DEXTRANASE ACTIVITY AND AUXIN-INDUCED CELL ELONGATION IN COLEOPTILES OF AVENA

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Dextranase activity, so far only found in microorganisms and animals, is found to be associated with coleoptiles of Avena. Dextranase breaks down α-glucans in which the glucose residues are mainly linked by α-1,6- linkages. This enzyme is found to be sensitive to the plant growth hormone, auxin. The cell walls of the coleoptiles, moreover, are found to contain dextran-like α-glucans which can be broken down by dextranase. This enzyme, therefore, seems to meet two main requirements of an intermediate in the plasticization of the cell wall in auxin-regulated cell elongation in coleoptiles.

A knowledge of enzymes which occur in elongating plant cells and which can act on cell wall substances is of interest in connection with the role of the cell wall and its plasticity (1, 2, 3) in auxin-regulated cell elongation. A systematic study has therefore been undertaken of such enzymes in coleoptiles of Avena, the traditional material for studies on auxin-induced cell elongation. In a previous article (4) four different β-glucanases have been studied. These enzymes, however, probably do not participate in hormone regulated cell elongation because they are not sensitive to the presence of the hormone in the tissue.

The present communication deals with the occurrence and behavior in this material of an α-glucanase, "dextranase." This enzyme acts on glucans in which the glucose residues are linked by α-1,6- and α-1,3- bonds, so-called dextrans. It is also shown that in the cell walls of coleoptiles, polysaccharides occur which can be broken down by pure dextranase. The importance of these findings for a further analysis of the mechanism of cell elongation is discussed.

MATERIALS AND METHODS

Coleoptiles of Avena sativa were grown in the dark. When the coleoptiles measured about $2\frac{1}{2}$ cm long, sections of 1 cm long were taken 4 mm below the tip. The sections were ground or homogenized under standard conditions, twice centrifuged at 2200 x g with intermittent washing in water. The pellets so obtained mainly consist of cell wall materials and were found to contain the enzyme activity, just as in the case of the β-glucanases studied before (4).

The specific substrate used for demonstrating dextranase activity was a natural dextran produced by Leuconostoc mesenteroides NRRL B512(F), 50B. This sample was received courtesy of Dr. A. Jeanes. It had a high purity and a molecular weight of 20-30 million. This dextran is reported to contain about 95% of α-1,6- bonds, the remaining bonds are α-1,3- linkages, which mainly constitute the branch points. One percent of the branches would be very long, the remaining branches are believed to be as short as 1 glucose residue (5, 6). In addition, a commercial dextran (Worthington Biochemical Corporation) of a molecular weight of 2 million was used.

Two percent solutions of these dextrans were incubated with the pellet derived from 20 or 30 coleoptile sections at 45°C and pH 5.0. An incubation time of 4 hours was found to be the most suitable.

The depolymerization of the dextran molecule was studied viscometrically in the usual way (6, 7) by the decrease in flowtime. A semi-micro viscomater of the Cannon-Manning type for volumes of 1 ml was used at 25.0°. Duplicate determinations did not vary more than 2 seconds. The flowtime of the solvent (water) was 68 seconds.

The products of the enzymatic degradation were studied by descending paper chromatography. The hydrolyzates were concentrated from 3 ml to 0.2 ml and streaked across the paper. The chromatograms were developed with isopropanol-acetic acid-water (54:8:18) in the conventional manner used for sugars. The locations of the products of hydrolysis were compared with those of pure samples of isomaltose, isomaltotriose, and glucose, which are the final products

Table 1

EFFECT OF HEATING AND OXYGEN ON THE STABILITY OF ELECTRON CARRIER AND NITROGENASE ACTIVITY OF RHIZOBIUM BACTEROID EXTRACTS

Treatment of bacteroid extract	Electron carrier activity[1]	Dithionite-dependent nitrogenase activity
	(Ethylene produced (mμmoles/min))	
None	34.1	92.5
100% O_2 for 2 hours	4.6	0.5
Heating at 60° for 10 min (under argon)	34.8	2.0

[1]The electron carrier activity of the treated extract was measured by its ability to support acetylene reduction in the coupled chloroplast-nitrogenase system described by Yoch and Arnon (1969).

The reaction mixture used to measure dithionite-dependent nitrogenase activity of the Rhizobium bacteroid extract contained, in addition to bacteroid extract (3.75 mg protein), treated as indicated, the following in μmoles: HEPES buffer, pH 7.4, 50; Mg^{++}, 5; creatine phosphate, 40; ATP, 4; creatine phosphokinase, 0.05 mg; and sodium dithionite, 20. To measure electron carrier activity, the above reaction mixture was modified by adding a washed A. vinelandii nitrogenase (4.6 mg protein) and replacing the sodium dithionite by spinach chloroplasts (300 μg chlorophyll); ascorbate, 10 μmoles; and 2,6-dichlorophenol indophenol, 0.05 μmoles. Final volume, 1.5 ml. Light intensity, 9,000 foot-candles. Gas phase, 73% argon and 27% acetylene. The reaction was carried out at 30° for 30 min.

The supernatant solution was used as the source of the electron carrier. Its effectiveness in coupling the reducing power generated by illuminated spinach chloroplasts to Azotobacter nitrogenase and to the native bacteroid nitrogenase is shown in Fig. 1. Activity of the washed Azotobacter nitrogenase had an absolute requirement for the bacteroid electron carrier. The crude bacteroid extract which contained both nitrogenase and electron carrier showed significant activity without added carrier. Nevertheless, the addition of carrier to the crude bacteroid extract gave a marked increase in nitrogenase activity, at a rate parallel to the carrier-dependent nitrogenase activity of Azotobacter.

A further purification of the bacteroid electron carrier is summarized in Table 2. The supernatant solution (after the heat treatment) was passed through a DEAE-cellulose column previously equilibrated with 0.02 M HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, pH 7.2. Most of the electron

from 10, 20, 30, and 40 coleoptiles, respectively. Heating of the pellet on a water bath before incubation reduced the lowering of viscosity in 15 minutes from 15.2% of the unheated pellet to 2.5%, and in 30 minutes to 0.0%.

These findings are in agreement with the conclusion that the decrease in

TABLE I

Dextranase Activity in Pellets of 20 Avena Coleoptile Sections from Normal and Decapitated Plants by Viscosity of Dextran Solutions After 4 Hours' Incubation, as Flow in Seconds and Percentage Decrease of Flowtime as Compared to Control Substrate. Each Figure is the Average of Two Viscosity Runs Which Varied Less Than 2 Seconds.

	2% Dextran mol. wt. 20-30 million			4% and 2% Dextran mol. wt. 2 million		
Substrate alone	447	450	444	460	475	260
Substrate digest with pellet from normal coleoptile	404	369	376	390	433	226
% Decrease	9.5%	18.0%	15.5%	15.2%	9.2%	13.1%
Substrate with pellet from decapitated coleoptile	445	423	-	-	475	247
% Decrease	0.0%	6.0%	-	-	0.0%	5.0%

TABLE II

Dextranase Activity in Pellets of 20 Avena Coleoptile Sections, Which Have Been Previously Treated or Not Treated with Auxin. Each Figure is the Average of Two Viscosity Runs Which Varied Less Than 2 Seconds.

	a. Coleoptiles in water		b. Coleoptiles dry	
Substrate alone	410	410	352	-
Substrate with pellet from hormone-treated sections	367	383	317	348
% Decrease	10.5%	7.0%	10.0%	-
Substrate with pellet from untreated sections	391	392	332	397
% Decrease	4.7%	4.7%	5.3%	-

viscosity is due to the activity of an enzyme acting on dextran. The general term "dextranase" activity will therefore be used for this effect.

In order to study the effect of the presence of the hormone, auxin, a comparison was made between the activity of pellets from sections of normal plants with those of plants that had been decapitated about $2\frac{1}{2}$ hours prior to the preparation of the pellet, so as to decrease the hormone content of the tissue. From Table I it is seen that the activity of the decapitated coleoptiles is much less than that of normal coleoptiles. The activity is 0.0% $2\frac{1}{2}$ hours after decapitation, and 6.0% $3\frac{1}{2}$ hours after decapitation. The higher value after $3\frac{1}{2}$ hours is no doubt due to the known regeneration of a hormone-producing tip.

The effect of the hormone was further studied by comparing the activity of sections which had been placed for 1 or 2 hours in water with or without 8 ppm auxin (Table II,a), and of sections which remained in the dry state, of which the tips had been powdered with some talcum with or without some indole acetic acid (Table II,b). In each of the two sets of two experiments the activity of the hormone-treated plants was larger, the average difference in viscosity being 24 seconds or about 6%. The average deviation from the mean flowtime with 4 comparable pellets from normal coleoptiles, incubated for 4 hours with 3 cc of a same dextran solution, was 3 seconds (0, 2, 4, 6 seconds).

Chromatography of dextranase hydrolyzates of a pure dextran and of coleoptile cell walls. The results of the paper chromatographic analysis of the products of hydrolysis of the natural dextran, 50B, by a purified dextranase are given in Table III. The mobilities R_g are represented as fractions of the mobility of glucose. By comparing these mobilities with those of pure sugars, the products of degradation by the purified enzyme could be identified as isomaltose, isomaltotriose, and in some cases also isomaltotetrose. Glucose was only present in a very low concentration. In addition, a spot consisting of substances of very low mobility was seen. These substances must be polysaccharides, some of which contain the α-1,3- linkages. The degradation products observed are typical for normal dextranase activity (8, 9, 10, 11, 12).

TABLE III

Hydrolysis Products of a Natural Dextran and of Cell Wall Material of Coleoptiles Acted on by a Purified Dextranase.

Component		Dextran mol.wt. 20-30 million	Coleoptile cell walls
R_g <0.20	Oligosaccharides	weak	weak
R_g 0.13	Isomaltotetrose	weak	none
R_g 0.26	Isomaltotriose	weak	medium
R_g 0.52	Isomaltose	strong	strong
R_g 1.00	Glucose	weak	medium
Unidentified spots:			
R_g 0.66		none	weak
R_g 1.13		none	medium

In order to determine whether or not substances are present in the cell walls of these coleoptiles which can be broken down by dextranase, cell wall pellets were digested for 4 or 12 hours at 45°C with the purified dextranase. The same products of hydrolysis resulted from the digestion of the pellets as from the digestion of pure dextrans by this enzyme (Table III). Particularly the isomaltotriose spot was clearly marked in the chromatogram from the pellet hydrolyzate. In addition to the spots of the isomaltose series, two unidentified weaker spots were present.

From these findings it may be concluded that dextran-like polysaccharides occur in these cell walls and that these substances can be acted upon by dextranase.

CONCLUSION

From the above experiments it may be concluded that dextranase activity is present in coleoptiles of Avena, and that the enzyme is sensitive to the presence of auxin, being higher at higher auxin concentration. The cell walls of the coleoptiles contain dextran-like substances in which the dextranase can act.

Natural dextrans are generally highly branched. In the cell wall these substances probably form a continuous network. The above experiments suggest that this network is broken down under the influence of the dextranase acting on the cell wall. This will lower the plasticity of the cell wall in a way similar to the lowering of the viscosity of the dextran solutions investigated.

It is concluded that a specific enzyme has been found which meets the two main requirements of an intermediate in the plasticization of the cell wall in auxin-regulated cell elongation in coleoptiles of Avena.

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