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Physiological Studies on Pea Tendrils. III. ATPase Activity and Contractility Associated with Coiling

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Summary. Extracts of the tendrils of *Pisum sativum*, Var. Alaska, exhibit adenosine triphosphatase activity which is inversely proportional to the amount the tendrils have coiled. The specific viscosity of the extract decreases when ATP is added. This evidence indicates a possible role of a contractile adenosine triphosphatase in coiling.

Recently, we have shown that ATP is an energy source in the coiling movements of pea tendrils (2). During contact coiling, the level of endogenous P_i increases, while the ATP titer decreases. The addition of ATP or ADP, but not AMP or adenosine to the bathing solution, increases the coiling of excised tendrils. Such observations indicate that ATPase activity may be associated with the coiling of pea tendrils, as it is with muscle contraction (4), the movements of the plasmodium of *Physarum polycephalum* (3) and the rapid movements of *Mimosa pudica* leaves (T. Sibaoka, personal communication).

The methods for measuring contact coiling in the tendrils of light grown Alaska pea plants have been previously reported (1). In these experiments, the tendrils were stimulated and allowed to coil in situ. For the ATPase determinations, 10 to 20 tendrils (ca 50 mg) were harvested, frozen and ground in a mortar with 30 ml of tris-maleate buffer (20 mM at pH 6.4). The ground material was centrifuged at 0° for 90 minutes at 2700 × *g* and the supernatant fluid made up to 30 ml. The rate of the reaction was linear for the first 30 minutes, hence the P_i content of 4.5 ml aliquots of each sample was determined before and after the incubation of the appropriate reaction mixture for 30 minutes at 24°. For the reaction mixture, 0.5 ml of 6 mM Na salt of ATP or other substrate was added to 4.5 ml of tendril extract. The reaction was stopped after 30 minutes incubation by immersing the tubes containing the reaction mixtures in boiling water for 15 minutes or by the addition of an equal volume of 1 M trichloroacetic acid. After centrifugation for 5 minutes at 2000 × *g*, P_i determinations were made on the supernatant fluid as previously described (2).

To obtain sufficient ATPase for viscosity measurements, 12 g of frozen tendrils (roughly 1200 tendrils) were ground in a frozen mortar, with 8 ml of gradually added extraction solution composed of 600 mM KCl, 40 mM $NaHCO_3$ and 10 mM Na_2CO_3 (6) and squeezed through cheesecloth to remove fibrous material. The grinding took about 1.5 hours and resulted in 7.0 ml of filtered extract. After storage at 16° for 30 minutes, the extract was centrifuged at 0° for 30 minutes at 2700 × *g*, providing 6.0 ml of an opaque green supernatant

Table I. *ATPase Activity in Extracts from Tendrils at Rest or Allowed to Coil for 20 or 30 Minutes*

The data represent increases in P_i after 30 minutes of incubation. P_i was determined at 650 nm after the addition of phosphomolybdate chromogenic reagent.

Enzymatic activity stopped by	Expt	Nanomoles P_i released per mg fr wt: Resting tendrils	Nanomoles P_i released per mg fr wt: Coiled tendrils	$\frac{\text{Coiled}}{\text{Resting}} \times 100$
Boiling	1	27.0	14.0	52
	2	2.9	1.5	52
	3	13.0	11.0	85
Trichloroacetic acid*	4	10.0	7.4	73
	5	11.4	8.1	71
	6	14.1	7.4	52

* Equal volume of 1 M trichloroacetic acid added to reaction mixture.

fluid. For viscosity measurements, Ostwald pipettes having a minimum volume of 3.0 ml were used. The delivery times of the extracting solution (t_1) and of the extract (t_2) were determined and the specific viscosity of the solution computed by the following formula (III).

$$\text{Specific viscosity} = \frac{t_2}{t_1} - 1 \qquad (I)$$

After temperature equilibration of extract and ATP solution, 0.4 ml of 20 mM ATP was added to the Ostwald pipette and mixed with the 3.0 ml of extract, yielding a final concentration of 1.18 mM ATP. Throughout the experiment, measurements were made at 2 or 3 minute intervals.

The extracts contained ATPase activity which was lower in tendrils that had coiled in situ for 20 or 30 minutes than in unstimulated tendrils (table I). The variation occurring when the reaction mixture was heated to stop the enzymatic activity was probably due to irregular accelerations of that activity as the mixture heated up. Thus halting the reaction with TCA is the preferred method. The kinetics of this decrease in ATPase activity were significantly correlated with the kinetics of coiling of tendrils in situ (fig 1). ITP, GTP and CTP were about 67 % and ADP about 44 % as effective as ATP. The addition of ATP to an extract of unstimulated tendrils resulted in a decrease in the specific viscosity of the extract (fig 2). This was not due to dilution of the extract, since addition of the same amount of water had no effect on the specific viscosity.

If contact coiling resulting in part from the contraction of the ventral surface of the tendril (1) is dependent on the utilization of ATP (2), then ATPase activity must be present, as here demonstrated. The decrease in ATPase activity observed during coiling is explicable if the ATPase retains AMP or some other product of its activity on its active sites, thus blocking them and rendering them unavailable for further action. Such a mecha-

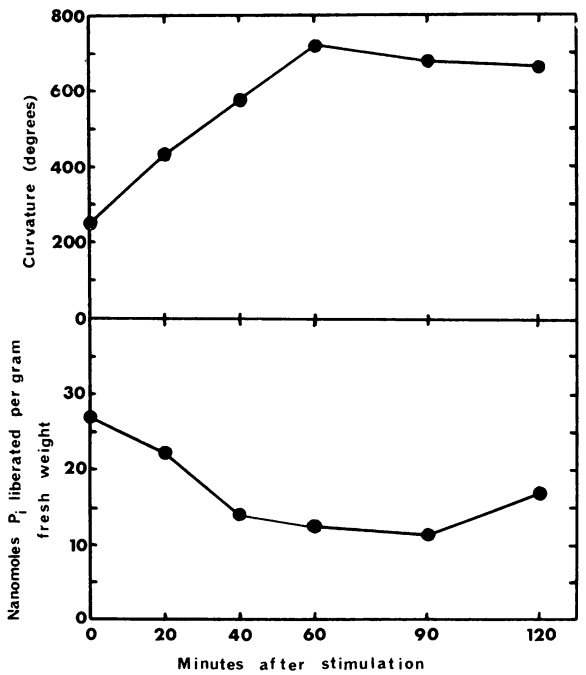

FIG. 1. Correlation of kinetics of coiling (above) with the kinetics of decrease in ATPase activity (below). For the ATPase assay, samples were extracted from coiling tendrils at various times after stimulation, the reaction mixtures incubated for 30 minutes and the P_i measured at 650 nm using the phosphomolybdate chromogenic reagent. The correlation coefficient between the 2 sets of data was —0.935 and was significant at the 1 % level (5).

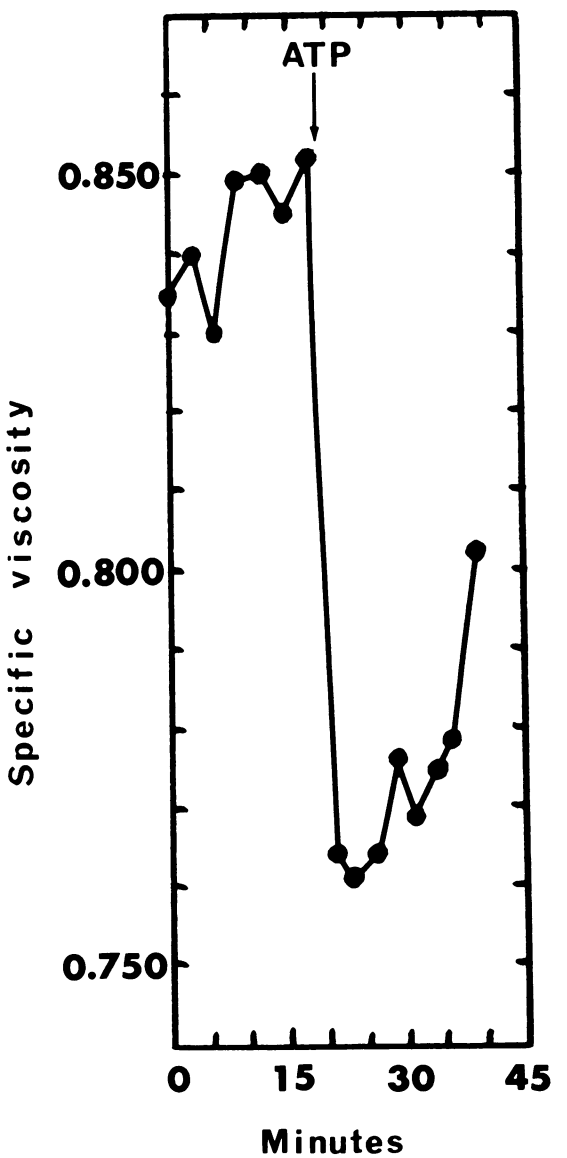

FIG. 2. The effect of a final concentration of 1.18 mM ATP on the specific viscosity of the tendril extract.

nism has been postulated for the decreased activity of muscle actomyosin (4). The ability of the crude enzyme preparation to utilize ADP, GTP, ITP and CTP as well as ATP is consonant with our previous findings (2) that these compounds are able to increase the rate of coiling in excised tendrils in the dark. Further evidence supporting the role of ATPase in coiling is the demonstration of ATPase activity in an extract that also displays the actomyosin-like property of viscosity decrease in the presence of ATP. To the best of our knowledge this is the first demonstration of a contractile ATPase associated with the rapidly moving organ of a higher plant, although it has been found to exist in a slime mold (3) and in the vascular tissue of the leaves of various species (6).

Acknowledgments

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