Short Communication

Masahiro SUGIURA*: Promotion of Chlorophyll Synthesis by Kinetin

杉浦昌弘*: カイネチンによるクロロフィル合成の促進

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In our previous papers, it has been reported that kinetin¹⁾ as well as red light²⁾ retards the fading in mature tobacco leaves. That red light is known to be a stimulant of chlorophyll synthesis³⁾ suggests a similar promoting action of kinetin on the synthesis. To examine this possibility, the effect of kinetin on the greening process in etiolated leaves exposed to light was investigated.

Soaked seeds of *Phaseolus vulgaris*, "Top Crop" were germinated in sand in the dark at 25° and grown for 6 days. Pairs of primary leaves, each with one cotyledon attached, were excised from the seedlings. They were immersed in 10⁻⁵ M kinetin solution for 2 hours and then placed on a filter paper, wetted by the same solution, for 16 hours in the dark at 25°. The kinetin solution was replaced with water in control run. The above manipulations were carried out under a green-safe light. After the tissues were exposed to ca. 3,000 lux light from day-light type fluorescent lamps for 3 hours at 25°, they were blotted to be estimated for their fresh weight and then their chlorophyll was extracted with 80% aqueous acetone to be assayed by the method of Arnon⁴⁾. Acetone insoluble fraction was determined for its protein content by a modified Biuret method of Racusen and Johnstone⁵⁾.

The results are given in Table 1. Chlorophyll accumulation in the primary leaves during the 3-hour illumination was promoted by ca. 60% by the kinetin treatment. Kinetin also elevated the levels of both fresh weight and protein. Little difference was, however, observed in chlorophyll contents between kinetin-treated and untreated samples when they were exposed for only 5 min. to light. As almost all the chlorophyll formed during this brief exposure to light may originate from protochlorophyll which has been accumulated by the start of illumination, it may be concluded that kinetin exerts no acceleration on the protochlorophyll formation in the dark. Accordingly, the observed stimulation of chlorophyll synthesis by kinetin in the longer (3 hours) illumination would not be explained in terms of promoted protochlorophyll accumulation during the dark culture. Essentially similar results were obtained with the cotyledons of *Pharbitis nil* seedlings.

Wolff and Price⁶⁾ have shown that excised primary leaves of *Phaseolus vulgaris*, "Great Northern" lose their chlorophyll synthesizing ability probably because of the exhaustion of the endogenous metabolites, the ability being restored by the addition of sugars. It was thought, therefore, interesting to examine the effect of kinetin on the chlorophyll formation in primary leaves detached from cotyledons in the presence or absence of added sugar. 0.2 M sucrose solution and/or 10⁻⁵ M kinetin were given to the isolated pairs of leaves by the same procedure as described above. As seen in Table 2, the leaves incubated on water for 18 hours in the dark lost markedly their chlorophyll synthesizing ability, whereas in the presence of 0.2 M sucrose the ability was kept at the same level as was observed in the leaves with attached cotyledon (Table 1). Kinetin could not induce any appreciable rise in chlorophyll

* Biological Institute, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya, Japan.
名古屋大学理学部生物学教室.

Table 1. Effect of kinetin on chlorophyll synthesis in bean primary leaves with cotyledons attached. Cotyledons of the samples were removed before the assays of fresh weight, protein and chlorophyll. Values are expressed as mg/leaf.

Kinetin	Illumination	Fresh weight	Protein	Chlorophyll
—	5 min.	33	1.4	0.5×10^{-3}
+	5 min.	32	1.5	0.4
—	3 hrs.	30	1.3	2.9
+	3 hrs.	43	1.8	4.6

Table 2. Effect of sucrose and kinetin on chlorophyll synthesis in decotyledonized bean primary leaves. Light was given for 3 hours. Values are expressed as mg/leaf.

Sucrose	Kinetin	Fresh weight	Protein	Chlorophyll
—	—	33	1.5	1.1×10^{-3}
—	+	30	1.4	0.8
+	—	29	1.5	2.8
+	+	31	1.5	2.9

content, fresh weight and protein content irrespective of sucrose addition. These indicate that among metabolites which may be transported from cotyledons, sugar would chiefly contribute to chlorophyll synthesis, and that in the first experiment if kinetin may hasten sugar-transport from cotyledons into primary leaves⁷⁾, it may not link directly with the promotion of chlorophyll synthesis. Some other cotyledonary reserves than sugar will be needed in order that kinetin exerts its stimulatory action on the synthesis.

Kinetin has been suggested to promote the synthesis of chloroplastic protein¹⁾. Hence, a most simple explanation of the present findings may be that kinetin accelerates the organization of chlorophyll synthesizing machinery in collaboration with light, provided that appropriate metabolites probably such as amino acids are available. According to Mego and Jagendorf⁸⁾, the increase in protein and fresh weight as well as in chlorophyll takes place in leaves upon short exposure to red light of whole seedlings of *Phaseolus vulgaris*, "Black Valentine". Further studies, however, will be necessary to compare the mechanism of kinetin action with that of red light action on chlorophyll synthesis.

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