ACTIVATION OF ADENYL CYCLASE IN THE EARLY PHASE OF GERMINATION*

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1. Introduction

Earlier studies with *Cicer arietinum* (Bengal gram, chick pea) seedlings have shown that cyclic 3′, 5′-adenosine monophosphate (cyclic AMP) mimics the stimulatory action of indolyl-3-acetic acid (IAA) on tryptophan oxygenase (EC 1.13.1.12) [1] and IAA increases cyclic AMP level by activating adenyl cyclase [2,3]. RNA and protein synthesis [4] and protein phosphorylation [5] are also stimulated by IAA or cyclic AMP in 72 hr seedlings. Furthermore, cyclic AMP or IAA can reverse the inhibitory effect of 8-azaadenine on the germination of *C. arietinum* [6]. These observations have led to the examination or regulatory role, if any, of cyclic AMP in triggering post imbibition events. In this communication are reported the changes in the activities of adenylcyclase, phosphodiesterase and proteinphosphokinase and the intracellular concentration of cyclic AMP during the early phase of germination of *C. arietinum.*

2. Methods and materials

C. arietinum seeds were surface sterilized and allowed to imbibe water at 4°C for 5 hr and then germinated moist beds of acid-washed sea sand covered with filter paper [2]. Adenyl cyclase activity was measured by labelling ATP in situ 8-[^{14}C] adenine and using the same for conversion into cyclic 8-[^{14}C] AMP [2,7]. Cyclic AMP phosphodiesterase was assayed according to a modification of the procedure described by Butcher and Sutherland [8] and protein phosphokinase was assayed according to Sahib et al. [9]. A relative estimate of intracellular concentration of cyclic AMP was obtained as described under table 1. The amount of unlabelled cyclic AMP used to isolate cyclic 8-[^{14}C] AMP was constant in all the time intervals.

3. Results

Results summarized in table 2 indicate that adenyl cyclase activity is detectable immediately after imbibition and continues to rise. Phosphodiesterase activity

Table 1
Formation of cyclic AMP during initial hours of germination of *C. arietinum* seeds

Hours of germination	picomoles of cyclic AMP/g seed
0	36
4	77
6	89
8	123

Surface sterilized seeds were imbibed in 25 ml sterile distilled water containing 50 μCi 8-[^{14}C] adenine for a period of 5 hr at 4°C, after imbibition the seeds were washed free from adhering radioactivity, planted on moist filter paper sheets in sterile petri dishes and allowed to germinate. At indicated time of germination the samples were taken out and after removing the testae, 1 g seeds were processed for the isolation of radioactive cyclic AMP [2].

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Table 2
Changes in adenyl cyclase, cyclic AMP phosphodiesterase and protein phosphokinase activities during early phase of germination of *C. arietinum*

	Enzyme activity				
	Time of germination (after 5 hr imbibition at 4°C				
	0	2	4	6	8
Adenyl cyclase	0	10	20	30	58
cAMP Phosphodiesterase	49	50	48	49	47
Protein phosphokinase	350	370	390	440	400

Adenyl cyclase activity = pmole 8-[^{14}C] adenine incorporated into cyclic 8-[^{14}C] AMP per g dry weight of seeds.
Cyclic AMP phosphodiesterase activity = nmoles of P_i liberated per mg protein per 60 min. Assay system in a final volume of 0.9 ml contained acetate buffer pH 5.0, 50 μmoles, cyclic AMP 0.36 μmoles, $MgSO_4$ 1.8 μmoles, snake venom (Naja Naja) 200 μg and enzyme extract. After incubation at 37°C for 60 min reaction was terminated by the addition of 0.1 ml 55% (w/v) TCA. P_i in protein protein-free supernatant estimated colorimetrically [10].
Protein phosphokinase activity = cpm [^{32}P] incorporated per mg protein.

was present in dormant seeds and this activity did not change significantly during the period studied. Protein phosphokinase activity also registers an increase during this period although not as appreciably as adenyl cyclase. A relative estimate of intracellular concentration of cyclic AMP is given in table 1 and it would be evident that during the first 8 hr the conditions seem to favour accumulation of this nucleotide.

4. Discussion

Activation of adenyl cyclase appears to be one of the significant happenings in the post imbibition period of germination of *C. arietinum*. Whether this is triggered by some hormonal factors released during imbibition of seeds needs to be investigated. Stimulation of adenyl cyclase would lead to increased synthesis of cyclic AMP. The intracellular concentration of cyclic AMP at any stage would represent the balance between synthesis by adenyl cyclase and degradation by cyclic AMP phosphodiesterase. The latter enzyme does not register any change in activity and hence it may assumed that the rate of degradation of cyclic AMP may be constant during this period. The relative concentration of the nucleotide, however, shows a significant rise during this period suggesting that conditions are more favourable for rapid synthesis and a build-up of adequate concentration of the nucleotide so that it could exert its action through phosphorylation of target proteins.

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