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Maria Ida De Michelis, Piera Lado

Effect of ethylene on auxin- and fusicoccin-induced growth of pea internode segments and on the secretion o f H^+ in the incubation medium

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Fisiologia vegetale. — *Effect of ethylene on auxin- and fusicoccin-induced growth of pea internode segments and on the secretion of* H^+ *in the incubation medium.* Nota di MARIA IDA DE MICHELIS e PIERA LADO (*), presentata (**) dal Corrisp. E. MARRÈ.

RIASSUNTO. — Nel presente lavoro è stato studiato l'effetto dell'etilene sulla crescita per distensione indotta da auxina e fusicoccina in segmenti di internodi di pisello e sulla estrusione di protoni nel mezzo, fenomeno che generalmente accompagna lo stimolo della crescita. La produzione di etilene nel tessuto è stata ottenuta sia con la somministrazione di acido 2–cloro–etilfosfonico, sia con alte concentrazioni di auxina.

I risultati ottenuti indicano che l'etilene pur modificando la direzione dello sviluppo della parete e riducendo lo stimolo alla distensione indotto da auxina e fusicoccina, non influenza affatto l'estrusione di H^+ nel mezzo.

INTRODUCTION

Growth by cell enlargement induced by IAA in etiolated pea segments is affected by ethylene both when supplied externally and when produced within the tissues by high concentrations of the hormone [1]. In the majority of tissues examined, ethylene converts the auxin-induced stimulation of growth mainly by cell elongation into a stimulation of growth in all directions This phenomenon is explained as due to a change in the orientation of the microfibrils in the cell wall [2]. The structural changes are accompanied by several alterations in the biochemical composition of the cell wall [3], but the interaction between them is still unclear.

Recent results have shown that the effect of auxin on the extensibility of the cell wall is mediated by a hormone-induced accumulation of H^+ within the cell wall itself [4, 5, 6, 7]. The mechanism of action of H^+ in the induction of cell enlargement is at present unknown.

These results raised the question as to whether ethylene, which depresses growth by cell enlargement induced by IAA, also affects the concurrent release of H^+.

In this research we have compared, in pea stem segments treated with IAA, the effects of ethylene on growth (both by elongation and by isodiametric expansion) as well as on the secretion of H^+ in the medium. Ethylene production within the tissues was obtained both by supplying CEPA [8, 9] and by treating the tissue with high concentrations of IAA [1].

(*) Centro di studio del C.N.R. per la Biologia Cellulare e Molecolare delle Piante. Istituto di Scienze Botaniche dell'Università di Milano.

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Abbreviations. IAA, indole–3–acetic acid; FC, fusicoccin; CEPA, 2–chloro–ethyl–phosphonic acid.

We also studied the effect of ethylene in the presence of fusicoccin, a diterpene glucoside which, like auxin, stimulates growth by cell enlargement in pea stem segments and induces massive release of H^+, but does not stimulate the synthesis of ethylene in these tissues [10, 4].

The results obtained indicate that in the presence of ethylene the expansion of the cell wall induced by both IAA and fusicoccin is depressed, whereas the release of H^+ is not affected.

MATERIALS AND METHODS

Pea seeds (*Pisum sativum* L., cv. *Alaska*) were allowed to germinate on moist sawdust for 5–6 days in the dark; 10 mm long segments were cut from the growing end of the distal internode.

Batches of 20 randomly chosen segments were preincubated for 2 hours in 5 ml distilled water or 10^{-4} M CEPA (pH 6). At the end of the preincubation, both fresh weight and length of the segments were measured and suitable aliquots of stock solutions of IAA and FC were added to the incubation medium. The samples were incubated at 25° C in the dark on a shaker operating at 50 spm. Immediately after the addition of the growth stimulators, the pH of the medium was measured (initial pH) and measurements were also taken at regular intervals during the duration of the treatment as well as at the end (final pH).

At the end of the incubation fresh weight and length were also measured. All other experimental conditions were as described previously [4].

The titration of the H^+ released in the incubation medium was achieved by adding amounts of 10^{-3} N NaOH sufficient to restore the pH of the medium to the value measured at the beginning of the incubation. N_2 gas was bubbled through the liquid during titration. The amount of H^+ corresponding to the CO_2 dissolved at the beginning of the treatment (when the medium was equilibrated with the atmospheric CO_2) was calculated and added to the amount of H^+ measured by titration.

RESULTS

a) *Effects of ethylene on growth induced by* IAA *and* FC.

The elongation induced by 10^{-6} M IAA in isolated pea internodes is inhibited to a considerable extent by 10^{-4} M CEPA (fig. 1).

The inhibitory effect becomes more consistent as incubation proceeds in agreement with the slow release of ethylene in contact with the tissue [8].

In the segments treated with fusicoccin, CEPA has an inhibitory effect of the same order of magnitude as the one shown in segments incubated in the presence of IAA. The FC-induced growth is therefore as sensitive to ethylene as the IAA-induced growth, even though FC, lacking the capacity to stimulate ethylene synthesis, is not able to determine itself the inhibitory

effect in the tissues. This result is a further confirmation of the similarity existing between FC- and IAA-induced growth [10].

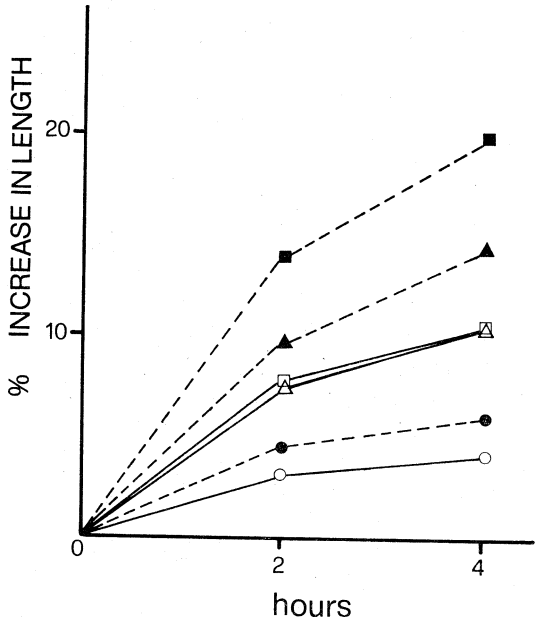

Fig. 1. – Effect of CEPA (10^{-4}M) on the increase in length of pea internode segments treated with 10^{-6} M IAA (triangles) or 1.5×10^{-5} M FC (squares). (20 segments/5 ml). Control in water (circles). Segments were preincubated for 2 hours ± CEPA. With CEPA: open symbols; without CEPA: closed symbols.

No inhibition of growth was obtained when the segments were incubated in the presence of 10^{-5} N NaCl and 10^{-5} M NaH_2PO_4 (presumably released in these amounts by CEPA under our experimental conditions [8]). We could therefore rule out the possibility that the inhibitory effect observed in the presence of CEPA was due to the products formed during the release of ethylene in the tissue.

TABLE I

Effect of CEPA (10^{-4}M) *on the increase in fresh weight, length and surface area of internode pea segments treated (20 segments/5 ml) with* IAA (10^{-6}M) *or* FC (1.5×10^{-5}M) *for 4 hours.*

	% Increase in fresh weight		% Increase in length		% Increase in surface area		Increase in length / Increase in diameter	
	—	+	—	+	—	+	—	+
H_2O	12.0	11.8	6.1	4.1	8.8	7.9	20.6	10.3
IAA (10^{-6}M) . . .	26.2	23.7	14.5	10.5	19.4	15.6	21.9	15.6
FC (1.5×10^{-5}M) .	34.2	25.9	20.1	10.6	25.8	17.6	23.0	9.6

The segments were preincubated for 2 hours ± CEPA. The measurements were made at the end of the preincubation and at the end of the treatment. The values at the end of the preincubation are: weight: 28.5 mg/segment; lenght: 11.48 mm/segment for the samples incubated in H_2O and weight: 28.7 mg/segment; lenght 11.36 mm/segment for the samples incubated in CEPA. + and — signs on top of columns indicate the absence or the presence of 10^{-4}M CEPA.

The data in Table I show that the inhibitory effect induced by CEPA occurs mainly on the elongation of the segments, without significant change in the stimulation of the increase in fresh weight by both IAA and FC (in some experiments we observed no change at all). As an increase in weight corresponds to an increase in volume, these results indicate that the reduction of growth by elongation brought about by ethylene corresponds to a stimulation of growth in all directions (see ratio between increase in length and increase in diameter in Table I). This is in agreement with the conclusions reported by other Authors [2].

However, the radial expansion induced by ethylene with no changes in the increase in volume must correspond to a relatively lower increase in the surface area of the segments (and therefore of their cells, whose number is not affected by IAA or ethylene [11]). CEPA does in fact significantly reduce the increase in surface area of the segments (calculated on the weight of the segments, assumed as having density = 1). This result demonstrates that ethylene not only alters the direction in which the expansion of the cell wall takes place, but also effectively reduces the extent of the increase in surface area of the cells.

b) *Effect of ethylene on changes in the* pH *of the medium induced by* IAA *and* FC.

Fig. 2 shows that the release of H^+, measured as the drop in the pH of the incubation medium during treatment with growth stimulators is not significantly affected by the presence of CEPA.

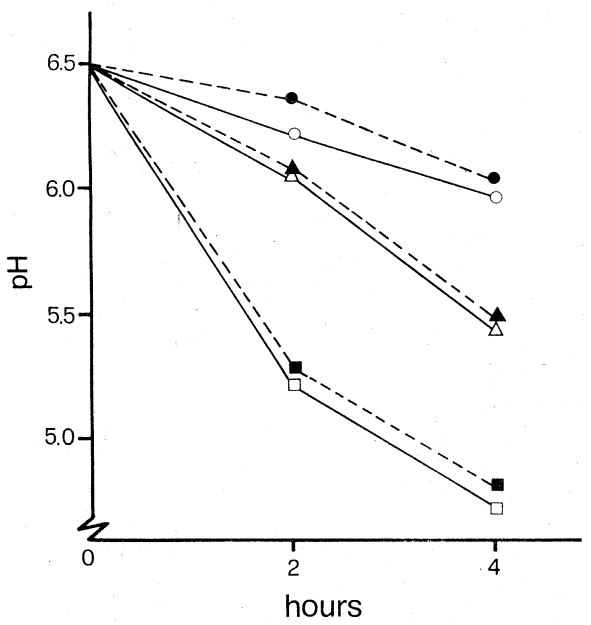

Fig. 2. – Effect of CEPA (10^{-4}M) on the pH of the incubation medium of pea internodes segments treated with 10^{-6} M IAA (triangles) or 1.5×10^{-5} M FC (squares). (20 segments/5 ml). Control in water (circles). Segments were preincubated for 2 hours ± CEPA. With CEPA: open symbols; without CEPA: closed symbols.

The values obtained by titrating to the initial pH values the incubation media at the end of the treatment are shown in Table II. Substances with buffering properties are secreted by the tissues: a simple measurement of the

pH therefore grossly understimates the true amount of H^+ released [4]. However the amount of H^+ released, as measured by titration, is the same for the segments incubated in the absence and in the presence of CEPA.

TABLE II

Effect of CEPA (10^{-4}M) *on proton extrusion in the incubation medium of pea internode segments treated (20 segments/5 ml) for 4 hours with* IAA (10^{-6}M) *or* FC (1.5×10^{-5}M).

	Initial pH	Final pH	mμeq. NaOH/ml for titration from final to initial pH
H_2O	6.70	6.05	42.5
H_2O + CEPA	6.60	5.94	55.0
IAA	6.75	5.59	104.5
IAA + CEPA	6.67	5.55	106.0
FC	6.69	5.10	169.0
FC + CEPA	6.60	5.01	174.5

Pea segments before the treatment were preincubated for 2 hours ± CEPA. The pH value was measured at the end of the preincubation (initial pH) and at the end of the treatment.

c) *Effect of high concentrations of* IAA *on growth and on changes in the* pH *of the medium.*

In internodes treated with increasing concentrations of IAA a similar if not identical pattern to that induced by CEPA can be observed.

At 10^{-3}M IAA, condition under which a considerable production of ethylene occurs [1], elongation is inhibited by 50 % and fresh weight only by 12 % (Table III). The decrease in the pH of the medium is much lower

TABLE III

Effect of different concentrations of IAA *on the increase in fresh weight; length and surface area in isolated pea internodes (4 hours of treatment).*

	% Increase in fresh weigth	% Increase in length	% Increase in surface area	Increase in length / Increase in diameter
H_2O	22.0	16.0	17.8	32.0
IAA (10^{-5}M) . .	33.0	22.0	25.9	27.5
IAA (10^{-4}M) . .	32.0	21.0	25.1	26.2
IAA (10^{-3}M) . .	31.7	19.0	23.9	19.0

in the presence of 10^{-3}M IAA than with 5×10^{-5}M IAA. Such a difference, however, appears to be due in part to a buffering effect of the hormone itself and also to different amounts of buffer substances released by the tissues in the two conditions. As shown in Table IV, the same amount of H^+ released was obtained by titration with NaOH in both cases.

TABLE IV

Proton extrusion in the incubation medium of pea internode segments treated (20 segments/5 ml) with IAA (5×10^{-5}M *or* 10^{-3}M) *for 3 hours.*

	Initial pH	Final pH	mμeq NaOH/ml for titration. from final to initial pH
IAA (5×10^{-5}M) . . .	6.55	5.48	70
IAA (10^{-3}M)	6.55	5.87	72

CONCLUSIONS

The results described above indicate that ethylene produced by treatment with CEPA or with high concentrations of IAA affects the enlargement of the cell wall both qualitatively, by turning it from elongation to expansion in all directions, and quantitatively, by reducing the increase in surface area. Ethylene was not however found to affect the release of H^+ in the medium, a phenomenon supposed to play an important role in the process of cell enlargement [4, 5, 6, 7]. These data suggest that in the sequence of events leading to the expansion of the cell wall, the effect of ethylene does not depend on the release of H^+.

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