THE ROLE OF WALL-BOUND HYDROXYPROLINE-RICH PROTEIN IN CELL EXTENSION

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SUMMARY

The influence of G.A. and I.A.A. on the hydroxyproline content of cell walls was examined with reference to the concept of wall-bound protein as a cross-linking agent regulating wall extensibility. It appeared that the hydroxyproline-rich protein content of cell walls of elongating cells from the stems of two pea varieties, Alaska and Rondo, differed remarkably. In accordance with Lamport's hypothesis, the dwarf variety (Rondo) had a much higher hydroxyproline content than the standard variety (Alaska). When G.A. was applied to the top of the slow growing dwarf variety, the growth rate increased until comparable to the standard variety and the hydroxyproline content of the cell walls decreased somewhat. However, no linear relationship between growth stimulation and decreased hydroxyproline content existed.

Experiments performed with excised elongating pea stem segments, grown in a culture solution containing phosphate buffer, showed that the hydroxyproline content of the cell walls increased considerably during an incubation time of 24 hours. IAA strongly inhibited this increase while stimulating elongation. However, a solution containing IAA and sugar caused the greatest elongation but also the greatest formation of hydroxyproline. It was concluded that cell extension and the hydroxyproline content of cell walls are not necessarily inversely correlated.

1. INTRODUCTION

In a review about the protein component of primary cell walls, LAMPORT (1965) emphasized the importance of hydroxyproline-rich protein for the mechanism of cell extension. He argued that in biological systems hydroxyproline is exclusively located in structural proteins. The hydroxyproline-rich protein which could be located in the cell wall (DOUGALL & SHIMBAYASHI 1960 and LAMPORT & NORTHCOTE 1960 a,b) must, therefore, play a structural role in the wall and consequently in cell extension. It might contribute to wall tensile strength by forming cross-links between wall polysaccharides. In this hypothesis loss of wall protein would decrease wall tensile strength. Though the experimental evidence supporting this is poor, it could be shown that walls containing little hydroxyproline have a lower tensile strength than walls rich in hydroxyproline. (LINSKENS 1964, TULECKE *et al.* 1962). Moreover it appeared that in general, walls of rapidly growing plants and young organs contain less hydroxyproline than walls of slowly growing plants and old organs. RIDGE & OSBORNE (1970) showed that ethylene caused an increase in wall-bound hydroxyproline in apical pea tissue and inhibited longitudinal growth of the cells. The question arose, whether the pea variety Alaska (standard) and the variety Rondo (dwarf) differ-

ed in the amount of wall-bound hydroxyproline. These peas differ very much in final plant length. The number of stem internodes is the same but each internode of the variety Rondo attains about half the length of an internode of the variety Alaska. According to Lamport's hypothesis the pea variety Alaska is likely to have a lower amount of wall-bound hydroxyproline than the Rondo variety.

Application of gibberellic acid to the top of the dwarf plants, increases the growth rate to one comparable with standard peas (BRIAN & HEMMING 1955), with only a slight effect on total cell number. This method made it possible to change experimentally in a physiological way and in a very short time the growth rate of the plants. It would be interesting to know whether or not the wall-bound hydroxyproline content changed too.

Lamport failed to change the wall-bound hydroxyproline content of the cells of sycamore suspension cultures by changing the culture conditions. In this publication experiments to check the questions raised above are described.

2. MATERIAL AND METHODS

Peas (*Pisum sativum*) of the varieties Alaska (standard) and Rondo (dwarf) were grown in pans containing water soaked vermiculite. The pans were placed in a dark room at 25 °C and high humidity. After 5 or more days a segment of 10 mm was cut out the third internode from each plant at a distance of 1 mm from the tip. Samples of 2 g were frozen in liquid nitrogen, ground in a mortar and extracted with Tris buffer (0.05 M, pH 7.5) on a boiling water bath for 15 min. After extraction, the cell wall was separated from the soluble fraction by filtration on a Buchner funnel and subsequently extracted with boiling 80% ethanol. For measuring dry weight of the residue (cell wall fraction) it was dried in an oven for one night at a temperature of 105 °C. Finally this cell wall fraction was hydrolysed with 6 N HCl in a glycerine bath at 115 °C for 18 hrs. The hydrolysate was neutralised with NaOH and tested for the amount of hydroxyproline according to a method derived from HUTTERER & SINGER (1960).

Gibberellic acid was added to the top of the plants as a lanolin paste or as a very small droplet hanged on to the top with the aid of a microsyringe.

3. RESULTS

In the first experiment, the amount of wall-bound hydroxyproline in elongating and mature cells of 7 days old Alaska peas was compared with those of Rondo peas of the same age. The length of the two varieties differed (*fig. 1*) at that age, but physiologically they were in the same phase. The results are shown in *fig. 2*.

Fig. 2 clearly shows that the hydroxyproline content of the cell walls of elongating and of matured cells of the dwarf variety (ro) is much higher than of the standard variety (al). The amount of cell wall as a percentage of dry weight of the cell did not differ significantly for the two varieties.

Fig. 2 also shows that the cell walls of matured cells contain much more hydroxyproline than the cell walls of elongating cells. This is in agreement with

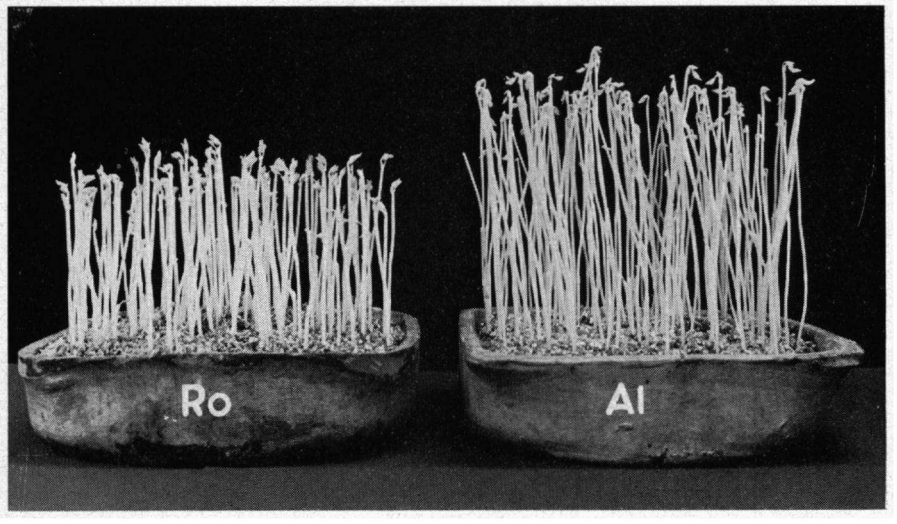

Fig. 1. 7 days old Rondo (Ro; dwarf) and Alaska (Al; standard) peas germinated in pans containing water soaked vermiculite.

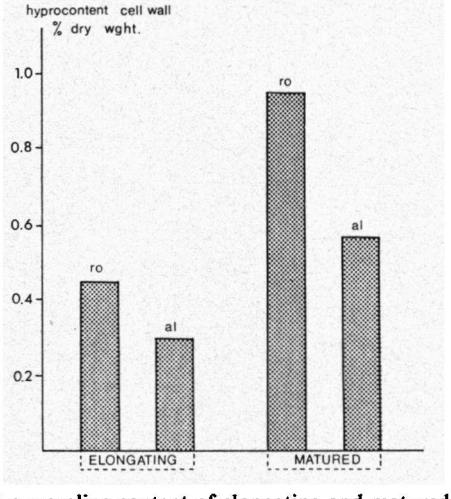

Fig. 2. Wall-bound hydroxyproline content of elongating and matured cells of pea stem segments. Rondo (ro) and Alaska (al.)

the results of CLELAND & KARLSNES (1967).

In the next experiment gibberellic acid was added to the top of part of the dwarf variety plants on the fifth day after germination and during several days 1 cm stem segments were cut out of the growth zone of the youngest internode (total fresh weight about 2 g) and analysed for wall-bound hydroxyproline content.

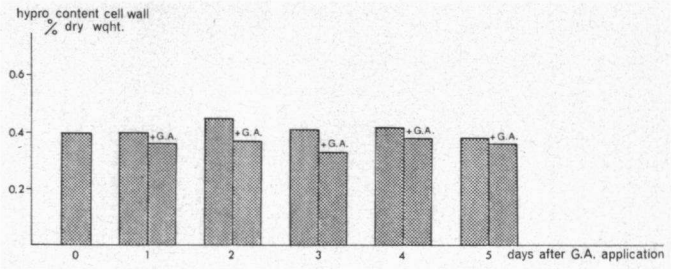

Fig. 3. The effect of gibberellic acid on the hydroxyproline content of cell walls of meristematic elongating cells of peas (Rondo).

Fig. 3 shows that the wall-bound hydroxyproline content of the growth zone of the epicotyls is slightly but significantly lower ($P < 0{,}05$) when gibberellic acid is applied to the top of the plants. At the same time the growth in length is strongly stimulated (*fig. 4*).

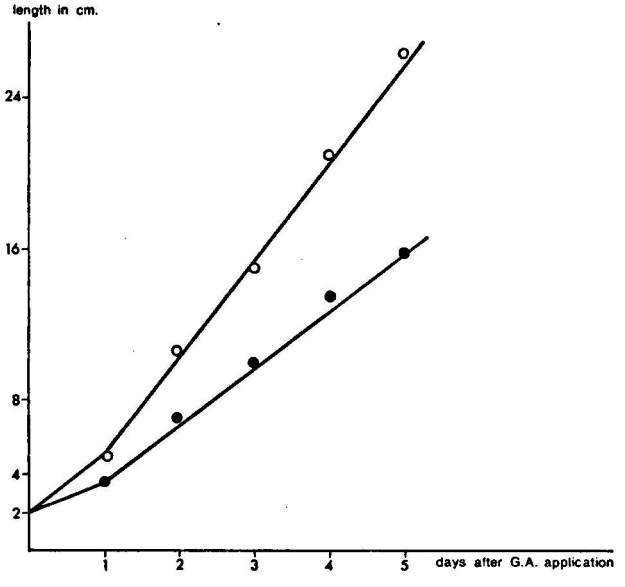

Fig. 4. The effect of gibberellic acid on the growth in length of pea (Rondo) plants.
o——o GA treated plants; ●——● control plants.

It appeared that no linear relationship between the stimulation in growth and the decrease in hydroxyproline content of the cell walls existed. Whereas the strongest decrease in hydroxyproline content is not more than 20% and much smaller after 5 days, the average stimulation of growth in length amounts to about 70%.

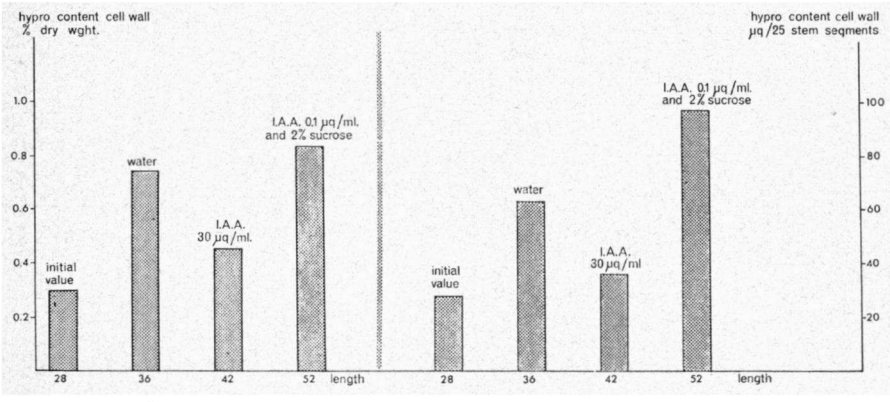

Fig. 5. Hydroxyproline content of cell walls and growth in length (arbitrary units) of pea stem segments (Alaska) after growth in various culture solutions for 24 hours.

In order to study the effect of IAA on hydroxyproline content of cell walls of elongating cells in relation to growth stimulation, excised stem segments of 5 mm length were grown for 24 hrs. in various culture solutions (*fig. 5*).

Fig. 5 shows that during the period in water (phosphate buffer added) the hydroxyproline content of the cell walls increased considerably whether expressed as a percentage of cell wall dry weight or as $\mu g/_{25}$ stem segments. When IAA is added at a concentration of 30 µg/ml, to stimulate the growth in length, the increase in hydroxyproline content was strongly inhibited. According to Lamport's hypothesis this is what could be expected.

However, when sugar is added to an IAA solution (0.1 µg/ml), the highest growth rate is found in spite of a high wall-bound hydroxyproline content. We conclude thereforet hat there is no causal relationship between the hydroxyproline content of elongating cells and their ability to elongate.

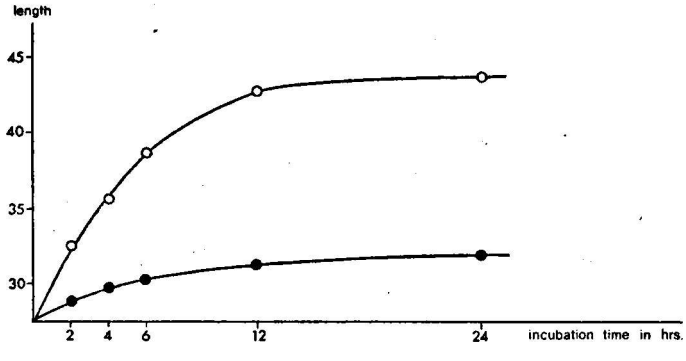

Fig. 6. Increase in length (arbitrary units) of pea stem segments (Alaska) incubated in culture solutions for various periods. ●——● water; o——o 0.1 µg/ml IAA and 2% sucrose.

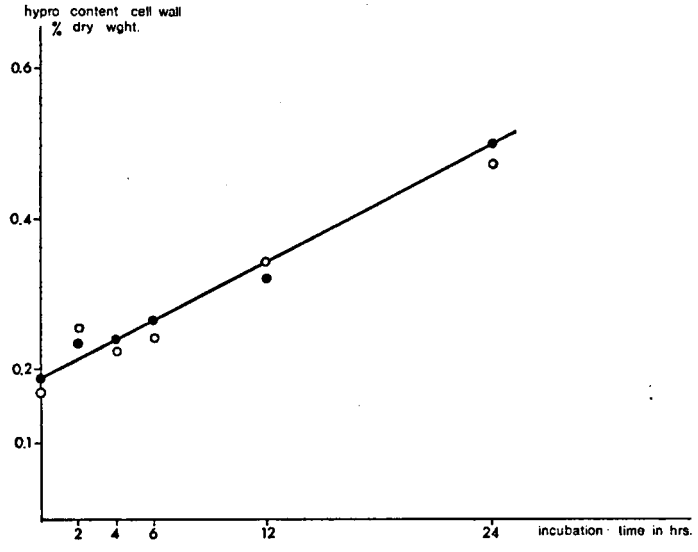

Fig. 7. Increase in wall-bound hydroxyproline content of pea stem segments (Alaska) incubated in culture solutions for various times.●——● water; o——o 0.1 μg/ml IAA and 2% sucrose.

However, in these experiments, increases in wall-bound hydroxyproline and in segment length were measured after a 24 hrs. incubation period. It is possible, however, that the growth response is much sooner completed than the formation of wall-bound hydroxyproline-rich protein, especially when IAA and sugar are added to the solution. Hydroxyproline formation might catch up after completion of growth in length. Therefore in the next experiment the course of the increases in length and hydroxyproline content of the cell wall was measured during a 24 hrs. period.

Fig. 6 shows that indeed the growth in length is almost completed after 12 hrs. especially when growth is strongly stimulated by the addition of IAA and sugar to the culture solution. However, the increase in hydroxyproline content of the cell walls is almost linear with time and is independent of the final length obtained.

The result means that a difference in growth (*fig. 6*) cannot be ascribed to a difference in wall-bound hydroxyproline-rich protein content. The general conclusion, therefore, is that the extent to which a cell is able to elongate (cell wall plasticity) was not correlated with the amount of wall-bound hydroxyproline-rich protein of the pea cells.

4. DISCUSSION

According to Lamport's hypothesis (figs. 1 and 2) an inverse correlation between growth rate and amount of wall-bound hydroxyproline-rich protein of elongat-

ing cells of two pea varieties should exist. However, when studying the effect of gibberellic acid on growth rate and hydroxyproline content of the cell walls of the dwarf pea (Rondo), it appeared that the growth rate could be strongly stimulated and brought to the growth rate of standard peas (Alaska), whereas the amount of wall-bound hydroxyproline was affected to a much lower degree. Though the growth rate was the same for these two varieties under these conditions, the large difference in hydroxyproline content remained. This result does not indicate a close relationship between the amount of wall-bound hydroxyproline and cell extension. However, this does not necessarily mean that cell cell wall protein plays no role in cell extension. In dwarf peas cell wall protein might not be the growth limiting factor. Dwarf peas possibly have a lower rate of gibberellic acid synthesis (BARENDSE 1970) and this might be a factor determining the osmoregulation of the cell and subsequently final cell length.

The experiments performed with excised pea stem segments showed (*fig. 5*) that IAA could retard the formation of wall-bound hydroxyproline which is formed during the incubation in phosphate buffer. At the same time the growth rate is strongly stimulated. This result is in agreement with Lamport's hypothesis.

CLELAND (1968) studied the effect of IAA on the incorporation of proline into the cell walls of excised *Avena* coleoptiles and concluded that IAA has little or no effect on wall-bound hydroxyproline formation. However, when IAA and sugar were both present in the culture solution, the incorporation of the C^{14} from proline into wall-bound hydroxyproline is strongly stimulated by IAA. Cleland's results do not exclude the possibility that the effect of IAA on wall-bound hydroxyproline formation is at least partly due to an effect on proline absorption by the excised coleoptiles.

GIESEN & KLÄMBT (1968) found a time dependent effect of NAA on C^{14} proline incorporation into the cell wall of wheat coleoptiles. A reduced incorporation during the first 2 hrs. is followed by an increased incorporation during the next 6 hrs. This increased incorporation is closely correlated with the stimulation of cell expansion. They assume that auxin may have a direct function in regulating cell wall protein synthesis.

Our results with peas indicate that when sugar is added to the culture solution, the retarding effect of IAA on wall-bound hydroxyproline formation is far less pronounced or even absent (*fig. 5*). However, no stimulation could be found. This result can be explained in the light of earlier results. WINTER (1967) concluded that the amount of available sugar in the cells of pea segments is limiting for the synthesis of cellulose. As it was found IAA strongly stimulated cellulose synthesis (WINTER 1966, AREF 1967) and this may mean that under certain experimental culture conditions a competition between cellulose synthesis and wall-bound hydroxyproline formation will occur. The sugar would not only be needed as a substrate for the protein but probably also for the galacto-araban moiety of the wall glycoprotein complex (LAMPORT 1967).

The result obtained when both IAA and sugar are added to the culture solution also means that in spite of the high rate of formation of wall-bound hydr-

oxyproline, the growth rate is highest. We also showed (*figs. 6* and *7*) that the wall-bound hydroxyproline is not formed after completion of growth in length. Furthermore the faster growing stem segments show the same increase in hydroxyproline during the period of greatest elongation as do the segments with a slower growth rate.

The general conclusion therefore is that growth rate (final length obtained) of cells is not always inversely correlated with the amount of wall-bound hydroxyproline. It therefore weakens Lamport's hypothesis, the evidence of which is primarily based on the relation between hydroxyproline content and growth rate.

However, it can be argued that this does not necessarily mean that wall-bound hydroxyproline-rich protein plays no role in determining cell wall plasticity and thus cell extension. The possibility remains that hydroxyproline-rich protein may be cross-linked to varying degrees with wall polysaccharides and the total amount of hydroxyproline may not be closely correlated with the number of cross-linkages.

IAA might affect directly or indirectly the amount of free hydroxyproline or hydroxyproline-arabinose (LAMPORT 1970) which is not connected with cellulose microfibrils.

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