KM, Delfert D and Junger KD: A direct colorimetric assay for Ca\(^{2+}\)-stimulated ATPase activity. Anal Biochem 157, 375–380 (1986)

12 Seino A, Furukawa K, Miura T, Yaginuma T, Momose K and Ohizumi Y: 3',3",5',5"-Tetoraiodophenolsulfonephthalein is a selective inhibitor of Ca\(^{2+}\)-pumping ATPase in intracellular Ca\(^{2+}\) store. J Biol Chem 269, 17550–17555 (1994)