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Indoleacetic Acid Stimulation of β 1-3 Glucanase Activity in Wheat Coleoptiles

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After fractionation of wheat coleoptile segments, the specific activity of β 1-3 glucanase seems to be strongly bound to the cell wall, and can be partly released from it by a detergent. The glucanase activity increases in all fractions by the treatment of coleoptile segments with auxin. IAA-stimulation of β 1-3 glucanase is reduced by 10 μg/ml of cycloheximide. The possible relation between the cell wall bound glucanase and wall extension is discussed.

Auxin-induced growth must involve two processes: wall loosening and wall extension (1). The wall loosening is thought to consist of some biochemical modification of the cell wall. This modification, changes the physical properties of the wall, so that when acted on by turgor the cell wall extends at a more rapid rate.

The role that polysaccharide hydrolases play in wall loosening seems very strong. In fact, such hydrolases do appear to be an essential part of wall extension in bacteria (13), in cells wich undergo tip growth such as pollen tubes (12), and fungal hyphae (15). A variety of polysaccharidases as β 1-3 glucanase (2), β 1-4 glucanase (5), β 1-6 glucanase (5), α 1-6 glucanase (6) and exogalactanases (7) exist in the cell walls. In the last years it has been shown that auxin is able to cause an increase in the activity of each of these enzymes in one or more systems (2, 3).

The following study was made to see the intracellular localization of β 1-3 glucanase [3.2.1.6. β 1-3 (4) glucan glucanohydrolase] in wheat coleoptile and the effect of auxin on its activity.

Materials and Methods

Plant material. Germinating seeds of wheat (*Triticum vulgare*, L) were grown in the dark at 25° C during 5 days. The apical 2 mm of each coleoptile 30±3 mm long were decapitated and 2 h later a 10 mm segment was excised from its upper region with the leaf removed. Coleoptile segments were floated for 30, 60 and 120 min on 0.25 M mannitol solution with or without addition of indole-3-acetic acid

(IAA) to the final concentration of 10 mg/l. The mannitol solution was used to inhibit cell elongation osmotically, to exclude the possibility that a change in the enzyme activity would be a consequence of increased cell elongation.

When used, cycloheximide was added at the same time that IAA, in a final concentration of 10 μg/ml.

Enzyme extraction. The method of MASUDA and YAMAMOTO (9) was followed. About 300 to 400 coleoptile segments were quickly frozen by introducing them in a freezer at —20° C, and throroughly ground for 5 min in 10 ml of a buffer solution composed of saline (0.15 M) and phosphate (0.05 M) at *p*H 7.5 (SPB). The homogenate was centrifuged for 30 min at 10,000 g. The supernatant dialyzed overnight against 0.05 M phosphate buffer (PB) *p*H 5.7 was referred to as fraction S. The sediment was washed×3 with SPB by centrifugation. Since the washed sediment, the crude cell wall fraction (W) may contain some membraneous matters in addition to the cell wall, it was treated with SPB containing 0.1 % deoxycholate (DOC) for 30 min and centrifuged. The supernatant was dialyzed overnight against PB *p*H 5.7 to obtain the DOC-soluble fraction (DS). The DOC-extracted sediment was washed×3 with SPB, the pellet obtained being referred as the cell wall fraction (CW). All the operations were done at 0° C-4° C. The four fractions S, DS, W and CW thus obtained were subjected to enzyme assay.

Enzyme assay. Each fraction was incubated with 0.1 % final concentration of laminarin (from Koch-Light Lab. Ltd.) in PB at 35° C for 60 min. Reducing sugars were determinad by SOMOGYI (14) method modified by NELSON (10). The enzyme activity was expressed as mg reducing sugars released per mg proteins determined by the LOWRY's (8) method. Proteins in W and CW were first extracted with 0.5 N NaOH and then determined as in the former fractions.

All the results are the average of at least three experiments.

Results

Table I shows the activity of β 1-3 glucanase in the four fractions obtained from wheat coleoptiles. Specific activity of glucanase is higher with DS than with S, and with CW than with W.

When coleoptile segments are treated with IAA (Table I) for 30, 60 and 120 min the β 1-3 glucanase activity in all fractions increases at about the same rate. This stimulatory effect of auxin on β 1-3 glucanase activity has been described previously in the literature (2, 3, 9).

Table II shows the effect of cycloheximide when added to the assay systems. The antibiotic reduced the enzymatic activity in the coleoptile segments treated with auxin. In the not treated segments, cycloheximide has almost no effect on the enzyme activity.

Table I. *Efect of IAA treatment of coleoptile segments on the specific activity of β 1-3 glucanase.*

Coleoptile segments were floated for 30, 60 and 120 min in 0.25 M mannitol solution with and without addition of 10 mg/l IAA and then homogenized and fractionated as described in Materials and Methods.

Fraction	Specific activity [1]		
	30 [2]	60	120
S	0.05+0.00 [3]	0.08±0.01	0.16±0.02
S+IAA	0.07±0.01	0.17±0.01	0.25±0.02
DS	0.16±0.02	0.21±0.02	0.31±0.02
DS+IAA	0.22±0.02	0.26±0.02	0.33±0.02
W	0.02±0.00	0.04±0.01	0.09±0.01
W+IAA	0.05±0.01	0.10±0.02	0.16±0.01
CW	0.05±0.01	0.10±0.01	0.15±0.02
CW+IAA	0.12±0.01	0.17±0.02	0.20±0.02

[1] mg sugar released per mg protein.
[2] Incubation time in min.
[3] Standard deviation.

Table II. *Effect of treatment of coleoptile segments with IAA and cycloheximide (CHI) on the specific activity of β 1-3 glucanase.* Coleoptile segments were floated for 30 min in 0.25 M mannitol solution with and without 10 mg/l and 10 μg/ml CHI and then homogenized and fractionated as described in Materials and Methods.

Treatment	Enzyme activity			
	S	DS	W	CW
Mannitol without IAA	0.05	0.16	0.02	0.05
Mannitol with IAA	0.07	0.22	0.05	0.12
Mannitol with CHI	0.04	0.15	0.02	0.04
Mannitol with IAA and CHI	0.04	0.15	0.02	0.07

Discussion

The results here reported agree with those reported by HEYN (5) and MASUDA and YAMAMOTO (9) that a considerable amount of β 1-3 glucanase found in coleoptiles is bound to the cell wall, and the especific activity of enzyme (Table I) increases after treatment of the cell wall with a detergent.

Cycloheximide at 10 μg/ml reduced the IAA-stimulation of β 1-3 glucanase activity, suggesting that auxin stimulation of glucanase activity is due to *de novo* protein synthesis. The same concentration of the antibiotic cause a 90 % inhibition in the incorporation of labelled amino acids into protein of wheat coleoptiles (11). These results seems to support the MASUDA's (9) hypothesis that auxin stimulated in the cytoplasm the synthesis of the enzyme, which is then transferred to the proximity of the cell wall. It is also possible that auxin stimulated not only the enzyme synthesis but also the binding of the enzyme to the cell wall, as founded for pectin methylesterase (4). However, our results together with those reported by MASUDA and YAMAMOTO (9) and HEYN (5) do not seems to prove, as many workers think, that the cell wall-bound enzyme is physiologically significant in the wall extension as a loosening factor. In fact, POPE and BLACK (11), although they recognise that growth depends on the availability of protein, conclude that IAA does not promote extension growth by inducing protein synthesis, since they found that IAA promotes cell extension in wheat coleoptiles in the absence of protein synthesis.

More work is needed in order to clear this intriguing problem.

Resumen

La enzima β 1-3 glucanasa parece estar fuertemente unida a la pared celular de los coleoptilos de trigo. La actividad glucanásica aumenta en todas las fracciones obtenidas por fraccionamiento de los segmentos de coleoptilo al tratarlas con auxina. Esta estimulación se reduce en las cuatro fracciones si se incuban también con 10 μg/ml de cicloheximida. Se discute la posible relación entre la glucanasa unida a la pared celular y el crecimiento de la pared.

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