

Detection of cell wall structural polysaccharides by cellulase-gold and chitinase-gold complexes

Detekce polysacharidů v buněčné stěně

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Abstract:

The rigid cell wall of *Chlorella vulgaris* (Trebouxiophyceae) is probably composed of chitin-like substance. Chitinase isolated from *Streptomyces griseus* is able to split covalent linkages on isolated microfibrils. On cross-sections of plastic embedded cell walls the covalent linkages are "masked" by the layer of amorphous polysaccharides. The presence of cellulose in the cell wall can not be excluded. It does not dominated the rigid part, but is probably part of almost amorphous cell wall constituents located in interfibrillar matrix. Inner polysaccharidal layer of *Scenedesmus quadricauda* (Chlorophyceae) cell wall is composed of cellulose. Neither algenan nor reticulate layer were labelled by cellulase-gold complex. Lorica of *Dinobryon divergens* (Chrysophyceae) is not composed of cellulosic microfibrils as it was previously thought (FRANKE & HERTH 1973), microfibrils are of chitinous nature. Cellulosic cell wall of *Bumilleriopsis filiformis* (Xanthophyceae) is multilayered, certain layers exhibit different intensity of labelling by cellulase-gold complex. Method of rigid cell wall polysaccharidal composition testing by cellulase-gold and chitinase-gold complexes can be considerate as reliable.

Introduction

Cell wall represents a complex structure composed of polysaccharides forming microfibrils, amorphous matrix polysaccharides, proteins, amino acids and glycoproteins. An important structural compound of the cell wall is water. Structural polysaccharides cellulose, chitin, β - 1,4 mannans and β - 1,4 xylans have been detected to form microfibrils in algal cell walls. Chemical structures of cellulose and chitin are very similar: cellulose is poly - β (1,4)-D-glucose and chitin is its 2-acetamido derivative, not surprisingly, that both polysaccharides form microfibrils similar in structure (BLACKWELL 1982).

In the past algal cell walls were generally supposed to consist of cellulose. These statements were mostly based on primitive cytological staining. Cellulose was reliably proved in only several taxa, most of them serving as model

organisms for cellulose-synthesis. Chitin was found in crosswalls of green filamentous alga *Pithophora oedogonia* (PEARMUTTER & LEMBI 1976), in appendages of centric diatoms *Thalassiosira* and *Cyclotella* (BLACKWELL et al. 1976), and in the lorica of *Poterioochromonas stipitata* (HERTH & SCHNEFF 1982). In the cell wall of the symbiotic *Chlorella* Pbi strain the chitin-like substance was proved (KAPAUN & REISSER 1995). TAKEDA studied a monosaccharidal composition of the rigid cell wall and matrix in chlorococcal genera *Chlorella* and *Scenedesmus* (TAKEDA 1993, 1996). Monosaccharidal composition of the rigid cell wall provides only limited information about chemical composition of structural polysaccharides forming microfibrils (position of the linkages and sequence of monomers is not known).

Cellulase-gold (chitinase-gold) complex can be used for cellulose (chitin) detection and localisation. The enzyme is bound in site, where the polysaccharide in question is restricted. When is the enzyme conjugated with a gold particle, the binding site is easily detectable on electronmicroscopic micrographs. Gold particles are visible as electrondense dots of defined size. Cellulase-gold and chitinase-gold labelling were successfully applied on epoxy resin sections (e.g. Spurr, Epon, Araldite).

As the models these organisms have been selected: 1) *Chlorella vulgaris* (class Trebouxiophyceae). Rigid cell wall of related strain *Chlorella* Pbi was found to be composed of chitin-like substance (KAPAUN & REISSER 1995). 2) *Scenedesmus quadricauda* (class Chlorophyceae). Inner layer was thought to be cellulosic (BISALPUTRA & WEIER 1963). TAKEDA (1996) found that the rigid cell wall of different *Scenedesmus* taxa is composed of monosaccharides glucose, mannose and galactose, glucosamine was not detected. 3) *Dinobryon divergens* (class Chrysophyceae). The microfibrils forming lorica were thought to be of cellulosic nature (FRANKE & HERTH 1973). On the other hand the lorica of related species *Poterioochromonas stipitata* was proved to be chitinous (HERTH et al. 1977). 4) *Bumilleriopsis filiformis* (class Xanthophyceae). Rigid cell wall composition is not known, cellulosic cell wall has been proved in filamentous taxa *Tribonema* (CLEARE & PERCIVAL 1972) and *Vaucheria* (MIZUTA & BROWN 1992).

Material and Methods

Algae strains were obtained from the Culture Collection of Algae at the Department of Botany, Faculty of Natural Science, Charles University in Prague (CAUP). The source of *Dinobryon divergens* was Černé Lake in Šumava Mts. *Mucor plumbeus* was obtained from the Culture Collection of Fungi (CCF) at the Department of Botany. Algal cultures were grown in 100 ml glass tubes filled with Bold's Basal Medium, aerated with filtered air under continuous illumination of light intensity 100 mW. cm⁻². Cells were cultivated at 25 °C for 4-8 days. *Mucor plumbeus* was cultivated in a Petri dish on 4 % agar at 25 °C

for 3 days. Preparation of the ultrathin sections, cell walls preparation and extraction were described in NĚMCOVÁ & KALINA 2000.

Preparation of cellulase-gold complex and labelling. 20 mg of cellulase from *Trichoderma viride* (Sigma) were dissolved in 2 ml double distilled water. 10 ml colloidal gold pH 5.5 (particle size 10 nm, Sigma), was coated with 250 μ l cellulase solution and further stabilised with 1 ml of 10 % bovine serum albumine. After centrifugation at 34 000 g for 30 min at 4 °C, the pellet was resuspended in 1 ml of 0.05 M citrate buffer, pH 5.0, containing 1 % BSA. PEVELING et al. (1992) and finally conventionally contrasted.

Preparation of chitinase-gold complex and labelling. 0.6 mg of chitinase from *Streptomyces griseus* (Sigma) was dissolved in 300 μ l double distilled water and linked to 10 ml colloidal gold (pH 7.0; particle size 10 nm, Sigma) by stirring. After 3 min the complex was stabilised by adding 250 μ l 1 % polyethylene glycol (PEG; m. w. 20 000). After centrifugation at 34 000 g for 30 min at 4 °C the red, mobile pellet was resuspended in 1 ml PBS (0.05 M, pH 6.0) containing 0.02 % PEG. Labelling was performed as described in SCHLARMANN et al. (1990) and conventionally contrasted.

Controls. 1) Sections were treated with uncoated colloidal gold. 2) Preceding section labelling, the enzyme-gold sol was first incubated with an equal portion of the corresponding substrates.

Results and discussion

Cellulase-gold complex was tested on *Oocystis solitaria* (Trebouxiphyceae). Cellulose is a structural polysaccharide of the cell wall (QUADER et al. 1978). Cell walls on cross-sections as well as isolated microfibrils were labelled strong and selectively by cellulase-gold complex. Chitinase-gold complex was tested on filamentous mycelium *Mucor plumbeus* (Eumycota, Zygomycetes). Chitin is a structural polysaccharide of the hyphal cell walls (PFYFFER 1998). Isolated microfibrils of *Mucor plumbeus* were labelled strong and selectively by chitinase-gold complex. Produced complexes provide selective labelling and they can be used to test polysaccharidal composition of microfibrils.

Chlorella vulgaris. *C. vulgaris* cell walls on cross-sections were weakly labelled while isolated microfibrils, embedded in the interfibrillar matrix, were relatively strong labelled by cellulase-gold complex. This is in congruence with KAPAUN & REISSER (1995) results. They revealed that the cell wall material of symbiotic *Chlorella Pbi* was also degraded by cellulase, although the data obtained by other methods did not indicate the presence of cellulose. It is conceivable that the cellulose-related structures do not dominate the rigid part, but might therefore consist of almost amorphous cell wall constituents. This should be generally applied to investigated *C. vulgaris* strain, since no glucose was detected in the rigid wall of this species (TAKEDA 1993). Chitinase-gold

complex did not leave any label on cell walls on cross-sections. Isolated microfibrils were labelled by chitinase-gold complex, which is in contradiction with previous statement. On cross-sections of plastic embedded cell walls the covalent linkages on "chitin" molecule are not accessible for chitinase. Chitinous microfibrils on sections are overcoated by the layer of amorphous polysaccharides, which prevent the chitinase from restricting the linkages. Consequently, the chitinase gold complex, when used on sections of plastic embedded cells, only strongly binds to the microfibrils lying parallel to the sectioning plane, whereas it weakly binds to the microfibrils lying oblique or transverse to the sectioning plane (BONFANTE-FASOLO et al. 1986). On the other hand isolated microfibrils are free of amorphous polysaccharides and most of covalent linkages are chitinase accessible. Positive labelling by chitinase-gold complex is than not surprising.

Scenedesmus quadricauda. Cellulase-gold complex bound to polysaccharidal layer of the cell wall. This is in congruence with previous knowledge, this layer was considered to be of cellulosic nature (BISALPUTRA & WEIER 1963). Neither algenan layer nor reticulate one were labelled. Cell walls on the cross-sections were not labelled by chitinase-gold complex. Isolated microfibrils were not investigated. Chemical structure of cell wall algenan in *Scenedesmus communis* was proposed by BLOKKER et al. (1998). The biopolymers are composed of long-chain even-carbon-numbered unsaturated ω -hydroxy fatty acid monomers varying in chain length from 30 to 34 carbon atoms. Chemical structure of the outer reticulate layer is still not known. The outer hexagonal structure is not water-soluble, it persists in the culture medium and it is even able to resist the hot acetolysis (ATKINSON et al. 1972). Chitinous nature of the hexagonal structure was not proved. It is speculative, whether the chemical composition of the outer hexagonal structure and algenan are similar.

Dinobryon divergens. The microfibrils forming were selectively labelled by chitinase gold complex, while cellulase-gold complex gave no label. Microfibrils were previously thought to be cellulosic on the bases of chlor-zinc-iodide reaction, the solubility in copper tetramine complexes, the alkali resistance of the fibrils and the electron microscopic appearance (FRANKE & HERTH 1973). Reactions mentioned above are not specific, e.g. chlor-zinc-iodide reacts with various structural polysaccharides. It is obvious that neither dimensions nor appearance of MFs can be used to determine whether they are composed of cellulose, chitin or other polysaccharides.

Bumilleriopsis filiformis. Cell walls were multilayered, single layers were not optically homogenous. Cell walls on cross-sections were labelled with cellulase-gold complex. Layers with microfibrillar appearance were intensely labelled in comparison with homogenous ones. From these observations can be concluded that *B. filiformis* cell wall is composed of layers, where cellulosic microfibrils dominated alternated with layers of other matrix polysaccharides. Cellulase-gold complex bound selectively to isolated microfibrils. Neither cell

walls on sections nor isolated microfibrils were labelled by chitinase-gold complex.

Method of rigid cell wall polysaccharidal composition testing by cellulase-gold and chitinase-gold complexes can be considerate as reliable. In case of no label on cross-sections of plastic embedded cells, I suggest to use the isolated microfibrils, where accessibility to enzyme is many times higher. According to presented results is seems probably that chitin as a structural polysaccharide of algal cell walls occurs more often than it was previously thought.

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