Action of some amino acids, nucleosides and 5-adenylic acid on IAA- induced elongation of sunflower hypocotyl sections

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INTRODUCTION

It has been repeatedly demonstrated that protein synthesis plays an essential role in auxin-induced growth. It is known (Thimann and Loos 1967; Masuda 1959; Galston et al., 1964; Key 1964; Key and Shannon 1964; Nooden and Thimann 1965, 1966; Penny and Galston 1966) that auxin induces elongation with a simultaneous stimulation of the synthesis of proteins or enzymes essential for growth. Fang (1965) found that IAA (in concentrations optimal for growth) induces incorporation of labelled glycine into proteins in pea sections. Kesser (1955) observed a decrease in the content of free endogenic amino acids in auxin-treated sunflower hypocotyls.

According to Bonner (1963) the IAA-induced increase in elongation is related with cell wall loosening caused by this agent. The investigations of Cleland (1958, 1959) have shown that the enhanced loosening of the cell wall in oat coleoptiles is due to increased plastic (irreversible) extensibility of the cell walls. The increase in extensibility of the cell walls induced by auxin requires synthesis of RNA (Morre 1965; Masuda and Wada 1966; Coartney et al. 1967). This last hypothesis is, however, inconsistent with that expressed by Cleland (1965) who suggests that RNA synthesis is not necessary for auxin-induced loosening of cell walls but is indispensable for other processes related to growth.

The purpose of present paper was to investigate the possible contribution of some amino acids, nucleosides and 5-adenylic acid (AMP) to auxin-induced elongation of sunflower hypocotyl sections.

METHODS

Seeds of sunflower (*Helianthus annuus* L.) were saked in water for 4 hours and sown in flowerpots filled with compost soil mixed with peat. The pots were placed in a thermostat and incubated in the dark at 25°. After 5 days, 15-mm sections were cut 5 mm below the cotyledon from

8 cm long hypocotyls. The sections were immersed into three-times redistilled bidistilled water and after measuring their length (with a binocular) they were put into the investigated solutions.

Groups of 10 sections were placed in 25-ml Erlenmayers flask containing 20 ml of 0,01 M potassium phosphate buffer, pH 5,2. When required the buffer also contained 10^{-5} M IAA, suitable amino acids, nucleosides or 5-adenylic acid. The stream of air that passed through the solutions in the Erlenmayers was strong enough to provide the solution with oxygen

Graph 1. Effect of IAA and AMP on reversible and irreversible elongation of sunflower hypocotyl sections.

a — reversible elongation; b — irreversible elongation

1 — Control, 2 — AMP 10^{-5} M, 3 — IAA 10^{-5} M, 4 — IAA 10^{-5} M + AMP 10^{-5} M

and to keep the solution in constant vibration in order to avoid geotropism. The Erlenmayers were placed in a thermostat and kept in the dark at 25° for 25 hours. Then the sections were taken out and the increment in their length was measured with an accuracy within 0,1 mm (under a binocular). The results given in Tables are arithmetical means of 10 replications. Each experiment was repeated at least three times.

Elastic (reversible) and plastic (irreversible) extensibility after 24-hour growth was measured by the method of Cleland (1958, 1960). Plasmolysis was run in 1,5 M mannitol over 120 minutes. All operations were carried out under weak green light.

The following substances were used: Indole-3 acetic acid (IAA) (Chemapot — Czechoslovakia), Dl-serine (Suchardt), l-leucine (Reanal — Hungary), l-cysteine (Loba-Chemie Wien—Fischamend). Nucleosides: adenosine, cytidine and guanosine (Schwarz Lab.) and 5-adenylic acid (Muscle) (AMP), (Reanal—Hungary).

Statistical analysis was performed by Student's *t* test. The significance of differences was statistically proved at the 5 per cent level if the computed $t \geq 2,12$.

RESULTS

The effect of amino acids, nucleosides and AMP on the auxin-induced elongation of sunflower hypocotyl sections was investigated. The optimal concentrations of the particular substances used in the experiments were found in preliminary experiments. Table 1 shows the results illustrating the effect of amino acids, nucleosides and AMP. It appeared that neither amino acids nor nucleosides or AMP when given alone exert any significant effect on the increment of sections in the course of 24-h incubation as compared with the control. However, they increased the elongation when given together with IAA, the only exception being cytidine. The auxin-induced elongation of sections is significantly greater in the presence of serine, leucine, cysteine, adenosine and AMP.

Table 1

Effect of aminoacids, nucleosides and 5 adenylic acid on IAA-induced elongation of sunflower hypocotyl sections

IAA in M	Increase in length				%
	0		10 ⁻⁵		
	mm	t	mm	t	
Control	2,9		7,2		148
Serine x10 ⁻⁴ M	3,4	1,27	9,4	3,71	176
Leucine x10 ⁻⁴ M	3,1	1,81	8,6	2,89	178
Cysteine x10 ⁻⁵ M	3,3	1,93	8,5	2,71	158
Control	2,7		6,6		144
Adenosine x10 ⁻⁴ M	3,1	1,33	9,0	3,48	190
Cytidine x10 ⁻⁴ M	2,8	0,29	6,8	0,35	143
Guanosine x10 ⁻⁴ M	3,1	1,14	8,8	5,26	184
AMP x10 ⁻⁵ M	3,3	1,62	10,3	5,94	212

It follows from Table 2 that auxin alone significantly increases the elastic extensibility, whereas all the other investigated substances (as compared with the control) exert no influence. Addition of the investigated substances to the solution of IAA did not change the effectiveness of auxin.

Table 3 shows that amino acids, nucleosides and AMP given without auxin exerted a slight effect on the irreversible elongation of cell wall. The differences, however, are not significant. Yet all the substances investigated (except cytidine) increase significantly the plastic extensibility if given together with IAA.

Table 2

Effect of aminoacids, nucleosides and 5 adenylic acid on IAA-induced elastic extensibility

IAA in M		Change in length				%
		0		10 ⁻⁵		
		mm	t	mm	t	
Control		1,1		1,9		73
Serine	10 ⁻⁴ M	1,2	1,32	1,9	1,12	58
Leucine	10 ⁻⁴ M	1,3	1,18	2,2	1,33	69
Cysteine	10 ⁻⁵ M	1,3	0,92	2,1	1,75	62
Control		1,2		1,8		50
Adenosine	10 ⁻⁴ M	1,3	0,82	1,8	1,63	38
Cytidine	10 ⁻⁴ M	1,3	1,17	1,8	1,42	38
Guanosine	10 ⁻⁴ M	1,4	1,27	1,9	1,12	36
AMP	10 ⁻⁵ M	1,4	1,56	2,1	1,87	50

Graph 1a and 1b shows the course of changes in extensibility during the 20-hour period of incubation. It follows that the increase in plastic extensibility caused by AMP+IAA occurred during a later phase of incubation, as compared with the effect of IAA alone. AMP given alone did not exert any effect on the extensibility of cell walls. From the course of the curves we can read that the effect of IAA alone is distinctly weakened after 10-hour incubation, and that AMP since that moment begins to intensify the effect of IAA.

Table 3

Effect of aminoacids, nucleosides and 5 adenylic acid on IAA-induced plastic extensibility

IAA in M		Change in length				%
		0		10 ⁻⁵		
		mm	t	mm	t	
Control		3,5		6,9		97
Serine	10 ⁻⁴ M	3,7	1,02	8,3	5,64	124
Leucine	10 ⁻⁴ M	3,6	0,31	8,5	3,18	138
Cysteine	10 ⁻⁵ M	3,7	1,12	8,9	4,12	140
Control		2,9		7,6		162
Adenosine	10 ⁻⁴ M	3,3	1,75	9,0	3,35	173
Cytidine	10 ⁻⁴ M	3,0	1,22	7,9	1,91	164
Guanosine	10 ⁻⁴ M	3,0	0,97	8,9	2,85	197
AMP	10 ⁻⁵ M	3,2	1,33	9,7	5,17	203

DISCUSSION

It follows from the above described experiments that auxin makes possible the utilization of exogenic amino acids, nucleosides and AMP in the process accompanying the elongation of sunflower hypocotyl

sections. This hypothesis results from the following facts: 1. A synergism was observed between IAA and the investigated substances (except cytidine). 2. It seems that owing to the action of IAA, the exogenic amino acids, nucleosides and AMP may be better utilized in the processes accompanying elongation. 3. Amino acids, nucleosides and AMP do not exert any effect on elongation of the sections if given without IAA. 4. The substances, precursors of RNA and protein synthesis increase IAA-induced elongation.

The above mentioned facts being consistent with the hypothesis of other authors allow to suppose that the auxin-induced elongation of hypocotyl or coleoptile sections is accompanied by nucleic acids and proteins synthesis. It also seems that IAA takes part in protein metabolism and in this way induces elongation. This last hypothesis results from the following data: 1. The auxin-induced stimulation of RNA and protein synthesis in plant cells is accompanied by stimulation of elongation (Masuda 1959; Galston et al. 1964; Key 1964; Galston 1965; Nooden and Thimann 1966; Penny and Galston 1966). 2. Amino acids, nucleosides and AMP used in our experiments being precursors of nucleic acids and protein synthesis increase the effectiveness of IAA in stimulation of elongation, but they do not contribute to elongation if given without IAA.

Therefore it seems very likely that the effect of exogenic amino acids, nucleosides and AMP is related to the fact that these substances act as precursors in IAA-controlled synthesis of protein and RNA. This conclusion is in line with the results obtained by Roychoudhury and Sen (1964), Bendana, Galston et al. (1965), Hamilton et al. (1965), Truelsen and Galston (1966). The above mentioned authors established that labelled precursors of nucleic acids synthesis may be incorporated in to the RNA in plant cells when IAA is given in concentrations optimal for growth.

It has also been found that exogenic amino acids, nucleosides or AMP, when given together with IAA, increase the plastic extensibility of cell walls, and that they do not exert any effect if given without IAA.

The fact that the increase in plastic extensibility due to IAA+AMP is during the first ten hours of incubation the same as that due to IAA, suggests two alternatives. Either the synthesis of nucleic acids is a or as it is more probable, the synthesis of nucleic acids is a process indispensable in auxin-induced elongation and increase in plastic extensibility of cell walls. In the latter case free endogenic precursors of nucleic acid are first used for synthesis of RNA, the exogenic precursors of RNA and protein synthesis being utilized later.

Since our experiments have shown that the increase in elongation and plastic extensibility due to amino acids, nucleosides and AMP takes places

solely in presence of IAA, it seems that the IAA-induced elongation of sunflower hypocotyl sections is associated with a simultaneous induction of nucleic acids (of RNA type) and protein synthesis. Galston (1965) interpretes the action of auxin in terms of its involvement in the metabolism of RNA or proteins. It seems therefore that the data obtained in our experiments may contribute further evidence to the theory formulated by Galston.

SUMMARY

The effect of amino acids: serine, leucine and cysteine, nucleosides: adenosine, cytidine and guanosine, and 5 adenylic acid on the auxin-induced elongation and cell walls extensibility of sunflower hypocotyl sections was investigated.

It was found that the investigated substances (except cytidine) given together with IAA increase elongation and plastic extensibility of the sections as compared with the effect of IAA alone, when given without IAA they have no effect on elongation and plastic extensibility. The same substances did not exert any effect on elastic extensibility whether given with or without IAA.

The experiments reported lead to the following conclusions:

1. Auxin makes possible the incorporation of some amino acids, nucleosides and of adenylic mononucleotide in the processes of protein synthesis, this process being related with elongation.

2. Protein synthesis is a process essential for the auxin-induced elongation and cell wall extensibility.

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Indukcja wydłużania wycinków hypocotyli słonecznika przez IAA w obecności niektórych aminokwasów, nukleozydów i kwasu 5-adenilowego

Streszczenie

Badano wpływ seryny, leucyny, cysteiny oraz adenozy, cytydyny, guanozy i kwasu 5-adenilowego na indukowane auksyną wydłużanie i rozciągliwość ścian komórkowych wycinków hypocotyli słonecznika.

Stwierdzono, że badane substancje, z wyjątkiem cytydyny, podane łącznie z IAA zwiększyły wydłużanie i plastyczną rozciągliwość wycinków w porównaniu z wpływem samego IAA. Te same substancje podane bez dodatku IAA nie miały wpływu na wydłużanie i zwiększenie plastycznej rozciągliwości.

W oparciu o przeprowadzone doświadczenia można wyciągnąć następujące wnioski:

1. Auksyna umożliwia włączenie pewnych aminokwasów nukleozydów czy AMP do procesów syntezy białka w wydłużających się tkankach słonecznika.

2. Synteza substancji białkowej jest istotnym procesem dla indukowanego auksyną wydłużania i zwiększenia rozciągliwości ścian komórkowych.