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Photosynthesis dependent acidification of perialgal vacuoles in the *Paramecium bursaria/Chlorella* symbiosis: visualization by monensin

Rapid Communication

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Summary. After treatment with the carboxylic ionophore monensin the *Chlorella* containing perialgal vacuoles of the "green" *Paramecium bursaria* swell. The *Paramecium* cells remain motile at this concentration for at least one day. The swelling is only observed in illuminated cells and can be inhibited by DCMU. We assume that during photosynthesis the perialgal vacuoles are acidified and that monensin exchanges H^+ ions against monovalent cations (here K^+). In consequence the osmotic value of the vacuoles increases. The proton gradient is believed to drive the transport of maltose from the symbiont into the host. Another but light independent effect of the monensin treatment is the swelling of peripheral alveoles of the ciliates, likewise indicating that the alveolar membrane contains an active proton pump.

Keywords: *Paramecium bursaria*; Endosymbiontic *Chlorella*; Perialgal vacuoles; Sugar transport; Photosynthesis; Proton pumps; Monensin.

Abbreviations: HEPES N-(2-hydroxyethyl)piperazine-N′-2-ethane sulfonic acid; DCMU 3-(3, 4-dichlorophenyl)-1,1-dimethylurea.

Introduction

Some *Chlorella* species live as endosymbionts in fresh water animals and protists (for survey, see Smith and Douglas 1987, Reisser 1986). They are photosynthetically active and supply their hosts with organic nutrients. The symbiontic consortium *Paramecium bursaria/Chlorella* behaves as an autotrophic organism in inorganic culture media. Experiments with isolated strains of symbiontic *Chlorella* of *Paramecium bursaria* have shown that the algae secrete high amounts of maltose (Muscatine 1967; Reisser 1981, 1986; Kessler et al. 1991). Up to 45% of the photosynthetically fixed carbon is released as maltose at a pH optimum of about 4.5, whereas at neutral pH only low amounts of sugars are secreted. In situ the algae transfer up to 57% to totally fixed carbon to the *Paramecium* cells (Reisser 1976). The low pH is the only factor known to increase sugar secretion. The pH dependence of sugar secretion with an optimum of about pH 5 is also reported for other isolated symbiontic *Chlorella* strains, e.g., from *Stentor polymorphus* (Reisser 1981), *Hydra viridis* (Cernichiari et al. 1969, Smith and Douglas 1987, Kessler et al. 1991), *Spongilla fluviatilis* (Fischer et al. 1989) and the heliozoan *Acanthocystis turfacae* (Matzke et al. 1990). For the latter two algae a pH dependent active transport of sugars against a concentration gradient was shown.

It is, however, not known as yet whether in situ the sugar transport depends also on a proton gradient. Within the host single endosymbiontic *Chlorella* cells are included in a perialgal vacuole with the vacuole membrane closely surrounding the alga in a distance of about 0.05 µm (Reisser 1986). We, therefore, tried to visualize an acidification of the perialgal vacuoles of *Paramecium bursaria* with the carboxylic ionophore monensin.

Monensin complexes monovalent cations and releases them after protonation (Sandeaux et al. 1982). In this way cations are exchanged against protons, and the

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acidification of a compartment is transformed into an increase of its osmotic value, resulting in its swelling. Monensin was thus used to demonstrate active proton pumps, e.g., in the Golgi apparatus (Mollenhauer et al. 1983, Boss 1984), in irradiated chloroplasts of *Funaria* protonemata (Schnepf 1983), and in granules of chromaffine cells (Geisow and Burgoyne 1982). The shrinkage of the mitochondrial matrix after treatment with monensin is probably due to the same mechanism; it does not affect ATP production (Tartakoff et al. 1981, Tartakoff 1983).

In addition we used DCMU, a blocker of electron flow in photosystem II (Kleinig and Sitte 1986) to characterize the monensin effects in more detail.

Material and methods

Paramecium bursaria, kindly supplied by Prof. Dr. W. Wiessner, Planzenphysiologisches Institut, Universität Göttingen, was cultivated in Erlenmeyer flasks at 20 °C and a 20 : 4 h light : dark regime in an inorganic medium [Knop solution, consisting of 0.25 g/l KH_2PO_4, 0.25 g/l KCl, 0.25 g/l $MgSO_4 \times 7H_2O$, 1 g/l $Ca(NO_3)_2 \times 4H_2O$ and 1 ml/l Fe-EDTA-complex (Jacobsen)] which was adjusted to pH 7.0 with 0.1 M HEPES (Serva, Heidelberg). Stock solutions of monensin (Calbiochem, La Jolla, California), DCMU (Serva, Heidelberg) (both 10^{-2} M in ethanol), were used to obtain media with final concentrations of 10^{-5}–10^{-4} M monensin and 10^{-5}–10^{-4} M DCMU. DCMU was given simultaneously or 3 h before monensin treament to inhibit photosynthesis. The *Paramecium* cells were incubated for up to 24 h, partly in light, partly in darkness. We used corresponding concentrations of ethanol for control experiments; they did not show any effect. For closer inspection and to photograph the cells they were immobilized by adding $NiCl_2$ from 20 mM stock solution to the medium just before observation on a final concentration of 4×10^{-4} M (Larsen and Satir 1991). This treatment did not affect the appearance of the *Paramecium* compartments.

Results

In concentrations higher than 10^{-4} M monensin damaged letally *Paramecium bursaria*. At a concentration of 10^{-4} M the ciliates remained motile for at least one day in darkness or at low light intensities. Treatment with 10^{-5} M monensin caused only slight effects. At concentrations of 10^{-4} and 5×10^{-5} M perialgal vacuoles of illuminated *Paramecium* cells began to swell within 3–4 h (Fig. 1). They continued to increase in size, became very big after 6 h in all cells (Fig. 2) and tended to fuse then (Fig. 3). The about 2.5-fold increase in diameter of the perialgal vacuole after 6 h corresponds to a 200-fold increase of the perialgal volume. The *Chlorella* cells were freely suspended within a vacuole, i.e., they were not in contact with the vacuolar membrane, and looked normally. After prolonged treatment with monensin the previously swollen perialgal vacuoles shrunk in some *Paramecium* cells.

The monensin induced swelling of the perialgal vacuoles was reversible. They became normal again within 12 h when the cells were retransferred into the normal culture medium. In *Paramecium* cells incubated with monensin in darkness the perialgal vacuoles did not swell (Figs. 4 and 5). The cells then showed cyclosis with algae in digestion and some swollen digestive vacuoles, as well as smaller clear vacuoles which partly were located in the periphery without any movement. Independent of the illumination was the formation of peripheral blebs, arising from peripheral alveoles (electron microscopy observations, not shown). At 5×10^{-5} M monensin, the first small protruding vesicles became visible within a few minutes. Later 1–4 big blebs were observed which reached a diameter of about 30 µm (nearly cell diameter) (Figs. 3–5). These vesicles had clear, translucent contents. After longer incubation the cells rounded off more and more. This was more expressed in illuminated cells where the swollen perialgal vacuoles promoted the rounding.

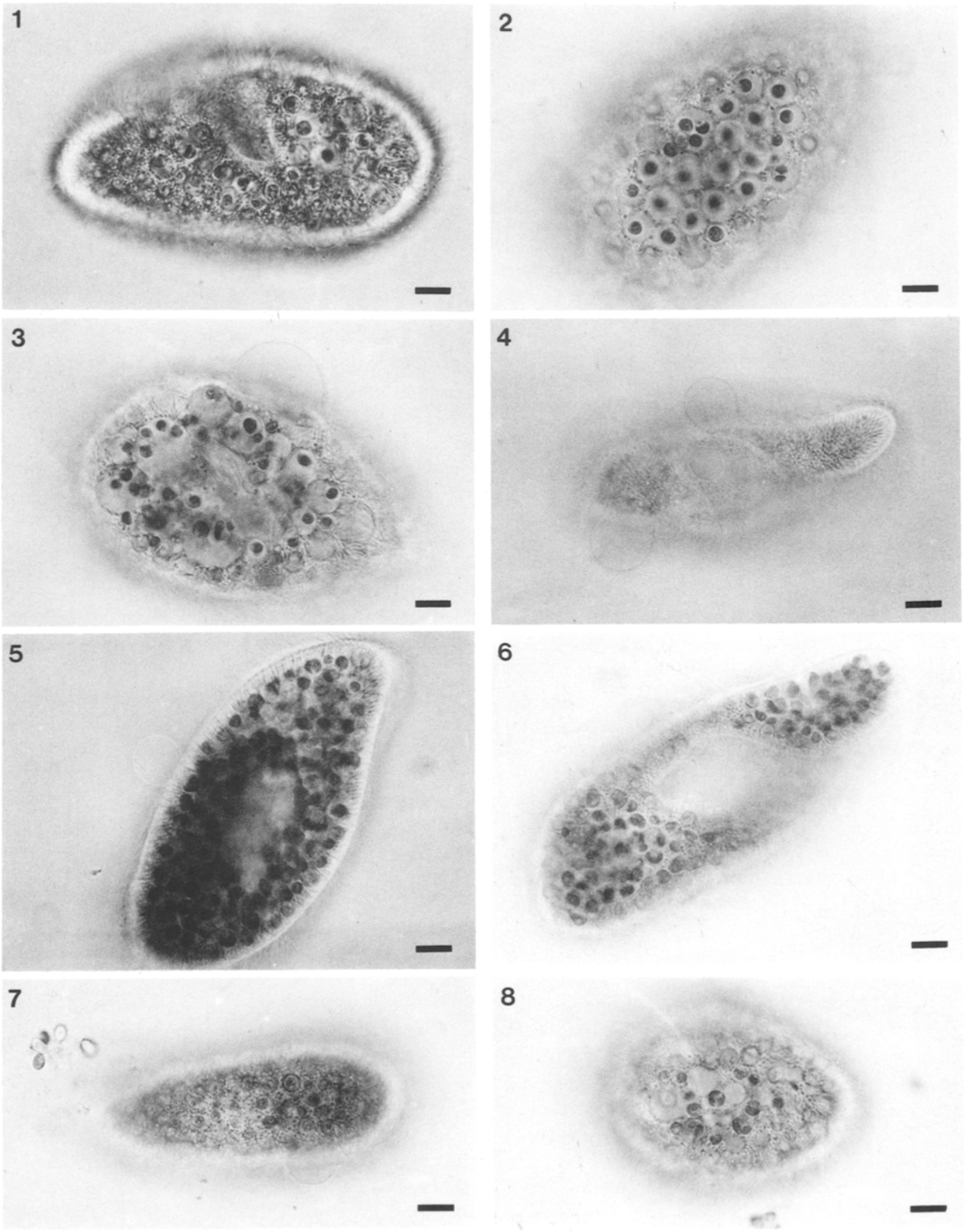
In the presence of DCMU (10^{-4}–10^{-5} M) the monensin induced swellings of the perialgal vacuoles were considerably reduced if both agents were supplied simultaneously and did not occur at all if the *Paramecium* cells were incubated 3 h with DCMU before monensin was added (Figs. 7 and 8). The effects were then similar as after monensin treatment in darkness: cyclosis and small clear vesicles occurred as well as swollen alveoles.

Discussion

The swelling of the perialgal vacuoles after monensin treatment indicates that they become acidified under conditions when the symbiontic *Chlorella* cells are photosynthetically active. This supports the assumption that also in situ maltose is transported via a sugar/proton antiport form the *Chlorella* cell into the perialgal vacuole. The transport from the perialgal vacuole into the cytosol of the *Paramecium* cells could be passive or be driven by a proton cotransport (Willenbrink 1987).

The absence of the swellings in darkness and after treatment with DMCU correlates with the strong decrease of maltose secretion under this conditions. After treatment with 10^{-6} M DCMU the rate of secreted maltose decreases to 5% (Reisser 1976). The source of the residual release could be the decomposition of starch.

It is probable that the proton gradient is formed by the *Chlorella* cells, since the swelling is coupled with photosynthesis. It can, however, not be excluded that the occurrence of maltose within the perialgal vacuole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triggers a proton pump in the vacuolar membrane. The observation that the *Chlorella* cells are not attached to the membrane of swelling perialgal vacuoles indicates that those contacts are perhaps less important than frequently suggested (Reisser 1990).

The swollen centrally located vacuoles within the *Paramecium* cells which are incubated in darkness or DMCU seem to be digesting vacuoles becoming acidified, meaning that the algae are not longer protected against digestion (Hohmann et al. 1982, Reisser 1986). Under these conditions the perialgal membrane becomes altered (Reisser 1976). The clear small vacuoles are perhaps swollen primary lysosomes.

The swelling of the peripheral alveoles after monensin treatment indicates that their membrane contains an active proton pump. This effect is indepentent of illumination and the presence of DCMU. The peripheral alveoles have been shown to sequester Ca^{2+} ions by a Ca^{2+}-ATPase (Stelly et al. 1991) and are believed to control calcium-regulated processes that take place in the immediate vicinity of the alveoles, for example trichocyst exocytosis stimulation, ciliary beating or cytoskeletal elements dynamics during division. The meaning of the acidification remains obscure.

Though monensin has a 10-fold higher affinity to Na^+ than to K^+ ions (Pressman 1976), in the present experiments K^+ ions are probably used for the exchange against protons. The medium does no contain Na^+ ions, and the tiny traces perhaps liberated from the glass vessels are surely not high enough to be osmotically effective.

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Figs. 1–8. *Paramecium bursaria*. Bars: 10 µm

Fig. 1. Monensin, 4 h, 5×10^{-5} M, light. First significant swellings of perialgal vacuoles

Fig. 2. Monensin, 6 h, 5×10^{-5} M, light. The swellings are stronger and first perialgal vacuoles have fused

Fig. 3. Monensin, 10 h, 5×10^{-5} M, light. Many big perialgal vacuoles, frequently fused, one peripheral bleb is in focus

Fig. 4. Monensin, 8 h, 5×10^{-5} M, darkness. Cell surface with some peripheral blebs

Fig. 5. Monensin, 8 h, 5×10^{-5} M, darkness. No swollen perialgal vacuoles and a peripheral bleb, algae also in the cell interior in digestion

Fig. 6. Control cell, 0.5% ethanol, 8 h

Fig. 7. Monensin, DCMU, 8 h, each 5×10^{-5} M, light, after 3 h preincubation with DCMU, 5×10^{-5} M. No swellings of the perialgal vacuoles, swollen peripheral alveoles

Fig. 8. As Fig. 7, some empty appearing vacuoles beside non-swollen perialgal vacuoles

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