Proceedings of the Iowa Academy of Science

Volume 76 | Annual Issue Article 6

1969

Carbohydrate Breakdown in Cell Walls of Collenchyma of Celery

Waldo S. Walker
Northwestern University

Thomas Hoeltgen
Northwestern University

Carbohydrate Breakdown in Cell Walls of Collenchyma of Celery[1]

WALDO S. WALKER AND THOMAS HOELTGEN[2]

Abstract. Celery plants (*Apium graveolens L.*) were placed in darkness for 18 days and the collenchyma cells of the petioles of these plants were subjected to acid hydrolysis and qualitative and quantitative analyses to determine the effect of prolonged dark treatment on the metabolism of the wall components. Chromatographic separation and spectrophotometric measurement of the amounts of sugars present after 18 days of treatment indicated a great reduction in amounts of galactose and arabinose, a smaller reduction of mannose and xylose, and no reduction of glucose. The changes reported are attributed to a possible metabolic turnover of wall materials brought about by the low carbohydrate level which existed under the experimental conditions. The role of the plant cell wall in the general metabolism of the cell is briefly discussed.

Plant cell walls are formed outside the plasma membrane and until recently it has been generally considered that the constituents of the vegetative cell walls do not re-enter the cell to participate in intracellular nutritional metabolism (Esau, 1965; Frey-Wyssling, 1959; Miller, 1957; Neumiller, 1958; and Roelofsen, 1965). Although cell wall breakdown is recognized as a common occurrence in such processes as abscission, the ripening of fruit, and the nutrition of germinating embryos, it was only recently that the possibility of metabolic recycling of wall materials of vegetative cells was suggested (Matchett and Nance, 1962; Setterfield and Bayley, 1961; Siegel, 1962). Little quantitative evidence has been presented to support this suggestion, however. Roelofson (1965), in a recent review of cell wall ultrastructure expressed the opinion "Breakdown of cell wall substances in primary walls, going beyond the plasticization of the amorphous interfibrillar substances, has, in our view not been proved". Albersheim (1965), on the other hand, considers that there is little doubt of *in vivo* enzyme activity in the cell walls of plants. His view is supported by results of studies by Buston (1935) and by Matchett and Nance (1962). Clark and Stone (1962) have proposed that enzyme systems may be responsible for functions such as seasonal removal of sieve tube callose. A similar proposal has been suggested to account for the insertion of the mother-cell wall of the newly formed cell plate following cell division (Der-Fong Fan and Maclachlan, 1966).

[1] This study was supported by National Science Foundation Grant G-13407 and National Institutes of Health Grant RG 9876. The technical assistance of Mrs. Alan McKnight is gratefully acknowledged.

[2] Present address: Northwestern University School of Medicine, 301 East Chicago Avenue, Chicago, Illinois.

Anatomical studies (Walker, 1960) have shown that the thickness of collenchyma cell walls in *Datura* may be reduced 60-75% as a result of carbohydrate starvation. It was suggested that, under these conditions, one or more of the wall constituents may be utilized as substrate in the respiration of the cells.

The purpose of the present study was to determine which, if any, constituents are removed from the cell walls under conditions of carbohydrate starvation.

Materials and Methods

Collenchyma of celery (*Apium graveolens L.*) was chosen as the subject of this investigation because the cells remain metabolically active when mature. The cells, which occur in discrete bundles, can be easily and cleanly separated from the other tissues of the petiole. The characteristically thick walls of individual cells are well suited to a correlation of morphological observations and changes in chemical composition. The cells contain little, if any starch; therefore, the carbohydrate component of dried cells is almost entirely derived from the cell wall fraction.

Preparation of tissue. Celery plants were grown in the greenhouse (21-26°C and a 15 hr photoperiod) for 75 days, by which time 6-8 medium size (20-25 cm in length) petioles had developed. Two comparable groups of 20 plants were selected for study. One group was maintained in total darkness for 18 days for comparison with the control group. Conditions other than light were similar for both groups. Sampling of collenchyma was restricted to the three central bundles of the four most mature petioles of each plant. Care was taken to collect equal lengths of tissue from each of the two groups. In this manner, an equivalent amount of wall material was obtained from each of the groups which could not have been accomplished by taking equivalent numbers of cells, for the cells vary in length. The collenchyma was oven dried (48 hr at 60°C), weighed and pulverized. The pulverized material was then suspended in water to remove water soluble components. The cell wall fraction was collected by centrifugation and was dried as before.

Analysis. A modification of the procedure used by Seaman, Moore and Millet (1960) for analysis of cell wall carbohydrates in xylem tissue was used in this study of collenchyma. Measured lengths of collenchyma bundles were hydrolyzed for 45 minutes in 72% H_2SO_4 at 30°C. Secondary hydrolysis was conducted by diluting the acid to 3% and refluxing this cell wall-acid mixture for 4 hours. Finely pulverized BaOH was added to adjust the pH to 5.5 and the resulting mixture was centrifuged. The solution (about 160 ml) was decanted from the precipitate. The precipitate was

washed and this solution was added to the original solution, the combined solutions were concentrated by vacuum evaporation at 40°C to 10 ml of hydrolysate. Separation of the components of the hydrolysate was conducted using paper chromatography (Whatman No. 1 paper; ethylacetate-pyridine-water solvent, 8:2:1). Positions of the sugars were determined by spraying the chromatograms with p-anisidine hydrochloride (for reducing sugars; Pridham, 1956) and with urea solution (for non-reducing sugars; L. B. Wise, personal communication). Separated monosaccharides, eluted with distilled water from a second set of chromatograms were prepared for quantitative analysis by the use of Somogyi's sugar reagent (1945) in conjunction with Nelson's chromagenic reagent (1944). Quantitative determinations of concentrations were made with a Beckman DU spectrophotometer by comparing the spectral absorption of the recovered sugars with appropriate standard concentration curves.

Results

Dark-treated plants showed symptoms of chlorosis at 4-5 days and were pale yellow at 14 days. Petioles of treated plants remained turgid up to 20-24 days. Petioles did not elongate significantly in the dark-treated group. Anatomical observations indicated that the thickness of the collenchyma cell walls was appreciably reduced as a result of the dark treatment.

Analysis of several groups of untreated plants showed that from 29-52% of the collenchyma cell wall was hydrolyzable to monosaccharides. The presence of glucose, galactose, arabinose, xylose and mannose as the basic monosaccharide components of these cell walls agrees with the findings of many others (e.g. Ray, 1963). Although the chromatograms indicated the presence of sugar acids in the hydrolysate, these were not analyzed quantitatively. A coagulum was formed early during secondary hydrolysis which was collected and gave a positive test for protein.

Analytical data presented in Table 1 represent averages taken from three repetitions of the experiment. The overall loss of monosaccharide carbohydrate from the collenchyma during the 18 day dark treatment amounted to more than 20% of the amount initially present. As Table I indicated, the greater part of this loss was shown as a reduction in the amount of galactose and arabinose and the remainder as xylose and mannose. The difference in glucose values between treated and control plants is not considered to be significant.

Discussion

Hydrolysis and subsequent analyses of the cell walls of collen-

Table I. Monosaccharides of hydrolyzed collenchyma cell walls of celery before and after prolonged dark treatment*.

Monosaccharide	g sugar/g of tissue before treatment (dry wt)	% of total wall monosaccharides	g sugar/g of tissue after treatment (dry wt)	% gain or loss as a result of treatment
Glucose	0.198**	55.73	0.201	+ 1.17
Galactose	0.055	15.34	0.023	—58.53
Arabinose	0.052	14.72	0.013	—75.14
Xylose	0.035	9.71	0.029	—15.65
Mannose	0.016	4.50	0.013	—21.87

*Treatment consisted of maintaining the plants in total darkness for 18 days.

**All values represent averages taken from 3 repetitions of the entire experiment.

chyma tissue of celery show the presence of five monosaccharides. These substances accounted for up to 52% of the collenchyma cell wall hydrolysate. It is thought that sugar acids and protein may have accounted for the greater part of the unanalyzed cell wall material, although this was not analyzed quantitatively. The protein may have originated from cell membranes intimately associated with the wall as suggested by Beevers (1961) or it may have originated from the wall itself, since recent evidence (Lamport, 1965) indicates that the primary wall may contain significant amounts of structural protein.

As in an earlier study with *Datura* (Walker, 1960) these experiments with celery have also shown that the thickness of collenchyma cell walls is reduced in dark-treated plants. Results shown in Table I indicate that the loss of carbohydrate, as measured in these experiments on collenchyma tissue of celery, amounted to more than 20% of the original amount.

Although it is well established (e.g. Setterfield and Bayley, 1961) that cellulose is the basic structural component of primary walls of plant cells, there is no uniform approach to the classification and analysis of cell wall materials. Categories based on differential solubilities (e.g. protopectin, pectin and hemicelluloses *a* and *b*) have been used frequently, however, as stated by Albersheim (1965) such arbitrary categories "have probably led more to confusion than to clarification". This being the case, it was decided to hydrolyze the cell walls in this analysis and compare the treated and control plants on the basis of released or free monosaccharides present in the hydrolysate.

The results show that the galactose and arabinose fractions are much reduced (58 and 75% respectively) in collenchyma cell walls

as a result of dark treatment. This provides strong support for the contention that cell wall material can be used as a source of respiratory substrate, at least under the conditions of this experiment. The reductions in the xylose and mannose fractions, though less pronounced, also support this interpretation.

The work of Matchett and Nance (1962) on ^{14}C metabolism in the stems of pea seedlings indicated that ^{14}C could be incorporated into cell walls and removed later to take part in metabolism which occurs in the cytoplasm. This is also indicated by the present study, where it appears that components of the walls of collenchyma cells have definitely been removed in dark treated plants. Although it remains for tracer studies of metabolic pathways to show how these components are broken down and utilized, it appears to be highly possible that they have contributed to the general metabolism of these cells. It would follow from the results obtained in these studies, either that the cells normally contain enzyme systems that are capable of wall degradation or they have the potential to develop such systems under unusual conditions. Bearing in mind the recent reports of cellulase enzyme activity in vegetative tissues of certain plants (Albersheim, 1965; Clark and Stone, 1962; and Maclachlan and Perrault, 1964) we incline to the view that such enzyme systems are normally present in plant cells. We believe that the normally synthetic activity of these systems can be reversed under certain conditions, as in the present experiment where there was extreme carbohydrate starvation, to provide an energy source for cellular metabolism.

References

Albersheim, P., 1965. Biogenesis of the cell wall. *In* Bonner, J. and Varner, J. E. Plant biochemistry. Academic Press, New York.

Beevers, H., 1961. Respiratory metabolism in plants. Row, Peterson and Co. New York.

Buston, H. W., 1935. Observations of the nature, distribution, and development of certain cell wall constituents of plants. Bioch. J. 29, 196-213.

Clark, A. E. & B. A. Stone, 1962. β-1, 3-glucan hydrolases from the grapevine (Vitis vinifera) and other plants. Phytochem. 1, 175-188.

Der-Fong Fan & G. A. Maclachlan, 1966. Control of cellulase activity by indoleacetic acid. Can. J Bot. 44, 1025-1035.

Esau, K., 1965. Plant anatomy. John Wiley and Sons Inc. New York.

Frey-Wyssling, A., 1959. Die Pflanzliche Zellwand. Springer-Verlag. Berlin.

Lamport, D. T. A., 1965. The protein component of primary cell walls. *In* Advances in botanical research (R. D. Preston, ed.) Vol. II Academic Press, New York.

Maclachlan, G. A. & J. Perrault, 1964. Cellulase from pea epicotylis. Nature 204, 81-82.

Matchett, W. H. & J. F. Nance, 1962. Cell wall breakdown and growth in pea seedling stems. Am. J. Bot. 49, 311-319.

Miller, E. V., 1957. The chemistry of plants. Waverly Press Inc. Baltimore.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem 153, 375-380.

NEUMILLER, G., 1958. Pentosans and hexosans. *In* Ruhland, W. Handbuch der Pflanzenphysiologie. Vol. VI. Springer-Verlag. Berlin.

PRIDHAM, J. B., 1956. Determination of sugars of paper chromatograms with p-anisidine hydrochloride. Anal. Chem. 28, 1967-1968.

RAY, P. M., 1963. Sugar composition of oat-coleoptile cell wall. Biochm. J. 89, 144.

ROELOFSEN, P. A., 1965. Ultrastructure of the wall in growing cells and its relation to the direction of growth. *In* Advances in botanical research (R. D. Preston, Ed.) Vol. II. Academic Press, New York.

SAEMAN, J. F., W. E. MOORE, & M. A. MILLET, 1960. Sugar units present . . . hydrolysis and quantitative paper chromatography. Forest Products Laboratories, Madison, Wisc. April Bull.

SETTERFIELD, G. & S. T. BAYLEY, 1961. Structure and physiology of cell walls. Ann. Rev. Pl. Physiol. 12, 35-62.

SIEGEL, S. M., 1962. The plant cell wall. Pergamon Press, New York.

SOMOGYI, M., 1945. Determination of blood sugar. J. Biol. Chem. 160, 69-73.

WALKER, W. S., 1960. The effects of mechanical stimulation and etiolation on the collenchyma of *Datura stramonium*. Am. J. Bot. 47, 717-724.

