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Determination of natural and wound-induced potato tuber suberin phenolics by thioglycolic acid derivatization and cupric oxide oxidation

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Summary

Application of two lignin chemistry techniques, CuO oxidation and thioglycolic acid derivatization, has confirmed that natural and wound-induced suberin does have a lignin-like component. The time course of suberin phenolic deposition could be monitored by using the thioglycolic acid technique. The thioglycolic acid procedure is easily performed, can be adapted readily to multiple samples, and thus could be an effective tool for studying rates of wound-induced suberization.

Introduction

The suberized cell walls of natural and wound-induced periderms of potato tubers function in protection against pathogens and in prevention of water loss (Kolattukudy, 1980, 1981). Although these functions are very important, relatively little is known about the biosynthesis and structure of suberin and the genetic control of the suberization process.

Suberin is a complex heteropolymer consisting of aliphatic and phenolic domains in association with waxes. Work by Kolattukudy and co-workers (Kolattukudy, 1980, 1981) has contributed greatly to the understanding of the structure and biosynthesis of suberin aliphatics. For example, Kolattukudy & Dean (1979) demonstrated that the aliphatic components of natural potato tuber periderm suberin are very similar to those found in wound-induced suberin, and that a reliable method for monitoring the suberization process can be based on the measurement of one of these aliphatic components.

Much less is known about the phenolic (aromatic) portion of the suberin polymer. Histochemical staining has suggested that the phenolic portion of suberin has a lignin-like character (Walter & Schadel, 1982). Recently, Cottle & Kolattukudy (1982a, b) have applied a traditional lignin chemistry technique, nitrobenzene oxidation, to the study of suberin phenolics. They found that nitrobenzene oxidation of wound-induced suberin from potato discs (Cottle & Kolattukudy, 1982a) or of abscisic acid (ABA) induced suberin from potato callus cultures (Cottle & Kolattukudy, 1982b), yielded *p*-hydroxybenzaldehyde and vanillin. Both of these phenolic aldehydes are expected products from polymers with a lignin-like character. The lack of syringaldehyde generation indicated that the material, although lignin-like, was different from the lignin found in the vascular

tissue of angiosperms (Freudenberg & Neish, 1968). Cottle & Kolattukudy (1982a) reported that measurement of the two phenolics over time provided a measure of the rate of deposition suberin aromatics and, therefore, a measure of the rate of suberization. The rate of phenolic deposition was also shown to parallel the deposition of polymeric suberin aliphatics and waxes (Cottle & Kolattukudy, 1982b).

Since Cottle & Kolattukudy (1982a, b) have demonstrated the lignin-like character of wound- and ABA-induced suberin, I have tested the use of another lignin chemistry technique, i.e. thioglycolic acid derivatization, as a method for determining suberin phenolics in natural and wound-induced suberin. I have also tested thioglycolic acid derivatization as a method for measuring suberization rates in wounded tuber tissue. Finally, I have confirmed Cottle & Kolattukudy's results which demonstrated that suberin phenolics yield *p*-hydroxybenzaldehyde and vanillin upon alkaline oxidation.

Materials and methods

Potato tubers stored at 8 ° C for 2–4 months were used in all experiments. Tubers were allowed to warm to room temperature for 24 hours prior to use in suberization experiments. For suberization experiments, tissue discs (2 cm diameter and 0.5 cm thick) were aseptically prepared from the central parenchymous tissue of the tubers (Hammerschmidt, 1984). The discs were then incubated in the dark at 22 ° C in inverted petri dishes containing 0.5 % water agar as described by Marcan et al. (1979).

At intervals after preparing the discs, the upper 0.5 mm of tissue was removed with a slicer. The tissue slices (typically 6 per time period) were extracted with absolute methanol (5 ml/disc, three changes of solvent) over a three-day period and then allowed to air-dry. The dried slices were ground to a fine powder prior to further analysis.

A suberin-enriched fraction was obtained by the method of Kolattukudy et al. (1975) from the natural periderm of potato tubers and from discs allowed to suberize for 12 days.

Analysis of suberin phenolics by the thioglycolic acid procedure (Whitmore, 1978; Hammerschmidt, 1984) was carried out on 50–60 mg of methanol-extracted tissue (equivalent to two slices) or 10 mg of a suberin-enriched fraction.

Cupric oxide (CuO) oxidation was carried out on 25 mg of pulverized suberin-enriched fraction by the method of Rhodes & Wooltorton (1973) as described by Hammerschmidt (1984). The CuO oxidation products were analysed for the presence of *p*-hydroxybenzaldehyde, syringaldehyde and vanillin (Rhodes & Wooltorton, 1973).

All experiments were repeated three times. Results presented are those of a typical experiment.

Results and discussion

Characterization of lignin-like components of suberin from potato tuber skin and wound-induced suberin

CuO oxidation of suberin from natural periderm or healed (12 days) wounds from two varieties yielded *p*-hydroxybenzaldehyde and vanillin (Table 1). The results from the healed tissue are very similar to those obtained by Cottle & Kolattukudy (1982a) by alkaline nitrobenzene oxidation, a technique similar to CuO oxidation. The type of

Table 1. CuO oxidation products of natural and wound-induced suberin-enriched fractions (SEF) from two cultivars.

Cultivar	SEF-type	Oxidation products (mg/g dry wt SEF)	
		p-hydroxybenzaldehyde	vanillin
Onaway	natural	7.62	10.22
	wound-induced	6.78	4.36
Russet Burbank	natural	7.10	8.33
	wound-induced	4.88	2.95

Table 2. Relative lignin thioglycolic acid (LTGA) yields from natural and wound-induced suberin-enriched fractions (SEF).

Cultivar	SEF type	Relative LTGA yield (A_{280}/mg SEF in 1 ml NaOH 0.5 mol/l)
Onaway	natural	4.59
	wound-induced	2.56
Russet Burbank	natural	4.48
	wound-induced	1.99

phenolic aldehydes released from healed tissue were the same as those released from natural periderm (Table 1). The amounts, however, were lower. This provides further evidence that the wound-induced and natural suberin are very similar in terms of phenolic as well as aliphatic constituents (Kolattukudy, 1980, 1981). Only traces of phenolic aldehydes were released from fresh tissue, and this agrees with the findings of Cottle & Kolattukudy (1982a) (data not shown).

Treatment of a suberin-enriched fraction from natural tuber periderm or tissue slices from 12-day-healed wounds with thioglycolic acid in HCl yielded an acid-insoluble, base-soluble material (a lignin thioglycolate or LTGA) (Table 2). Lower relative amounts of LTGA were released from the wound-induced suberin-enriched fraction as compared to the natural suberin. This is in agreement with results obtained with CuO oxidation (Table 1), and suggests that suberization may not have been completed at the time of sampling. Only a small amount of absorbance was found in the samples taken from fresh tissue. These results indicate strongly that, as proposed by Kolattukudy (1980, 1981), suberin does contain phenolic domains that are very lignin-like in nature. The thioglycolic acid procedure is quite specific for lignin, and no product would have been produced if a material with the intermolecular linkages found in lignin were not present (Freudenberg & Neish, 1968).

Time course of suberin phenolics deposition in wounded potato-tuber tissue

Since thioglycolic acid derivatizations and CuO oxidations reported in this paper have confirmed the previous work of Cottle & Kolattukudy (1982a, b), the thioglycolic acid procedure was used to monitor deposition of suberin phenolics in wounded tuber tissue

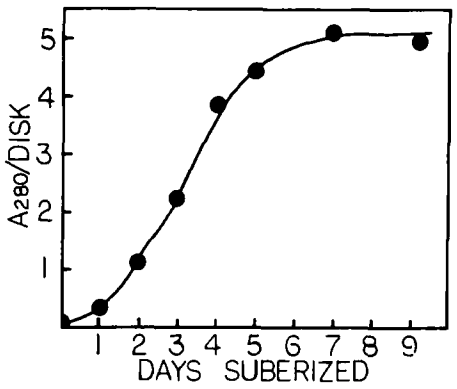

Fig. 1. Rate of suberin phenolics deposition in wounded Onaway-tuber tissue as determined by the thioglycolic acid method. Relative suberin phenolic content was expressed as A_{280} per disc in 5 ml 0.5 mol/l NaOH. Each point represents the mean of three determinations from one representative experiment.

as a function of time. Fig. 1 shows the rate of accumulation of LTGA-generating phenolics in suberizing Onaway tuber tissue as a function of time. Increase in LTGA was detected by day 1. The greatest rate of increase occurred between days 2 and 6. The rates of suberization as revealed by the thioglycolic acid method is very similar to published rates of suberin deposition determined by measuring increases in diffusive resistance (Kolattukudy & Dean, 1974; Jarvis & Duncan, 1979), aliphatics (Kolattukudy & Dean, 1974), and phenolics (Cottle & Kolattukudy, 1982a).

The results of this study have confirmed that suberin does have a lignin-like phenolic component and that the amount of this phenolic component can be determined by two traditional lignin chemistry techniques. The thioglycolic acid procedure has also been shown to be potentially useful as a method for monitoring suberin deposition. This method is easy to perform and can be readily applied to multiple samples. Since the thioglycolic acid procedure extracts lignin from cell walls with relatively little structural change (Freudenberg & Neish, 1968), this technique might also be useful in preparing the phenolic domains of suberin for structural studies.

Acknowledgements

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