Promotion by a Peptidyl Growth Factor, Phytosulfokine, of Chlorophyll Formation in Etiolated Cotyledon of Cucumber

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We examined the effects of a peptidyl growth factor, phytosulfokine-α (PSK-α), isolated from conditioned medium and known to induce the proliferation of single mesophyll cells of asparagus, and its derivatives on chlorophyll formation in etiolated cotyledons of cucumber after illumination. The chlorophyll content was increased by PSK-α treatment. Chlorophyll-content increases were not observed following either [2-5]PSK or Tyr-SO₃H treatment. There was no difference between the PSK-α treatments and water controls on increasing rate of fresh weights of cotyledons. These results suggest that PSK-α has the effect of elevating the chlorophyll content in the cells.

Key words: phytosulfokine; peptidyl growth factor; cucumber; chlorophyll formation

The proliferation of plant cells in a dispersed culture is strictly dependent on the initial cell density. Mitotic activity in low-density suspension cell cultures cannot be stimulated by supplementation with known plant hormones or defined nutrients, but it can be induced by the addition of conditioned medium (CM) prepared from rapidly growing cells in culture.1) This phenomenon suggests that CM confers unknown mitogenic advantages on plant cells. Phytosulfokines (PSKs) are sulfated peptides that have been isolated from the CM and found to have the active factor inducing the proliferation of single mesophyll cells of Asparagus officinalis L.2) PSKs are the first peptidyl growth factors to be characterized among higher plants.

Two types of PSK, PSK-α and PSK-β, have previously been characterized by a bioassay system.3) PSK-α, H-Tyr(SO₃H)-Ile-Tyr(SO₃H)-Thr-Gln-OH, showed a 10-fold higher activity than PSK-β, H-Tyr(SO₃H)-Ile-Tyr(SO₃H)-Thr-OH, which differs from PSK-α by only a truncated C-terminal Gln residue.

The roots of higher plants are known to control aspects of the development of above-ground organs, such as the greening of leaves and the formation of flower buds, possibly via the action of cytokinin and other growth-related compounds contained in xylem sap.4) Because the xylem sap produced by the roots may be thought of as a kind of culture medium for the above-ground organs, we hypothesized that PSKs might be synthesized in the roots, and transported by xylem sap to the above-ground organs, where they could then play various roles as signal molecules.

In this paper, we examined the effects of PSK-α and its derivatives on chlorophyll formation in etiolated cucumber cotyledons, and showed a difference between the promotive effects of PSK-α and kinetin based on the increase of weight of cotyledons.

The assay was accomplished following the method of Fletcher et al.5) Cucumber (Cucumis sativus L. Kagaonagafushinari) seeds were germinated in the dark at 28°C for 6 days. Ten cotyledons were excised in dim green light and placed in 5-cm petri dishes containing 2 ml of sample solutions. The dishes were incubated in the dark at 28°C for 14 h and then moved under fluorescent light with an intensity of 40 μmol photons/m² s⁻¹. After 5 h the chlorophyll was extracted from 10 cotyledons using 10 ml of 80% cold acetone, following the method of Tanaka et al.6) The chlorophyll levels were measured spectrophotometrically using the formula: Chl. (μg/cotyledon) = 20.2 × A₆6₀⁺ + 8.02 × A₆₄₅.7)

The results of the assay are shown in Table 1. The cotyledons that were treated by 10⁻⁶ m PSK-α had up to 18% more chlorophyll than the water-treated controls. This result indicated that PSK-α increased the chlorophyll levels in etiolated cucumber cotyledons. Parallel experiments with [2-5]PSK, which is the N-terminal truncated tetrapeptide of PSK-α, and Tyr-SO₃H showed these derivatives to be ineffective. These results

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Concentration (μM)</th>
<th>Content of chlorophyll (mg/cotyledon)</th>
<th>Increase of chlorophyll content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water control</td>
<td>—</td>
<td>112 (5.4)</td>
<td>0 (4.9)</td>
</tr>
<tr>
<td>PSK-α</td>
<td>10⁻⁶</td>
<td>116 (4.3)</td>
<td>3.7 (3.9)</td>
</tr>
<tr>
<td></td>
<td>10⁻³</td>
<td>132 (7.8)</td>
<td>18 (7.0)</td>
</tr>
<tr>
<td>[2-5]PSK</td>
<td>10⁻³</td>
<td>112 (4.0)</td>
<td>-0.3 (4.3)</td>
</tr>
<tr>
<td>Tyr-SO₃H</td>
<td>10⁻³</td>
<td>111 (3.8)</td>
<td>-1.3 (4.6)</td>
</tr>
</tbody>
</table>

In the central column, nonparenthetical values represent the contents of chlorophyll per cotyledon and parenthetical values are the SD of ten samples. Ten cotyledons were used as one sample. The chlorophyll content in the water-treated control is 100%.

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Abbreviations: PSK, phytosulfokine; CM, conditioned medium
indicated that the N-terminal sulfated Tyr residue of PSK-α is essential for the expression of its activity, and that the sulfate group alone does not induce this expression. Regarding the structure-activity relationships of PSK, we have previously examined these relationships in the growth of mesophyll cells of asparagus and in the adventitious root formation of cucumber hypocotyls. These results agree with these former reports. The N-terminal structure of PSK would appear to be essential for binding the putative receptors and inducing a molecular change in the receptors that initiates physiological events inside the target cells.

From these results, we can draw two hypotheses about the mechanism by which the chlorophyll level is apparently elevated by PSK-α. One is that the content of chlorophyll is increased in each cell with the change of chlorophyll metabolism. The other is that the total amount of chlorophyll in the cotyledon is increased as the result of growth and proliferation of cells, and the content of chlorophyll in each cell is not changed. Therefore, we next examined the photo-irradiation-induced increase in the fresh weights of cotyledons to clarify the effects of PSK-α on this phenomenon. Kinetin, one of the cytokinins known to induce a strong increase in chlorophyll level, was assayed here for comparison. The cotyledons were excised and incubated in sample solutions under the same conditions as for the above assay. Following incubation, they were moved under fluorescent light, and in 5 and 23 h, they were collected and their fresh weights were measured. The results are shown in Fig. 1.

The weights of cotyledons increased with increasing time of incubation in all samples (Fig. 1), kinetin-treated cotyledons showed apparent differences in weight relative to the water-treated controls and PSK-α-treated cotyledons. In case of kinetin treatment, the increasing rate of weight was apparently higher than that in water-treated controls and the differences between them were increased with the progress of time. This result indicates that both cell proliferation and chlorophyll formation were activated by kinetin treatment. In case of PSK-α treatment, in contrast, no statistically significant difference was observed between the PSK-α-treated samples and the water-treated controls throughout the trial. This result indicates that the increases of chlorophyll contents by PSK-α was mainly caused by the activation of chlorophyll formation itself.

In addition, we examined the synergism between PSK-α and kinetin by the assay of chlorophyll formation. When the cotyledons were treated with suboptimal concentration (10⁻⁷ M) of kinetin for greening, a 35.0% (SD: 10.1) increase of chlorophyll formation was observed. When cotyledons were treated with a mixed solution of 10⁻⁷ M kinetin and 10⁻⁷ M PSK-α, the chlorophyll formation increased by 52.5% (SD: 7.8). This value coincided with the sum of the increases in chlorophyll content by 10⁻⁷ M PSK-α treatment and by 10⁻⁷ M kinetin treatment. These results indicated that there was neither synergism nor antagonism between PSK-α and kinetin. These results also suggest that the signalling pathways of PSK-α and kinetin are independent of each other.

In a previous study, we isolated PSKs and demonstrated their active promotion of cell proliferation. In this paper, we demonstrated that PSK-α also has a promotive effect on chlorophyll formation. This result suggests that PSK may not only act as a growth factor, but may play a key role in various events of plant growth and differentiation. The effect of PSK-α on chlorophyll formation is lower than that of cytokinin (data not shown). These results suggest the possibility that PSK-α would affect the chlorophyll formation indirectly. Further physiological study is required for the elucidation of the function of PSK-α.

In mammalian cells, many peptidyl growth factors have been identified, and investigations into their physiological roles have clarified the mechanism of cell growth and differentiation. But in higher plants, peptidyl signal molecules have been identified in only a few reports and peptides have generally been considered less important in plant than in animal physiology. Therefore, additional research on PSKs will provide a novel viewpoint on the mechanisms of controlling plant cell growth and differentiation, in addition to filling in many gaps in our knowledge of plant physiology.

In our previous study using cultured asparagus cells, PSKs were detected in the CM only, not in the cell-fraction. Our immunological study using squash showed that there were few compounds that reacted with anti-PSK-α antibody in the xylem sap, which can be thought to be a culture medium in vivo. To further clarify the role of PSKs in plants, further studies will need to analyze the system of production and transportation of PSKs in intact plants. Further studies on PSKs should provide much useful information on both plant physiology and plant molecular biology.

Acknowledgments

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![Fig. 1. Increases of Fresh Weights of Cotyledons by Treatments of PSK-α and Kinetin.](image-url)
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References