Cell wall development in *Oocystis solitaria* in the presence of polysaccharide binding dyes

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**Abstract.** Cell wall and thus cellulose microfibril formation in the presence of Congo red or Calcofluor white by *Oocystis solitaria* autospores was investigated ultrastructurally and chemically. The prevention of microfibril formation by both substances is accompanied by drastic changes of microfibril synthesis and orientation as well as the morphology of plasma-membrane-associated E-face terminal complexes. Removal of Congo red and Calcofluor white from the culture medium results in the recovery of microfibril formation, of a normal patterned microfibril arrangement, and of terminal complexes with extending microfibril imprints.

**Key words:** Calcofluor white – Cellulose synthesis – Congo red – *Oocystis* – Plasma membrane – Terminal complexes (plasma membrane).

**Introduction**

Cell wall synthesis in the unicellular green alga *Oocystis solitaria* is completed within 50 h after autospore production. This wall is composed of thick, highly crystalline cellulose microfibrils which are deposited in alternating layers (Quader and Robinson 1981). Each layer is completed in about one hour and is laid down with a directional change of 90° to the preceeding one (Quader et al. 1978). Through the application of a range of differently working substances, we have been able to show that cortical microtubules mediate this alteration in microfibril deposition (Quader 1981; Robinson and Quader 1982).

Structural features of the plasma membrane, as revealed by freeze-fracturing, implicate the role of intramembrane particles in microfibril deposition, both in *Oocystis* (Robinson and Preston 1972; Brown and Montezinos 1976; Robinson and Quader 1981 b) and elsewhere (Gooday 1983; Williams 1983). Characteristic for *Oocystis* are the so-called “terminal complexes” (TCs) present on the E-face, and “granule bands” (GB) on the P-face. TCs are initially paired together but with the onset of microfibril synthesis they part and occur singly at the ends of the imprints of microfibrils in the E-face. This situation remains essentially unchanged after microtubule-drug treatment, but is dramatically affected through the addition of calcofluor white (CW) and congo red (CR) (Robinson and Quader 1981 b).

CW and CR possess a strong affinity for linear β-hexapyranosyl polysaccharides (Maeda and Ischida 1967) and, as a result, prevent their crystallization into microfibrils (Robinson 1981). This paper is the extention of a previous short communication (Quader 1981) on the effects of these substances on *Oocystis*, with particular emphasis on the reversibility of their action.

**Material and methods**

Growth conditions for the algae as well as the procedures for fixation, embedding, sectioning, and staining for the electron microscopical examination of thin sections were as given previously (Quader et al. 1978). The methods used in the freeze-fracture studies were as described by Robinson and Quader (1981 b).

All the experiments were carried out under normal growth conditions. Congo red (CR; purchased from Serva, Heidelberg FRG) and Calcofluor white (CW; from American Cynamid Co., Bound Brook USA) were directly dissolved in the culture medium which had a cell density of 3–4 × 10⁶ cells/ml. For recovery experiments, treated cultures were extensively washed with
four changes of fresh medium by centrifugation which was accomplished within 15 min.

Cellulose was determined according to Updegraff (1969). Non-cellulosic material was removed by incubating the pelleted cells from a 5 or 10 ml aliquot suspension culture with 3 ml acetic-nitric acid mixture for 1 h at 100°C. The residue was dissolved and hydrolyzed in sulfuric acid and the released glucose was measured with the anthrone reagent (Roe 1955). Chlorophyll was determined according to Arnon (1949).

Results

Cellulose synthesis in the presence of Congo red and Calcofluor white. Young autospores are still surrounded by a rigid parental cell wall when developing their own cell wall. Therefore, in attempting to measure newly synthesized cellulose by autospores, one must take into consideration the cellulose of the mother cell wall which accounts for initial high background values. Using Updegraff’s method (1969), we have examined the change in the amount of cellulose during autospore development in the presence and absence of CR and CW. The first samples were taken between the 6th and 10th hour of the light period following cell division, which occurs in the dark. Depending on the synchrony of the experimental culture, most often in the range between 60–80%, a doubling or tripling of the measurable cellulose is to be expected, since under our culture conditions, mother cells produce two or four autospores. The amount of cellulose obtained is expressed relative to the chlorophyll content in order to allow a better comparison of results from different experiments. This appeared appropriate since, interestingly, chlorophyll synthesis only occurs on the third day after autospore formation (Fig. 1, insert), a period in which less than 5% of the cells are not engaged in wall production, the rest not having yet divided.

Within the first 10 h of culture, the increase of cellulose is moderate after which it is drastically enhanced. Fourteen hours later the amount of cellulose has almost tripled (Fig. 1). In contrast to the control cultures, CR- and CW-treated cultures did not show such an increase in cellulose content (Fig. 1), this amount being not much more than that seen at the beginning of the experiment, i.e., 28 mg mg⁻¹ chlorophyll.