Note

Promotion of Barley Root Elongation under Hypoxic Conditions by Alginate Lyase-Lysate (A.L.L.)

Yoshihisa Tomoda, Kenji Umemura, and Takashi Adachi

Meiji Sekka Kaisha Ltd., Bio-Science Laboratories, 5–3–1 Chiyoda, Sakado-shi, Saitama 350–02, Japan

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Alginate lyase-lysate (A.L.L., Algin-Oligo®), which had been prepared by degrading sodium alginate by alginate lyase, was found to have a growth-promoting effect on the elongation of barley roots, and especially that of the radicle, the effective concentration of A.L.L. for elongation of the roots being 100–3000 µg/ml, with no inhibition at the highest concentration. When a radicle was brought into contact with A.L.L., it responded by initiating elongation within 2 to 4 h. The elongation rate increased from 2.9 mm/h to 5.3 mm/h. Treatment with A.L.L. resulted in about a 2-fold increase in the alcohol dehydrogenase activity of the control under hypoxic conditions.

There have recently been developments in elucidating of the function of certain oligosaccharides in plants; for example, as a signal for a defensive response, for regulating growth and development, and for signal transduction.\(^{1,2}\)

We found that only A.L.L. (Algin-Oligo®) had a growth-promoting effect on the elongation of barley roots among tested saccharides.

Sodium alginate, with a molecular weight of 9600 ± 300 daltons (Kimitsu Chemical Industries Co., Tokyo, Japan), was depolymerized by treating with alginate lyase that had been prepared from a culture broth. *Ateromonas macleodii* (LB-102, FERM P-9218) was cultured in a medium containing 1.0% sodium alginate, 1.0% peptone, 0.1% yeast extract, 0.05% NaCl, 0.2% CaCl\(_2\), 2H\(_2\)O, 2.0% MgSO\(_4\)·7H\(_2\)O, 0.2% KHPO\(_4\), and 0.0144% Clewcox® compound (a mixture of a metal and EDTA; Nagase Sangyo Co., Tokyo, Japan) by incubating at 25°C for 17 h with rotary shaking (180 rpm). The alginate lyase activity was monitored by spectrophotometry as described by Brown and Preston.\(^{3,4}\) The enzymatic reaction was carried out in a mixture (500 ml) containing 14.3% sodium alginate and the culture broth (1000 units/ml). After incubating at 35°C and pH 7.0 for 20 h, the mixture was heated at 95°C for 15 min. The average degree of polymerization of the alginate oligomer (A.L.L.) was estimated to be four by the periodate oxidation method and the phenol-sulfuric acid method.\(^{5,6}\) Sodium alginate was also treated similarly with a heat-inactivated culture broth and was used as a negative control.

To monitor the elongation of the roots, we used an elongation ratio that compared the root length of A.L.L.-treated barley with that of the untreated control. Figure 1 shows the relative length of barley roots at the each concentration of A.L.L., the relative length increasing almost linearly with increasing concentrations of A.L.L. up to 800 µg/ml, and then remaining constant (180%) up to 3000 µg/ml. In contrast, the length of the leaves was similar for both the control and A.L.L.-treated seedlings. Sodium alginate treated with the heat-inactivated broth did not have any similar activity. A comparison of root growth (Fig. 2) shows that A.L.L. had strong root-elongation activity.

To examine how the barley roots responded to A.L.L., we measured the time required for changes in the morphology and growth rate. Barley seedlings were cultured on stainless-steel mesh for 3 days prior to the treatment, and then the culture solution was replaced by one supplemented with or without 300 µg/ml of A.L.L. The lengths of the longest roots were then measured at 2 h intervals (Fig. 3), and the rate of elongation was calculated in terms of the increase in length per hour. Before exchanging the culture solution, the rate of elongation was 2.9 mm/h. When the nutrient solution was exchanged, the roots were subjected to “solution-exchange stress,” and the elongation rate dropped to about 1.0 mm/h for three hours. However, three hours later the elongation rate had recovered to the original value. With A.L.L. present in the nutrient solution, “solution-exchange stress” was perceived in the same way as with the control seedlings, but the

**Fig. 1.** Effects of A.L.L. on the Elongation of Barley Roots.

Barley seeds (*Hordeum vulgare* L.) were surface-sterilized with 0.05% (v/v) sodium hypochlorite for 30 min, and then allowed to germinate in a tray at 25°C in darkness. The germinated seeds after about 16 hours were placed on an agar bed (0.8%, w/v), prepared with a 1:1000-fold diluted Hypinox® solution (Hypinox, Japan) containing each concentration of A.L.L. For the 4-day-old seedlings, the longest radicles were measured (twelve seedlings) and the values averaged. Growth is expressed in terms of the relative root length, and vertical bars represent S.E.M.

**Fig. 2.** Effects of A.L.L. on the Growth of Barley Seedlings.

Germinated seeds were grown on the agar medium as described in Fig. 1. The numbers represent the duration of the culture in days.

**Abbreviations:** A.L.L., alginate lyase-lysate; ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase; PDC, pyruvate decarboxylase.
Fig. 3. Response of Barley Roots to A.L.L.

Barley seeds germinated as described in Fig. 1 were placed on stainless-steel mesh over the surface of a culture solutions prepared with 1/1000-fold diluted Hypone® solution. After three days, the culture solutions was replaced with a fresh one that had been prepared to include the sample solution. The elongation of the longest roots was followed for 48 hours, and the lengths of the roots from ten seedlings were averaged at each time with (O) or without (●) the A.L.L. treatment. Vertical bars represent S.E.M.

Table Effects of A. L. L. on Enzymatic Activities

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity</th>
<th>ADH*</th>
<th>LDH*</th>
<th>PDC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.6 ± 0.48</td>
<td>2.8 ± 0.32</td>
<td>3.9 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>A.L.L. (300 µg/ml)</td>
<td>14.8 ± 0.84</td>
<td>3.1 ± 0.28</td>
<td>2.8 ± 0.23</td>
<td></td>
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</tbody>
</table>

* See the text for a definition of the abbreviations (units/root × 10³).

Four-day-old barley roots (ca. 2 g fresh weight) were cut and ground with two parts by weight of 50 mM sodium phosphate buffer (pH 7.0) that contained 5 mM 2-mercaptoethanol and sea sand in an ice-cold mortar. The homogenate was centrifuged at 10,000 × g for 20 min at 4°C, and the supernatant was used for the assays. The activity of alcohol dehydrogenase (ADH) was measured spectrophotometrically as described by Kadowaki et al. The activity of lactate dehydrogenase (LDH) was measured spectrophotometrically as described by Hoffmann et al. The activity of pyruvate decarboxylase (PDC) was measured spectrophotometrically as described by Laszlo and St. Lawrence. Values are the mean ± S.E.M. (n = 3).

The elongation rate subsequently increased from 2.9 mm/h to 5.3 mm/h (Fig. 3). From 2 to 4 h were needed to change the elongation rate or morphology, the effect of A.L.L. on the elongation rate becoming evident in this period. These phenomena with respect to the response time seem reasonable since, for example, when we consider gene expression, about 10 to 30 min are needed for the expression of a specific gene as a functional protein in the case of HSP90 or auxin-induced H+-ATPase.

The barley seedlings used for the bioassay were cultured on agar. While a few roots elongated straight into the agar control medium, almost all the roots elongated straight into the A.L.L.-containing medium. The amount of dissolved oxygen (D.O.) in an agar medium is known to be very low, as it is in microbial stab culture, the measured D.O. being lower than 1 ppm. As a rule, with plantlets cultured under hypoxic conditions (e.g., submerged or flooded), the roots are subjected to hypoxic stress, and certain stress-related proteins are induced; for example, twenty or more proteins have been observed in maize. It is said that ADH, LDH, and PDC are induced under hypoxic conditions and have a very important role in the regeneration of NAD⁺. In comparison of the activities of these enzymes, the LDH and PDC activity of A.L.L.-treated roots was equivalent to the control values, but the ADH activity was specifically enhanced (Table). On the other hand, under aerobic conditions the enzymatic activity was little induced in both the A.L.L.-treated roots and the control roots (data not shown). The reason for this phenomenon is unknown, but under hypoxic conditions, it was apparent that A.L.L. enabled the roots to elongate into the agar medium, and that the ADH activity of the roots was enhanced. Thus, it may be possible that A.L.L. promotes a certain resistance to hypoxic stress or mitigates it. Furthermore, in potato tubers, such elicitors as salicylic acid and arachidonic acid induced transcription of ADH mRNA and a significant ADH activity. These observations are interesting with respect to perception of stress and the response to it. In its practical application, A.L.L. may act to mitigate the effects of hypoxic stress on a plant.

Farmer et al. have suggested that some kinds of uronide-Ca²⁺ complexes (including polygaluronic acid) might possibly initiate certain signal-transduction pathways. Since alginate consists of guluronic acid and mannuronic acid, A.L.L. may function in such a pathway. Alginate is found mainly in marine algae, e.g., Laminaria japonica, and farmers have used marine algae for centuries, plowing it into their fields prior to cultivating crops. There is a possibility that alginate may be degraded by the lyases of soil bacteria to yield oligosaccharides and be further metabolized, which may contribute to the increasing the yield of crops.

References