ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVII — nr 2
1968

Effect of metal ions on IAA- and EDTA-induced elongation of sunflower hypocotyl sections and on their absorption of water

J. BUCZEK and Z. KONARZEWSKI

INTRODUCTION

Although it has been previously demonstrated (Bennet-Clark 1956; Heath and Clark 1956; Weinstein et al. 1956; Ng and Carr 1959) that disodium versenate induces elongation of the coleoptile and hypocotyl sections, the mechanism of this phenomenon, however, is not well known. The results of comparative investigations (Buczek 1965 and Buczek 1968a, 1968b) have shown a similarity in the effect exerted by IAA and EDTA, however, it may be concluded that those effects are due to different mechanisms.

The purpose of the present paper was to investigate the effect of metallic ions on the elongation and water absorption (in sunflower hypocotyl sections) induced by IAA and EDTA, as well as the effect of these agents on the extensibility of cell walls.

MATERIAL AND METHODS

The material and methods have been described in detail in a former paper (Buczek 1968c), we shall, therefore, confine ourselves to present briefly the course of the experiment.

Sunflower (*Helianthus annuus* L.) hypocotyl sections were used in the experiment. The 15-mm sections cut 5 mm below the cotyledon were soaked in 0,01 m K-phosphate buffer at pH 5,2 (control). When required the solution contained also 10^{-5} M β-indolylacetic acid (IAA) or 10^{-5} M disodium salt of ethylenediamine tetraacetic acid (EDTA) and ions given in the form of sulphates (three times recrystallized) namely: Fe^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}. The concentration of the individual ions was equimolar with respect to the investigated IAA and EDTA concentrations. The sections were incubated in 25-ml erlenmayer flasks filled with the investigated solutions (20 ml), each containing 10 sections.

A stream of air was continously bubbled through the solutions. The experiment was conducted in the dark at 25°. The length and increase

in fresh weight were measured in green light after 24 hrs. Plasmolysis was conducted in 1,5 M mannitol in the coures of 120 min. Each combination consisted of 10 replications, each experiment was repeated at least three times.

Tests of significance of differences between means were made by calculating the standard error of the difference from the standard errors of two means, by means of the expression:

$$\text{S.E. of difference} = \sqrt{(\text{S.E}_1)^2+(\text{S.E}_2)^2},$$

differences which exceeded twice the standard error were deemed to be significant at the 5 per cent level.

RESULTS

As may be read from the data given in Table 1, EDTA (like IAA) induced both elongation and absorption of water measured by the increase in fresh weight. One observes that both processes run almost parallely. Although auxin-induced elongation exceeded several times the growth of sections induced by sodium versenate (Table 1), nevertheless, the induction observed for EDTA was significant.

Table 1

Effect of 10^{-5} M IAA and 10^{-5} M EDTA on elongation and absorption of water

	Elongation			Absorption of water		
	Increase in length		As % of initial length	Increase in fr. wt		As % of initial fr. wt
	mm	SE		mg	SE	
Control	1.5	0.22	9.1	7.4	0.90	10.9
IAA	8.8	0.38	53.7	36.7	1.64	53.9
EDTA	2.8	0.28	17.5	12.1	0.92	17.2

In the above described experiments we investigated the direct effect of metals belonging to the Martell-Calvin series (1952) on the elongation and absorption of water by hypocotyl sections as well as the effect of those metals on the effeciveness of IAA and EDTA in stimulation of growth. The concentrations of particular metal ions were equimolar with respect to the investigated concentrations of IAA and EDTA. The data referring to the effects of the investigated ions on the elongation and absorption of water by hypocotyl sections are given in Tables 2 and 3. As seen Fe^{3+}, Cu^{2+}, Mn^{2+}, Ca^{2+} or Mg^{2+} ions added to the buffer caused slight (non significant) increases in elongation and in fresh weight, whereas Ni^{2+} and Co^{2+} ions increased both processes significantly.

Table 2

Effect of 10^{-5} M metal ions on 10^{-5} M EDTA-induced elongation and increase in fresh weight

	Increase in length					Increase in fresh weight				
	Buffer		EDTA		due to EDTA	Buffer		EDTA		due to EDTA
	mm	SE	mm	SE		mg	SE	mg	SE	
O	2,0	0,20	3,5	0,22	+1,5	10,3	0,66	16,8	0,72	+ 6,5
Fe^{3+}	2,5	0,18	1,8	0,15	—0,7	11,8	0,78	10,5	0,81	— 1,3
Cu^{2+}	2,5	0,28	1,7	0,22	—0,8	10,5	0,33	9,4	0,80	— 1,1
Ni^{2+}	5,0	0,58	2,3	0,24	—2,7	22,8	2,86	10,8	1,26	—12,0
Co^{2+}	5,3	0,63	2,8	0,25	—2,4	24,8	0,69	14,9	1,49	— 8,9
Mn^{2+}	2,1	0,17	1,6	0,22	—0,5	10,9	0,51	7,1	0,71	— 3,8
Ca^{2+}	2,0	0,21	2,0	0,19	0,0	11,0	0,42	11,8	1,35	+ 0,8
Mg^{2+}	2,3	0,27	2,1	0,18	—0,2	11,8	1,65	12,6	1,11	+ 0,8

According to our expectation (Table 2) the solution of EDTA induced growth and an increase in fresh weight. The addition of individual metal ions (except Co) brought about a significant inhibition in the effectiveness of EDTA, as compared with the effect of EDTA alone. Similarly, equimolar solutions of EDTA+individual metal ions (except Ca and Mg) significantly inhibited the elongation and water absorption as compared with the effect of ions given alone. The solutions of EDTA+Ca or EDTA+Mg exerted the same effect on elongation as Ca or Mg ions given alone, and caused an insignificant increase in fresh weight.

Table 3 shows the relationship between the elongation and absorption of water by sunflower hypocotyl sections in presence of metal ions and IAA. Equimolar solutions of IAA and the investigated ions (except Ni and Co) did not bring any significant change in the elongation and water

Table 3

Effect of 10^{-5} M metal ions on 10^{-5} M IAA-induced elongation and increase in fresh weight

	Increase in length					Increase in fresh weight				
	Buffer		IAA		Change due to IAA	Buffer		IAA		Change due to IAA
	mm	SE	mm	SE		mg	SE	mg	SE	
O	2,2	0,32	8,0	0,57	5,8	11,0	0,80	37,8	2,42	26,8
Fe^{3+}	2,7	0,55	9,2	0,73	6,5	14,6	1,98	41,6	2,40	27,0
Cu^{2+}	2,6	0,48	8,2	0,63	5,6	11,3	0,82	39,8	2,24	27,5
Ni^{2+}	6,3	0,69	14,9	0,72	8,6	27,6	1,86	64,3	2,58	36,7
Co^{2+}	5,3	0,53	15,3	0,87	10,0	26,8	0,78	64,9	3,85	38,1
Mn^{2+}	2,4	0,71	8,6	0,49	6,2	12,0	0,77	39,5	2,44	27,5
Ca^{2+}	2,3	0,47	8,1	0,62	5,8	13,1	1.13	38,6	0,86	25,5
Mg^{2+}	2,3	0,51	9,7	0,81	9,7	14,3	1,62	44,2	3,80	29,9

Table 4

Effect of 10^{-5} M metal ions on 10^{-5} M EDTA-induced elongation

	Buffer		IAA			Buffer		EDTA		
	Increase in length mm	SE	Increase in length mm	SE	Change due to IAA	Increase in length mm	SE	Increase in length mm	SE	Change due to EDTA
None	1,9	0,41	9,5	0,62	7,6	1,8	0,16	3,8	0,19	+2,0
Fe^{3+}	2,7	0,62	11,3	0,83	8,6	2,1	0,22	1,2	0,15	—0,9
Co^{2+}	5,4	0,93	16,2	0,77	10,8	4,1	0,52	3,0	0,27	—1,7
$Fe^{3+}+Co^{2+}$	5,7	0,87	16,1	0,62	10,4	3,8	0,62	4,0	0,44	+1,0
Ca^{2+}	2,0	0,71	9,6	0,33	7,6	1,8	0,21	1,8	0,25	0,0
$Co^{2} + Ca^{2+}$	5,8	0,82	16,7	0,91	10,9	4,9	0,37	1,9	0,22	—3,0

absorption as compared with the auxin given alone. Co and Ni ions given alone increased both processes significantly.

In former experiments we found that addition of equimolar quantities of metal to the solutions of EDTA removes the inductive effect of the chelate on the elongation and water absorption. This suggests that metals form with EDTA some complexes which are not active in induction of elongation. To verify this hypothesis we carried out a series of experiments with solutions of the following metal ions: Fe^{3+}, Co^{2+}, Ca^{2+}, $Fe^{3+}+Co^{2+}$ and $Co^{2+}+Ca^{2+}$, according to the assumption that of the cations arranged in the Martell-Calvin series, the one which lies nearer should release from the EDTA complex the cation occupying a more distant place.

From the data in able 4 we may read that the solutions of $Fe^{3+}+Co^{2+}+$EDTA (all components given in 10^{-5} M concentration) induced elongation, whereas the solution of $Co^{2+}+Ca^{2+}+$EDTA proved to be inactive. Sections placed in this solution increased in length to the same

Table 5

Effect of 10^{-5} M IAA and 10^{-5} M EDTA on reversible and irreversible elongation
Mean of 7 experiments

	Reversible elongation			Irreversible elongation		
	Change in length mm	SE	As % of control	Change in length mm	SE	As % of control
None	1,2	0,13		1,9	0,15	
IAA	1,9	0,28	+58	8,4	0,37	+342
EDTA	1,1	0,22	— 8	2,7	0,21	+ 42

degree as in the solution of Ca alone. In experiments with IAA such relations were not observed (Table 4).

Parallelly to the previously described investigations we conducted experiments on the effect of EDTA on plastic (irreversible) and elastic (reversible) extensibility of cell walls. Extensibility was measured after a 24-hr growth. (Results are the means of 7 separate experiments).

The data in Table 5 show that EDTA caused an icrease in plastic extensibility of cell walls and did not exert any effect on the elastic extensibility, whereas IAA induced both plastic and elastic extensibility.

Table 6

Effect of metal ions on IAA- and EDTA-induced irreversible and reversible elongation of Helianthus hypocotyl sections after 24 h. period of growth
Concentration of metals, IAA and EDTA — 10^{-5} M

		Buffer		IAA		Buffer		EDTA	
		Change in length mm	SE	Change in length mm	SE	Change in length mm	SE	Change in length mm	SE
Reversible elongation	None	1,3	0,11	2,2	0,22	1,4	0,08	1,3	0,22
	Fe^{3+}	1,5	0,16	2,5	0,19	1,4	0,09	1,0	0,11
	Co^{2+}	2,6	0,23	3,1	0,12	2,2	0,18	1,6	0,14
	$Fe^{3+}+Co^{+2}$	2,6	0,20	3,3	0,23	1,9	0,21	2,1	0,19
	Ca^{2+}	1,6	0,18	2,1	0,14	1,3	0,08	1,4	0,05
	$Co^{2+}+Ca^{2+}$	2,8	0,22	3,7	0,08	2,1	0,20	1,2	0,15
Irreversible elongation	None	2,3	0,12	10,4	0,42	2,4	0,19	3,6	0,15
	Fe^{3+}	2,8	0,32	10,9	0,37	2,9	0,18	2,5	0,17
	Co^{2+}	5,9	0,28	16,4	0,29	4,4	0,37	3,1	0,28
	$Fe^{3+}+Co^{2+}$	6,2	0,32	15,9	0,45	3,5	0,28	4,2	0,45
	Ca^{2+}	2,5	0,21	9,6	0,32	2,6	0,21	2,5	0,18
	$Co^{2+}+Ca^{2+}$	6,1	0,37	16,1	0,25	4,4	0,40	2,5	0,21

Plastic and elastic extensibilities were determined simultaneously with the measurements of elongation (Table 4). The results are given in Table 6. It appears that Fe^{3+} and Ca^{2+} ions did not cause any significant changes in elastic extensibility (as compared with control) whereas Co^{2+}, $Fe^{3+}+Co^{2+}$ or $Co^{2+}+Ca^{2+}$ increased significantly the reversible extensibility of cell walls. EDTA added to the solution of the investigated ions brought about a significant loss of elastic extensibility as compared with the effect of ions given alone, Ca^{2+} + EDTA and $Fe^{3+}+Co^{2+}$ + EDTA solutions being an exception. IAA given alone and in combinations with all ions increased significantly elastic extensibility.

Of all the metals investigated only Co ions increased significantly plastic extensibility (Table 6). Addition of Co ions to the solutions of IAA

resulted in a significant increase in irreversible extensibility as compared with the effect of IAA given alone. Fe^{3+} or Ca^{2+} ions added to the solutions of EDTA distinctly inhibited the effect of this agent. Co^{2+} ions seemed to be indifferent. Plastic extensibility was increased when the sunflower section were incubated in EDTA+ Fe^{3+}+ Co^{2+} solution (Table 6). Plastic extensibility in this solution approaches the value obtained for the solution of Co alone. In Co^{2+}+Ca^{2+}+ EDTA solution irreversible elongation decreases approaching the value obtained for the solution of Ca^{2+} alone.

DISCUSION

Analysis of experiment described above indicates that sodium versenate given in concentration 10^{-5} induces elongation growth and water absorption in sunflower hypocotyl sections. Although the effect of EDTA is rather small as compared with that of IAA, nevertheless it is distinct with respect to control sections.

It would seem that EDTA-induced elongation is related with the chelating properties of this agent, and that IAA exerts this effect through an entirely different mechanism. The above hypothesis is supported by the following facts: 1° The effect of EDTA depends on the metal ions present in the external solution. Equimolar quantities of metal ions added to the solutions of EDTA abolish the inductive effect of versenate 2° Solutions of EDTA+two different ions, of which one forms a stable complex with the chelate brings about an effect characteristic of the second non complexed ion. 3° IAA acts independently of metallic ions added to the incubation medium.

The above facts may be interpreted in following way: Complex compounds of EDTA with metal ions of the Martel and Calvin series are inactive. This fact has been confirmed by Weinstein et al. (1956). According to these authors, elongation of the hypocotyls may be stimulated only by non complexed EDTA (in contrast to FeEDTA). Of two solutions: Fe^{3+}+Co^{2+}+EDTA and Co^{2+}+Ca^{2+}+EDTA the first is active and the second inactive in induction of elongation. Since, according to Martel and Calvin a stable complex with EDTA is first formed by Fe^{3+} ion then in our case the stimulation of elongation observed in the Fe^{3+}+Co^{2+}+EDTA solution should result from the effect of Co^{2+} ion. As it follows from the former experiments and according to the observations of Thimann (1956) and Thimann and Takahashi (1963), Co ion plays a specific part in induction of elongation, although the mechanism through which this ion acts is not entirely clear. In solution of Co^{2+}+Ca^{2+}+EDTA the effect of Co ion was not manifested, since this ion was probably chelated by EDTA. As established in our experiments, Ca^{2+} ions are indifferent in the process of elongation, thus no

increase in elongation was observed. The inductive influence of EDTA on elongation and water absorption was not manifested in sections incubated in $Fe^{3+}+Co^{2+}+$EDTA and $Co^{2+}+Ca^{2+}+$EDTA solutions. This was probably due to chelation of those metal ions which form more stable complexes with EDTA. It seems, therefore, that the effect of EDTA consists in the chelation of ions essential for growth and water absorption.

According to the hypothesis of Bennet-Clark (1956), Heath and Clark (1956) and Weinstein et al. (1956) EDTA induces elongation by elimination of Ca ions from the cell walls. It follows from the investigations conducted by Anderson and Anderson (1958) and Tagawa and Bonner (1957) that Ca ions contribute to the rigidity of the cell walls and thereby they restrain their extensibility. The overall extensibility of cell walls is the sum of elastic and plastic extensibilities (Cleland 1960). It is known (Ruge 1938) that plastic extensibility depends on the swelling of intracellular colloids excreted to the cell walls by the protoplast. The above processes are actively regulated by auxin. According to the hypothesis of Masuda and Wada (1966), Morre 1965) and Coartney et al. (1967) contradicting that of Cleland (1965), the auxin-induced extensibility of cell wall requires the synthesis of RNA.

The results obtained in our experiments, namely that EDTA does not exert any effect on elastic extensibility but induces irreversible elongation, are inconsistent with the calcium-bridge hypothesis of EDTA action. Finally, from the investigations of Ng and Carr (1959), Carr and Ng (1959), Cleland (1960), and Buczek (1968 b), it follows that EDTA-induced elongation and increase in fresh weight are not related with the elimination of Ca ions from the cell walls. In the light of the above facts it seems quite probable that the pathway by which EDTA induces elongation in the sunflower hypocotyl sections is entirely different.

EDTA given in concentrations higher than 10^{-3} M causes degradation of RNA in plant tissues (Ts'O 1958 and Hanson 1959). This degradation may be almost completely arrested by addition of Ca or Mg ions. It seems possible that EDTA (in our experiments 10^{-5} M) affects the metabolism of nucleoproteides by way of chelation of bivalent ions (Ca^{2+}, Mg^{2+}) binding protein with nucleotides, and in this way it takes part in the induction of elongation growth. The above hypothesis requires further investigations and will be the subject of another paper.

SUMMARY

The authors investigated the effect of EDTA on elongation and water absporption in sunflower hypocotyl sections and compared it with that of IAA. The effect of metal ions on the effectiveness of IAA and EDTA was also studied.

A detailed study of elongation, water absorption and increase in extensibility of sunflower hypocotyl sections has revealed that, although the physiological effects of IAA and EDTA are similar, the modes of action of these two types of compounds are different.

It was found:

1. The effect of EDTA, being several times smaller than that of IAA, significantly exceeds, however, the elongation and water absorption observed in control sections.

2. The effect of EDTA depends on the presence of metal ions. Equimolar quantities of metal ions added to EDTA solutions inhibit its activity.

3. The effect of IAA does not depend on metal ions present in solution.

4. EDTA increases plastic extensibility, of cell walls showing no effect on elastic extensibility, while IAA increases both plastic and elastic extensibility.

5. Of the investigated cations only the solutions of Ca^{2+} + EDTA and Mg^{2+} + EDTA do not inhibit elongation and increase in fresh weight as compared with the effect of cations given alone.

The experiments carried out suggest the following hypothesis: EDTA-induced elongation and increase in fresh weight are related with chelation of some metal ions essential for elongation and water absorption and taking probably part in nucleic acid metabolism.

Department of Plant Physiology
University, Wrocław
Kanonia 6/8

(Entered: September 14, 1967)

REFERENCES

Anderson D., H. Anderson, 1958, Auxin action on coleoptiles in the presence of nitrogen and at low temperature, Science 128:532—533.

Bennet-Clark T. A., 1956, The chemistry and mode of action of plant growth substances, Butterworth Scientific Publications, London.

Buczek J., 1965, Comparative investigations on the rooting of twigs of *Salix vinimalis* due to IAA and EDTA, Acta Soc. Bot. Pol. 34:389—397.

Buczek J., 1968a, The role of Na_2-EDTA and metal ions on the induction of adventitious roots, Acta Soc. Bot. Pol., 37:101—110.

Buczek J., 1968b, Comparative investigations on the effect of IAA and EDTA on water uptake and respiration of potato discs during and after resting period, Acta Soc. Bot. Pol., 37:111—118.

Buczek J., 1968 c, Action of some amonoacids, nucleosides and 5 adenylic acid on IAA-induced elongation of sunflower hypocotyl sections, Acta Soc. Bot. Pol., In Press.

Carr D. J., E. K. Ng., 1959, The sequestration of caltium from preparations of wheat coleoptile cell walls, Physiol. Plantarum, 12:264—274.

Cleland R., 1960, Effect of auxin upon loss of calcium from cell walls, Plant Physiol. 35:581—584.

Cleland R., 1965, Auxin-induced cell wall loosening in the presence of actinomycin D. Plant Physiol. 40:595—600.

Coartney J. S., D. J. Moore., J. L. Key., 1967, Inhibition of RNA synthesis and auxin-induced cell wall extensibility and growth by actinomycin D., Plant Physiol. 42:434—439.

Hanson J. B., 1960, Impairment of respiration, ion accumulation, and ion retention in root tissue treated with ribonuclease and ethylenediamine tetraacetic acid, Plant Physiol. 35:3732—379.

Heath O. V. S., J. E. Clark, 1956, Chelating agents as plant growth substances, Nature 177:1118—1121.

Martell A. E., M. Calvin., 1952, Chemistry of the metal chelate compounds, Prentice Hall, New York.

Masuda Y., S. Wada, 1966, Requirement of RNA for the auxin-induced elongation of oat coleoptile, Physiol. Plant. 19:1055—1063.

Morre D. J., 1965, Changes in tissue deformability accompanying actinomycin D inhibition of plant growth and ribonucleic acid synthesis, Plant Physiol. 40: 615—619.

Ng E. K., D. J. Carr, 1959, Effect of pH on the activity of chelating agents and auxin in cell extensions, Physiol. Plant. 12:275—287.

Ruge U., 1938, Untersuchunged über die Anderungen der osmotischen Zustandsgrossen und der Membraneigenschaften des Hypokotyls von *Helianthus annuus* beim normalen Streckungwachstums, Planta 27:352—366.

Tagawa T., J. Bonner 1957, Mechanical properties of the *Avena* coleoptile as related to auxin and to ionic interactions, Plant Physiol. 32:207—212.

Thimann K., V., 1956, Studies on the growth and inhibition of isolated plant parts. V. The effects of cobalt and other metals, Amer. Jour. Bot. 43:241—250.

Thimann K. V., N. Takahashi, 1963, Interrelationships between metallic ions and auxin action, and the growth promoting action of chelating agents [In:] Plant Growth Regulation. Ames, Iowa, U.S.A.

Ts'O P. O. P., 1958, Structure of microsomal nucleoprotein particles from pea seedlings. [In:] Microsomal Particles and Protein Synthesis. P. 156—168. Symposium Biophys. Soc., Washington. Acad. Sci., Washington, D. C.

Weinstein L. H., M. Meiss, R. L. Uhler, E. R. Purvis, 1956, Growth--promoting effects of ethylenediamine tetraacetic acid, Nature 178:1188.

Wpływ jonów metali na indukowane przy pomocy IAA i EDTA wydłużanie wycinków hipokotylu słonecznika i pobieranie przez nich wody

Streszczenie

Badano wpływ EDTA na wydłużanie i pobieranie wody przez wycinki hipokotyli słonecznika w porównaniu z efektem IAA oraz wpływów jonów metali na efektywność auksyny i wersenianu sodowego.

Badania nad wydłużaniem, absorpcją wody i rozciągliwością wycinków słonecznika wykazały, że sposób działania IAA i EDTA jest różny, aczkolwiek efekt fizjologiczny uzyskany pod wpływem tych dwu substancji jest podobny.

Stwierdzono, że: 1. Wpływ EDTA jest kilkakrotnie słabszy od działania IAA, jednakże przewyższa istotnie wydłużanie i pobieranie wody przez wycinki kontrolne. 2. Działanie EDTA jest zależne od obecnych w roztworze inkubacyjnym jonów metali. Dodanie do roztworu EDTA ekwimolarnych ilości jonów metali hamuje aktywność EDTA. 3. Działanie IAA jest niezależne od obecnych w roztworze ionów metali. 4. EDTA zwiększa plastyczną rozciągliwość, nie ma natomiast

wpływu na zwiększenie elastycznej rozciągliwości ścian komórkowych, podczas gdy IAA zwiększa tak plastyczną, jak elastyczną rozciągliwość. 5. Spośród przebadanych jonów metali jedynie roztwory Ca^{2+} + EDTA i Mg^{2+} + EDTA nie hamowały wydłużania i przyrostu świeżej masy w porównaniu z efektem samych kationów.

Wysunięto hipotezę, że wzrost wycinków i przyrost świeżej masy indukowany wersenianem jest rezultatem chelatowania przez ten czynnik istotnych dla wydłużania jonów metali biorących prawdopodobnie udział w metabolizmie kwasów nukleinowych.