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Ferulic Acid as a Component of a Glycoprotein from Wheat Flour

IT has already been shown that the water-soluble wheat flour pentosans can be separated by DEAE-cellulose chromatography into five fractions: a pure arabinoxylan and four glycoprotein fractions[1]. For the peculiar gelation of aqueous flour extracts by oxidizing agents[2] the principal glycoprotein fraction (No. 2) could be responsible[3,4]. This fraction contains 80–90 per cent polysaccharide (components: xylose, arabinose and galactose) and 10–20 per cent proteins. The ultra-violet absorption spectra show in addition to the protein maximum a weak maximum at 320 mμ which disappears on oxidation[3]. It was suspected that polyphenolic compounds are responsible for this maximum.

Various degradation reactions were investigated, therefore, in order to obtain low-molecular weight fragments containing phenolic components. Degradation of the glycoprotein-2 by pure proteolytic enzymes released no phenolic compounds but showed that they were most probably linked to the xylan chain rather than to the protein part. Degradation by these enzymes produced two polysaccharide fragments of entirely different composition, indicating that in glycoprotein-2 the two polysaccharides are linked together by a polypeptide bridge[5].

Phenolic compounds could be split off from the glycoproteins (unfractionated flour pentosans), however, by treatment with aqueous 0·5 M potassium hydroxide at 60° C during 90 min under nitrogen. The hydrolysate was acidified (pH 3·5), extracted with ether followed by a bicarbonate extraction. The ether extract from the acidified bicarbonate solution was investigated by thin-layer chromatography. Three phenol positive spots were detected (solvent[6]: benzene–methanol–acetic acid 90 : 16 : 8, R_F values 0·46, 0·58 and 0·66). One of the spots (R_F 0·58) could be positively identified as ferulic acid (R_F values in various solvents, colour reactions, ultra-violet spectra of the extracted spot). When the flour pentosans were gelled by addition of hydrogen peroxide prior to hydrolysis with alkali, no ferulic acid could be detected. It is, therefore, very likely that ferulic acid and possibly other phenolic compounds in the soluble pentosans take part in the oxidative gelation of aqueous flour extracts and therefore constitute other types of compounds in wheat flour readily attacked by oxidizing agents.

It is tentatively assumed that ferulic acid is linked to the xylan chain via ester bonds. The deposition of toxic ferulic acid[7] and possibly other phenolic compounds in cell wall polysaccharides might be another way of detoxication of these compounds in certain plants, formation of simple glycosides being, of course, the most common way[8]. Aliphatic acids (formic and acetic acid) have long been known to be deposited as esters in certain plant cell wall polysaccharides[8].

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Sulphatases and Sulphated Polysaccharides in the Viscera of Marine Molluscs

GLYCOSULPHATASES are present in tropical[1] and British[2–4] molluscs. Molluscan preparations also contain chondrosulphatase[2,4], which may be an oligosaccharide sulphatase; but few examples of polysaccharide-sulphatases are known[5].

Recently the sulphatase activities of preparations from marine molluscs collected on the North Wales shore with respect to various sulphated carbohydrates of both high and low molecular weight have been studied. The viscera (1·0 g) of each mollusc was macerated in 0·2 M sodium acetate–acetic acid buffer, pH 5·5 (10 ml.), and incubated with the carbohydrate substrate for 48 h. Liberated sulphate was detected turbidimetrically[6]. Preparations from *Calliostoma zizyphinum* and *Patella vulgata* showed the widest range of activity, each liberating sulphate from D-glucose- and D-galactose-6-sulphate, chondroitin sulphate *A* and fucoidin. Other polysaccharides, keratosulphate, carrageenin and mucilages from *Dilsea edulis* and *Porphyra umbilicalis*, were not desulphated. *Littorina littorea*[3] and *Mytilus edulis* preparations contained glycosulphatase, but were inactive against chondroitin sulphate *A* and the algal polysaccharide sulphates. *Buccinum undatum* and *Gibbula cineraris* were without action on the foregoing substrates.

It was of interest to consider the nature of the natural substrates for molluscan sulphatases and a further investigation had led to the isolation, from *Patella vulgata* viscera, of a sulphated polysaccharide(s), which is a substrate for the sulphatase preparation obtained from the same source. The polysaccharide material was obtained by extraction of *Patella* viscera with 5 per cent potassium chloride followed by trypsin digestion, Sevag deproteinization, dialysis and alcohol precipitation. It was further purified by precipitation with cetyl pyridinium chloride[7]. Sugars detected when the preparation was hydrolysed with acid were fucose, xylose and galactose. Fucose and xylose are not usually encountered in other than trace amounts in animal tissues and it is considered likely that the polysaccharides, in *Patella* viscera are derived, partially at least, from algal sources (where these sugars are common) which are ingested by the mollusc[8]. *Patella* sulphatases are thus regarded tentatively as digestive enzymes.

Patella viscera also contains a glucan, which is obtained by extraction with 10 per cent trichloroacetic acid, as well as enzymes, which degrade starch, maltose and cellobiose.

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