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Lignin-carbohydrate complexes of Natural Fibres

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All the natural plant fibres other than cotton are ligno-cellulosic in nature. The nature of linkages in cellulose and hemicellulose are more or less clearly known, but the nature of the third important constituent, viz., lignin is not yet clearly known. Particularly the bonds between lignin and carbohydrates are only partly known. In order to understand these bonds, studies on lignin-carbohydrate complexes (LCC) have become very important.

Research activity in this topic has remained at a significant level on a world wide basis for many years and several hundred papers have been published in the past 20 years. Despite this however, there have been no dramatic development which could be described as a breakthrough.

In the case of natural plant fibres little work appears to have been done on this aspect. However if we could have understood these linkages perhaps a new range of chemical treatments might have been possible on these fibres. Woollenisation of Jute fibres, discolouration on exposure to light and many other phenomena are still to be clearly understood. The chemicals successfully applied for the fire retardancy of cotton fabrics, fail to produce desirable results for jute fabrics. While cotton can not be dyed with basic dyes without a mordant, jute has got high affinity for basic dyes. Many methods have been developed to improve the jute fabric feel and light fastness by partial removal of lignin as chlorolignin or by acetylation. IJIRA[1] had developed a procedure to chlorinate the jute fabric by passing it through hypochlorite solution at pH-6 obtained by adding dil. HCl gradually. Recently I have found (to be published) that addition of ammonium oxalate (1% o.w.f.) to the hypochlorite solution produces the same grade of light fastness and the fabric feel is much improved. Moreover, this procedure eliminates the sudden chlorine hazard in the IJIRA procedure due to increase in local concentration of acid. The work is in progress, and we would like to know the possible mechanism of this reaction. Today a brief survey of the methods of isolation of LCC's and the nature of the evidence for the covalent linkage will be made and the scope of its application on plant fibres will be discussed.

The structural investigation on the hemicelluloses present in jute was initiated by Sarkar[2] and coworkers. Partial hydrolysis of the D-xylan with dil. H_2SO_4 yielded an aldobiouronic acid[3], 3a. The acidic counterpart of which was erroneously reported as 3-O-methyl-D-glucuronic acid. But later on Srivastava and Adams[4] converted the glucuronic acid to the corresponding neutral sugar which was conclusively shown to be 4-O-methyl-D-glucose. And on the basis of methylation studies it was shown that the aldobiouronic acid was 2-O-(4-O-methyl-α-D-glucopy-ranosyluronic acid)-D-xylose. In a parallel communication Duttan and Rogers[5,6] proved the same structure. From the hydrolysis of fully methylated D-xylan, Aspinall and Dasgupta[7] along with other evidences assigned a structure to the xylan consisting of (1—▷4)-linked D-xylopyranosyl residues, the 4-O-methyl glucopyranosyl residues appear as branch points. This structure has also been found in the fibres of Mesta[8], Roselle[9], Sisal[10], Agave[11] fibres. In my own studies, I have found that the hemicellulose of Sansevieria trifasciata[12, 13] contains(1—▷4)-linked D-xylan backbone chain. In another study I have noted peculiar structure in the hemicelluloses of pineapple fibres [14, 15]. I found that arabinofuranosyl reduces residues are linked to the xylan chain as non-reducing and groups. D-xylan in flax[16, 17], has a small proportion of (1—▷3)-L-rhamnosyl residues as insertions to the backbone of (1—▷4)-linked D-xylan chain. The hemicellulosic content of sunn hemp[18] is unique in that the major portion of it is a glucomannan.

Some work has also been done at JTRL 18a, 18b IJIRA and Indian Association for the Cultivation of Science on the ester linkages between uronic acid carboxyl groups of glucuronoxylans and lignin hydroxyls. Such linkages are readily split by alkali or by borohydride, in the latter case with the production of 4-O-methyl glucose from 4-O-methyl glucuronic acid.

The trends in past five years have been changed

and many new methods have been tried for obtaining the highest yield of LCC's with the combination of several mild isolation procedures [19-36] providing several lignin fractions. Five fractions can be successively obtained by a recently developed procedure.

1. The finely ground fibres were extracted exhaustively with Ethanol: Toluene (1:2 V/V), ethanol and water respectively in a soxhlet extraction apparatus, then freeze-dried. Then it was ultraground in a ball mill.
2. The extractive free material was extracted with 90% aqueous dionane (Bjorkman) for 24h (liq. ratio 1:10) in dark, the extraction was repeated once and the mixture was centrifuged at 10,000 r.p.m. at 4°C for 10 mins. The extracted LCC fraction was purified by precipitation in 5% Na_2SO_4 solution. The precipitate was redissolved in the minimum volume of glacial acetic acid and reprecipitated in diethyl ether. After centrifugation LM was freeze-dried.
3. The residue after LM extraction was thoroughly washed with distilled water (3 times). The washings were mixed and freeze dried (LCC [H_2O]).
4. The residue was further dispersed in a solution of commercial cellulase "Cellulase onozuka R-10" at 37° for 72h in the dark (liq. ratio 1:20). The concentration of the enzymatic solution was 4mg/ml in 0·5M acetate buffer (final pH 4·5) with 10 p.p.m. (W/V) streptomycin and tetracycline. From the residue of the hydrolysed material LE was extracted by using the same procedure as for LM isolution. The enzyme containing supernatants were deproteinised by treating with pronase for 3 days at room temperature at pH 7·5 using phosphate buffer. The mixtures were centrituged and the supernatant was concentrated, dialysed and freeze-dried LCC (Enz).
5. After extraction of LM & LE, the residue was freeze-dried and ultraground again like in (1). From the ultraground material, the last fraction (LR) was isolated in the same way as for LM. The final residue LR was freeze-dried.

There is no reason to doubt that the nature of linkages between the polysaccharides and the lignin fragments is the same as in the original cell wall. But some scientists are of the view that the formation of free radicals during ball milling may lead to the covalent linkages between lignin and carbohydrates. Now-a-days, freezer-milling is being utilised which is supposed to be least harmful to the cell wall structure, as the use of liquid nitrogen (−190°C) avoided problems caused by localised heating. Spex 6700 Freezer-mill was used for 3 minutes and the average size of the particles was reduced to 10-40 μm diameter. Such particles swelled and dispersed in dimethyl sulphoxide (DMSO) and could be directly methylated by Hakomori's method[36].

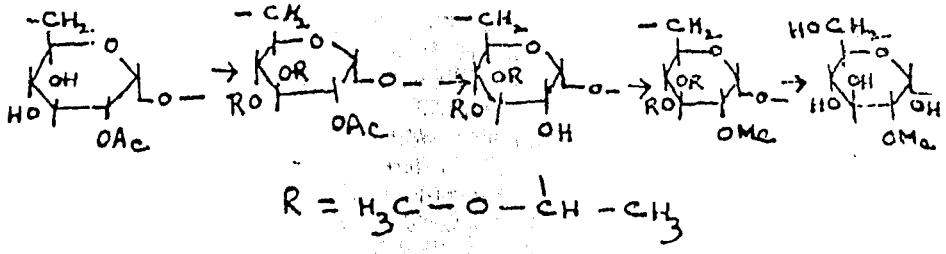
In the Hakomori's method the polyalkoxide of the substrate in anhydrous DMSO is prepared first by reaction with dimsyl sodium and then with methyl iodide to effect methylation. This method gives, in many cases, complete methylation in one step; the most important factor being the anhydrous condition of the reaction mixture. However, this powerful methylation technique destroys alkali-labile linkages that may be important in the overall architecture of the cell wall, particularly the secondary wall.

The reaction which has been found to be very useful in the determination of alkali-labile linkages is Acetalation[37, 38]. This reaction provides the results just opposite to methylation. The idea is to protect the free hydroxyl groups by methyl vinyl ether and then the substrate is methylated at the sites of alkali-labile substitution.

The dried sample (~20 mg) is dissolved in 2 ml of DMSO in a 20 ml tube sealed with rubber septum and 20 mg of p-toluene sulphonic acid is added to it. The tube is flushed with argon gas via two injection needles. The sissolution of the samples is often facilitated by treatment in an ultrasonic bath. About 2 ml of methyl vinyl ether is added via an injection needle using a syringe. The tube is then kept at 15°C for 3·5h and then the red coloured mixture is centrifuged. The pellet obtained is washed with acetone (3 times) and after drying pellet in vacuo over P_2O_5, it is permet-hylated by Hakomori's method.

Isolation of lignases and polysaccharases and their application on the LCC's will certainly lead to very much conclusive approach for the interpretation of linkages.

The presence of ether linkages between lignin and carbohydrates has often been assummed, but untill very recently there was never any direct evidence of such linkages in the cell wall. In support of this concept, Koshijima[39] and co-workers have now



shown that LCC oligomers from MWL (milled wood lignin) may be degraded by 2,3-dichloro-5, 6-dicyano-p-benzoquinone (DDQ) to yield galactose, arabinose, xylose and glucose. Since the conditions of the DDQ reaction are specific for scission of benzyl ethers these results appear to give the best evidence to date for ether linkages between lignin and carbohydrates.

Enzymes lebelled with colloidal gold as an electron dense marker, are applied on ultrathin sections of glutardehyde-fixed and resin embedded cells. A specific localisation of xylan[30] was obtained in the secondary walls whereas primary walls and cytoplasm were free of label. In all examined samples the polysaccharide substrates were not altered during tissue processing and a strong binding occurred combined to a good ultrastructural preservation.

Kosikova and co-workers[31] have investigated the fine structure of the LCC in the cell wall by electron microscopy and this approach in combination with aforementioned methods will certainly provide conclusive results.

The most conclusive evidence of covalent lignin-carbohydrate linkages relates to the behaviour of *solutions* of LCC's especially in gel permeation chromatography. The most widely used gels are based on a dextran matrix which is known to interact with a wide range of aromatic compounds. Such interactions retard the passage of the solute through the column so that the elution volume is many times greater than that of a non-interacting solute of the same molecular weight. Carbohydrates do not show this type of interaction with dextran therefore when a macromolecular (non-dialysable) LCC in aqueous solution is passed through a dextran gel column and the carbohydrate component elutes with the lignin component considerably latter than the inclusion volume at which a fully permeable low molecular weight non-interacting solute would elute, the only reasonable conclusion is that the carbohydrate was covalently linked to the aromatic components.

It has been recently shown (J. P. Joseleau, France, personal communication) that the use of lithium chloride in the gel chromatography will considerably reduce the dextran-aromatic interaction. In such systems the LCC's will elute within the normal fractionation range of the column and when in such chromatography the carbohydrate and aromatic profiles coincide this is also presumptive evidence of lignin-carbohydrate linkages.

In the case of natural fibres these methods may be very much useful to understand the linkages between lignin and carbohydrates.

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