Phytochemistry 1974, Vol 13, pp 1295 to 1296 Pergamon Press Printed in England

RADIOIMMUNOLOGICAL EVIDENCE FOR THE PRESENCE OF CYCLIC-AMP IN *HORDEUM* SEEDS

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(*Received* 4 *September* 1973)

Key Word Index—*Hordeum vulgare*, Gramineoe, barley half-seeds, cyclic-AMP, radioimmunoassay

Abstract—Cyclic-AMP was found in extracts of barley half-seeds incubated in H_2O or GA_3 for 0 5 or 1·5 hr This nucleotide varied from 0 2 to 2 nmol/g seed The cyclic-AMP was virtually eliminated when the extracts were incubated with cyclic-AMP phosphodiesterase

INTRODUCTION

CYCLIC-AMP has been shown to mimic the action of GA_3 in a variety of responses mediated by this hormone in barley seeds[1–3] and it has been suggested that cyclic-AMP is involved in the regulation of metabolism in these seeds. In this report we cite evidence to show that cyclic-AMP is a natural constituent of these seeds.

RESULTS AND DISCUSSION

The amount of cyclic-AMP found in barley seeds after incubation for 0·5 or 1·5 hr in either H_2O or GA_3 (1 μM) is shown in Table 1. The data shows that: (1) cyclic-AMP is present in these seeds at 0·2–2 nmol/g; (2) the amount found in these seeds, although differing between experiments, agrees with previously published values for cyclic-AMP in plant tissue;[8,9] (3) the data show no consistent effect of GA_3 on this nucleotide.

Cyclic-AMP phosphodiesterase treatment of barley seed extracts virtually eliminated the presumed cyclic-AMP within the seeds After 1·5 hr incubation in the presence of the

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enzyme 8 and 4 pmol of cyclic-AMP was found per g tissue, compared with 2100 and 2091 pmol in its absence

TABLE 1. AMOUNT OF CYCLIC-AMP PRESENT IN BARLEY HALF-SEEDS AS DETERMINED BY RADIOIMMUNOASSAY

Incubation time	pmol cyclic-AMP g seed Expt I	Expt II
0·5 hr H_2O	209	217
0·5 hr GA_3 (1 μM)	400	201
1·5 hr H_2O	300	2100
1·5 hr GA_3 (1 μM)	725	2091

EXPERIMENTAL

Seeds of *Hordeum vulgare* L. var. "Himalaya" were sterilized by soaking in a 1:4 dilution of "Chlorox" for 20 min, washed 10× with sterile distilled H_2O and allowed to soak in this for 20 hr at 4°. The seeds were then cut in half along their short axes and the half without the embryo transferred to 125-ml flasks (100 half-seeds/flask) containing 7 ml of incubation medium with 12 μg of chloromycetin, in addition to the substances tested. The flasks were then allowed to incubate for the appropriate time at 30°. Sterile conditions were used throughout this procedure. After this incubation period the cyclic-AMP was extracted from the seeds following the procedure of Alveraz[4] and Azhar and Murti.[5]

The seeds were homogenized in 50 vol. cold TCA(5%) for 4 min. The liquid suspension was poured off and centrifuged at 30000 *g* for 15 min at 4°. The resulting supernatant was then washed 2× with 2 vol. H_2O-saturated ether. The aq. layer was then subjected twice to a $ZnSO_4$–$Ba(OH)_2$ co-precipitation to remove most of the non-cyclic adenine nucleotides.[6] (0·1 ml of 0·3 M $ZnSO_4$ and $Ba(OH)_2$ were added for each ml of the aq. layer). All of these procedures were carried out in the cold room. The resulting supernatant was then evaporated at 65° the residue taken up in 2 ml deionized H_2O and transferred to small glass vials. The vials were placed in a N_2 evaporator and the liquid reduced to 0·1 ml. The vials were packed in ice while the evaporation was taking place. The residue was then transferred to small test tubes, covered with parafilm, and stored in a Dewar flask containing acetone and dry ice which provided sufficient vol. to cover the areas of the test tubes which contained the residues.

For the radioimmunoassay, the extracts were dissolved in 2 ml 0·05 M NaOAc buffer pH 6·2. The radioimmunoassay used was that of Steiner *et al.*[7], and the materials for this radioimmunoassay were purchased from Collaborative Research Inc., Waltham, Mass. The assay was performed in the following manner. 0·1 ml of properly diluted stock cyclic-AMP antiserum was added to 10 × 75 mm test tubes, followed by 0·1 ml extract and 0·1 ml properly diluted stock ^{125}I SCAMP TME (radioactive cyclic-AMP antigen) (0·1 μCi/ml). The mixture was then allowed to incubate for 4 hr at 4°. After this time 0·1 ml of properly diluted stock anti-rabbit IgG was added to each tube. The solution was then incubated for 16 hr at 4° and 2 ml cold 0·05 M NaOAc buffer pH 6·2 was added. The mixture was then centrifuged at 4000 rpm for 20 min at 4°, the resulting supernatant removed, and the precipitate counted on a Packard Model 5112 Automatic Auto-Gamma Spectrophotometer. A standard cyclic-AMP curve ranging 0·01–25 pmol cyclic-AMP (Sigma) was run in a similar manner.

For the experiments in which cyclic-AMP phosphodiesterase (Sigma) was used, 0·2 mg protein (or about 0·1 unit phosphodiesterase) was incubated with 0·1 ml barley seed extract at pH 7·5 for 4 min at 30° prior to testing for cyclic-AMP.

Acknowledgements—The authors wish to thank Dr. A. Nalbandov (University of Illinois) for the use of his gamma spectrophotometer and Drs. G. Foxcroft and D. Dickinson (University of Illinois) for their help and advice in setting up these experiments.

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