Plant Physiol. (1973) 51, 17–18

The Role of ATP in Mechanically Stimulated Rapid Closure of the Venus's-Flytrap[1]

Received for publication March 10, 1972

M. J. JAFFE
Department of Botany, Ohio University, Athens, Ohio 45701

ABSTRACT

When the midribs of untreated traps of *Dionaea muscipula* are frozen in liquid nitrogen after rapid closure, they contain significantly less ATP than those frozen before closure. Exogenous ATP causes a significant increase in the rate of mechanically stimulated trap closure. Illuminated traps close faster than those kept in the dark. The traps of plants placed in 100% O_2 close much faster than do air controls, while 100% CO_2 inhibits closure. It is concluded that ATP is probably the native source of potential energy for contraction of the trap's midrib, and that if the endogenous ATP titer is increased by oxidative phosphorylation or an exogenous source, the trap will close faster.

When a fly or other insect lands on the upper, abaxial surface of the leaf trap of *Dionaea muscipula* (Venus's-flytrap), its movements cause it to rub against one or more of the sensitive trigger hairs, three of which are to be found in the center of each half of the lamina. These hairs are true sensory organs, and produce receptor potentials when they are stimulated mechanically (2, 7). This receptor potentially precedes a nonvectorial action potential which spreads out concentrically from the point of stimulation (10), and when it reaches the midrib, seems to be responsible for causing the abaxial portion of the midrib to contract. This region contains motor cells which are similar to those found in the primary pulvinus of *Mimosa pudica* (H. Toriyama, personal communication), and it may be expected that they probably act in a parallel manner. The anatomy and the electrical characteristics of the very rapid contraction of the midrib, and therefore the closure of the trap have been studied (7, 10, 11), as have the digestive processes that occur subsequently (9). However, the biochemical changes that are responsible for mechanically stimulated trap closure are essentially unknown. The biochemistry of rapid movement in the motor organs of pea tendrils (5) and the sensitive Mimosa (11) have been studied, and several similar processes have emerged. The most striking of these is that both systems contain a contractile ATPase (4, 5, 11) both consume ATP during contraction (3, 8), and in both cases there is an increased efflux of monovalent cations from the motor tissue during or immediately preceding movement (6, 12). Since the trap of *D. muscipula* is another well documented mechanically stimulable rapid plant movement, it seemed appropriate to determine if its molecular mechanism is similar to those which have already been studied. The present study deals with the participation of ATP in this mechanism.

[1] This research was supported by National Science Foundation Grant GB 20474.

MATERIALS AND METHODS

Bulbs of *Dionaea muscipula* (Venus's-flytrap) were obtained from the Carolina Biological Supply Co., Burlington, North Carolina and grown in well drained peat moss in plastic pots.

Table I. *Effects of Various Treatments on the Rate of Trap Closure (Midrib Contraction) of Mature Traps of Dionaea muscipula*

Each datum is followed by its standard error. In order to stimulate the traps, the sensory hairs on the inner laminar surface were rubbed quickly five times with the point of a sharp pencil with care being taken not to touch the outer spines (1). In all cases, the rate of trap closure was measured with an electronic stop watch and was computed by measuring the abaxial laminar angle before and after movement. In order to test the effect of O_2 and CO_2 on movement of the trap, the pots were placed in gas-tight chambers, and the air was gently flushed with 100% O_2, 100% CO_2, or laboratory air as a control. One hundred um AMP or ATP were made up in 0.1% aqueous Tween 20 and applied with a Pasteur pipette to the upper surface of trap midribs. The Tween 20 solution without nucleotide was used as a control. Such solutions were allowed to remain for 30 min on the midribs before the traps were mechanically stimulated. To test the effect of light and darkness on the rate of closure of the trap, plants were held in the dark for 20 hr and then incubated either in darkness or under incandescent plus fluorescent light (3000 ft-c) for 30 min prior to mechanical stimulation.

Treatment	Trap Closure[1]
	degrees per second
Pretreated for 20 hr in the dark, then, prior to mechanical stimulation, held for 30 min in the air in:	
Darkness	39 ± 19
Light	129 ± 37
Following a pretreatment in the dark in the same atmosphere for 30 min, stimulated in the dark in:	
Air (0.03% CO_2, 20.5% O_2)	20 ± 2
100% CO_2	2 ± 0
100% O_2	82 ± 30
Stimulated in the dark under standard conditions after 30-min topical pretreatment on the midrib with aqueous solutions (containing 0.01% Tween 20) of:	
Water (control)	40 ± 5
100 μM AMP	34 ± 9
100 μM ATP	55 ± 8

[1] N = 20, LSD at 5% = 7.

One bulb was planted per pot and, during use, each plant usually had from two to five usable traps. The traps were considered mature when they opened fairly flat and the upper surface was a bright red color. The experiments testing the effects

of O_2, CO_2, AMP, and ATP on mechanically stimulated trap closure are as described in the legend to Table I. When the rate of trap opening was measured, observations were made at 5-min intervals. The "openness" of the trap, from which the rates of opening or closure were determined, was defined in terms of the angle formed by the upper surfaces of the two halves of the trap.

In order to freeze the midribs of the traps for ATP extraction, a thread was tied to the base of the leaf, and the leaf was excised below the ligature. By means of the thread, the trap was lowered into liquid nitrogen and held there until completely frozen. The midrib was then cut out with a scalpel and homogenized with 2.0 ml of ion free water. Only midribs that were from 9 to 13 mm long (about 10 mg per midrib) were used. The homogenate was centrifugated at 3 C for 10 min at 5400*g*, and the supernatant fluid was kept in the cold until use as the source of ATP. ATP was measured by the luciferin-lucinferase method as previously described by Yunghans and Jaffe (13).

RESULTS AND DISCUSSION

It normally took a trap less than 1 sec to close completely and less than 24 hr to open and be ready to be mechanically stimulated and close again. The midrib could not go through more than two or three successive contractions and recoveries.

The effects of various pretreatments on the rate of mechanically stimulated trap closure is shown in Table I. After a prolonged dark period, the traps closed over three times more rapidly in the light than in the dark. In 100% CO_2, the traps closed only one-tenth as rapidly as in air, whereas in 100% O_2, they closed four times faster than in the air control. Although pretreatment with AMP did not appreciably change the rate of trap closure, a 30-min topical application of 100 μM ATP caused a significant increase in contraction of the midrib. It might also be noted that the effects of the latter addendum on recovery (opening) of the trap were to produce average rates of 39, 37, and 55° per 24 hr, after treatments with water, AMP, and ATP, respectively.

Further indication that ATP is involved in the energetics of contraction (closure) of the trap midrib is shown by the measurement of endogenous ATP before and after stimulation and closure. There was 950 $\pm$ 40 μM ATP per midrib in midribs obtained before stimulation, whereas after stimulation and closure, the amount of ATP per midrib was 650 $\pm$ 50 μM. The least significant difference at 5% was 29, and so the two values were significantly different from each other.

The main observations made in the present study are that mechanically stimulated contraction of the trap midrib of *Dionaea muscipula* produces a dramatic drop in the endogenous titer of ATP, and that added ATP or environmental treatments which would tend to increase the natural ATP level, also cause an increase in the rate of closure of the trap. Thus, increasing the O_2 content of the air increases the rate of trap closure, whereas removing O_2 and increasing the CO_2 level, decreases it. These observations indicate that ATP is a native source of energy for the closing process, a conclusion supported by similar data reported for other mechanically stimulable rapid plant movements such as pea tendrils (3, 5) and the primary motor pulvinus of *Mimosa pudica* (8). The mechanism which uses ATP to produce such rapid movements is not known. However, the Mimosa and tendril systems are known to contain contractile, actomyosin-like ATPase (4, 11) which, at least in the case of the pea tendrils, becomes less active as contraction of the organ progresses (4). Thus, it may be that a similar ATPase also exists in the trap midrib of *D. muscipula* and is involved in the closing mechanism.

Acknowledgment: I thank Carolyn Roundhouse for her technical assistance.

LITERATURE CITED

1. DiPalma, J. R., R. McMicheal, and M. DiPalma. 1966. Touch receptor of Venus Fly Trap, *Dionaea muscipula*. Science 152: 539-540.
2. Jacobson, S. L. 1965. Receptor response in Venus Fly-Trap. J. Gen. Physiology 49: 117-129.
3. Jaffe, M. J. and A. W. Galston. 1966. Physiological studies on pea tendrils. II. The role of light and ATP in contact coiling. Plant Physiol. 41: 1152-1158.
4. Jaffe, M. J. and A. W. Galston. 1967. Physiological studies on pea tendrils. III. ATPase activity and contractility associated with cooling. Plant Physiol. 42: 845-847.
5. Jaffe, M. J. and A. W. Galston. 1968. The physiology of tendrils. Annu. Rev. Plant Physiol. 19: 417-434.
6. Jaffe, M. J. and A. W. Galston. 1968. Physiological studies on pea tendrils. V. Membrane changes and water movement associated with contact coiling. Plant Physiol. 43: 537-543.
7. Lloyd, F. E. 1942. The Carnivorous Plants. The Ronald Press, New York.
8. Lyubimova, M. N., N. S. Demyanovskaya, I. B. Fedorovich, and I. V. Itomlenskite. 1964. Participation of ATP in the motor function of the *Mimosa pudica* leaf. Biokhimiya 29: 663-667 (English translation).
9. Schwab, D. W., E. Simons, and J. Scala. 1969. Fine structure changes during function of the digestive gland of Venus Fly Trap. Amer. J. Bot. 56: 88-100.
10. Sibaoka, T. 1966. Action potentials in plant organs in nervous and hormonal mechanisms of integration. XX. Symp. Soc. Exp. Biol. 20: 49-74.
11. Sibaoka, T. 1969. Rapid movements in plants. Annu. Rev. Plant Physiol. 20: 165-184.
12. Toriyama, H. 1955. Observational and experimental studies of sensitive plants. VI. The migration of potassium in the primary pulvinus. Cytologia 20: 367-377.
13. Yunghans, H. and M. J. Jaffe. 1972. Rapid respiratory changes due to red light or acetylcholine during the early events of phytochrome-mediated photomorphogenesis. Plant Physiol. 49: 1-7.