The Mechanism of Inhibition of the Ca\(^{2+}\)-ATPase by Mastoparan

**MASTOPARAN ABOLISHES COOPERATIVE Ca\(^{2+}\) BINDING**

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The amphiphilic peptide mastoparan, isolated from wasp venom, is a potent inhibitor of the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase. At pH 7.2, ATPase activity is inhibited with an inhibitory constant \((K_i)\) of 1 ± 0.13 \(\mu M\). Mastoparan shifts the E2-E1 equilibrium toward E1 and may affect the regulatory ATP binding site. The peptide also decreases the affinity of the ATPase for Ca\(^{2+}\) and abolishes the cooperativity of Ca\(^{2+}\) binding. In the presence of mastoparan, the two Ca\(^{2+}\) ions bind independently of one another. Our results appear to support the model that describes the relationship between the two Ca\(^{2+}\) binding sites as “side-by-side,” because this model allows the possibility of independent Ca\(^{2+}\) entry to the two sites. Mastoparan shifts the steady-state equilibrium between E1-Ca and E1-Ca-P toward E1-Ca-P, by possibly affecting the conformational change that follows ATP binding. The peptide also causes a reduction in the levels of phosphoenzyme formed from \([^{32}\text{P}]{\text{ATP}}\).

Some analogues of mastoparan were also tested and were found to cause inhibition of the Ca\(^{2+}\)-ATPase in the range of 2–4 \(\mu M\). The inhibitory action of mastoparan and its analogues appears dependent on their ability to form \(\alpha\)-helices in membranes.

The sarcoplasmic reticulum Ca\(^{2+}\)-ATPase transports Ca\(^{2+}\) from the cytosol to the lumen of the sarcoplasmic reticulum (SR)/endoplasmic reticulum (ER). The mechanism of this Ca\(^{2+}\)-ATPase is usually discussed in terms of the model proposed by de Meis and Vianna (1). The model postulates two major conformational states of the enzyme, E1 and E2. These two states differ in that the affinity for Ca\(^{2+}\) is high in the E1 conformation but low in the E2 conformation, and the Ca\(^{2+}\)-binding sites are exposed on the cytoplasmic side of the SR in E1 but exposed to the lumen in E2.

It has been demonstrated that the binding of Ca\(^{2+}\) to the ATPase is both sequential and co-operative (2). This suggests that binding of the first Ca\(^{2+}\) ion is followed by a slow conformational change (E1Ca → E1′Ca), which allows binding of the second Ca\(^{2+}\) ion. The second site is only formed on transition to E1′Ca (2).

The Ca\(^{2+}\)-ATPase belongs to a family of enzymes known as the P\(_2\)-type ATPases (3). Several peptide toxins have been shown to inhibit the action of these enzymes. Both myotoxin a, from rattle snake venom, and mellitin, isolated from bee venom, are basic peptides that inhibit the SR Ca\(^{2+}\)-ATPase (4–6). Mellitin is also a potent inhibitor of the E1′K\(^+\)-ATPase and the Na\(^+\)/K\(^+\)-ATPase (7–10).

Mastoparan (MP) is an amphiphilic tetradecapeptide isolated from wasp venom (11). It is known to possess a variety of biological activities including mast cell degranulation, mobilization of Ca\(^{2+}\) from cerebellar microsomes and sarcoplasmic reticulum, activation of the ryanodine receptor and modulation of various enzymes, for example the Na\(^+\)/K\(^+\)-ATPase of rat brain (12–14).

In aqueous solutions, mastoparan forms a random structure, however, in a lipid environment, the peptide adopts an amphiphilic \(\alpha\)-helical structure, which is thought to be crucial for its interaction with biological membranes (15). In a previous study (13), we showed that mastoparan and a number of closely related analogues inhibit the SR Ca\(^{2+}\)-ATPase. Here we elucidate the mechanism of this inhibition.

**MATERIALS AND METHODS**

Mastoparan (MP), mastoparan X (MPX), mastoparan 7 (MP7), and mastoparan 17 (MP17) were all obtained from Bachem. \([^{45}\text{Ca}]\text{Cl}_2\), \([^{3}\text{H}]\text{glucose}, [\gamma-^{32}\text{P}]\text{ATP}\) and \([^{32}\text{P}]\text{Pi}\), were obtained from Amersham Pharmacia Biotech. All other reagents were purchased from Sigma.

SR and the purified Ca\(^{2+}\)-ATPase were prepared from rabbit fast-twitch skeletal muscle as described by Michelangeli and Munkonge (16). Ca\(^{2+}\)-ATPase activities were determined using the coupled enzyme method described by Michelangeli and Munkonge (16) and monitored in a buffer containing 40 mM Hepes/KOH (pH 7.2), 1 mM EGTA, 5 mM MgSO\(_4\), 2 mM ATP, 0.42 mM phosphoenolpyruvate, 0.15 mM NADH, 7.5 mM pyruvate kinase, and 18 mM lactate dehydrogenase. Ca\(^{2+}\)-ATPase (15 \(\mu\)g) was incubated for 10 min at 37 °C in 2.5 ml of assay buffer. ATPase activity was initiated by the addition of 1 mM CaCl\(_2\) to give a free Ca\(^{2+}\) concentration of 6.5 \(\mu M\).

The Ca\(^{2+}\)-ATPase/SR was labeled with nitrobenzo-2-oxa-1,3-diazole (NBD) as described by Henderson et al. (17). The Ca\(^{2+}\)-ATPase was labeled with fluorescein 5′-isothiocyanate (FITC) at a ratio of FITC to ATPase of 0.5:1 according to the method of Michelangeli et al. (18).

Fluorescence measurements were performed at 25 °C using a Perkin-Elmer LS-50B fluorimeter. Measurements of NBD fluorescence were made at excitation and emission wavelengths of 430 and 510 nm, respectively, in a buffer containing either 150 mM Mops/Tris, 0.3 mM EGTA, 100 mM choline chloride at pH 7.2, or 150 mM Mes/Tris, 0.3 mM EGTA, 100 mM choline chloride at pH 6.0. Trypsinophan fluorescence was monitored by exciting at 295 nm and measuring the emission at 330 nm. These measurements were made in a buffer containing 20 mM Hepes/Tris, 100 mM KCl, 5 mM MgSO\(_4\), 100 \(\mu M\) Ca\(^{2+}\) at pH 7.2.

Rapid kinetic fluorescence measurements were performed using a stopped-flow spectrophotofluorimeter (Applied Photophysics, Model SX17 MV). The sample handling unit possesses two syringes, A and B (drive ratio 10:1), which are driven by a pneumatic ram. Trypsinophan fluorescence was monitored (at 25 °C) by exciting the sample at 280 nm and measuring the emission above 320 nm using a cut-off filter. Ca\(^{2+}\) binding was measured at pH 7.2 in 20 mM Hepes/Tris, 100 mM KCl, 5 mM MgSO\(_4\), 50 \(\mu M\) EGTA, plus 1 mM Ca\(^{2+}\) from syringe B, whereas Ca\(^{2+}\) dissociation was measured at pH 7.2 in 20 mM Hepes/Tris, 100 mM KCl, 5 mM MgSO\(_4\), 100 \(\mu M\) Ca\(^{2+}\), plus 2 mM EGTA from syringe B, all values...
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Fig. 1. A, the effect of mastoparan on SR Ca$^{2+}$-ATPase activity. Activity of the purified ATPase (0.05 μM) was measured at pH 7.2 and 37 °C. Each data point is the mean ± S.D. of three determinations. The effect of mastoparan (1 μM) on SR Ca$^{2+}$-ATPase activity as a function of Ca$^{2+}$ (B), ATP (C), and Mg$^{2+}$ (D) concentration. Activity of the purified ATPase (0.05 μM) was measured at pH 7.2 and 37 °C. Each data point is the mean ± S.D. of three determinations.

being final concentration.

Ca$^{2+}$ binding to the ATPase was measured using the dual labeling technique of Michelangeli et al. (19). ATPase (0.1 mg) was incubated at 25 °C in 1 ml of buffer containing 20 mM Hepes/Tris, 100 mM KCl, 5 mM MgSO$_4$, 100 μM EGTA, and 500 μM H$^3$H-glucose (0.2 Ci/mol) and $^{45}$CaCl$_2$ (3 Ci/mol) to give the required free Ca$^{2+}$ concentration at pH 7.2. Samples were then rapidly filtered through Millipore HAWP filters (0.45 μm). Filters were left to dry, after which 8 ml of scintillant was added. The filters were then counted for both $^3$H and $^{45}$Ca$^{2+}$. The amount of $^3$Hglucose trapped on each filter was used to calculate the wetting volume for the filter, and the amount of Ca$^{2+}$ trapped in this volume was subtracted from the total Ca$^{2+}$ bound to the filter to give that bound to the ATPase. A correction was also applied for nonspecific binding of Ca$^{2+}$ to the filter.

Equilibrium levels of phosphorylation of the ATPase by $[^3$P]P$_i$ were measured in 150 mM Mes/Tris (pH 6.2), containing 5 mM EGTA, 10 mM MgSO$_4$, and 10 μM P$_i$ (10 Ci/mol), at 25 °C and a protein concentration of 0.9 mg/ml. Samples were incubated for 20 s and then quenched with 10% trichloroacetic acid, 0.2 M H$_3$PO$_4$. The precipitate was collected by rapid filtration through Whatman GF/C filters, washed with 30 ml of 12% trichloroacetic acid, 0.2 M H$_3$PO$_4$, and then counted.

Steady-state levels of phosphorylation of the ATPase by $[^3$P]ATP were carried out in a similar manner as above. Experiments were carried out at 25 °C in 20 mM Hepes/Tris (pH 7.2) containing 100 mM KCl, 5 mM MgSO$_4$, 100 μM CaCl$_2$, and 0.075 mg/ml ATPase. Two stocks of labeled ATP were made up to cover the range of ATP concentrations up to 100 μM, with specific activities of 10 and 100 Ci/mol. The reaction was initiated by addition of $[^3$P]ATP and quenched as described above after 10 s. The samples were then filtered, washed, and counted.

Dual wavelength spectrophotometry was performed on a Shimadzu UV-3000 dual wavelength-recording spectrophotometer. Experiments were carried out at 25 °C in 20% (w/v) sucrose, 50 mM Mops/KOH (pH 7) containing 1 mM CaCl$_2$ and 0.8 mg/ml ATPase. Titration of the Ca$^{2+}$-ATPase with trinitrophenyl adenosine diphosphate (TNP-ADP) was then monitored by recording the absorbance difference at 422 nm and 390 nm, as described by Coll and Murphy (20).

RESULTS

Ca$^{2+}$-ATPase Inhibition—Fig. 1A shows the effect of mastoparan on purified, fully uncoupled, Ca$^{2+}$-ATPase activity. The inhibitory constant is determined to be 1 ± 0.13 μM mastoparan. In sealed SR vesicles, ATPase activity is low due to high Ca$^{2+}$ concentrations in the vesicle lumen, and the inhibition can be relieved by addition of the Ca$^{2+}$-ionophore A23187. Effects of mastoparan on ATPase activity of SR vesicles in the presence of A23187 are very similar to those determined for the purified ATPase (data not shown).

However, in the absence of A23187, low concentrations of mastoparan (<1 μM) actually increase the activity of the ATPase. Thus mastoparan increases the permeability of the SR membrane to Ca$^{2+}$, a phenomenon previously reported by Longland et al. (13). At higher concentrations of mastoparan (≥1 μM), ATPase activity in sealed SR vesicles decreases due to inhibition of the pump.

Fig. 1B shows the effect of mastoparan on the Ca$^{2+}$ dependence of ATPase activity. A bell-shaped curve was obtained both in the presence and absence of mastoparan. The $K_m$ value for the high-affinity (activatory) Ca$^{2+}$ sites was increased from 0.16 μM in the absence of peptide to 0.27 μM in the presence of mastoparan. In contrast, the $K_m$ value for the low-affinity (inhibitory) sites (0.2 mM) was not significantly affected by the inhibitor. In addition, maximum ATPase activities were observed at similar free Ca$^{2+}$ concentrations in both the presence and absence of mastoparan (i.e., 6.5 μM).

The effect of mastoparan on the dependence of ATPase activity on the concentration of ATP is shown in Fig. 1C. The data are fitted to a modified form of the Michaelis-Menten equation, assuming that ATP interacts at 2 sites: a high-affinity (catalytic) site and a low affinity (regulatory) site (21).

Addition of mastoparan had little effect on ATPase activity at low ATP concentrations but considerably inhibited the pump at higher ATP concentrations. In the absence of peptide, the data could be fitted assuming $K_m$ and $V_{max}$ values for the catalytic site of 0.85 μM and 1.68 IU/mg, respectively, and $K_m$ and $V_{max}$ values for the regulatory site of 0.15 mM and 4.34 IU/mg, respectively. In the presence of mastoparan, the data could be fitted assuming the same values for the catalytic site and the same $K_m$ value for the regulatory site. The $V_{max}$ value for the regulatory site, however, decreased to 1.24 IU/mg.

Fig. 1D shows the effect of mastoparan on the Mg$^{2+}$ dependence of ATPase activity. In the absence of mastoparan, ATPase activity decreases with increasing concentrations of Mg$^{2+}$. At low concentrations of Mg$^{2+}$, the presence of mastoparan results in strong inhibition of the ATPase. As the Mg$^{2+}$ concentration
is increased from 2–10 mM, stimulation of ATPase activity is then observed followed by inhibition.

**NBD and FITC Fluorescence**—It has been shown that the E2-E1 equilibrium for the ATPase can be monitored by changes in the fluorescence intensity of the ATPase labeled with NBD (22). The fluorescence intensity of the labeled ATPase is higher in the E1 conformation than in the E2 conformation (22).

Addition of mastoparan to NBD-labeled SR at pH 7.2 (Fig. 2) results in an increase in fluorescence intensity with an apparent $K_d$ value of 1.8 mM. The maximal fluorescence increase observed is 17–18%. Therefore mastoparan shifts the E2-E1 equilibrium toward E1.

It has been suggested that the E2-E1 equilibrium is pH-dependent with low pH favoring the E2 form (23). Consequently pH can also be used to trigger the E2-E1 transition. The addition of mastoparan to NBD-labeled SR at pH 6.0 (Fig. 2) also causes an increase in fluorescence intensity, however, with a higher apparent $K_d$ (14.1 μM) and a maximal fluorescence increase of ~31%.

The E2-E1 equilibrium can also be studied by monitoring changes in the fluorescence intensity of FITC-labeled ATPase. In contrast to NBD-labeled ATPase, addition of Ca$^{2+}$ to FITC-labeled ATPase results in a decrease in fluorescence, which has been attributed to the E1 conformational state (23).

At pH 7.0, where the E1/E2 ratio has been determined to be 0.5 (23), calcium induces a decrease in fluorescence of 6%, whereas in the presence of 20 μM mastoparan, this decrease is reduced to 3%. This is consistent with mastoparan having shifted the E2-E1 equilibrium toward the E1 conformation. Furthermore, addition of 20 μM mastoparan to FITC-labeled ATPase in the absence of calcium also caused a 3% decrease in fluorescence.

**Calcium Binding and Dissociation**—Table I shows the level of Ca$^{2+}$ bound to the ATPase at a free Ca$^{2+}$ concentration of 50 μM, a concentration at which both high-affinity Ca$^{2+}$ binding sites should be fully saturated. This level is unchanged in the presence of 30 μM mastoparan (added either before or after the labeled Ca$^{2+}$), demonstrating that mastoparan does not affect the stoichiometry of Ca$^{2+}$ binding. Levels of Ca$^{2+}$ binding to the native ATPase are higher than expected as a result of nonspecific binding of Ca$^{2+}$ to the ATPase and associated lipids (19).

Fig. 3 shows Ca$^{2+}$ binding to the ATPase as a function of Ca$^{2+}$ concentration. It clearly shows that mastoparan reduces the affinity of Ca$^{2+}$ binding to the ATPase, increasing the $K_d$ from 0.6 to 3.7 μM. In the absence of mastoparan, binding of Ca$^{2+}$ to the ATPase is cooperative, as expected, with a Hill coefficient of 1.60. In the presence of mastoparan, this cooperativity is no longer observed, with the Hill coefficient being reduced to 0.9.

Calcium binding and dissociation can also be studied through changes in the tryptophan fluorescence of the ATPase. On addition of Ca$^{2+}$ to the ATPase, there is an increase in tryptophan fluorescence that has been attributed to the E1Ca-E1′Ca transition, with the E2, E1, and E1′Ca forms having relatively low tryptophan intensities and the E1Ca and E1′Ca, forms having higher fluorescence intensities (24).

Fig. 4 shows that the addition of 20 μM mastoparan to the ATPase causes a shift in the Ca$^{2+}$ concentration dependence of this transition to higher Ca$^{2+}$ concentrations. The apparent $K_d$ value is increased from 1.4 to 25 μM, suggesting once again that mastoparan may decrease the affinity of the ATPase for Ca$^{2+}$.

The presence of the peptide also halves the maximum change in the fluorescence intensity observed.

It has been shown in stopped-flow experiments with the Ca$^{2+}$-ATPase that both Ca$^{2+}$ binding and dissociation are biphasic in nature (25). Fig. 5 shows the binding (A) and dissociation (B) of calcium to and from the ATPase in the presence of 30 μM mastoparan. The kinetic parameters obtained from these experiments are given in Table II. In the absence of peptide, the data for both Ca$^{2+}$ binding and dissociation can be fitted to the biexponential equation (fitting these data to a monoeponential equation resulted in much larger $\chi^2$ values, see Table II)

$$\Delta F = A_1(1 - \exp^{-kt_1}) + A_2(1 - \exp^{-kt_2})$$  \hspace{1cm} (Eq. 1)

where $\Delta F$ equals fluorescence change, $A_1$, $A_2$, $k_1$, and $k_2$ are the amplitudes and rate constants for the fast and slow phases of Ca$^{2+}$ binding/dissociation, respectively, and $t$ is time (s).
The presence of mastoparan therefore shifts the equilibrium toward E1. Similar to mastoparan, these two analogues in- 
hibit the ATPase, with Ki values of 4.4 and 2.2 μM, respectively. Similar to mastoparan, these two analogues in-
crease the fluorescence intensity of NBD-labeled ATPase, im-
pacting that they too shift the E2-E1 equilibrium toward E1.

**Conclusion**

A number of P2-type ATPases, including the Ca2+-ATPase, Na+/K+-ATPase, and Na+/K+-ATPase are inhib-
ited by peptide toxins such as mastoparan and melittin (4–10, which two amino acid substitutions reduce α-helix formation as well as decrease the hydrophobic moment of the peptide (15). It can be seen that this peptide is a far weaker inhibitor of the ATPase. The Ks value for MP17 is extrapolated, because at 30 μM MP17 only 10% inhibition was observed. This peptide also has no effect on the fluorescence intensity of NBD-labeled ATPase.

MPX and MP7 retain the ability to form α-helices in lipids and have similar hydrophobic moments to mastoparan. They also inhibit the ATPase, with Ks values of 4.4 and 2.2 μM, respectively. Similar to mastoparan, these two analogues in-
crease the fluorescence intensity of NBD-labeled ATPase, implying that they too shift the E2-E1 equilibrium toward E1.

**Conclusion** —A number of P2-type ATPases, including the Ca2+-ATPase, H+/K+-ATPase, and Na+/K+-ATPase are inhibited by peptide toxins such as mastoparan and melittin (4–10,
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The data are fitted to either a monoexponential or biexponential equation, as described in the text.

| Experiment                          | Fast, \(k_{\text{obs}}\) (s\textsuperscript{-1}) | Slow, \(k_{\text{obs}}\) (s\textsuperscript{-1}) | Amplitude, \(\chi^2\) |
|------------------------------------|---------------------------------------------|---------------------------------------------|----------------------|
| Ca\textsuperscript{2+} binding      | 49.87 ± 2.75                                | 6.62 ± 0.19                                | 3.52 ± 0.11          |
| Ca\textsuperscript{2+} binding + mastoparan | 19.00 ± 0.34                                | 3.98 ± 0.10                                | 0.13 (1.2)*          |
| Ca\textsuperscript{2+} dissociation | 19.11 ± 1.72                                | 3.36 ± 0.11                                | -2.03 ± 0.16         |
| Ca\textsuperscript{2+} dissociation + mastoparan | 37.51 ± 0.91                                | 4.06 ± 0.01                                | 0.16 (0.3)*          |

* This value corresponds to the \(\chi^2\) obtained if the alternative equation was fitted to the data.

The data from the rapid kinetic measurements clearly show that in the absence of mastoparan both Ca\textsuperscript{2+} binding and dissociation to and from the ATPase are biphasic in nature. In the presence of mastoparan these processes are now monophasic, lending further weight to the argument that Ca\textsuperscript{2+} binding/
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**TABLE III**

Comparison of the effects of mastoparan analogues on the ATPase with mastoparan

$\mu$ corresponds to the hydrophobic moment of the peptide (a measurement of the asymmetry with which hydrophobicity is distributed around the axis of a helix). Fractional $\alpha$-helical contents and hydrophobic moments are derived from other studies (see Refs. 15, 29, and 30).

| Peptide (sequence)    | $\alpha$-Helix in lipid | $\mu$ | $\mu_m$ | $\%$ | Increase in NBD fluorescence
|-----------------------|------------------------|-------|---------|------|-----------------------------|
| MP (INLKALAKKIL-NH$_3$) | 61$^c$ | 0.33$^c$ | 1.00 ± 0.13 | 8.50 ± 0.34 |
| MPX (INWKGIAAMKKLL-NH$_3$) | 71–86$^{4c}$ | 0.32$^c$ | 4.42 ± 0.16 | 9.00 ± 0.66 |
| MP$^7$ (INLKALAKKIL-NH$_3$) | 69$^c$ | 0.27$^c$ | 2.24 ± 0.11 | 11.25 ± 0.19 |
| MP17 (INLKAKAAKLL-OMe) | 31$^c$ | 0.15$^c$ | >300 | 0 ± 0.30 |

* Measured at pH 7.2 and 37 °C.
* Effect of peptides (10 $\mu$m) on the fluorescence intensity of NBD-labeled ATPase, measured at pH 7.2 and 25 °C.
* See Ref. 15.
* See Ref. 29.
* See Ref. 30.

**SCHEME 1.** A and B represent the stacked and side-by-side models that have been proposed to describe the relationship between the two Ca$^{2+}$ binding sites (I and II). Large arrows indicate the major route of Ca$^{2+}$ translocation, whereas the smaller arrows indicate possible other sites of Ca$^{2+}$ entry and exit. C represents the two sites in the presence of mastoparan. Ca$^{2+}$ binding is now seen to be independent.

Dissociation is independent in the presence of the peptide.

Mg$^{2+}$ is an essential activator of the SR Ca$^{2+}$-ATPase. It is required for several of the steps, which together make up the catalytic cycle of the enzyme. However, Mg$^{2+}$ is also in competition with Ca$^{2+}$ for binding at the two Ca$^{2+}$ binding sites. Consequently, ATPase activity decreases with increasing concentrations of Mg$^{2+}$ in the absence of mastoparan. At low concentrations of Ca$^{2+}$, the presence of mastoparan results in strong inhibition of the ATPase. As the Mg$^{2+}$ concentration is increased from 2 to 10 mM, stimulation of ATPase activity is then observed, followed by inhibition. Perhaps the stimulatory effects of Mg$^{2+}$ (which are usually masked by the competition between Ca$^{2+}$ and Mg$^{2+}$) are due to the effect of mastoparan on the Ca$^{2+}$ binding sites. We have determined that the affinity of the Ca$^{2+}$ binding sites for Mg$^{2+}$ is reduced in the presence of mastoparan, it could therefore follow that the affinity of these sites for Mg$^{2+}$ will also be reduced. For the stimulatory effects of Mg$^{2+}$ to be observed, the affinity of the Ca$^{2+}$ binding sites for Mg$^{2+}$ must have been more greatly reduced than they were for Ca$^{2+}$.

The effect of mastoparan on the steady-state levels of phosphorylation of the ATPase by [$\gamma$-32P]ATP was studied. The results suggest that mastoparan increases the affinity of the ATPase for ATP and that the steady-state equilibrium between E1-Ca and E1′Ca$_3$P is pushed toward E1′Ca$_3$P. However, mastoparan was shown not to effect the binding of ATP to the catalytic site (see Fig. 1C) and was also shown to have no effect on the affinity of the enzyme for TNP-ADP. Petithory and Jencks (35) have suggested that phosphorylation of the ATPase by ATP is a two-step process in which ATP binding is followed by a conformational change. This active conformation of the enzyme is then able to undergo rapid phosphorylation.

Because binding of ATP is unaffected by mastoparan, perhaps the peptide effects the conformational change associated with ATP binding, thus decreasing the apparent $K_m$ for ATP in the phosphorylation experiment.

Mastoparan causes a reduction in the levels of phosphoryl-enzyme formed from 1 mM [32P]P$_i$ thereby pushing the E2 ↔ E2-P$_i$ equilibrium toward E2. The reduction in levels of phosphoryl-enzyme could be due to reduced levels of E2 as a result of mastoparan shifting the E2-E1 transition toward E1.

The two mastoparan analogues, MPX and MP7, have similar effects on ATPase activity as mastoparan itself. MP17, however does not. MP17 has a reduced $\alpha$-helical content in membranes and a much smaller hydrophobic moment. Thus, the ability of mastoparan analogues to interact with and inhibit the Ca$^{2+}$-ATPase appears to correlate with their ability to adopt ordered conformations in membranes as well as their amphiphilicity.

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