A Possible Role for Cyclic AMP in Gibberellic Acid Triggered Release of α-Amylase in Barley Endosperm Slices

α-Amylase is released by the aleurone layer into the starchy endosperm during barley germination. In the de-embryonated grain this release can be effected by the addition of the plant hormone gibberellic acid and has been shown to involve a de novo synthesis of the enzyme[1].

3′ 5′ Cyclic adenosine monophosphate (cyclic-AMP) has recently been implicated as an intermediate in the action of a number of hormones[2–4]. In addition it has been shown to cause secretion of α-amylase from the rat salivary gland[5].

The concentration of cyclic-AMP in the cell is a function of its rate of formation from ATP by adenyl cyclase and its rate of breakdown via a specific 3′ 5′ cyclic diesterase to AMP. Aminophyllin (theophyllin) is a competitive inhibitor[6] of the diesterase and has been shown to cause an intracellular accumulation of cyclic-AMP[7]. Thus any effect due to the action of cyclic-AMP might be expected to be increased in the presence of this inhibitor.

The present work indicates that cyclic-AMP can trigger α-amylase release in barley endosperm slices. It has further been shown that aminophyllin has a similar effect.

The barley used was a sample of Maris Baldric, dehusked by treatment with 50% H_2SO_4 and stored at 4 °C. 2 mm endosperm slices in groups of 10, were incubated for 18 h at 25 °C in 4 ml of solution as indicated below. The slices were preincubated for $1^1/_2$ h at 25 °C with 4 ml distilled water which was decanted off just before addition of the solutions. 1 ml *M* NaCl was added to the solutions before homogenizing in a glass (hand) homogenizer. The homogenates were left to stand for 1 h at room temperature before centrifuging. (M.S.E. bench centrifuge speed 10.) α-Amylase activity in the supernatant was assayed at 25 °C by the iodine-dextrin colour method of BRIGGS[8].

The results, tabulated below, indicate the relative activities of α-amylase released, expressed in enzyme units/g fresh weight of tissue as described by DUFFUS.[9]

Cyclic AMP at a concentration of $10^{-5}M$ stimulates gibberellic acid controlled release of α-amylase. Aminophyllin, on the other hand, has no significant effect. This suggests that the local concentration of cyclic AMP is so high that effective competition with aminophyllin is prevented.

Enzyme release due to the action of cyclic AMP and aminophyllin, separately and in combination, is about 10% that with gibberellic acid. The fact that there is no additive action is noteworthy. Furthermore, α-amylase release due to cyclic AMP increases with increase in concentration reaching a value of 111 enzyme units/g fresh weight of tissue at $10^{-3}M$ cyclic AMP. No α-amylase release was observed using adenosine 5′ monophosphate (5′-AMP) at a concentration of $10^{-5}M$.

The results would suggest that cyclic AMP is a possible intermediate between gibberellic acid stimulus and α-amylase release. Aminophyllin acts presumably through inhibition of the 3′ 5′ cyclic diesterase thus preventing cyclic AMP breakdown. (Neither cyclic AMP nor aminophyllin has any direct effect on the activity of α-amylase in the assay system used.)

Part of the action of gibberellic acid may be analogous to that of other hormones which apparently act by stimulation of a membrane bound adenyl cyclase[10,11] to give an increase in intracellular cyclic AMP concentration. That gibberellic acid may have some effect on the membrane was suggested by MACLEOD et al.[12]

It is probable, however, that gibberellic acid does more than increase the cyclic AMP level since the maximum rate of enzyme release with cyclic AMP is only 25% of that with gibberellic acid. This of course may be merely a function of the relative ease of penetration of the 2 compounds.

How this cyclic AMP effect relates to the stimulation of nucleic acid and protein synthesis by gibberellic acid[1,13] remains to be elucidated[14].

Relative activities of α-amylase released by action of gibberellic acid, cyclic AMP and aminophyllin

Addition		α-amylase activity in E U/g fresh weight
Gibberellic acid ($10^{-5}M$)		421 ± 80
	+ cyclic AMP ($10^{-5}M$)	711 ± 50
	+ Aminophyllin ($10^{-5}M$)	385 ± 170
Cyclic AMP ($10^{-5}M$)		36 ± 18
	+ Aminophyllin ($10^{-5}M$)	24 ± 4
Aminophyllin ($10^{-5}M$)		19 ± 7
Cyclic AMP ($10^{-3}M$)		111 ± 25
5′-AMP ($10^{-5}M$)		< 5
Distilled water		< 5

Each result is the mean of at least 3 experiments ± standard deviation.

Résumé. L'adénosine monophosphate cyclique peut agir comme intermédiaire dans la synthèse, controlée par l'acide gibberellique, de l'α-amylase (α-1,4-glucan-4-glucanohydrolase) dans des tranches de grains d'orge sans embryon.

CAROL M. DUFFUS and J. H. DUFFUS

Biochemistry Department, School of Agriculture, and Department of Zoology, University of Edinburgh, Edinburgh 9 (Scotland), 3 December 1968.

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