The Interaction of a Gibberellin and a Kinin in the Control of Chlorophyll Synthesis

Chlorophyll formation in etiolated plant material is a light requiring process. In darkness the immediate precursor of chlorophyll, protochlorophyll, accumulates and this is photoreduced to chlorophyll. This terminal step is at the end of a complex biosynthetic pathway, and the continuing production of chlorophyll is associated with a parallel development of chloroplast thylakoid structure¹. Many physiological and biochemical treatments have an apparent effect on chlorophyll formation in a variety of known and unknown ways. A few examples of this are the phytochrome-mediated abolition of the lag phase of chlorophyll synthesis²⁻⁵, the inhibition of chlorophyll formation by a range of antibiotics 6-9, and a range of responses elicited by growth regulators. Gibberellins 10-13 and kinins 14-16 have been reported to promote 10, 11, 14, 15 and inhibit 12, 13, 16 chlorophyll formation in a variety of plant materials.

In this preliminary report we have investigated the effects of a gibberellin (gibberellic acid, GA_8) and a kinin (6-benzyl-aminopurine, 6-BAP) on chlorophyll formation in etiolated mung bean leaves.

The treated plant material consisted of the two primary leaves with petioles and the minute apical bud. These were detached from seedlings which had been grown in total darkness on vermiculite for 5 days at 25 °C. Four uniform samples of 5 leaf pairs for each treatment were placed on Whatman number 3 filter paper in 9 cm crystallizing dishes, containing 10 ml of the appropriate solutions. GA₃ was prepared in aqueous solution and 6-BAP contained 0.05% dimethyl formamide at 10 mg/l. This was used to initially dissolve the cytokinin and did not effect chlorophyll synthesis. After 4 h dark incubation, the leaf pairs were illuminated at 1000 lux for 48 h at 25 °C. Chlorophyll was extracted in 80% acetone and the concentration determined using the method of Arnon 17.

The results in the Table reveal a synergistic promotion of chlorophyll synthesis at 1,5 and 10 mg/l 6-BAP in combination with 0.01, 0.1, 1.0 and 10 mg/l GA₃. A peak for this enhancement occured at 5 mg/l GA₃. Synergistic inhibition of chlorophyll formation was also observed with a maximum effect at 0.01 mg/l 6-BAP in the presence of 0.001 mg/l GA₃. The effect of these hormones on their own was small. A slight promotion was observed with most GA₃ concentrations, while 6-BAP showed an inhibition at the lower concentrations and a promotion at 5 mg/l.

The effect of a gibberellin (GA_3) and a kinin (6-BAP) on chlorophyll formation in etiolated bean leaves

Gibberellic ac GA ₃ (mg/l)	id 6-Ber 0	ızyl-amiı 0.001	~	ne (mg/ 0.1	l) 1.0	5.0	10.0
0	100	84	71	94	95	112	105
0.001	110	77	49	87	109	1 19	11
0.01	110	86	56	85	120	169	131
0.1	103	77	56	105	129	174	120
1.0	107	83	90	100	143	1 69	117
10	117	94	95	103	126	156	129

The chlorophyll content per leaf is expressed as a percentage of the control level.

The relationship of the concentrations of GA_3 and 6-BAP which produced an optimum promotion showed that as the concentration of 6-BAP was increased, the concentration of GA_3 necessary for this optimum effect decreased. A similar interrelationship between GA_3 and kinetin was found by Roth-Bejerano and Lips ¹⁸ for the induction of nitrate reductase activity in summer-grown tobacco. The addition of both hormones to discs of dwarf french bean leaves resulted in a stimulation of expansion, but their action was additive rather than synergistic ¹⁹. Kursanov et al. ²⁰, however, elicited a synergistic response with 10 mg/l BAP + 1 mg/l GA_3 on the expansion of isolated pumpkin cotyledons.

The results reported above were obtained with leaves detached from 5-day-old seedlings, and these were the maximal responses elicited over a wider age range. It is possible that this maximal effect was related to the internal hormonal and substrate status of the leaves, which was determined, prior to detachment by the physiological interaction of leaves, cotyledons, hypocotyl and roots.

Resumé. La promotion synergétique et l'inhibition de la synthèse de la chlorophylle par la gibberelline (GA_3) et par la 6-benzylaminopurine (6-BAP) de concentrations diverses, fut observée. L'augmentation de la concentration de 6-BAP réduisit la concentration de GA_3 nécessaire pour obtenir la promotion maximum.

C. C. Hole and A. D. Dodge

School of Biological Sciences, University of Bath, Claverton Down, Bath (Somerset, England), 15 March 1972.

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