Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Piera Lado, Franca Rasi Caldogno, Roberta Colombo, Erasmo Marrè

Regulation of pH in the plant cell wall and cell enlargement. II. Auxin-induced decrease in pH of the medium of incubation of pea internode segments

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **53** (1972), n.6, p. 583–588.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1972_8_53_6_583_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1972.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Fisiologia vegetale. — *Regulation of* pH *in the plant cell wall and cell enlargement.* II. *Auxin-induced decrease in* pH *of the medium of incubation of pea internode segments* (*). Nota di PIERA LADO, FRANCA RASI CALDOGNO, ROBERTA COLOMBO e ERASMO MARRÈ, presentata (**) dal Corrisp. E. MARRÈ.

RIASSUNTO. — Segmenti di internodio di pisello incubati in acqua stabilizzano in due ore il pH del mezzo su di un valore di circa 6,5. L'aggiunta di acido indol-3-acetico dopo raggiunta la stabilizzazione determina, parallelamente allo stimolo della crescita per distensione, un abbassamento del pH del mezzo ad un valore di circa 5,4 in 5 ore. L'effetto di abbassamento di pH non è imputabile all'accumulo di CO_2 respiratoria nel mezzo. L'interpretazione proposta è che l'ormone agendo sul metabolismo cellulare promuova l'estrusione di protoni dal citoplasma alla parete cellulare, prima, e quindi, per diffusione, al mezzo. L'abbassamento del pH nella parete potrebbe contribuire, coerentemente a dati e ipotesi di altri Autori, all'effetto di distensione cellulare, in quanto quest'ultimo è conseguenza d'un aumento della distensibilità plastica (pH dipendente) della parete.

INTRODUCTION

In the first paper of this series [1] we reported a metabolism-dependent decrease of pH from a value of 6 to a value of ca. 4.5 in the medium where pea internode segments were stimulated to cell enlargement by fusicoccin (FC), a toxin inducing in this material effects on growth and metabolism markedly greater than those induced by the natural auxin IAA [2, 3, 4, 5]. As the simplest interpretation of this effect of fusicoccin we suggested that the accumulation of protons in the medium was the consequence of the activation of a mechanism of active transfer of protons from the cytoplasm to the cell wall space, followed by diffusion of the protons (and presumably also of the accompanying anions) from the cell wall space to the medium. If this interpretation is correct, the mechanism of action of FC on cell enlargement would involve, as a necessary step, the lowering of pH in the cell wall to a value appropriate to induce an increase of cell wall extensibility. In fact, recent work in other laboratories shows that low pH increases, *in vivo* as well as *in vitro*, the extensibility of the cell wall, a necessary condition for cell enlargement [6, 7, 8, 9, 10].

(*) Laboratorio di Fisiologia Vegetale, Istituto di Scienze Botaniche, Università di Milano, Centro di Studio del C.N.R. per la Biologia Cellulare e Molecolare delle Piante, Milano (Italia).

(**) Nella seduta dell'11 novembre 1972.

The data reported in the present paper suggest that also the natural auxin indole–3–acetic acid (IAA) induces in the medium where the pea internode segments are incubated an accumulation of H^+ similar (even if less marked) to that induced by fusicoccin.

Materials and methods

Pea internode segments preparation and all experimental conditions were as previously described [1] unless as otherwise specified in the text.

10^{-3} M IAA stock solution was obtained by dissolving IAA in 2×10^{-3} M NaOH and adjusting to the pH values indicated for the single experiments with HCl. The segments were incubated at 25° C, with a volume of 0.25 ml/segment when not specified in the table.

Experiments and results

I) *Preliminary experiments of the effects of* IAA *on* pH *of the incubation medium.*

A first series of experiments, run under conditions similar to those previously described while studying the effects of fusicoccin, showed that the pH of the medium of incubation of the internode segments after 3 to 6 hours of treatment with IAA was reproducibly lower than in the case of incubation in distilled water. This difference, however, was due to the fact that pH tended to increase with time at a higher rate in the controls than in the IAA containing system, rather than to a net accumulation of H^+ in the presence of the hormone. A possible interpretation of this finding is that the segments, in the absence of the hormone, tend to stabilize the pH of the medium on a pH higher than that of distilled water equilibrated with CO_2 in air (under our conditions *ca.* 5.7). This would implicate some kind of physiological reaction presumably localized at the level of the two cut surfaces of the segments, in direct contact with the medium. In this case, the change of pH in the medium would be the result of two opposite components: a first one, arising from the tissue close to the cut surface, tending to increase the pH of the medium, and a second one, arising from the inner part of the segment (stimulated to grow by IAA) leading to the accumulation of protons in the cell wall and to their release in the medium. If this (or a similar) mechanism was acting, the pH changes in the medium should be affected by changing the ratio $\frac{\text{mass of intact internode tissue}}{\text{total cut surface}}$. This can be checked by varying the length of the segments and keeping constant the amount of medium per pea segment. The experiments of Table I show that indeed the final pH values observed in the medium become progressively lower, particularly for the IAA treated segments, when the $\frac{\text{mass of tissue}}{\text{total cut surface}}$ ratio is increased from a value of 63 to one of 168.

TABLE I

The effects of the ratio $\frac{\text{mass of tissue}}{\text{total cut surface}}$ *on the increase in fresh weight of pea internode segments treated for 3 hours with water,* IAA (5×10^{-5}M) *or* FC (5×10^{-5}M), *and on* pH *changes in the medium of incubation.*

Segment length (mm)	Mass of tissue (mg fresh weight) / total cut area (mm^2)	Increase in fresh weight from the initial value (%)			ΔpH (units) from initial pH 6.0		
		H_2O	IAA	FC	H_2O	IAA	FC
5	63.3	7.2	28.7	48.1	+0.37	+0.09	—0.38
9	114	6.7	27.8	49.7	+0.26	—0.20	—0.70
13	168	8.9	27.3	42	+0.17	—0.33	—0.87

The segments were incubated for 3 hours with a volume of medium of 0.5 ml per internode segment.

II) *Identification of a condition suitable for the observation of an* IAA*-induced decrease of* pH *in the medium.*

In order to investigate the effects of auxin on H^+ accumulation, it was necessary to find a condition of pH stability in the incubation medium (distilled water) of the control samples. The experiments of fig. 1 show that this condition is achieved by simple preincubation of the segments in distilled

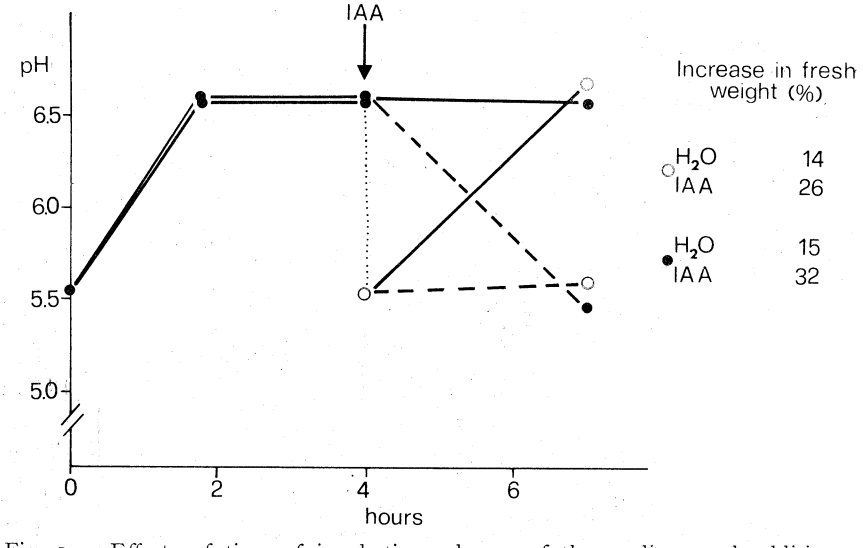

Fig. 1. – Effects of time of incubation, change of the medium and addition of IAA on the pH of the incubation medium. Solid circles: segments were incubated in the same medium through the whole period of the experiment. Open circles: segments were transferred in fresh medium (pH 5,5) at the 4th hour. IAA was added at the 4th hour. Continuous line: water (control). Broken line: IAA 5×10^{-5}M.

water: in this case, the pH value rapidly rises from a the initial value of *ca.* 5.6 to one 6.5–6.6, and then appears stabilized within a narrow variation range (± 0.15 pH units) for at least 5 hours. When the segments after 4 hours of incubation are transported in fresh distilled water (pH of *ca.* 5.6) the pH rapidly rises again to a value close to that of the segments maintained for the same period in the initial medium, thus suggesting an interesting tendency of the tissue to regulate the external pH on a given value. Auxin, when added at the 4 th hour to the segments stabilized at pH 6.6, induces a drop of pH, in 3 hours, to a value of *ca.* 5.4. On the other hand, when the hormone is added after the same period of preincubation, but following the transfer of the segments into the new medium (i.e., at pH 5.6) the hormone suppresses the pH rise detectable in the control samples.

III) *The effects of auxin and* FC *on the final* pH *values, measured in equilibrium with* CO_2 *in air and after removal of* CO_2*–bicarbonate.*

On the basis of the experiments described above, in the following experiments the effects of IAA and (for a comparison) of fusicoccin on $[H^+]$ changes in the incubation medium were performed by adding appropriate amounts of concentrated (10^{-3} M) solutions of the two substances after 2 hours of

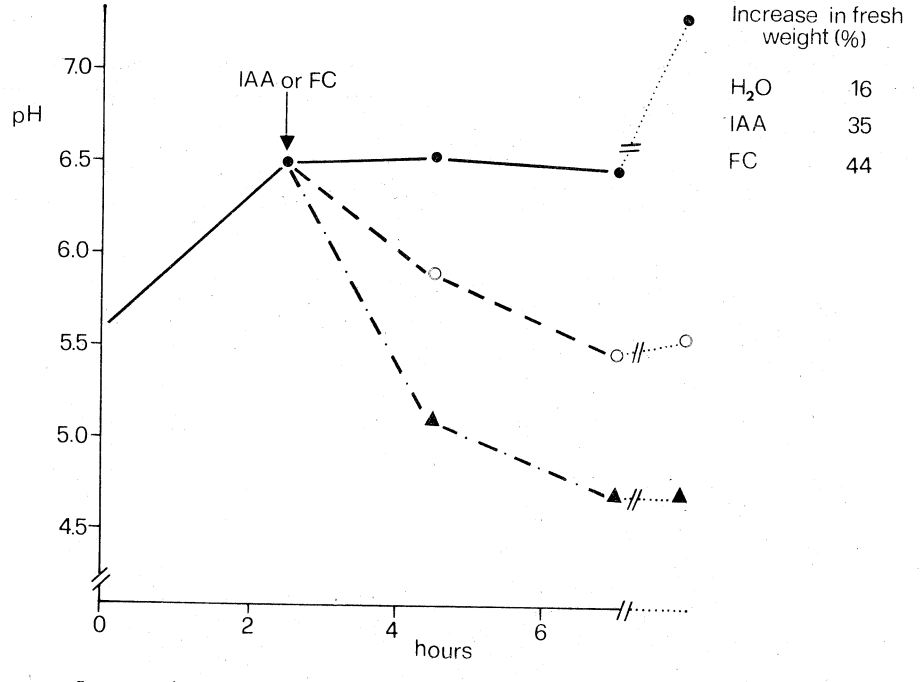

Fig. 2. – Increase in fresh weight of pea internode segments treated with IAA (5×10^{-5} M) or FC (5×10^{-5} M) and pH changes in the medium of incubation. IAA or FC were added after 2 h of pretreatment in water. (●—●) water (o– – –o) IAA (▲–·–·–▲) FC. pH was measured both in the medium in equilibrium with CO_2 in air and after removal of CO_2–bicarbonate by alternate treatment with N_2 bubbling and vacuum (·····).

preincubation of the segments in water, i.e. when the pH value was stabilized on a value of *ca.* 6.5. pH was measured 5 minutes after the additions and after 3 and 5 hours of incubation at 25° C under agitation, in open Erlenmeyer flasks. The contribution of bicarbonate to the pH changes was evaluated by measuring the pH in the medium, under N_2 flux, after removal of CO_2–bicarbonate by alternating bubbling N_2 and vacuum. The data of fig. 2 show that: *a*) both IAA and fusicoccin induce a reproducible, progressive decrease in pH of the incubation medium, this effect being markedly greater for FC than for IAA; *b*) also the effect on cell enlargement is larger for FC than for IAA; *c*) the removal of CO_2–bicarbonate from the medium does not significantly affect the low pH values of the medium of the FC and the IAA treated segments, while a rise from pH 6.45 to pH 7.30 is observed for the medium of the control sample. This shows that some acid different from carbonic acid is responsible for the decrease of pH induced by IAA and (as already shown, [1]) by FC in the medium.

Discussion and conclusions

The results presented above show that also the natural hormone, IAA, is able to induce in the medium, where the internode segments are incubated, a decrease of pH similar to that already shown for fusicoccin. Preliminary experiments of titration of the incubation media at the end of the treatment indicate that the amount of protons required to lower the pH from an initial value of 6.5 to the final value of 5.4 (as observed for IAA treatment) is of *ca.* 0.07 μ equivalents of HCl per ml of medium, i.e. *ca.* 50 % of the amount of protons required to produce the pH decrease observed with fusicoccin (from 6.5 to 4.7, experiments of fig. 2).

The fact that IAA is less active than FC in lowering the external pH is in suggestive correlation with its markedly lower effect in promoting cell enlargement. On the other hand, the more likely interpretation of the IAA induced drop of external pH appears the same already proposed for the similar effect of FC. IAA acting at cytoplasmic, or cell membrane level, would accelerate the energy dependent output of H^+ (and corresponding anions) into the cell wall, and the protons would diffuse from the cell wall space to the medium.

The attractiveness of this interpretation is that it would very nicely fit with the following well established data: *a*) the effect of auxins on cell enlargement is usually accompanied by an increase of respiration, and suppressed by respiration and phosphorylation inhibitors; *b*) low pH increases (in the absence of auxin) both the rate of cell enlargement and the extensibility of the cell wall, *in vivo*, [6, 7, 8, 9] and the extensibility of the cell wall, *in vitro* [10]; *c*) the effect of auxin on cell enlargement is accompanied by (and presumably depends on) an increase of cell wall extensibility.

The present data, if our interpretation is confirmed, would add a missing link, that is: *d*) auxin acts on cell wall extensibility and thus on cell enlar-

gement by actively regulating the pH in the cell wall on an appropriately low value.

A series of more detailed investigations on the effects of inhibitors of respiratory and of biosynthetic metabolism, and on the correlation between the effect of growth stimulating substances on external pH and on cell enlargement, are being reported elsewhere.

References

[1] E. Marrè, P. Lado, F. Rasi Caldogno and R. Colombo, « Rendic. Acc. Naz. Lincei » (Nota I), *53*, 453 (1972).
[2] E. Marrè, P. Lado, F. Rasi Caldogno and R. Colombo, « Rend. Acc. Naz. Lincei », *50*, 45 (1971).
[3] P. Lado, A. Pennacchioni, F. Rasi Caldogno, S. Russi and V. Silano, « Physiological Plant Pathology », *2*, 75 (1972).
[4] P. Lado, F. Rasi Caldogno, A. Pennacchioni and E. Marrè, « Planta (Berl.) », in press (1973).
[5] P. Lado, F. Rasi Caldogno and M. C. Cocucci, « Giorn. Bot. It. », *103*, 617 (1969).
[6] D. L. Rayle and R. Cleland, « Plant Physiol. », *46*, 250 (1970).
[7] A. Hager, H. Henzel and A. Krauss, « Planta (Berl.) », *100*, 47 (1971).
[8] M. L. Evans, P. M. Ray and L. Reinhold, « Plant Physiol. », *47*, 335 (1971).
[9] D. L. Rayle and R. Cleland, « Planta », *104*, 282 (1972).
[10] D. L. Rayle, P. M. Haughton and R. Cleland, « Proc. Nat. Acad. Sci. (U.S.) », *67*, 1814 (1970).