A Correlation between Detergent Tolerance and Cell Wall Structure in Green Algae

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12 strains of the geni *Chlorella, Scenedesmus, Chlamydomonas,* and *Dunaliella* were tested for their tolerance against the anionic detergent "LAS" (linear alkylbenzene sulfonate). Cellular parameters (cell titer and chlorophyll content) were monitored for 28 days after addition of LAS (0.01–2 mM). A 100-fold difference in sensitivity toward LAS was detected for the algal strains analyzed. Electron microscopic investigations revealed that LAS-resistance is correlated with both, the presence of thick cell walls and sporopollenin layers. It is speculated that this structure acts as a protecting coat preventing the chemical attack of detergents on algal cells. An application for selecting algae by detergents is proposed.

Introduction

Linear alkylbenzene sulfonate (LAS) is inhibitory or even toxic to a variety of aquatic organisms. Roberts [1] reported toxic effects on rainbow trouts *(Salmo gairdnerii), Gammarus pulex,* and the pond weed *(Potamogeton densus).* Hynes and Roberts [2] showed different inhibitory effects on higher plants: *Ranunculus pseudofluitans* and *Potamogeton pectinatus* were seriously affected by 2.5 mg/l alkyl benzene sulfonate (ABS); *Cladophora glomerata* exhibited chloroplast destruction, chlorophyll loss and cell death within three weeks at ABS concentrations from 10 to 50 mg/l.

LAS is also toxic to other photosynthetic organisms: algae showed reduction of photosynthetic activities and complete lysis at higher LAS concentrations, particularly under anaerobic conditions [2–4]. In waste waters a concentration of LAS from 10 to 20 mg/l is still tolerated by the microflora, whereas higher concentrations of detergents damage the activated sludge [5]. The ecological significance of these pollutants is evident, since remarkably high concentrations of LAS occur in our environment, especially in sewage sludges: worldwide about 1.1×10^6 metric tons of LAS are released to aquatic systems; in 1982 four million metric tons of surfactants were produced and released [6].

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On the other hand, higher tolerance for detergents was shown for the green alga *Chlorella fusca* 211-8b utilizing LAS as only sulfur source for growth at 100 mg/l [7]. We further noticed stability toward LAS in some green algae up to 520 mg/l initiating this study. As structural parameters may be the essential factor for phytotoxicity of surfactants, 12 algal strains were investigated for tolerance against detergents. In parallel these algae were analyzed by electron microscopy if LAS resistance is correlated with specific structural parameters.

Materials and Methods

Organisms

The following strains were obtained from the algal collection of Göttingen and cultured as described previously [8]: *Chlorella fusca* 211-8b and 211-11n, *Chlorella saccharophila* 211-9a, *Chlorella sorokiniana* 211-8k, *Chlorella vulgaris* 211-1e, *Haematococcus vulgaris* 211-11c, *Chlamydomonas reinhardii* 11-32a, *Scenedesmus obliquus* 276-3a, *Scenedesmus armatus* 276-4a, and *Scenedesmus communis* 276-4b. *Chlorella* k and *Dunaliella tertiolecta* were gifts from Prof. Dr. O. Kandler and Dr. H.-P. Köst (Botanical Institute, München). Algae were grown in 300 ml Erlenmeyer flasks containing 100 ml of medium at 25 °C and 4000 lux. Since normal aeration leads to foam formation due to the detergents added, algae were cultivated in a rotary shaker.

Detergent

Linear alkylbenzene sulfonate (LAS) with average alkyl chains of 10 carbon atoms [7] was obtained from Henkel (Düsseldorf, W-Germany).

Electron microscopy

For electron microscopic investigations algae were cultured for 5 days in 1 l Pirson flasks under constant aeration (normal air) at 28 °C and 10,000 lux. Cells were harvested by centrifugation, fixed with 3% glutardialdehyde in 50 mM cacodylate buffer pH 7 and postfixed in 2% OsO_4 as described previously [9]. Sporopollenin was determined by its morphological arrangement according to Atkinson *et al.* [10, 11].

Results and Discussion

Growth parameters

Cultivating the green alga *Chlorella sorokiniana* 211-8k on different LAS concentrations (0.01–1 mM) demonstrated a defined threshold for LAS with severe effects on algal growth above 0.15 mM LAS (Table I). Below this growth limiting concentration the algal growth was comparable to controls up to 28 days as indicated by similar cell titer, chlorophyll content, and general appearance. Higher LAS concentrations caused inhibitory effects referred to chlorophyll content and algal number (lysis). The green homogen cultures bleached within one day in parallel with algal sedimentation caused by clumping. However, algal sediments can be regenerated to exponential growing cultures after transfer to normal medium.

Chlorella fusca 211-8b – known to contain sporopollenin in the cell wall – showed a tenfold higher LAS tolerance and a growth limiting concentration of 1.5 mM (Table II). This surprising difference in LAS tolerance within the same genus led to further investigations on LAS resistance using 12 different species of green algae and a concentration range of 0.001 to 2 mM LAS. The effects can be generalized as follows: below the threshold concentration algal growth is similar to control cultures and above the threshold concentration cell division was inhibited, the cells bleached and finally sedimentated. As shown in Table II the growth limiting concentrations differed widely spanning two decades. It is confirmed that within the geni *Chlorella* and *Scenedesmus* a tenfold difference in tolerance for LAS is given. It is obviously a genetic property of the species itself and not of the genus.

Structural observations

For a structural comparison, normal grown algae were analyzed by electron microscopy in the logarithmic phase. Despite of individual and strain specific differences in cellular architecture a correlation of cellular parameters with LAS sensitivity was not observed. However, a striking correlation is evident in regard to cell wall structure: Typical for the LAS resistant *Chlorella fusca* 211-8b, *Chlorella fusca* 211-11n, *Scenedesmus obliquus* 276-3a, and *Scenedesmus communis* 276-4b 8 (Fig. 1–4) are thick cell walls (120 to 200 nm) with an electron-translucent

Table I. Cell number and chlorophyll content of *Chlorella sorokiniana* 211-8k grown on different LAS-concentrations. Cells were grown under different LAS-concentrations and cell titer and chlorophyll content were measured in relation to cultivation time.

LAS concentration [mM]	Inoculum	3.5 days	7 days	28 days	Chlorophyll (mg/200 ml culture)
	Cell titer × 10^{-6}				
Control	11 ± 3	293 ± 52	1024 ± 93	1651 ± 91	0.75 mg
0.10 mM	11 ± 3	306 ± 52	1239 ± 89	1700 ± 126	0.72 mg
0.15 mM	11 ± 3	188 ± 54	860 ± 98	1620 ± 102	0.70 mg
0.20 mM	11 ± 3	14 ± 2*	3 ± 2*	0	0
0.30 mM	11 ± 3	13 ± 2*	2 ± 1*	0	0

* Discoloured cells and white precipitate.

Table II. Correlation between LAS-tolerance and cell wall structure Algae were grown with increasing LAS-concentrations of 0.01 to 2 mM. The growth limiting concentration was defined as the threshold diminishing chlorophyll content and cell titer. The presence of a continuous sporopollenin layer was determined by electron microscopic analysis.

Organism	Growth limiting concentration [mM]	Tolerance against 1 mM LAS	Sporopollenin layer (continuous)
Chlorella fusca 211-8b	1.5	+	+
Chlorella fusca 211-11n	1.5	+	+
Chlorella saccharophila 211-9a	0.25	−	−
Chlorella sorokiniana 211-8k	0.15	−	−
Chlorella vulgaris 211-1e	0.15	−	−
Chlorella k	0.15	−	−
Haematococcus vulgaris 211-11c	0.15	−	−
Scenedesmus obliquus 276-3a	> 1.0	+	+
Scenedesmus armatus 276-4a	0.1	−	−
Scenedesmus communis 276-4b	> 1.0	+	+
Chlamydomonas reinhardii 11-32a	0.08	−	−
Dunaliella tertiolecta	0.01	−	−

cuticle-like outer layer, composed of sporopollenins [10, 11]. In contrast algae which are sensitive to LAS (*Chlorella sorokiniana* 211-8k, *Chlorella* k, *Chlamydomonas reinhardii* 11-32a) have thin cell walls (15 to 40 nm) and no nosporopollenin layer (Fig. 5–7). From these observations it is not surprising that *Dunaliella tertiolecta* with the highest sensitivity to LAS has neither a sporopollenin layer nor a cell wall at all (Fig. 8). One would expect consequently that *Scenedesmus armatus* with its typical sporopollenin "spines" (= *armatus*) is LAS resistant. However, as shown in Table II *Scenedesmus armatus* is very sensitive to this detergent. Ultrastructural studies demonstrate that indeed a sporopollenin layer is present, but not continuous (Fig. 9 and 10). The sporopollenin layer seems to be restricted to small areas around the spines (open arrows in Fig. 10). In addition the cell wall is thin in comparison with to that of *Scenedesmus communis* (Fig. 4).

Conclusion

Disruption of cellular membranes is the critical factor for detergent toxicity. Bacteria, *Hydra,* and fishes are damaged already at very low detergent concentrations, probably due to the absence of protecting cell walls and cuticles [7]. In accordance the cellwall-free *Dunaliella tertiolecta* is very sensitive to LAS-concentrations. The 10- to 100-fold higher resistance of other green algae is obviously caused by two structural parameters: a) a thick cell wall and b) a sporopollenin layer, which in general is a very potent protective agent as known from pollen grains. In addition the chemical composition of cell walls might be of importance as well.

Especially the sporopollenins known to be chemically resistant may protect algae against the effect of detergents. Thus, in searching detergent resistant algae for biological waste water treatment it may be promising to screen for sporopollenin-containing algae. However, the mere biochemical analysis is not sufficient, since *Scenedesmus armatus* clearly contains sporopollenin, but not in a continuous layer. Only the morphological arrangement of sporopollenins in the cell wall in a closed structure protects against detergent effects, extending the ecological advantages of sporopollenins to man-made selection pressure.

Additional remark: Since eubacteria and cyanobacteria are very sensitive toward detergents, appropriate detergent concentrations might be used for selecting green algae from accompanying microorganisms. This procedure was successfully applicated in our laboratory to isolate *Chlorella fusca* 211-11n from a non-axenic culture using a concentration of 0.5 mM LAS.

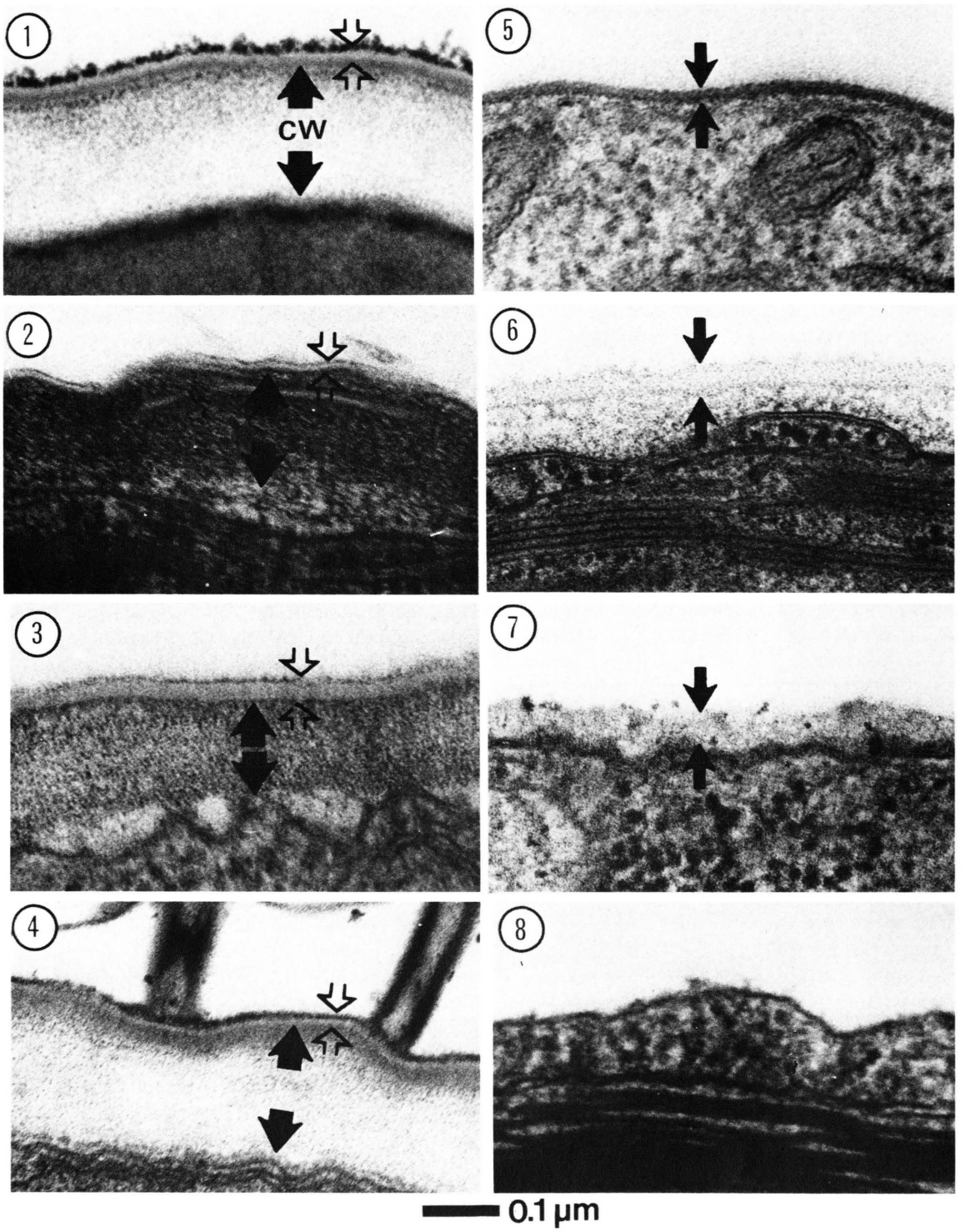

Fig. 1–8. Electron micrographs of cell wall architecture of LAS-resistant green algae (Fig. 1–4) and LAS-sensitive strains (Fig. 5–8). LAS-resistant cells have thick cell walls (CW, black arrows) with an outer sporopollenin layer (open arrows): 1 *Chlorella fusca* 211-8b; 2 *Chlorella fusca* 211-11n; 3 *Scenedesmus obliquus* 276-3a; 4 *Scenedesmus communis* 276-4b. Typical for sensitive strains are thin cell walls (arrows) without sporopollenin additions: 5 *Chlorella sorokiniana* 211-8k; 6 *Chlorella* k; 7 *Chlamydomonas reinhardii* 11-32a. *Dunaliella tertiolecta* (8) with highest LAS-sensitivity has neither a sporopollenin layer nor a cell wall.

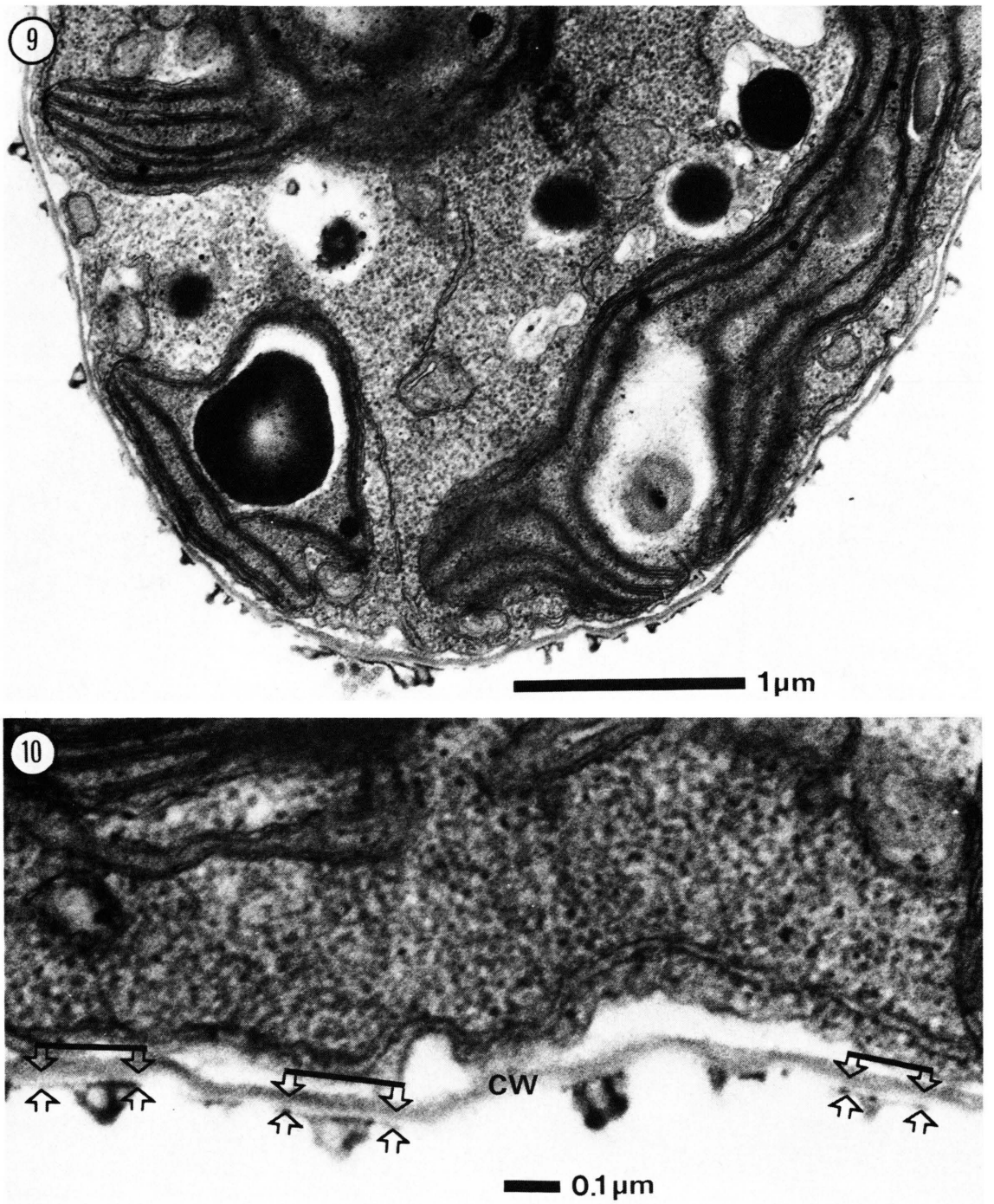

Fig. 9–10. Electron micrographs of *Scenedesmus armatus* 276-4a. This strain is sensitive to LAS, although a sporopollenin layer and cell wall (CW) are present. However, the sporopollenin layer (open arrows) is restricted to areas around the spines leaving parts of the cell wall without protection (Fig. 10).

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