Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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

Regulation of pH in the plant cell wall and cell enlargement. I. Decrease in pH of the medium of incubation of pea internode segments treated with Fusicoccin

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **53** (1972), n.5, p. 453–459.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1972_8_53_5_453_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.* I. *Decrease in* pH *of the medium of incubation of pea internode segments treated with Fusicoccin* (*). Nota di ERASMO MARRÈ, PIERA LADO, FRANCA RASI CALDOGNO e ROBERTA COLOMBO, presentata (**) dal Corrisp. E. MARRÈ.

RIASSUNTO. — La fusicoccina (FC), sostanza naturale che induce in segmenti isolati di internodio di pisello una stimolazione della crescita per distensione simile, ma quantitativamente maggiore a quella indotta dall'ormone naturale, l'auxina, determina parallelamente allo stimolo della crescita una caduta da 6,0 a 4,5 del pH del mezzo di incubazione. Il dato viene interpretato come indicazione della capacità della FC di stimolare il trasporto di protoni dal citoplasma alla parete cellulare. Da quest'ultima i protoni diffonderebbero al mezzo. Coerentemente a recenti dati di altri Autori, l'abbassamento indotto da FC del pH a livello delle pareti potrebbe costituire uno dei fattori determinanti l'aumento della distensibilità plastica della parete stessa, e di conseguenza anche della crescita per distensione cellulare.

INTRODUCTION

Fusicoccin (FC), the toxin produced by *Fusicoccum amygdali*, is very active in promoting water uptake and cell enlargement in a number of plant materials such as leaf discs [1, 2], germinating seed cotyledons [3], oat and corn coleoptiles [3] and etiolated pea internode segments [1, 4, 5, 6]. In the latter material the investigation of the effects of FC on cell enlargement [1, 6], respiration, and metabolism [7, 8] showed that in all cases these effects are similar to the effect of the natural hormone indole-3-acetic acid (IAA), but quantitatively much larger, thus suggesting: *a*) that the final steps of the mechanism mediating the promotion of cell enlargement are in common for FC and for IAA; *b*) that the greater amplitude of the effects induced by FC makes this drug an appropriate tool for the study of the physiological control of cell enlargement by natural hormones [4, 6].

The present paper deals with the capacity of the FC-treated pea internode segments to lower the pH of the incubation medium. The interest of this apparent extrusion of hydrogen ions accompanying growth stimulation

(*) 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.

is discussed in relationship with the recent demonstration by other Authors of the effects of low pH (3–4) in stimulating cell enlargement *in vivo* and in increasing cell wall extensibility both *in vivo* and *in vitro* [9, 10, 11, 12, 13].

Materials and methods

Pea seeds (*Pisum sativum*, L., cv. Alaska) were grown on poplar sawdust in the dark at 25° C. 6–7 days after planting 10–mm segments were cut from the apical part of the still elongating uppermost internode.

The segments were washed in distilled water for 30 min. and randomized batches of 20 segments were transferred into Erlenmeyer flasks containing the volumes of incubation media indicated in the individual experiments.

The segments were incubated in a water thermoregulated bath at 25° C with shaking at 50 s.p.m. Fusicoccin solutions were obtained by diluting 10^{-3} M stock solution, containing 10^{-1} M ethanol, prepared as previously described [1].

The pH of all solutions was adjusted with micromolar amounts of HCl or NaOH to a value of 6.0.

pH measurements were performed with a Radiometer pH meter 28.

Fusicoccin, isolated from *Fusicoccum amygdali* Del. was a gift of Prof. A. Ballio.

Experiments and results

1) *The effects of the ratio* $\frac{\textit{number of internode segments}}{\textit{volume of medium}}$ *on the* FC*–induced cell enlargement.*

A first indication of modifications in the incubation medium comes from the experiments of Table I, showing that the rate of FC–induced cell enlargement is markedly increased when the volume of medium available per internode segment is reduced from 1 to 0.12 ml.

Table I

The effect of FC *on cell enlargement of pea internodes as a function of the ratio number of segments/volume-of medium.*

N. of segments (10 mm)	ml of medium per segment	% Increase in fresh weight from the initial value (2 h)	
		H_2O	FC 1.5×10^{-5} M
25	1	8.8	21.6
25	0.5	8.6	24.5
25	0.12	8.5	34.9

2) pH *changes in the medium accompanying growth stimulation by* FC.

The data of fig. 1 show the results of an experiment in which the $\frac{\text{number of segments}}{\text{volume of medium}}$ ratio was of 1 segment/0.5 ml. The data show that, under this condition, a reproducible decrease in pH from an initial value of ca. 6.0 in the unbuffered medium to a value of ca. 5.3 accompanies the stimulation of growth by FC; while no decrease of pH, or even a slight tendency

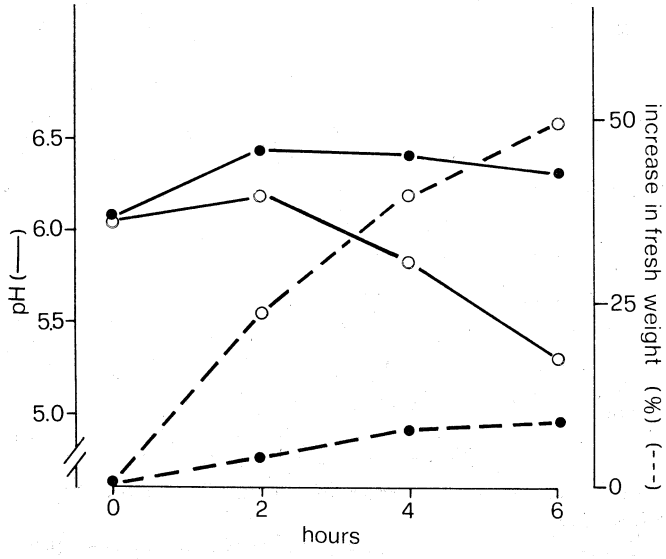

Fig. 1. – Time course of the increase in fresh weight and of pH changes in the medium of incubation of pea internode segments incubated in H_2O (solid circles) or in 10^{-5} M FC (open circles).

to increase, is observed in the medium where the segments are incubated whithout FC. Other experiments of this type show that the effect of FC in lowering the pH is increased (final values down to 5–4.5) when the volume of (unbuffered) medium per internode segment is adjusted to 1 segment/0.25 ml. This condition was thus chosen for the following experiments.

3) *Elimination of possible interferences due to microorganisms associated with the pea internode segments.*

The experiments of Table II show that no pH change is observed after incubation for 7 hours of: 1) mixtures containing FC, ethanol and a suspension in distilled water of the particulate fraction obtained by centrifugation at 12,000 × g of an homogenate from internode segments; 2) FC and the medium of incubation of pea segments in distilled water (" washing liquid "); 3) FC and a combination 1/1 by volume of the suspension of the particulate fraction

and the " washing liquid ". It seems therefore that the FC induced drop of pH is not due to degradation of FC to acidic compounds by microorganisms associated with the segments.

TABLE II

pH *stability of solutions of* FC *incubated with the " washing liquid " and with the particulate fraction from internode tissue homogenates.*

SYSTEM	Initial pH	pH after	
		2 h	6 h
A (*)	6.45	6.62	6.60
A + FC (10^{-3} M)	6.46	6.60	6.54
B (**)	6.20	6.51	6.52
B + FC (10^{-3} M)	6.30	6.52	6.58
A + B	6.42	6.50	6.48
A + B + FC (10^{-3} M)	6.42	6.52	6.41

(*) A – Suspension in distilled water of the particulate fraction obtained from pea internode segments homogenate.

(**) B – Medium of incubation of 2 g (100 segments) fresh weight of pea internode segments shaked for 2 hours at 100 s.p.m. in 25 ml of distilled water (" washing liquid "). Ethanol 10^{-1} M (required to prepare the FC solution) was present in all samples.

4) *Influence of bicarbonate and lack of influence of* K^+, Na^+ *and* Cl^- *ions on the final* pH *values.*

The data of Table III show that the presence of 5×10^{-4} M KCl, 5×10^{-4} M NaCl does not influence the pH changes in the incubation medium. This might be taken as a first indication that the FC–induced increase of H^+ in the medium is accompanied by the parallel increase of some anion extruded by the segments, rather than correspond to a H^+–cation exchange. As to the nature of the anion, the relatively low final pH of the medium of the FC–treated segments already indicates that the participation of the bicarbonate anion to the phenomenon should be almost negligeable ($pK_{\text{carbonic acid}} = 6.3$). This conclusion is confirmed by experiments in which the final pH was measured within the liquid either in equilibrium with CO_2 in air or after accurate removal of CO_2 and bicarbonate by means of alternate N_2 bubbling and vacuum (and the measurement made under N_2 flux).

As shown by Table III, no change of the final pH (4.5) is observed for the FC containing samples, while an increase from pH 6.45 to pH 7.30 is seen for the control medium, obviously containing a measurable amount of bicarbonate.

TABLE III

The effects of monovalent ions and of 2-4 DNP *on the* FC-*induced decrease of* pH, *measured before and after the removal of* CO_2-*bicarbonate from the medium.*

	Initial pH	Final pH (*)		
		Open flasks equilibrated with CO_2 in air	Δ pH	After removal of carbonate
H_2O	6.10	6.45	+0.35	7.30
NaCl (5×10^{-4} M) + KCl (5×10^{-4} M)	6.05	6.35	+0.30	7.05
FC (5×10^{-5} M)	6.08	4.50	—1.58	4.50
FC + NaCl + KCl	6.13	4.55	—1.67	4.55
H_2O + 2-4 DNP (10^{-4} M)	6.00	6.40	+0.40	7.30
FC + 2-4 DNP (10^{-4} M)	6.00	6.22	+0.22	7.15

(*) After 5 hours of incubation.

5) *Effects of an uncoupler of oxidative phosphorylation on the* FC *induced decrease of* pH.

As shown in Table III 2-4 DNP, an uncoupler of oxidative phosphorylation which at the concentration used stimulates oxygen uptake and almost completely inhibits cell enlargement in this material [6, 8, 14] also prevents the decrease of pH in the incubation medium, thus indicating that the phenomenon depends on the availability of high energy phoshate and of oxidative-phosphorylative metabolism.

DISCUSSION AND CONCLUSIONS

The results reported above show that the FC induced activation of cell enlargement in the pea internode segments is accompanied by an accumulation of H^+ in the incubation medium. In the case of a drop in pH from ca. 6.0 to 4.5 the accumulation of H^+ would be of 0.031 μ equivalents/ml of liquid, if no buffering substances such as bicarbonate and organic acids

were present. Preliminary experiments of titration with HCl and NaOH of the incubation medium at the end of the period of treatment in the pH range from 6.5 to 4.5 show the presence of small amounts of weak acids extruded by segments, beside bicarbonate, in quantities practically identical in the controls as in the FC treated samples. Due to the buffering activity of these compounds the amount of H^+ required to decrease the pH from 6.0 to 4.5 rises to ca. 0.14 μ equivalents/ml of liquid, when the ratio internode tissue/volume of medium is of 100 mg/ml. The FC–induced decrease of pH seems therefore to correspond to the extrusion of this amount of protons from the segments.

The possibility that the accumulation of H^+ in the medium is due to causes different from a metabolism-dependent extrusion of H^+ (and corresponding anions) from the pea internode segments appears, at the moment, remote. In fact, the above reported experiments seem to rule out the hypothesis of a degradation of FC to acidic compounds by microorganisms associated with the segments, and the inhibition of the pH effect by 2–4 dinitrophenol indicates its dependency on normal oxidative phosphorylation.

The hypothesis that in our opinion covers more satisfactorily all the available data is that the H^+ accumulation in the medium is due to a FC activated, high energy phosphate dependent extrusion of protons from the cytoplasm through the cell membrane into the cell wall space, followed by diffusion into the medium.

The mechanism of proton extrusion might be of the type postulated for Na^+ and H^+ active transport by ion-activated anisotrope ATPase through muscle cell and eritrocyte membranes [15]. The very interesting data on the nucleotide triphosphate-induced stimulation of cell wall extension under anaerobic conditions reported by Hager *et al.* [10] should be carefully considered in this connection. At the present moment, even the discussion whether the extrusion of protons is preceeded or followed by a corresponding extrusion of anions (as required for the maintenance of electroneutrality) appears premature speculation.

Some characteristics of the phenomenon must first be investigated, such as the quantitative measurement of the amount of H^+ extruded, the chemical identification of the substances leaking out from the segments into the medium, the correlation between the effects of FC on growth, on metabolism and on pH, the generalization of the phenomenon to other materials responding to FC with a cell enlargement reaction.

On the other hand, if our interpretation of the present data is correct, the physiological relevance of the phenomenon would be obvious. The demonstration that a cell enlargement promoting substance induces an increase of $[H^+]$ in the cell wall compartment would link the very important work recently carried out in other laboratories, showing that low pH (3 to 4.5) increases the extensibility of the cell wall *in vitro* [9, 10, 12], and promotes cell enlargement *in vivo* [11, 13] on one hand, with the relevant amount of data showing that auxins induce cell enlargment mainly by a metabolism

dependent capacity to increase, once again, cell wall extensibility on the other. In the second paper of this series we shall report some data indicating that also in the case of auxins, a in that of FC, the effect on cell enlargement is accompanied by an accumulation of protons in the medium.

REFERENCES

[1] P. LADO, A. PENNACCHIONI, F. RASI CALDOGNO, S. RUSSI and V. SILANO, « Physiological Plant Pathology », *2*, 75 (1972).
[2] A. BALLIO, A. GRANITI, F. POCCHIARI and V. SILANO, « Life Sciences », *7*, 751 (1968).
[3] R. COLOMBO, P. LADO and F. RASI CALDOGNO, « Proceedings of National Meeting of Italian Society of Plant Physiology », Modena, Italy (1972).
[4] E. MARRÈ, P. LADO, F. RASI CALDOGNO and R. COLOMBO, « Rend. Acc. Naz. Lincei », *50*, 45 (1971).
[5] A. BALLIO, F. POCCHIARI, S. RUSSI and V. SILANO, « Physiological Plant Pathology », *1*, 95 (1971).
[6] P. LADO, F. RASI CALDOGNO, A. PENNACCHIONI and E. MARRÈ, « Planta (Berl.) », in press.
[7] P. LADO, F. RASI CALDOGNO and M. C. COCUCCI, « Giorn. Bot. It. », *103*, 617 (1969).
[8] F. RASI CALDOGNO, P. LADO, R. COLOMBO and E. MARRÈ, *unpublished data.*
[9] M. L. EVANS, P. M. RAY and L. REINHOLD, « Plant Physiol. », *47*, 335 (1971).
[10] A. HAGHER, H. HENZEL and A. KRAUSS, « Planta (Berl. », *100*, 47 (1971).
[11] D. L. RAYLE, P. M. HAUGHTON, and R. CLELAND, « Proc. Nat. Acad. Sci. (U.S.» , *67*, 1814 (1970).
[12] D. L. RAYLE and R. CLELAND, « Plant. Physiol », *46*, 250 (1970).
[13] D. L. RAYLE and R. CLELAND, « Planta (Berl.) », *104*, 282 (1972).
[14] E. MARRÈ and G. FORTI, « Physiol. Plantarum », *55*, 36 (1958).
[15] D. C. TOSTESON, « Fed. Proc. », *22*, 19 (1963).