ATP- and Calcium-Controlled Contraction in a Saponin Model of Physarum polycephalum

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ABSTRACT. Saponin models of the plasmodial strand of Physarum polycephalum were constructed to study how Ca\(^{2+}\) and ATP regulate the generation of tension. ATP-induced isometric tension in a saponin model increased with an increase in ATP concentration until maximum tension (0.3–1.7 mg) was reached at about 1 mM. The sensitivity of the model to ATP was heightened three to five times in a basic solution containing an ATP-regenerating system, the maximum tension (0.3–0.6 mg) being reached at 0.2 to 0.3 mM ATP. Contraction of the model also depended on the Ca\(^{2+}\) concentration irrespective of the presence or absence of the ATP-regenerating system. The optimal pCa was 7.0, and tension decreased with a Ca\(^{2+}\) concentration above or below this value. These results indicate that the tension generated in the plasmodial strand of Physarum in vivo may be regulated by ATP and/or Ca\(^{2+}\).

A plasmodial strand excised from the network region of a Physarum plasmodium contracts and relaxes actively with a particular rhythm. An effective approach for determining the mechanism of tension generation is to construct a suitable model of the plasmodial strand and to study its dynamic characteristics.

The contractile properties of Physarum plasmodia have been studied using glycerinated models (4, 15), actomyosin threads (2, 8) and a triton model (13). All these models showed that contraction requires ATP as the energy source. Matsumura et al. (8), who reconstituted an actomyosin thread from Physarum myosin and actin, demonstrated clearly that a considerable amount of tension is produced by the thread as a function of the ATP concentration. No distinct Ca\(^{2+}\) dependency, however, has yet been found with the actomyosin thread or other models of Physarum. Recently, we constructed saponin models of the plasmodial strand and found that the tension generated in them clearly depends on both the ATP and Ca\(^{2+}\) concentrations.

MATERIALS AND METHODS

The Physarum polycephalum plasmodium was cultured with compressed oats. To prepare specimens with the least individual deviation, we quickly excised 8 to 10 segments of plas-
modial strands of similar diameters (0.7-0.9 mm) and lengths (a few centimeters) taken from the same area of the plasmodium spreading over an agar surface. These segments were conditioned by being hung in a moist chamber for 1 h with a load of 10 mg by means of a fine glass hook attached to each end of the segment. Segments then were laid on an agar plate and held between a pair of setting pins with glass hooks.

The strand segments on an agar plate were treated at room temperature for 30 min with 3 × 10⁻⁴ g/ml saponin dissolved in a basic solution of pCa 7.0 composed of 20 mM KCl, 5 mM Ca-EGTA buffer (pCa 5.5–8.5), 6 mM MgCl₂, 6 mM DTT and 10 mM PIPES (pH 7.0). The Ca²⁺ concentration was varied from pCa 5.5 to 8.5 in pCa 0.5 steps by changing the ratio of CaCl₂ to EGTA (5 mM). The apparent association constant of Ca-EGTA at pH 7.0 was taken as 10⁶.68 (1). The strand segments then were washed for 30 min with a basic solution of pCa 7.0 containing no saponin and stored in the same solution with ice-cooling after removal of the glass hooks.

The prepared model, which maintained its ability to contract for about 3 days, was mounted on a very sensitive horizontal-type electromagnetic tensiometer (5, 18). The noise level and drift, respectively, were less than 0.1 mg and 0.05 mg/h. The model was held horizontally in the basic solution by its terminals which were attached to the two fine-glass rods of the tensiometer with alkyl-α-cyanoacrylate, a surgical adhesive agent (Aron Alpha A, Sankyo Co., Tokyo).

**RESULTS**

*Dependence of tensile force production on the ATP concentration.* We measured the isometric tension generated by the saponin model when ATP was added to the basic solution (pCa 7.0) in which the model was immersed. The time course of the isometric contraction of the model induced by 1 mM ATP is shown in Fig. 1. The tensile force increased somewhat slowly on the addition of ATP and reached maximal tension (0.3–1.7 mg) in 5–20 min. The tensile force produced did not decrease on the removal of ATP; hence, the process was irreversible.

The maximal values of the ATP-induced tensile force of the strand segments varied widely with the mother plasmodium and the time of isolation when the mother plasmodium was the same. This fluctuation could be minimized by isolating samples

![Fig. 1. Isometric contraction of the saponin model induced by 1 mM ATP (pCa 7.0). During the period indicated between the two arrows, the basic solution was replaced with ATP solution.](image)
from the same area of a plasmodium over a short period of time. For this reason, we used a set of specimens belonging to the same series to compare the tension generated at ATP concentrations ranging from 0 to 1.4 mM at 0.2-mM intervals.

The dependency of the tension on the ATP concentration is shown in Fig. 2. The tensile force (F) was normalized with respect to its maximal value (F_{max}), and the mean of the three normalized values from different series of experiments was plotted against the ATP concentration. Tensile force increased as the ATP concentration increased until the maximum was reached at about 1 mM ATP.

**Tensile force produced in the presence of an ATP-regenerating system.** An example of isometric contraction in a solution containing an ATP-regenerating system is shown in Fig. 3. The concentration of ATP was 0.8 mM and that of creatine phosphate 20 times this value, the creatine phosphokinase content being 36 units/ml (0.2 mg/ml). As shown in this record, the model first contracted but soon relaxed spontaneously. The reason for this is not clear.

Peak values of tension in an ATP-regenerating system are given in Fig. 4. The

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**Fig. 2.** ATP dependency of tensile force generation in the saponin model. Tension is indicated by the ratio of tensile force (F) to the maximal value (F_{max}). Bars represent standard deviations (n=3).

**Fig. 3.** Isometric contraction of a model induced in the ATP-regenerating system (0.8 mM ATP, 16 mM creatine phosphate, 36 units/ml creatine phosphokinase, pCa 7.0). Contraction was followed by spontaneous relaxation.
sensitivity to ATP was 3- to 5-fold that of the control which had no regenerating system. Maximum tension without the regenerating system was found at about 1 mM ATP, as described above, whereas the maximum tension produced in the presence of the regenerating system was found between 0.2 and 0.3 mM ATP.

**Ca\(^{2+}\) dependency.** To examine the relation between tension generation and Ca\(^{2+}\), the Ca\(^{2+}\) concentration in the basic solution containing 1 mM ATP was varied from pCa 5.5 to 8.5 in 0.5 steps by modifying the ratio of CaCl\(_2\) to EGTA (5 mM). The optimum pCa was about 7.0 (Fig. 5). The tensile force decreased whether the Ca\(^{2+}\) concentration was high or low.

**Fig. 5.** Ca\(^{2+}\) dependency of ATP-induced contraction in the saponin model. The abscissa gives the pCas of the ATP solution in which the model is submerged. The solution contained no ATP-regenerating system, but the pattern is the same as when it was present. Bars represent standard deviations (n=3).
concentration was above or below this value, decreasing steeply when the concentration was increased and reaching zero at about pCa 5.5. A small tensile force remained, however, when the Ca²⁺ concentration was lowered. This Ca²⁺ dependency of tension generation also was found in the model with the ATP-regenerating system. These results show clearly that the tension generated in the model in the presence of a sufficient amount of ATP can be controlled by Ca²⁺.

In another series of experiments we stored the strand model in ice-cooled basic solution containing a comparatively high concentration of Ca²⁺ (pCa 5.5). No appreciable tension was generated even when a sufficient amount of ATP (1 mM) was added; but, when the Ca²⁺ concentration was lowered from pCa 5.5 in the presence of 1 mM ATP, the saponin model began to generate a tensile force. Tension increased until the Ca²⁺ concentration was lowered to pCa 7.0 (Fig. 6). Below 7.0, the tension decreased again. The relation of the tension output to pCa was the same whether the Ca²⁺ concentration was modified before or after ATP was added (Figs. 5 and 6).

Our findings indicate that the tension generated by the saponin model of the plasmodial strand segment was regulated by both ATP and Ca²⁺, ATP being the energy donor and Ca²⁺ the controller of the energy output.

**DISCUSSION**

Previously (20), we reported that the intracellular ATP concentration oscillated with the same period as cyclic tension production in a phase relationship in which the ATP concentration increased as the tension increased and decreased as the tension decreased. This is consistent with the ATP dependency of the tension developed in the saponin model described in the present report.

In the presence of an ATP-regenerating system, the sensitivity of the model to ATP increased three to five times. An increase in ATP sensitivity also has been found in
the reconstituted actomyosin thread of *Physarum*. Maximum tension was present at about 200 \( \mu \text{M} \) ATP, whereas maximum tension with the ATP-regenerating system was reached at 10–20 \( \mu \text{M} \) ATP (8). The sensitivity of the actomyosin thread to ATP was higher by one order than that of the saponin model. The reason for this is not clear.

Isometric contraction of the reconstituted *Physarum* actomyosin thread without an ATP-regenerating system is irreversible, but contraction with the regenerating system is reversible (8). In the saponin models, contraction is irreversible irrespective of the presence or absence of the regenerating system. In the presence of the regenerating system, the model first contracts, but soon relaxes spontaneously. This transient contraction of the saponin model may have its structural counterpart in structural changes in microfilament aggregation. Cyclic changes corresponding to the phase of contraction and relaxation cycle of the strand are known to occur in the morphology and aggregation pattern of microfilaments (3, 11, 12, 17). Although observations have not always been unanimous because of the experimental conditions used (isometric or isotonic), actin filaments that form bundles seem to disaggregate after maximal contraction, but to become reorganized prior to the next contraction. If the saponin model loses its ability to rearrange actin filaments, it produces only one transient contraction with no cyclic tension changes.

Many reports have cited \( \text{Ca}^{2+} \) inhibition of various activities of the contractile proteins of *Physarum*. Kohama *et al.* (6) prepared myosin B of *Physarum* which was prevented from superprecipitating by \( \text{Ca}^{2+} \). At pCa 4.0, superprecipitation was completely inhibited. Kohama (7) went a step further and separated a factor responsible for this inhibition from myosin B. Nachamias (9, 10) reported that the actin-activated ATPase activity of purified *Physarum* myosin was inhibited by \( \text{Ca}^{2+} \). More recently, Ogihara *et al.* (14) reported that the superprecipitation activity of phosphorylated *Physarum* myosin was conspicuously inhibited by \( \text{Ca}^{2+} \). Using reconstituted actomyosin threads of *Physarum* (8), Sugino and Matsumura (16) showed that the isometric contraction of reconstituted actomyosin threads associated with fragmin was inhibited by \( \text{Ca}^{2+} \). The results of those investigations agree well with our findings.

We previously reported that \( \text{Ca}^{2+} \) leakage from a permeabilized plasmodial strand occurs cyclically with the same period as the contraction-relaxation cycle but in anti-phase to it (19). Although the absolute intracellular \( \text{Ca}^{2+} \) concentration of the plasmodium has yet to be measured exactly, a \( \text{Ca}^{2+} \) concentration below pCa 7.0 is unlikely to constitute a physiological condition. If we assume that the \( \text{Ca}^{2+} \) concentration in the plasmodial strand *in vivo* changes cyclically above pCa 7.0 with cyclic changes in tension production, the results found with our saponin model mean that the tension output is regulated by \( \text{Ca}^{2+} \) in an inhibitory manner. This conclusion agrees well with the phase relation between \( \text{Ca}^{2+} \) and tension oscillation.

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