Inhibition of Cell Elongation in Avena Coleoptile by Hydroxyproline

Robert Cleland
Department of Botany, University of Washington, Seattle, Washington 98105

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Summary. A study has been made of the hydroxyproline-induced inhibition of elongation of Avena coleoptile tissues. The isomers of 4-hydroxyproline differ in their effectiveness; only the L isomers are growth inhibitors with the cis form (allohydroxyproline) being more effective than the trans form (hydroxyproline).

Hydroxyproline differs from other amino acid antagonists and protein synthesis inhibitors in respect to 2 characteristics of the growth inhibition. First, a certain increment of auxin-induced elongation must take place following addition of hydroxyproline before the growth is inhibited. In contrast, pretreatment with other amino acid antagonists or protein synthesis inhibitors completely eliminates the ability of Avena coleoptile sections to respond to auxin. Secondly, sucrose markedly increases the magnitude of the hydroxyproline inhibition; i.e., sucrose acts to inhibit rather than promote growth when in the presence of hydroxyproline.

It appears that hydroxyproline is a specific inhibitor for the synthesis of some factor which is utilized in elongation. Following addition of hydroxyproline, auxin-induced elongation continues until the pool of this factor is exhausted; then elongation is inhibited.

Auxin-induced growth can be inhibited by a wide variety of agents (3). One group of inhibitors are the amino acid antagonists. Both division and elongation of plant cells are inhibited by antagonists such as ethionine (2, 12, 14), canavanine (1) and p-fluorophenylalanine (8).

In 1958, Steward et al. (14) reported that the free amino acid hydroxyproline would inhibit the growth of carrot callus tissues. Cleland extended this result by showing that cell elongation in Avena coleoptile sections was also blocked by hydroxyproline (4). In both cases, hydroxyproline seemed to be acting as an antagonist of proline metabolism since the addition of proline completely reversed the hydroxyproline-induced inhibition.

Many amino acid antagonists act by being incorporated into proteins in place of their corresponding amino acid with the result that inactive proteins are formed (7, 10). This mode of action seemed unlikely for hydroxyproline in light of the evidence obtained from both plant (9) and animal systems (13) that free hydroxyproline is not incorporated into proteins. In order to determine the mode of action of hydroxyproline on cell elongation, an investigation of the effects of hydroxyproline on growth and metabolism has been undertaken. This paper extends the earlier observations (4) by providing new information on the effects of hydroxyproline on auxin-induced cell elongation in Avena coleoptile sections.

Materials and Methods

Avena seedlings were grown as detailed earlier (2). Sections 5 mm in length were cut from the region 3 to 8 mm below the tip of 2.5 to 3.25 cm long coleoptiles. Groups of 10 sections were placed in test tubes with 5 ml of solution. The test tubes were rotated at 1 rpm on a Rollardrum and after the desired length of time, the sections were measured with a microscope fitted with an eyepiece micrometer. All manipulations and incubations were carried out under dim red light.

The basic medium contained K-maleate buffer (2.5 mM, pH 4.8) and sucrose (55 mM). To this solution was added, when needed, IAA (5 μg/ml), hydroxy-L-proline (1 mM), and L-proline. All chemicals were obtained from the California Corporation for Biochemical Research and were used without purification. Fresh solutions were made up weekly. All solutions were adjusted to pH 4.8 before use.

The plants were grown and all manipulations and incubations were carried out under dim red light. Treatments were run in duplicate; values reported here are the average of the 20 sections. Experiments were run at least 3 times.

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Results

The isomers of 4-hydroxyproline differ in their ability to antagonize auxin-induced growth (fig 1). Only the L-isomers are growth inhibitors with the cis form (allohydroxyproline) being a more effective inhibitor than the trans form (hydroxyproline). For example, a 50% inhibition of growth was induced by 0.05 mM allohydroxy-L-proline while 0.15 mM of the hydroxy-L-proline was needed to produce the same effect.

The inhibitory effects of both isomers of hydroxy-L-proline can be almost completely reversed by 1 mM proline (data not given). No other amino acid caused more than a slight reversal of the inhibition.

It has been shown earlier (4) that upon addition of 1 mM hydroxyproline to coleoptile sections, auxin-induced elongation proceeds unaffected for 2 to 3 hours before any inhibition of growth takes place; then the auxin effect on elongation is completely eliminated. The onset of this inhibition is governed by the amount of elongation which has occurred following addition of hydroxyproline rather than the time that has elapsed. This was shown as follows (fig 2). Hydroxyproline was added to one set of auxin-treated control sections and growth was followed for 24 hours. Auxin-induced growth proceeded unchanged for 3 hours and then was completely blocked. To a duplicate set, 0.065 M mannitol was added with the hydroxyproline in order to retard osmotically the growth rate. In this case, auxin-induced growth continued unaffected for 12 hours, but the total amount of auxin-induced growth which took place prior to the inhibition was the same in both cases.

The hydroxyproline concentration affects both the amount of growth prior to the inhibition and the growth rate after inhibition (fig 3). For in-

![Fig. 1. Relative abilities of 4-hydroxyproline isomers to inhibit auxin-induced elongation of Avena coleoptiles. Sections incubated 24 hrs with varying levels of trans-hydroxy-L-proline (--0--), cis-hydroxy-L-proline (- - - - - -) or cis-hydroxy-N-proline. Initial length 3.00 mm. All solutions contained K-maleate, 1AA and sucrose.](image1)

![Fig. 2. Effect of osmotically retarding the growth rate on the onset of hydroxyproline growth inhibition. Sections incubated with or without 5 μg/ml IAA in water (---), 1 mM hydroxyproline (- - - - - -), 0.065 M mannitol (----), or mannitol + hydroxyproline (---). Difference in length between auxin- and non-auxin-treated sections (auxin growth) determined after 0 to 24 hours.](image2)

![Fig. 3. Effect of hydroxyproline concentration on the time-course of the growth inhibition. Sections incubated 0 to 27 hrs with 1 mM hydroxyproline (- - - - - -), 0.1 mM hydroxyproline (----) or no hydroxyproline (---). All solutions contained K-maleate buffer, sucrose and 1AA.](image3)
stance, in the presence of 1 mM hydroxyproline, 0.75 mm of growth occurred prior to inhibition and the subsequent growth rate was 0.02 mm/hour. When the hydroxyproline level was lowered to 0.1 mM, the growth prior to inhibition increased to 2.0 mm and the subsequent growth rate was 0.06 mm/hour.

Pretreatment of tissues with hydroxyproline does not affect the requirement for a certain amount of elongation prior to the inhibition of growth (4). It was suggested that this is an unique characteristic of hydroxyproline but evidence to support this was not available. To obtain this information, the following experiments were performed. Sections were treated 0 to 22 hours in basal medium (K-maleate buffer + sucrose) or in basal medium plus one of the following: hydroxyproline (1 mM), puromycin (0.3 mM), cycloheximide (4 μg/ml), KCN (0.4 mM), p-fluorophenylalanine (3 mM) or β-thienylalanine (0.5 mM). Then IAA was added to one half of the sections in each treatment and the incubations were continued for an additional 8 hours. At the end of this time, the difference in length between auxin and non-auxin treated sections was determined (fig 4).

Pretreatment of coleoptile sections with puromycin, cycloheximide, KCN, p-fluorophenylalanine and β-thienylalanine resulted in a progressive loss in the ability of the section to respond to auxin.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Sucrose</th>
<th>Growth-mm</th>
<th>Sucrose effect-%**</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>+</td>
<td>4.93</td>
<td>+ 148%</td>
</tr>
<tr>
<td>Puromycin</td>
<td>+</td>
<td>1.04</td>
<td>+ 86</td>
</tr>
<tr>
<td>0.3 mm</td>
<td>-</td>
<td>0.56</td>
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<tr>
<td>PFPA, 3mm</td>
<td>+</td>
<td>1.76</td>
<td>+ 82</td>
</tr>
</tbody>
</table>

* Sections incubated 22 hrs in K-maleate (2.5 mM, pH 4.8), IAA (5 μg/ml), ± sucrose (55 mM) and inhibitors as noted. Initial length 5.00 mm.
** % increase in length + sucrose as compared with — sucrose controls.
*** p-Fluorophenylalanine.

Fig. 4. Effect of inhibitor pretreatment on the ability of sections to show a growth response to auxin. Sections pretreated 0 to 24 hrs with 1 mM hydroxyproline (— ● —), 0.3 mM puromycin (— ▲ —), 3 mM p-fluorophenylalanine (—△ — —) or water ( — ○ —). Auxin then added to half of the sections and difference in length between auxin- and non-auxin-treated sections determined 8 hours later. All solutions contained K-maleate buffer and sucrose. Initial length 5.00 mm.

After a certain length of pretreatment (e.g., 3 hr for cycloheximide, 9 hr for p-fluorophenylalanine), the tissues were unable to undergo any auxin-induced growth. In contrast, even after a 24 hour pretreatment with hydroxyproline, a significant growth response was induced by auxin. Lengthy pretreatment with hydroxyproline did result in a decrease in the ability of the sections to respond to auxin, but the parallel decrease in the water-pretreated sections indicates that it was the length.

Fig. 5. Interaction between sucrose and hydroxyproline in the inhibition of growth. Sections incubated 0 to 24 hours in: water (— □ —); 1 mM hydroxyproline (— ○ —); 5 μg/ml IAA (— ▲ —); IAA + hydroxyproline (—△ — —); IAA + 55 mM sucrose (—● —); or IAA, sucrose and hydroxyproline ( — × —). Initial length 5.00 mm.
of the pretreatment rather than the hydroxyproline which caused this decrease.

The previous experiments were carried out in a medium that contained 2% sucrose. It was shown by Schneider (11) that if the sucrose is omitted, the initial rate of auxin-induced growth is unaffected but the growth rate can not be maintained for more than a few hours. The result is that the final length is decreased if sucrose is omitted. Even in the presence of inhibitors such as puromycin and p-fluorophenylalanine, omission of the sucrose results in a decrease in the final lengths of auxin-treated sections (table 1). In contrast, hydroxyproline-treated sections obtain a greater final length when treated with auxin in the absence of sucrose; i.e., sucrose accentuates the inhibition by hydroxyproline (fig. 5). The amount of the growth which occurs prior to the onset of the inhibition is unaffected by the presence or absence of sucrose but the subsequent growth rate is markedly depressed by sucrose.

**Discussion**

Hydroxyproline is an effective growth inhibitor in *Avena* coleoptile, carrot callus (14), sycamore cambium callus (6) and tobacco callus cells (Olson, personal communication). In each case, the inhibition can be reversed by proline. It is apparent that hydroxyproline is acting as an antagonist of some facet of proline metabolism. Not all tissues respond to hydroxyproline, however: the germination of bean seeds was unaffected by this compound (5).

The hydroxyproline-induced growth inhibition in *Avena* coleoptile possesses 2 characteristics which differentiate it from the growth inhibitions caused by other amino acid antagonists or protein synthesis inhibitors. First, the capacity of the tissue to undergo some auxin-induced growth cannot be eliminated by pretreatment with hydroxyproline. In contrast, pretreatment with p-fluorophenylalanine, β-thienylalanine, puromycin or cycloheximide results in a complete loss in the ability of the tissues to respond to auxin. Secondly, hydroxyproline shows a unique interaction with sucrose in regard to the growth inhibition. In the absence of inhibitors or in the presence of protein synthesis antagonists such as puromycin and p-fluorophenylalanine, sucrose enhances the final length which is obtained by auxin-treated sections. But with hydroxyproline the inhibition is made more severe by the addition of sucrose. Such a situation has not been reported previously.

The present results are consistent with the hypothesis that *Avena* coleoptile contain a substance which is required for elongation and which is used up in this process. The amount of growth which the tissue can undergo depends upon the pool of this substance. Normally, this pool is being constantly replenished.

Hydroxyproline blocks the synthesis of this compound, but normal auxin-induced growth can continue until the pool is exhausted; then growth ceases. In the presence of suboptimal levels of hydroxyproline, the synthesis of this substance will be only partially blocked. The result will be that it will take longer for the pool to be depleted and even then the partial synthesis of this compound will allow some elongation to occur.

Although conjectures could be made concerning the nature of this substance, they must wait until evidence is presented concerning the biochemical inhibitions of hydroxyproline.

**Literature Cited**