Acetyl Groups in Cell-Wall Preparations from Higher Plants

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O-Acetyl groups were detected by i.r. spectroscopy in cell-wall preparations from grasses and other higher plants and their presence was confirmed chemically. The amounts present are likely to influence both the physical state of the cell-wall polysaccharides and also their digestion by enzymes.

The technique of preparing cell walls by breaking the cells mechanically and then washing out the contents, first extensively used with bacteria, was soon afterwards applied to yeasts and unicellular algae, but despite some early publications (e.g. Kivilaan *et al.*, 1961) cell walls of higher plants have not often been prepared and analysed (cf. Rogers & Perkins, 1968). In most cases the walls have been derived from immature tissues (e.g. oat coleoptile; Ray, 1963) or from suspension-cultured cells. The most comprehensive studies are those of Albersheim and his colleagues on sycamore and other callus cultures (Burke *et al.*, 1974).

During an investigation of the digestion of plant cell walls by ruminants we have attempted to prepare cell walls from mature samples of several grasses, including leaves, leaf sheaths and stems. Two types of preparation were made, type 1 being a mixture of cell walls from vascular and fibrous elements and epidermis, type 2 consisting chiefly of walls from mesophyll cells (see under 'Experimental' for details).

The i.r. spectra of preparations of both types showed a medium-intensity absorption at $8.0\mu\text{m}$ attributable to acetate, which was associated also with ester carbonyl absorption at $5.75\mu\text{m}$ (Fig. 1, spectra *a* and *b*). Samples were therefore treated with 1 M-NaOH at 20°C for 3 days and portions of the extract acidified and steam-distilled. G.l.c. of the distillates showed that 90–100% of the acid present was acetic acid, and titration indicated the presence of 1.5–2.0% acetyl groups in the cell walls (Table 1). Morrison (1973) has noted the presence of an acid, tentatively identified as acetic acid, in alkaline hydrolysates of lignin-carbohydrate complexes from Italian rye grass; his estimates would suggest 2–3% acetyl groups.

I.r. spectroscopy thus provides a sensitive, simple and quick indication of the presence of acetyl groups in plant material (cf. Matsuo & Mizuno, 1974), and using it we have found evidence for acetyl groups, in amounts comparable with or greater than those in grass cell walls, in cell-wall preparations from leaves

of lettuce (*Lactuca sativa*), lime (*Tilia* sp.) and beech (*Fagus sylvatica*), and from root tissue of beet (*Beta vulgaris*) and swede (*Brassica napus* var. *napobrassica*). Several of these preparations contained considerably more pectin than did grass cell walls, and removal of pectin decreased but did not eliminate the acetyl absorption bands (compare spectra *d* and *e*, Fig. 1, of beet root cell walls before and after removal of pectin). I.r. spectra have also indicated that acetyl groups are stable to the conditions used in preparing holocellulose as described by Wise *et al.* (1946) but not to those used in preparing neutral-detergent fibre as described by Van Soest & Wine (1967); the latter is therefore not equivalent in all respects to native cell wall.

Acetyl groups have long been known to be present in pectins (Kertesz, 1963) and in woods (Timell, 1964, 1965). In the latter case Hägglund *et al.* (1956) showed that acetyl groups were part of the hemicellulose fraction, and Bouveng (1961) developed techniques by which they were assigned to C-2 and C-3 of the xylose residues in a hard-wood hemicellulose. In these polysaccharides, on average, every second xylose residue is acetylated.

Although it is recognized that acetyl groups occur generally in hemicelluloses (cf. Northcote, 1972), little consideration appears to have been given to the part that they might play in biochemical processes involving the plant cell wall, such as its biosynthesis (Lampert, 1970) or its degradation by micro-organisms (Bailey, 1973). Of particular interest is the possibility that they may decrease the digestibility of cell walls in the ruminant. Waite & Gorrod (1959*a,b*), in attempting to provide a complete analysis of dried grass samples, determined the acetyl contents of holocelluloses prepared from them. The degree of acetylation increased with the maturity of the grass, and animal tests on similar samples (Waite *et al.*, 1964) showed an accompanying fall in the digestibility, particularly of the pentosan fraction. If the acetyl groups were all associated with the pentosan fraction, then in the most mature

Fig. 1. *I.r. absorption spectra of cell-wall preparations*

(a) Type 2 preparation (Italian rye grass); (b) type 1 preparation (perennial rye grass); (c) type 1 preparation from sheep faeces; (d) type 1 preparation from beet root; (e) type 1 preparation from beet root after removal of pectin by extraction with aqueous ammonium oxalate.

samples, where the acetyl content reached as high as 2.7% of the dry matter, on average every second residue must be acetylated. By analogy with the lower activity of cellulase on CM-cellulose of a similar degree of substitution (Holden & Tracey, 1950), such a degree of acetylation could greatly impede enzymic digestion. In support of this proposal cell walls isolated from faeces of sheep were found still to be acetylated (Fig. 1, trace c, and Table 1; see also Beveridge & Richards, 1973a). Acetylation therefore deserves serious consideration as a factor which may contribute to the fall in digestibility as forages mature.

Apart from the obstruction to enzymic action, acetylation (as little as one group per 12 residues in salep mannan) can hinder the packing of polysaccharide molecules and so increase their solubility in water. Salep mannan, from the tubers of some Orchidaceae (Néel, 1965), and a glucomannan from bulbs of the

Table 1. *Acetyl contents of some fractions derived from grasses*

The sample (100–200mg) was suspended in 25ml of 1M-NaOH and left at 20°C for 3 days. Insoluble material was removed by centrifugation and the volume made up to 50ml. Samples (5ml) were acidified with 1.3M-H₂SO₄ containing 2M-MgSO₄ and distilled in a Markham apparatus (Markham, 1942). About 75ml of distillate was collected and titrated with 0.01M-NaOH, with Phenol Red as indicator, with stirring with a stream of CO₂-free air.

Description of sample	Acetyl content (% dry wt.)
Whole grass, commercially dried, high digestibility	1.7
Whole grass, commercially dried, low digestibility	2.0
Cell-wall preparation (type 1) from dried grass*	2.0
Cell-wall preparation from sheep faeces*	2.1
Total insoluble fraction of sheep faeces†	2.2
Cell-wall preparation (type 1) from Italian rye grass (<i>Lolium multiflorum</i> Lam.), grown in glasshouse	1.7
Cell-wall preparation (type 2) from perennial rye grass (<i>Lolium perenne</i> L.), grown in field	1.4

* This dried grass constituted the sole diet of the sheep.

† Insoluble material was 94% of the dry weight of the faeces.

Easter lily (*Lilium longiflorum*) (Matsuo & Mizuno, 1974) both become insoluble in water after removal of the acetyl groups present in the native polysaccharide. Such findings raise the possibility that studies of enzymic digestion of polysaccharides isolated by the use of alkaline reagents may not be relevant to the digestion of these polysaccharides in their native states; see, e.g., Beveridge & Richards (1973b) and references therein.

Experimental

I.r. spectra. These were measured on air-dry or, preferably, freeze-dried samples (1mg) in 12mm KBr discs.

G.l.c. of carboxylic acids. The acids were separated by a method similar to that of Fell *et al.* (1968) on a column of 20% neopentyl glycol adipate with 3% (w/v) orthophosphoric acid on 60–85-mesh Chromosorb W.

Cell-wall preparations. Fresh or dried material (10–30g of plant tissues, rumen digesta, faeces) was ground in a mortar with liquid N₂ (Bean & Ordin, 1961) and, after thawing, the powder was ground for a further 30min in water containing a detergent (0.1% Triton X-100; BDH Ltd., Poole, Dorset,

U.K.), and an anti-bacterial substance [0.1% Givgard DXN (6-acetoxy-2,4-dimethyl-1,3-dioxan); Givaudan and Co. Ltd., Whyteleafe, Surrey CR30YE, U.K.]. The resulting suspension was subjected to an elutriation procedure in which the suspending medium was pumped up a vertical glass tube (56 mm internal diameter) in which a plastic cylinder (45 mm external diameter) revolved at 25 rev./min. The annular space had a volume of about 450 ml and pumping was at 220 ml/h. After 5–6 h most of the green debris had passed out of the top. The almost colourless material remaining suspended in the column was collected by centrifugation at 1500 g for 10 min, ground in a mortar and again subjected to the elutriation treatment. Finally, the residue was washed with water, and either freeze-dried in aqueous suspension or dehydrated by successive treatments with ethanol, acetone and light petroleum (b.p. 40–60°C) on a sintered-glass funnel. A grass sample of fresh weight 30 g yielded about 1 g of dry residue (type 1 preparation).

Microscopic examination showed that this residue consisted chiefly of walls derived from clumps of cells, predominantly vascular tissue, fibre elements and epidermis. Very few walls derived from mesophyll cells were identified and it was found that many of these passed unbroken out of the top of the column.

Mesophyll cells were therefore pressed out of fresh grass leaves by gentle grinding in a mortar (cf. Edwards & Black, 1971) and washed by centrifugation. They were then passed through a French pressure cell (American Instrument Co. Inc., from V. A. Howe, London S.W.6, U.K.), which produced more than 90% breakage at 25000 kPa. The walls were washed repeatedly with water by centrifugation and dried as described above (type 2 preparation).

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