when acting alone. In both parasitic fungi, *Coniothyrium minitans* and *Trichoderma viride*, the main component was an exo- β -(1 $\rightarrow$ 3)-glucanase (cf. Chesters & Bull, 1963). Since an exoglucanase from a basidiomycete had been shown by others to degrade sclerotan completely (Buck *et al.*, 1968), we have tested the glucanases on glucan extracted from the fungus.

Sclerotia of *Sclerotinia sclerotiorum* or autoclaved mycelia of *Sclerotium rolfii* were homogenized and centrifuged at 100000g. A water-clear colourless gel was decanted, which with 0.2 vol. of ethanol could be compressed to a small volume at 100000g. Resuspension and repetition of the process gave a product that could be freeze-dried without affecting its properties.

The purified exo- β -(1 $\rightarrow$ 3)-glucanase from *Coniothyrium minitans* acted on it to give the expected products (glucose and gentiobiose in molar ratio 2:1) but degradation beyond 30–35% was very slow. After dissolution in dimethyl sulphoxide and recovery the resistance was unchanged.

Purified endo- β -(1 $\rightarrow$ 3)-glucanase, from *Coniothyrium minitans* or *Cytophaga johnsonii*, had a very restricted action on the preparation, the products being mainly glucose and a suspected trisaccharide, but no gentiobiose; the viscosity was not affected.

Simultaneous action of the two enzymes brought about almost 100% conversion into glucose and gentiobiose, with small amounts of other oligosaccharides.

The resistance of the cell walls to the β -glucanases used singly is thus partly attributable to resistance by the sclerotan component. The resistance shown by our preparations (and by one from *Claviceps purpurea* kindly provided by Dr. K. W. Buck) may be due to occasional structural 'errors' that impede the exoglucanase, or possibly to non-glycosidic linkages; treatment with hot 3% (w/v) NaOH renders the glucan more susceptible to exoglucanase.

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Double-Helical Conformations of Sulphated Polysaccharides and their Potential Biological Function

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Many ionic polysaccharides exist naturally in a gel state. With the carrageenans, which are a group of sulphated polysaccharides present in the intercellular matrix of certain species of red seaweeds, the predominant mechanism of gel formation involves the twisting together of linear polysaccharide molecules to form double-helical tie points (Rees *et al.*, 1969). The extent and stability of the double-helical regions are dependent on the primary structure of the polysaccharide. Enzymic alteration of the primary structure would seem to provide a means of metabolically controlling the polysaccharide conformation and hence the properties of the intercellular gel matrix.

The primary structures of the carrageenans can also be chemically altered to yield sulphated regular linear co-polymers of 3,6-anhydro- α -D-galactopyranose and β -D-galactopyranose, linked 1,3 and 1,4 respectively. The different carrageenans each have their own characteristic sulphation pattern, and the non-sulphated hydroxyl groups are available for hydrogen-bonding, which is responsible for holding the double helices together. Breaking of these hydrogen bonds results in a denaturation of the double helix to yield two separate polysaccharide chains with more random conformations. These conformations and conformational transitions can be conveniently studied by the use of optical-rotation techniques, which have been shown to give good correlations between theoretical and observed rotation values (Rees *et al.*, 1970).

The acidic sulphate half ester groups confer interesting ion-exchange properties to the gels. Studies of ligand-induced conformational transitions and selective cation-binding suggest that the double-helical carrageenans may have a biological function in maintaining a suitable intercellular ionic environment, and they may act as allosteric ion-exchange agents involved in the uptake and transport of specific ions. Calcium binding studies suggest that certain carrageenans may play an active role in the calcification of these seaweeds.

Studies of the distribution and conformations of ionic polysaccharides within the native tissue are possible by using metachromatic fluorescent 'reporter' molecules, which are cationic histochemical dyes that bind to polyanions and exhibit changes in their optical properties that reflect the nature of the polymer binding site.

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