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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT PHYSIOLOGY ON SEPTEMBER 7, 1973

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The Structure of (1→3)-β-D-glucans

In the plant kingdom, (1→3)-β-D-glucans, i.e. chains of (1→3)-β-linked D-glucose residues, are of widespread occurrence. They can serve as a storage product, e.g. in the shape of the paramylon grains of Euglenophyta, or may have a function in the cell wall, which is the case in particular in many fungi. In higher plants (1→3)-β-D-glucans are found e.g. in the callus-cushions on the sieve plates of older sieve tubes.

Although it is well known that the (1→3)-β-D-glucans may occur in microcrystalline condition and may form microfibrils, their physical properties are poorly explored.

The cell walls of baker's yeast contain a glucan with a molecular structure in which (1→3)-β-D-glucans chains are supposed to form the branches of a main chain of (1→6)-β-linked glucose residues (MANNERS & PATTERSON 1966). This glucan is of poor crystallinity. KREGER & KOPECKÁ (1972) observed that in the native wall this glucan exhibits a fine microfibrillar network, whereas naked yeast protoplasts in the first stage of regeneration of a new wall produce a much coarser net structure, with microfibrils containing a high percentage of free chains of (1→3)-β-D-glucans of rather high crystallinity.

The present study deals with

1) The crystallization of (1→3)-β-D-glucan.

2) The conformation of the (1→3)-β-chain, in relation to the formation of microfibrils.

Ad. 1). Crystallized (1→3)-β-D-glucan ("hydroglucan", c.f. HOUWINK & KREGER 1953) was prepared by first isolating the ramified yeast glucan (see above) as the residue of extraction of yeast cells with hot dilute alkali, and subsequent partial hydrolysis by boiling in 2% HCl fort 3 hours. By this treatment c. 50 per cent of the wall glucan dissolves in the acid (HOUWINK & KREGER 1953). The hydroglucan is soluble in alkali and can be purified now by dissolution in and reprecipation from dilute alkali. The amorphous precipate can be recrystallized by boiling again in 2% HCl. It turned out that by this treatment again c. 50 per cent of the purified hydroglucan dissolved in the acid.

Our hypothesis is, that yeast glucan from native walls cannot crystallize well because of its branched nature. When it is boiled with dilute acid, the (1→6)-β-linkages and part of the (1→3)-β-linkages are hydrolysed (c. 50 per cent of the wall glucan dissolves) and the remaining, linear (1→3)-β-chains can crystallize now. As crystallites they are protected from hydrolysis. When redissolved and precipated, part of it dissolves again on boiling in dilute acid, until crystallization has taken place.

If this hypothesis is correct, it should be possible also to recrystallize the unbranched (1→3)-β-D-glucans chains by boiling in water instead of dilute acid, but without loss of material by hydrolysis. Indeed the X-ray diagrams of the water-boiled precipate showed a sharp hydroglucan pattern while the loss of material was less than 10 per cent.

Another property observed of purified, crystalline hydroglucan is the conversion by X-ray irradiation into a different crystal modification, corresponding to the B-modification of paramylon. Therefore, paramylon-B and hydroglucan appear to be chemically identical. Since also the conversion of paramylon-A to paramylon-B has been observed (KREGER, unpublished), hydroglucan, paramylon-A and paramylon-B represent three crystal modifications of one and the same substance, of which the paramylon-B modification is probably the most stable one.

Ad 2). Data about the conformation of (1→3)-β-chains can be derived form an X-ray fibre diagram of the crystallized chains. We have therefore tried to obtain hydroglucan fibers with suitable dimensions for X-ray examination. For this purpose solutions of purified hydroglucan were squirted through a small hole into precipitating media. This method failed, probably because of the low degree of polymerisation of the chains. Besides, we have tried to obtain X-ray fibre diagrams from fibrous fungal tissues, and along these lines such diagrams have indeed been produced. The present work concerns improvement and interpretation of the diagram.

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MEETING OF THE SECTION FOR PLANT TAXONOMY AND GEOGRAPHY ON NOVEMBER 2, 1973

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The year cycle of Petalonia fascia (Muell.) Kuntze in The Netherlands

Petalonia fascia, a marine brown alga of the order Scytosiphonales, grows in the tidal belt. The blade-like thalli produce plurilocular sporangia.

In Japan, NAKAMURA (1965) discovered the existence of a crustlike thallus with unilocular sporangia in the life history of the species. In culture experiments WYNNE (1969) and EDELSTEIN *et al.* (1970) showed that swarmers from several *Ralfsia*-species from the coasts of America (*Ralfsia californica* S & G, *Ralfsia clavata* Crouan and *Ralfsia Borneti* Kuck.) gave rise to *Petalonia* blades. Sexual processes never have been observed.

On european coasts only DANGEARD (1969) sampled the (sterile) crustlike thallus of *Petalonia fascia*, so it seemed worthwhile to start an investigation to get supplementary information from this part of the world.

The species was studied in nature in a tidal pool along the Eastern Scheldt. The pool was visited every two months from September 1970–September 1971 and monthly from October 1971–November 1972. The blades of *Petalonia* here appeared in the course of November, showed an optimum in February-April and disappeared at the end of May.

At first the crustlike thallus could not be found in nature. However, during the summer months when no *Petalonia* blades were present, swarmers were caught on artificial substrata placed in the tidal pool.

In culture these swarmers gave rise to *Petalonia* plants. In November 1972 fertile crustlike thalli were sampled on mussel shells. These crusts were identified as *Ralfsia clavata* (Carm.) Crouan sensu Farlow. From this time they could be found regularly.

In culture the influences of temperature and photoperiod on the development of both *Petalonia* and *Ralfsia* were studied. At low temperatures and a short day photoperiod almost only bladelike thallus arose. At high temperatures and with a long day photoperiod mainly crustlike thallus developed from the swarmers. These results agree with the observations on material from the coast of America.

Also the rate of development was studied at different temperatures and photoperiods. The highest rate of development is found at 20 °C, the lowest at 4 °C. Besides, the development appeared to pass faster at a short day photoperiod, especially at the lower temperatures.

A general outline is given, based on the results of culture experiments. Probably *Petalonia* may produce 3 generations of blades and 3–4 generations of crusts a year.

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