STIMULATION OF RNA SYNTHESIS IN *CICER ARIETINUM* SEEDLINGS BY CYCLIC 3′:5′ ADENOSINE MONOPHOSPHATE*

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1. Introduction

IAA induces tryptophan oxygenase (EC 1.13.1.12) in *Cicer arietinum* seedlings and cAMP mimics this action [1]. In seedlings preincubated with IAA, there is also a 3–3.5-fold activation of adenyl cyclase as evident from increased incorporation of [8-^{14}C]adenine into [^{14}C]cAMP [2]. Furthermore, the inhibitory effect on germination of *C. arietinum* caused by inclusion of 8-azadenine in the imbibition medium is readily reversed by either IAA or cAMP [3]. These findings are suggestive of a regulatory role played by IAA in the early phase of seed germination. Evidence presented now demonstrates that cAMP and IAA stimulate *in vitro* RNA and protein synthesis in *C. arietinum* without affecting DNA synthesis.

2. Methods and materials

C. arietinum seeds after surface sterilization were allowed to germinate on moist beds of acid washed sea sand covered with filter paper [1]. Seedlings were separated from the cotyledons at the required time and used within an hour of their preparation. Seedlings were cut into approx. 1 cm long segments as described by Trewavas [4] and incubated in the appropriate medium. Labelling of macromolecules was carried out by exposure of the seedlings for 15 min to the appropriate labelled precursor and subsequently processing them for the isolation of DNA, RNA and proteins according to the procedures of Trewavas [4] and Ogur and Rosen [5]. The labelled compounds were obtained from the Isotope Division, Bhabha Atomic Research Centre, Trombay, Bombay-86. Radioactivity counts were made in a Packard Tricarb liquid scintillation spectrometer.

3. Results

Results summarised in table 1 indicate that neither cAMP nor IAA has any effect on the synthesis of DNA in 72 hr seedlings. However, as evident from table 1 and 2, IAA and cAMP stimulated protein and RNA synthesis. The stimulation of RNA synthesis by cAMP and IAA was not additive. 5′ AMP, 3′AMP, ATP or ADP did not stimulate RNA synthesis.

4. Discussion

IAA has been reported to activate synthesis of RNA in root sections, isolated nuclei and chromatin and apparently new species of proteins are made as a result of this activation [6]. Such enhancement of RNA synthesis by IAA could be due to an activation of endogenous RNA polymerase [7] or its synthesis [8]. The present findings on *C. arietinum* indicate that the modulatory effect of IAA on RNA synthesis is presumably mediated through the agency of cAMP. In consonance with the current concepts concerning cAMP action, we have also shown that proteins are phosphorylated to a greater extent in seedlings of *C. arietinum* exposed to IAA than in controls (Azhar et al., unpublished work) although the nature of the target proteins is not clear at present.

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Abbreviations: IAA = indolyl-3-acetic acid; cAMP = cyclic 3′:5′ adenosine monophosphate.

Table 1
Effect of IAA and cAMP on the synthesis of DNA and protein by *C. arietinum*.

Additions	(cpm/mg DNA)	(cpm/mg protein)
None	920	4700
IAA 50 μM	987	8400
cAMP 100 μM	954	7200

The incorporation medium contained 1 g seedling sections in 10 ml Na Tris medium, 2500 units of penicillin G and 100 μg streptomycin. After 2 hr incubation 30 μCi [^{3}H]thymidine (specific activity 6600 mCi/mmole) or 5 μCi *Chlorella* [U-^{14}C] protein hydrolysate (specific activity 13 mCi/matom) were added and incubation continued for 15 min. Seedlings were processed for DNA or proteins and counted. DNA content of samples was measured colorimetrically according to Schneider [9] and protein according to Itzhaki and Gill [10].

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Table 2
Effect of IAA and cAMP on synthesis of RNA and protein by *C. arietinum*.

Addition to medium	(cpm/mg RNA)
Expt. 1. None	4500
IAA 50 μM	14000
cAMP 100 μM	12000
Expt. 2. None	4600
IAA 10 nM	8500
IAA 100 nM	9300
IAA 10 μM	10600
IAA 100 μM	11200
Expt. 3. None	3900
cAMP 100 nM	7800
cAMP 1 μM	9000
cAMP 10 μM	10000
cAMP 100 μM	12000
Expt. 4. None	3400
ATP 100 μM	3420
ADP 100 μM	4010
5′ AMP 100 μM	4040
3′ AMP 100 μM	3950
cAMP 100 μM	13400
Expt. 5. None	3500
IAA 50 μM	11250
cAMP 100 μM	12480
IAA 50 μM + cAMP 100 μM	10520

The incorporation medium was similar to the one described under table 1. 5 μCi[2-^{14}C]uracil (specific activity 13.43 mCi/mmole) replaced [^{3}H]thymidine or *Chlorella* [U-^{14}C]protein hydrolysate. Incoropration was allowed to proceed for 15 min, the sections processed for isolation of RNA and the samples counted. RNA was estimated colorimetrically according to Ceriotti as modified by Pilet and Braun [11].

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