The influence of Congo red on the cell wall and (1→3)-β-D-glucan microfibril biogenesis in *Saccharomyces cerevisiae*

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Abstract. Congo red was applied to growing yeast cells and regenerating protoplasts in order to study its effects on wall biogenesis and cell morphogenesis. In the presence of the dye, the whole yeast cells grew and divided to form chains of connected cells showing aberrant wall structures on both sides of the septum. The wall-less protoplasts in solid medium with the dye exhibited an abnormal increase in volume, regeneration of aberrant cell walls and inability to carry out cytokinesis or protoplast reversion to cells. In liquid medium, the protoplasts synthesized glucan nets composed mainly of thin fibrils orientated at random, whereas normally, in the absence of dye, the nets consist of rather thick fibrils, 10 to 20 nm in width, assembled into broad ribbons. These fibrils are known to consist of triple 6/1 helical strands of (1→3)-β-D-glucan aggregated laterally in crystalline packing. The thin fibrils (c. 4 to 8 nm wide) can contain only a few triple helical strands (c. 1.6 nm wide) and are supposed to be prevented from further aggregation and crystallization by complexing with Congo red on their surfaces. Some loose triple 6/1 helical strands (native elementary fibrils) are also discernible. They represent the first native (1→3)-β-D-glucan elementary fibrils depicted by electron microscopy.

The effects of Congo red on growth and the wall structure in normal cells and regenerating protoplasts in solid medium can be explained by the presence of a complex which the dye forms with (helical) chain parts of the glucan network and which results in a loss of rigidity by a blocked lateral interaction between the helices.

Key words: *Saccharomyces cerevisiae* – Yeast cells – Yeast protoplasts – Cell wall – Congo red – (1→3)-β-D-glucan microfibrils – Cytokinesis – Reversion of walled protoplasts to cells

The principal structural components of the cell wall in yeasts are (i) microfibrils forming a submicroscopic network of thin filaments (Houwink and Kreger 1953; Kopecká et al. 1974 a, b) and (ii) an amorphous matrix substance (Kopecká 1985). The microfibrillar network is found on the inner side of the wall and is built of (1→3)-β-D-glucan chains interlinked by (1→6)-glucan branches, and a small proportion of (1→6)-β-D-glucan. The matrix, a highly ramified component, is made up of mannan, glucan and protein (cf. Bacon 1981; Ballou 1988). The composition of the wall allows polydisperse colloidal dyes, such as Congo red, calcofluor white and primulin, to enter the matrix and associate with the microfibrils, thus visualizing the characteristic features of the wall (cf. Strciblová 1983).

In higher plants Congo red and calcofluor white were shown to adhere, in an oriented manner, to the surface of microfibrils and to cause dichroism in the cell envelopes (Frey-Wyssling 1976). They also have a strong affinity to the nascent stages of cellulose and chitin, which enables their polymerization taking place in the plasma membrane to be separated from their extracellular crystallization into microfibrils (Benziman et al. 1980; Haigler et al. 1980; Herth 1980; Roberts et al. 1982). In some algae (Herth 1980) and in *Acetobacter xylinum* (Colvin and Witter 1983) complexes between dyes and microfibrillar intermediates have been detected and characterized.

This paper investigates the influence of Congo red on growth of *Saccharomyces cerevisiae* cells and protoplasts. In contrast to the papers mentioned above, in the cell walls studied here the main structural polysaccharide was not cellulose but (1→3)-β-D-glucan. The studies on wall formation in *S. cerevisiae* published earlier (Vannini et al. 1983, 1987; Pancaldi et al. 1985) have not included the effects of Congo red on wall regeneration in protoplasts. Moreover, the interpretation of their results concerning the effects of the dye observed with normal cell walls differs from the conclusions drawn from our experiments.
Materials and methods

Strain and protoplasts

Saccharomyces cerevisiae, strain CCY 21.4-59 (Czechoslovak Collection of Yeasts and Yeast-Like Microorganisms, Bratislava), was grown in malt extract medium. Protoplasts were prepared from the exponential culture with the use of snail enzymes (Edfy and Williamson 1957). After digestion of cell walls, the protoplasts were washed free from the enzymes with 0.8 M mannitol in 50 mM citrate-phosphate buffer (pH 5.5) and used immediately.

Treatment of the whole cells with Congo red

The cells from a culture grown for 48 h at 28 °C were inoculated into flasks containing 100 ml of malt extract with concentrations of Congo red ranging from 10 to 0.01 mg/ml. The stock solution of Congo red (Lachema, Brno, Czechoslovakia) was prepared by dissolving the dye in distilled water at 40 °C (50 mg/ml). Cell counts were made in a Bürker chamber.

Treatmcit of regenerating protoplasts with Congo red

Regeneration of the whole cell walls was followed in protoplasts embedded in N1 medium (5 g glucose, 1.25 g asparagine, 1.25 g KH2PO4, 0.425 g MgSO4, 50 ml distilled water, pH 5.4; Kellett et al. 1954) supplemented with 30% gelatin (Notas 1961) and 1% Congo red and warmed to 36 °C.

Formation of fibrillar nets was studied in freshly prepared protoplasts transferred to YNBG medium (Johnson 1967) stabilized osmotically with 0.8 M mannitol or to N1 medium at 25 °C. Selected Congo red concentrations in the range of 1 to 0.001% were added to each medium.

Light microscopy

The samples were observed by phase-contrast microscopy (Amplival, Zeiss JenA, FRG) and fluorescence microscopy (Jenalumar, Zeiss JenA, FRG).

Electron microscopy

Freeze-etching. Congo red exposed cells were packed by centrifugation, transferred onto copper discs, frozen in Freon 22 and stored in liquid nitrogen. Replicas were made after fracturing at —100 °C in a Balzers BA 360 M apparatus (Moor and Mühlethaler 1963).

Sectioning. Congo red exposed whole cells were fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.1) and post-fixed with 2% OsO4 for 2 h (Gabriel 1968). After dehydration in a graded acetone series, the fixed cells were embedded in Durcupan and sectioned using a Reichert ultramicrotome.

Preparation of isolated cell walls. Whole cells treated with Congo red were centrifuged, the pellets were washed with 0.5% sodium dodecyl sulphate to remove the cytoplasm at 50 °C for 10 min (Kopčká 1976; Kopčká and Farkas 1979) and centrifuged again. The isolated cell walls were washed 3 times with distilled water (50 °C) and placed onto copper discs covered with a formvar film. After drying the specimens were shadowed with platinum at a 10° angle.

Preparation of regeneratcd walls and fibrillar nets of protoplasts. Regenerating protoplasts were released from the gelatin medium by warming it to 37 °C and diluting with distilled water. The walls were isolated and further processed by the procedures described above.

Preparations were examined and photographed in a Tesla BS 500 electron microscope.

Results

The influence of Congo red on yeast cells growing in malt extract

The cells of Saccharomyces cerevisiae growing in malt extract medium showed a normal growth rate (Fig. 1) and an almost regular budding pattern, septation and cell separation, comparable to that in the control cells (Fig. 2) when the concentration of Congo red was lower than 0.01%. The presence of Congo red at concentrations of 0.01% to 0.1% reduced the growth rate (Fig. 1) and affected septation and cell separation processes (Fig. 3). After 7 h the cells still kept growing but their morphology was aberrant: fully grown buds had developed but failed to separate, which resulted in cell chains of varying length. With increasing Congo red concentration the number of cells in a chains decreased from nine to two (Fig. 4). At a concentration of 1% the proliferation of cells was almost blocked (Fig. 1). Only some of the cells formed one to two buds. Congo red-stained material accumulated near the septum regions (Figs. 4, 5) and the dye made bud scars clearly visible (Fig. 5). However, the resolution was not high enough to show if the septa were closed completely.

It could be clearly seen that Congo red stained predominantly the secondary septum material (Fig. 5) while the primary septum region and the normal wall remained unstained (Fig. 5). The colour of the aberrant, Congo red-stained wall material varied from pink to reddish. In dead cells, however, the cytoplasm was generally coloured red. This can be explained by apparent permeabilization of the plasma membrane with the dye.

Fig. 1. The effect of Congo red on cell proliferation. Control cells (K) ○, cells growing in malt extract medium with Congo red at concentrations of 0.001% ●, 0.01% ★, 0.1% ▲, 1.0% ■. Each initial culture contained about 7.5 x 105 cell/ml of medium

Fig. 2. Control cells grown in malt extract medium without Congo red for 24 h. The cells are already in the stationary phase (arrow). Phase contrast × 2000. Bar, 10 µm

Fig. 3. Cells grown in malt extract medium with 0.1% Congo red. Phase contrast × 2000. Bar, 10 µm

Fig. 4. Cells grown in malt extract medium with 0.5% Congo red for 30 h. Secondarу septum region (full arrow) is discernible from the primary septum (small arrow). Phase contrast × 2000. Bar, 10 µm

Fig. 5. Cells grown in malt extract medium with 0.25% Congo red for 30 h. The primary septum is not stained with Congo red (small arrow), the secondary septum stains red (full arrow). Dead cells show deep red staining, × 2000. Bar, 10 µm

Fig. 6-8. Fluorescence microscopy of cells grown in malt extract with different concentrations of Congo red for 30 h. Small arrow indicates primary septum, full arrows secondary septa. Low fluorescence versus bright fluorescence in primary and secondary sep
ta, respectively. × 2000 Bars, 10 µm

Fig. 6. Congo red concentration of 0.25%

Fig. 7. Congo red concentration of 0.5%

Fig. 8. Congo red concentration of 1.0%