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Short communication

THE EFFECT OF AUXINS (IAA AND 4-CL-IAA) ON THE REDOX ACTIVITY AND MEDIUM pH OF *Zea mays* L. ROOT SEGMENTS

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Abstract: Indole-3-acetic acid (IAA) and 4-chloroindole-3-acetic acid (4-Cl-IAA) were tested at different concentrations and times for their capacity to change the redox activity and medium pH of maize root segments. The dose-response surfaces (dose-response curves as a function of time) plotted for redox activity and changes in medium pH (expressed as ΔpH) had a similar shape for both auxins, but differed significantly at the optimal concentrations. With 4-Cl-IAA, the maximal values of redox activity and medium pH changes were observed at 10^{-10} M, which was a 100-fold lower concentration than with IAA. Correlations were observed between redox activity and medium pH changes at the optimal concentrations of both IAA and 4-Cl-IAA. The results are discussed herein, taking into account both the concentration of the auxins and the effects produced by them.

INTRODUCTION

Ever since plasma membrane (PM) redox activity was discovered in eukaryotes [1], much evidence has been reported for its involvement in several physiological processes [2-4]. Research on plasma membrane redox activity suggests that plant cells are able to reduce exogenous electron acceptors; hexacyanoferrate III (HCF III) is much used in such studies [5-8]. Ferricyanide reduction by plant cells is accompanied by proton release [9-11] and plasma

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Abbreviations used: HCF (III) – hexacyanoferrate III, IAA – indole-3-acetic acid, 4-Cl-IAA – 4-chloroindole-3-acetic acid

membrane depolarization [12, 13]. It has also been reported that plant hormones of the auxin class change the rate of HCF III reduction and proton extrusion by plant cells [14, 15]. It is generally accepted that indole-3-acetic acid (IAA) is the main and most abundant auxin in plants. IAA exerts control over many processes of plant growth and development [16, 17]. Chlorinated auxin, 4-Cl-IAA, occurs naturally in a number of plants, mainly members of the *Fabaceae* [18-23], but it has not been found in maize [22]. 4-Cl-IAA has been tested in many different bioassays that have shown its exceptionally high level of biological activity as compared to IAA [24-32]. The aim of this investigation was to compare the effect of two auxins (IAA and 4-Cl-IAA), at a wide range of concentrations (10^{-12}-10^{-6} M), on the redox activity and proton extrusion in maize root segments.

MATERIALS AND METHODS

Seeds of maize (*Zea mays* L.) cv. K33xF2 were soaked in tap water for 2 h, sown on wet perlite in plastic boxes and placed for 4 days in a growth chamber at 27ºC. After a wash in distilled water, 10-mm long segments were excised 3 mm from the tip of the roots. These root segments were then washed for 1 h in distilled water, and transfered immediately afterwards to 1 mM Tris-HCl (pH 6.0) containing 0.5 mM $CaCl_2$ and 50 mM KCl (incubation medium). 300 mg of root segments were incubated in 3 ml of this incubation solution. After shaking for 30 min on a rotary shaker at 100 rpm, HCF III, at a final concentration of 1 mM, and either IAA or 4-Cl-IAA were added to the incubation medium. For the next 2 h, the segments were shaken at 100 rpm, and every 30 min, the level of ferricyanide reduction and the pH of the incubation medium were measured. HCF III reduction was monitored spectrophotometrically at 420 nm as per the method described previously by Federico and Giartosio [6], and expressed in µM of reduced-form HCF III calculated per g of fresh weight (FW).

The pH measurements were performed with a type N-517 pH-meter (Mera-Elwro, Poland) and OSH 10-10 pH electrode (Metron, Poland). pH changes in the incubation medium were expressed as ΔpH, where ΔpH means the difference between the medium pH measured every 30 min (over a 2-h period) and the pH measured immediately after the addition of the growth effectors and HCF III. Negative ΔpH values indicate extrusion of protons to the incubation medium. The results are the means of eight independent experiments.

Chemicals

A 1 mM aqueous stock solution of indole-3-acetic acid (Serva, Heidelberg, Germany) was prepared using the potassium salt of IAA, since it dissolves rapidly in distilled water. 4-Chloroindole-3-acetic acid (Sigma, St Louis, USA) was dissolved in a small volume of isopropanol, and stored as a 0.1 mM stock solution in which the concentration of isopropanol did not exceed 0.2%. These stock solutions were used for the preparation of appropriate concentrations of

both auxins, which were always added in the same volumes (100 μL/ml of the incubation medium).

RESULTS

Fig. 1 shows the dose-response surfaces (dose-response curves as a function of time) for IAA- and 4-Cl-IAA-mediated redox activity in maize root segments. The results indicate that the redox activity in the root segments depended on both the concentrations of auxins and the time after their addition to the incubation medium. With both IAA and 4-Cl-IAA, the dose-response surfaces had a similar shape, but differed at the optimal concentrations. For 4-Cl-IAA, the maximal value of redox activity was observed at 10^{-10} M, which was a 100-fold lower concentration than that for IAA. As the optimal IAA and 4-Cl-IAA concentrations were exceeded, the redox activity began to decline.
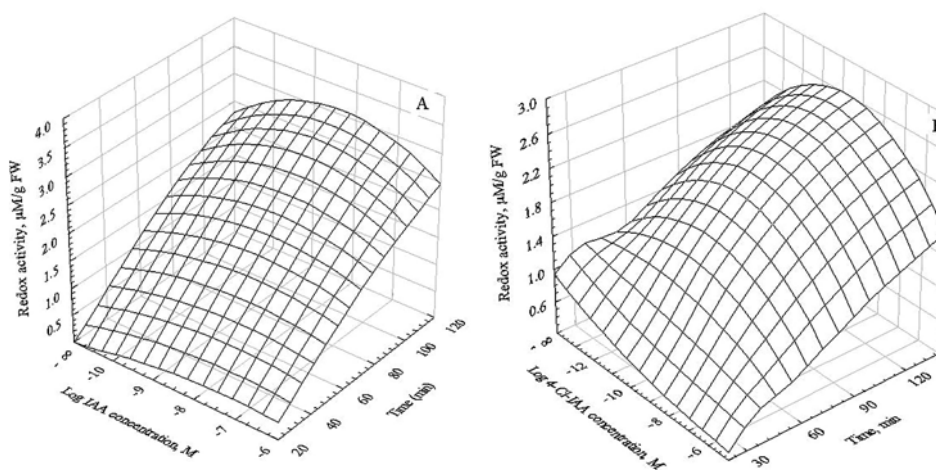

Fig. 1. The redox activity over time in maize root segments in solutions with various IAA (A) and 4-Cl-IAA (B) concentrations.

Fig. 2 shows the pH changes of the incubation medium expressed as ΔpH (see Materials and methods) measured synchronously with the redox activity, using the same tissue sample. As can be seen in Fig. 2, the optimal concentrations for the two auxins differed: for 4-Cl-IAA, the maximal value of ΔpH was observed at 10^{-10} M, whereas for IAA, it was at 10^{-8} M.

Fig. 3 shows the comparison between the effects of IAA and 4-Cl-IAA on redox activity and medium pH changes (expressed as ΔpH) in the maize root segments. The curves in Fig. 3 were constructed from the data obtained at the end (after 2 h) of the experiment, and expressed as a percentage of the results for the control (root segments in the incubation medium without the growth effectors). As can be seen in Fig. 3, the redox activity and proton extrusion at the optimal

4-Cl-IAA concentration were at a similar level (stimulation by ca. 20 %), whereas at the optimal IAA concentration (10^{-8} M), there was a higher stimulation of proton extrusion than of redox activity.
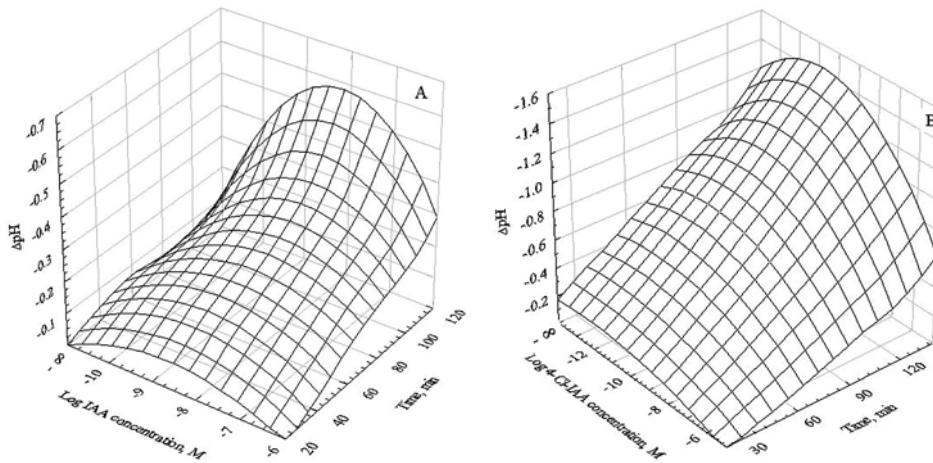

Fig. 2. Changes in medium pH (expressed as ΔpH) over time of maize root segments incubated in solutions with various IAA (A) and 4-Cl-IAA (B) concentrations.
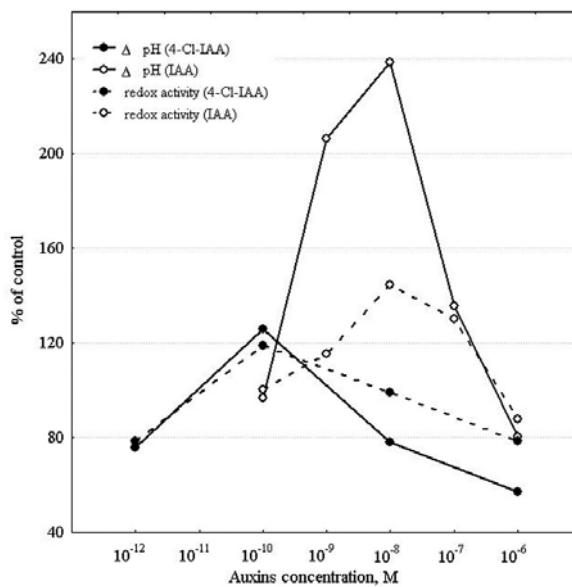

Fig. 3.   The redox activity and medium pH changes (Δ pH) of maize root segments expressed as a % of the results for the control (root segments in the incubation medium without the auxins). The results are the means of eight independent experiments. The SE did not exceed 8%.

DISCUSSION

The main strategy of the experiments described in this paper was the addition of either IAA or 4-Cl-IAA and hexacyanoferrate III (HCF III), a nonpermeable electron acceptor to the incubation medium, and the simultaneous measurement (using the same tissue sample) of HCF III reduction and proton extrusion. The dose-response surfaces (dose-response curves as a function of time) for IAA and 4-Cl-IAA-mediated redox activity and proton extrusion in maize root segments were obtained (Figs 1, 2 and 3). It was found here that the dose-response surfaces for the two auxins differed significantly at their optimal concentrations. With 4-Cl-IAA, the maximal values of the redox activity and medium pH changes were observed at 10^{-10} M, which was a 100-fold lower concentration than that for IAA. Although 4-Cl-IAA was more active than IAA in terms of the lowest concentrations needed to induce redox activity and medium pH changes, it produced much weaker changes in both processes. For example, after 2 h, only 20% more redox activity and change in medium pH could be detected with 4-Cl-IAA, whereas in the presence of IAA, a 140% higher ΔpH and 40% higher redox activity were observed as compared to the control.

It was previously shown that plasma membrane redox activity measured in the presence of ferricyanide stimulates net H^+ extrusion by maize root segments [6, 9]. It was also found that fusicoccin (a fungal toxin produced by *Fusicoccin amygdali*), a growth substance which mimics the effect of auxin (IAA) on the elongation growth of plant cells, stimulates the reduction of ferricyanide, accompanied by a decrease in the external pH [6]. The effect of plant hormones (IAA) on redox activity and proton extrusion was also the subject of the experiments performed with intact maize roots [33-35]. These experiments showed that at concentrations higher than 10^{-8} M, IAA inhibits growth, proton extrusion and transmembrane electron transfer in intact maize roots. Experiments performed with maize coleoptile segments, the classic object of elongation growth research, showed that an impermeable electron acceptor (hexachloroiridate IV) transiently inhibits naphthaleneacetic acid-stimulated growth and proton extrusion [36]. The IAA-mediated stimulation of redox activity and proton extrusion in maize root segments seen here concurs well with the findings of Böttger and Hilgendorf [33]. As far as is known, there is a lack of data in the literature concerning the effect of 4-Cl-IAA on redox activity in maize roots.

In conclusion, the experiments reported on in this paper showed that in maize root segments, 4-Cl-IAA is much more active than IAA in terms of the lowest concentrations needed to mediate redox activity and proton extrusion, although at its optimal concentration, this chloro-derivative induced much weaker effects than IAA.

REFERENCES

1. Crane, F.L., Goldenberg, H. and Morré, D.J. Dehydrogenases of the plasma membrane. **Subcell. Biochem.** <u>6</u> (1979) 345-399.
2. Lüthje S., Döring, O., Heuer, S., Lüthen, H. and Böttger, M. Oxidoreductases in plant plasma membranes. **Biochim. Biophys. Acta** <u>1331</u> (1997) 81-102.
3. Medina, M.A., Del Castillo-Olivares, A. and NúÑez De Castro, I. Multifunctional plasma membrane redox systems. **BioEssays** <u>19</u> (1997) 977-984.
4. Bérczi, A. and Møller, I.M. Redox enzymes in the plant plasma membrane and their possible roles. **Plant Cell Env.** <u>23</u> (2000) 1287-1302.
5. Craig, T.A. and Crane, F.L. Evidence for transplasma membrane electron transport system in plant cells. **Proc. Ind. Acad. Sci.** <u>90</u> (1981) 150-155.
6. Federico, R. and Giartosio, C.E. A transplasmamembrane electron transport system in maize roots. **Plant Physiol.** <u>73</u> (1983) 182-184.
7. Rubinstein, B., Stern, A.I. and Stout, R.G. Redox activity at the surface of oat root cells. **Plant Physiol.** <u>76</u> (1984) 386-391.
8. Menckhoff, M. and Lüthje, S. Transmembrane electron transport in sealed and NAD(P)H-loaded right-side-out plasma membrane vesicles isolated from maize (*Zea mays* L.) roots. **J. Exp. Bot.** <u>55</u> (2004) 1343-1349.
9. Rubinstein, B. and Stern, A.I. Relationship of transplasmamembrane redox activity to proton and solute transport by roots of *Zea mays*. **Plant Physiol.** <u>80</u> (1986) 805-811.
10. Böttger, M. and Lüthen, H. Possible linkage between NADH-oxidation and proton secretion in *Zea mays* L. roots. **J. Exp. Bot.** <u>37</u> (1986) 666-675.
11. Barr, R. The possible role of redox-associated protons in growth of plant cells. **J. Bioenerg. Biomemb.** <u>23</u> (1991) 443-467.
12. Döring, O., Lüthje, S., Hilgendorf, F. and Böttger, M. Membrane depolarization by hexacyanoferrate (III), hexabromoiridiate (IV) and hexachloroiridiate (IV). **J. Exp. Bot.** <u>41</u> (1990) 1055-1061.
13. Grabov, A. and Böttger, M. Are redox reactions involved in regulation of K^+ channels in the plasma membrane of *Limnobium stoloniferum* root hairs? **Plant Physiol.** <u>105</u> (1994) 927-935.
14. Barr., R. and Böttger, M. The effect of chloro-derivatives of indoleacitic acid on plasma membrane electron transport and proton excretion. **Proc. Ind. Acad. Sci.** <u>99</u> (1991) 129-136.
15. Carrasco-Luna, J., Calatayud, A., González-Darós, F. and del Valle-Tascón, S. Hexacyanoferrate (III) stimulation of elongation in coleoptile segments from *Zea mays* L. **Protoplasma**. <u>184</u> (1995) 63-71
16. Davies, P.J. Plant hormones. **Biosynthesis, signal transduction, action.** eds. Kluwer Academic Publishers, (2004) 204-220.
17. Taiz, L. and Zeiger, E. **Plant Physiology.** 3rd edn. Sinauer Associates, Inc., Publishers (2002).

18. Engvild, K.C. Natural chlorinated auxins labelled with radioactive chloride in immature seeds. **Physiol. Plant.** 34 (1975) 286-287.
19. Engvild, K.C. Simple identification of natural chlorinated auxin in pea by thin layer chromatography. **Physiol. Plant.** 48 (1980) 435-437.
20. Engvild, K.C., Egsgaard, H. and Larsen, E. Gass chromatographic-mass spectrometric identification of 4-chloroindole-3-acetic acid methyl ester in immature green peas. **Physiol. Plant.** 42 (1978) 365-368.
21. Engvild, K.C., Egsgaard, H. and Larsen, E. Determination of 4-chloroindole-3-acetic acid methyl ester in *Lathyrus*, *Vicia* and *Pisum* by gas chromatography-mass spectrometry. **Physiol. Plant.** 48 (1980) 499-503.
22. Hofinger, M. and Böttger, M. Identification by GC-MS of 4-chloroindolilacetic acid and its methyl ester in immature *Vicia faba* seeds. **Phytochem.** 18 (1979) 653-654.
23. Katayama, M., Thiruvikraman, S.V. and Marumo, S. Identification of 4-chloroindole-3-acetic acid and its methyl ester in immature seeds of *Vicia amurensis* (the tribe *Viciaeae*) and their absence from three species of *Phaseoleae*. **Plant Cell Physiol.** 28 (1987) 383-386.
24. Böttger, M., Engvild, K.C. and Soll, H. Growth of *Avena* coleoptiles and pH drop of protoplast suspensions induced by chlorinated indoleacetic acids. **Planta** 140 (1978) 89-92.
25. Pless, T., Böttger, M., Hedden, P. and Grabe, J. Occurrence of 4-Cl-indoleacetic acid in broad beans and correlation of its levels with seeds development. **Plant Physiol.** 74 (1984) 320-323.
26. Ahmad, A., Anderson, A.S. and Engvild, K.C. Rooting, growth and ethylene evolution of pea cuttings in response to chloroindole auxins. **Physiol. Plant.** 69 (1987) 137-140.
27. Hatano, T., Katayama, M. and Marumo, S. 5,6-dichloroindole-3-acetic acid as a potent auxin: its synthesis and biological activity. **Experientia** 43 (1987) 1237-1239.
28. Fischer, C., Lüthen, H., Böttger, M. and Hertel, R. Initial transient growth inhibition in maize coleoptiles following auxin application. **J. Plant Physiol.** 141 (1992) 88-92.
29. Rescher, U., Walther, A., Schiebl, C. and Klämbt, D. *In vitro* binding affinities of 4-chloro-, 2-methyl-, 4-methyl-, and 4-ethyl-indoleacetic acid to auxin-binding protein 1 (ABP1) correlate with their growth- stimulating activities. **J. Plant Growth Reg.** 15 (1996) 1-3.
30. Karcz,W., Lüthen, H. and Böttger, M. Comparative investigation of IAA and 4-Cl-IAA-induced growth and proton secretion in maize coleoptile segments. **Plant Physiol. and Biochem. Spec. Iss**. Abstract S01-14, (1996) 7
31. Karcz, W., Lüthen, H. and Böttger, M. Effect of IAA and 4-Cl-IAA on growth rate in maize coleoptile segments. **Acta Physiol. Plant.** 21 (1999) 133-139.

32. Karcz, W. and Burdach, Z. A comparision of the effects of IAA and 4-Cl-IAA on growth, proton secretion and membrane potential in maize coleoptile segments. **J. Exp. Bot.** 53 (2002) 1089-1098.

33. Böttger, M. and Hilgendorf, F. Hormone action on transmembrane electron and H^+ transport. **Plant Physiol.** 86 (1988) 1038-1043

34. Lüthen, H., Hilgendorf, F. and Böttger, M. Effect of auxin on growth, proton secretion and transmembrane electron transfer in intact maize roots. in: **Structural and functional aspects of transport in roots** (Loughman B.C. *et al*., Eds.), Kluwer Academic Publishers, 1989, 63-67.

35. Hilgendorf, F. and Böttger, M. Influence of temperature on proton secrection and hexacyanoferrate (III) reduction of *Zea mays L.* roots. **Plant Physiol.** 101 (1993) 1340-1353.

36. Lüthen, H. and Böttger, M. Induction of elongation in maize coleoptiles by hexachloroiridate and its interrelation with auxin and fusicoccin action. **Physiol. Plant.** 89 (1993) 77-86.