EFFECT OF INDOLE-3-ACETIC ACID ON THE SYNTHESIS OF CYCLIC 3'-5' ADENOSINE PHOSPHATE BY BENGAL GRAM SEEDS*

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Indole-3-acetic acid (IAA) was found to exert a two fold stimulation of the synthesis of cyclic 3'-5' adenosine phosphate (cyclic-AMP) in 72 hr seedlings of Bengal gram as judged from the increased incorporation of 8-C^{14}-adenine into cyclic-AMP in presence of the hormone.

Tryptophan oxygenase activity (L-Tryptophan : oxygen oxidoreductase EC 1.13.1.12) of 48-96 hr seedlings of Bengal gram (chick pea: *Cicer arietinum*) is stimulated two to three fold by the inclusion of 1×10^{-6} M IAA in the medium used for inducing the enzyme (Azhar and Krishna Murti, 1971) Since cyclic-AMP mimicks the action of IAA in activating the *de novo* synthesis of tryptophan oxygenase in the developing tissues of the plant, IAA presumably plays its regulatory role on enzyme synthesis and, as a consequence of that, on the growth of the plant through the agency of cyclic nucleotides. It would be of interest, therefore, to find out whether IAA has any direct effect on the synthesis of cyclic-AMP during enzyme induction in the seedlings. Evidence is presented in this communication to show that IAA indeed stimulates the incorporation of 8-C^{14} adenine into cyclic-AMP in 72 hr seedlings of Bengal gram.

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Materials and methods

Treatment of seeds Surface sterilization of the seeds was achieved by immersing them first in 0.1 % w/v aqueous $HgCl_2$ for 5 min followed by immersion for 20 min in a fungicide solution made up of 50 % v/v ethanol containing 200 mg thymol and 500 mg glycerol per ml (Parekh et.al 1969) The seeds were then washed thoroughly with sterile distilled water.

Germination The washed seeds were planted on moist filter paper laid on acid washed sea sand and allowed to germinate for 72 hr in the dark at 22 ± 1^{o} C. Testae and cotyledons were detached from the seeds and the residual embryos cut into 2 mm sections as described by Trewavas (1968) and used immediately in the incorporation experiments.

Incorporation of 8-C^{14} adenine The procedure adopted was a modification of that described by Pollard (1970) for barley endosperm slices under the influence of gibberrellic acid. 1 gm aliquots of the sections of 72 hr seedlings were shaken at 37 ± 1^{o} C for 2.5 hr in 4.5 ml Krebs Renger phosphate buffer pH 7.2 (calcium content reduced to half the indicated amount) containing 6 mM glucose, 5 μc 8-C^{14} adenine (Bhaba Atomic Research Centre, Trombay, India), 1.12 mg dihydrostreptomycin sulphate and 1125 units of penicillin G . A duplicate set of flasks contained in addition 0.18 M IAA. After the initial incubation of 2.5 hr, theophylline was added to the medium to a concentration of 5 mM and the flasks shaken for a further period of 2.5 hr. The sections were now separated from the medium by filtration through cheese cloth and washed

thoroughly with chilled KRP buffer and finally with chilled 1×10^{-5} M unlabelled adenine sulphate.

Extraction of labelled cyclic-AMP The sections were ground in four volumes of chilled 5 % w/v trichloroacetic acid in prechilled glass mortars with the aid of acid washed sea sand. The extracts obtained thus were strained through cloth and centrifuged at 1500 g for 30 min. The supernates were taken in glass stoppered tubes and extracted five times with two volumes of benzene followed by five volumes of ethyl ether. The aqueous layer was then mixed with active charcoal. The adsorbed nucleotides were eluted from the charcoal with 50 % v/v ethanol containing 2 % concentrated ammonia and the eluates lyophilised.

Identification of cyclic-AMP The lyophilised material was mixed with a small amount of unlabelled cyclic-AMP and subjected to paper chromatography in different solvent systems. Cyclic-AMP was visualized as violet bluish fluorescent spots with the aid of a Mineralight lamp. Areas corresponding to cyclic-AMP were cut out of the paper and counted in a Packard Liquid Scintillation Spectrometer.

Chromatography of cyclic-AMP on Dowex-50H$^+$ Charcoal eluates from the incorporation experiments were mixed with unlabelled cyclic-AMP and fractionated on columns (0.4×3.3 cm) of Dowex-50H$^+$ (200-400 mesh) essentially as described by Gopalkrishna et al (1968). The fractions rich in cyclic-AMP (predetermined from pilot runs with unlabelled cyclic-AMP, ATP, ADP and 5'-AMP) were pooled and treated twice with $ZnSO_4$ and $Ba(OH)_2$. The nucleotides recovered from the Ba-Zn supernates were rechromatographed on Dowex-50H$^+$ and the

radioactivity eluted counted as before.

Chemical identification of labelled cyclic-AMP $Ba(OH)_2$ hydrolysis to convert cyclic-AMP into 5' and 3' AMP was carried out according to Berger (1957). Conversion of cyclic-AMP into cyclic-IMP was brought about with the aid of acetic acid and $NaNO_2$ according to Kaplan (1957). Enzymic hydrolysis of cyclic-AMP to the nucleotides and their dephosphorylation to adenosine was carried out using an enzyme preparation from carrot leaves (Daucus carota) containing both phosphodiesterase and nucleotidase (Becker and Pollard, 1969).

Results and discussion

The effect of IAA in stimulating the incorporation of 8-C^{14} adenine into labelled cyclic-AMP is brought out in the results given in Table 1.

Table 1. Effect of IAA on the incorporation of 8-C^{14} adenine into cyclic-AMP by 72 hr Bengal gram seedlings.

	c.p.m per gm tissue	
	Without IAA	With IAA
Charcoal eluates directly chromatographed in solvent system A	25,100	51,700
Charcoal eluates directly chromatographed in solvent system B	17,100	48,000
Pooled fraction from Dowex-50H	28,300	55,600

Solvent system A: n-butanol, acetone, glacial acetic acid, water and concentrated ammonia 14:10:6:9:1
Solvent system B: n-butanol, methanol, ethyl acetate and ammonia 7:3:4:4 v/v

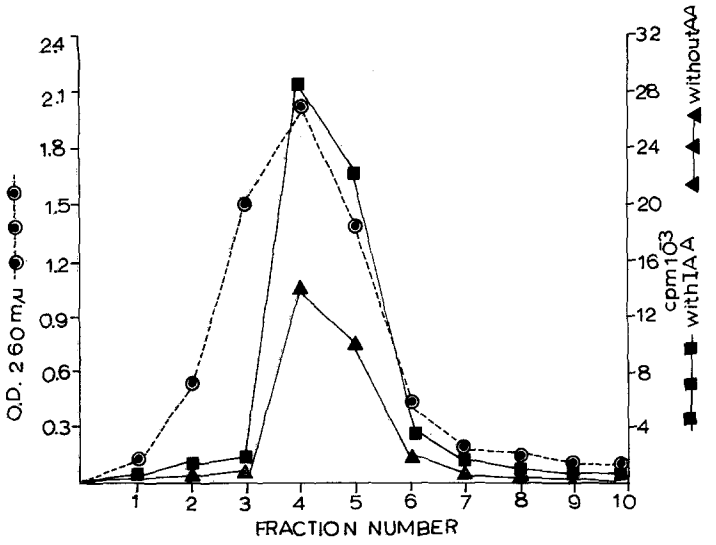

Fig 1. Elution profile of cyclic-AMP syntheiszed by Bengal gram

The elution pattern of the labelled cyclic-AMP is illustrated in Fig 1. The identity of the labelled material recovered from Dowex-50H$^+$ column with cyclic-AMP was established by both co-chromatography on paper with three different solvent systems and by chemical and enzymic degradation. The relevant results are summarised in Tables 2 and 3.

Table 2. Paper chromatography of cyclic-AMP recovered from Dowex-50H columns.

	c.p.m	
	Counts applied	Counts recovered
Solvent system A	7900	6300
Solvent system B	7400	6100
Solvent system C	5500	4800

Solvent systems A and B as under Table 1. Solvent system C: 95 % ethanol, ammonium acetate 75:25 v/v pH 7.5

Table 3. Chemical and enzymic degradation of cyclic-AMP

Treatment	c.p.m used	c.p.m recovered
Sat. $Ba(OH)_2$	3800	3' AMP 1700 5' AMP 1900
Acetic acid and $NaNO_2$	5900	IMP 5300
Carrot leaf enzyme	11,600	Adeno-9500 sine

Earlier studies have shown that the stimulation of tryptophan oxygenase by IAA or cyclic-AMP in 48-96 hr seedlings is through de novo synthesis of the enzyme and also that IAA while inducing enzyme activity in 72 hr seedlings atimulates the incorporation of C^{14}-valine into proteins (Azhar and Krishna Murti, under publication). The present report provides evidence for the stimulation of the synthesis of cyclic-AMP by IAA in 72 hr seedlings. The action of IAA as a growth hormone of Bengal gram may not be confined to the stimulation of tryptophan oxygenase . Other biosynthetic enzymes similarly affected remain to be explored. The evidence adduced thus far, however, leads to the conclusion that the growth enhancement arising out of the regulatory action of IAA is expressed through the molecular agency of cyclic-AMP. In the context of the known effects of cyclicAMP in translating hormonal action in mammals (Rall et al. 1957; Robison et al. 1968),or in regulating the synthesis and repression of catabolic enzymes in unicellular organisms (Perlman and Pastan,1969; Chambers and Zubay, 1969; deCrombrugg he et al. 1969; Perlman et al. 1969), the present findings as also those of Pollard (1970) would suggest that cyclic-AMP is perhaps one of the unitary molecular agents mediating homeostatic regulation of metabolism uniformly in bacteria, plants and mammals.

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