Formation of Hydrocarbons by Photobleaching Cyanobacterium, *Anacystis nidulans*

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The cyanobacterium *Anacystis nidulans* is bleached when subjected to both light and O_2 together with suitable (pre)treatment of the cells such as incubation at high (≧48 °C) or low (≦17 °C) temperatures, or in presence of metabolic inhibitors, or of substances forming complexes with divalent cations. Concomitantly degradation of the intracellular membranes is observed (G. Schmetterer, G. A. Peschek, Biochem. Physiol. Pflanzen **176**, 90–100 (1981)). The same three conditions cause formation of hydrocarbons, mostly ethane, a characteristic product of lipid peroxidation. Ethane production is unchanged and still light-sensitive even when no more pigments can be detected in the cells. In "white" cells light-dependent O_2-uptake is also observed. The action spectrum of this process suggests that "completely" bleached cells retain very small amounts of residual chlorophyll, which must be unusually resistant to photooxidation.

Introduction

Photooxidative bleaching of chloroplasts under the action of herbicides (metabolic inhibitors) is a well known phenomenon [1–4]. It is accompanied by lipid peroxidation and degradation of the thylakoid membranes [2–7]. Lipid peroxidation can be assayed by the production of ethane, as originally found by Riely *et al.* [8] in mammalian cells. Also in chloroplast bleaching a correlation between lipid peroxidation and ethane formation was found [2, 3, 5–7, 9, 11].

Recently it has been shown that the cyanobacterium (blue-green alga) *Anacystis nidulans* becomes liable to photooxidative bleaching upon a number of treatments, namely low (≦17 °C) or high (≧48 °C) temperature, addition of metabolic inhibitors (*e.g.* CCCP or DCMU) or agents strongly binding divalent cations (*e.g.* EDTA or phosphate buffer) [12]. Loss of photosynthetic pigments is accompanied by a degradation of the intracellular membranes (thylakoids), as seen in the electron microscope [13].

The present paper demonstrates the formation of hydrocarbons, mainly ethane, during bleaching of *A. nidulans*. Degradation of the intracellular membranes in *A. nidulans* can therefore be ascribed to photosensitized lipid peroxidation.

Abbreviations: DCMU, 3-(3′,4′-dichlorphenyl)-1,1-dimethylurea; CCCP, carbonyl cyanide m-chlorophenyl-hydrazone; EDTA, ethylenediamine tetraacetic acid; HEPES, N-2-hydroxyethylpiperazine-N′2-ethanesulfonic acid.

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Materials and Methods

Anacystis nidulans (strain L 1402-1 of the Culture Collection of Algae and Protozoa in Göttingen, FRG) was grown as described previously [13]. The formation of hydrocarbons was determined in an apparatus shown schematically in Fig. 1. The cells were suspended in 800 ml of different media as described in the legends to the figures at a cell density of 0.5 µl packed cells/ml. Cell suspensions could be gassed either with CO_2-free air or with pure N_2. Traces of hydrocarbons in the air were removed effectively by leading the air through a freezing trap cooled with liquid nitrogen. After passage through the cell suspension, all gas tubing was made of glass to prevent loss of hydrocarbons diffusing through rubber. The formed (volatile) hydrocarbons were frozen out in an exchangeable trap filled with the adsorbent Li-Chrosorb RP 8 (Merck). After removal of the trap the gas (mainly argon and O_2) was pumped into an evacuated bulb while still being cooled. After thawing the trap, the gas was again pumped into an evacuated bulb, this fraction now containing most of the hydrocarbons. All fractions were transferred to a Carlo Erba gas chromatograph equipped with a Poropak Q column of 1 m length and an FID detector. The retention times for methane, ethane, and propane were 2.0, 4.5, and 13 min, respectively. Identification of the gasses was by comparing the retention times and co-chromatography with a standard mixture of hydrocarbons.

Chlorophyll was determined by extraction into 80% aqueous aceton [14]. The detection limit was 5×10^{-4} µg chlorophyll/µl packed cells.

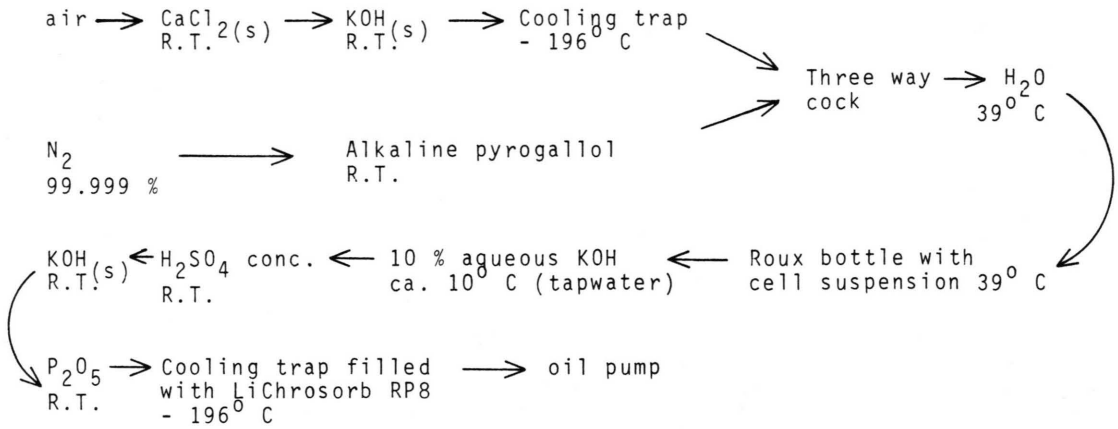

Fig. 1. Schematic representation of the apparatus used for determination of hydrocarbons formed by *A. nidulans*. The cell suspension could be illuminated with 20 W/m² of cool white fluorescent light. R.T. = room temperature.

Results and Discussion

A. nidulans cells suspended in phosphate buffer bleached in the presence of both light and O_2 [12]. Fig. 2a shows that this process was accompanied by the formation of considerable amounts of hydrocarbons, mostly ethane, but also some methane and propane. Smaller amounts of higher hydrocarbons were also occasionally found (not shown). Like bleaching, production of hydrocarbons was dependent on light and O_2 (Fig. 2b). Virtually no hydrocarbon was formed in the dark, either in air or in N_2 atmosphere. Substitution of air by N_2 in the light caused a significant decrease of total hydrocarbon formation, yet a rise of propane evolution was consistently observed. The cause of the latter is not clear, at present.

The formation of all hydrocarbons was found to be linear from the beginning for at least a week. This is in contrast to the results of Schobert and Elstner, who found that ethane production by bleaching of *Phaeodactylum tricornutum* cells leveled off within one day [2]. Their experiments, however, were carried out in sealed vessels, so that during photooxidation the concentration of O_2 slowly diminished. By contrast, the flow-through apparatus used for the present work ensured a constant partial pressure of O_2 during the whole experiment.

Even in the light and in the presence of O_2, bleaching of *A. nidulans* occurred only, when the cells additionally were exposed to suitable (pre)treatment [12]. Similarly, ethane was produced only if the cells were incubated in suitable "bleaching media" (Fig. 3). In Medium D without carbonate the cells neither grew nor bleached even after prolonged incubation [12]. In 50 mM phosphate buffer or in Medium D containing DCMU the cells bleached within 3–4 days (compare Fig. 2a and [12]). Similar amounts of ethane formed, when the cells were bleached by other methods (see [12]), *e.g.* incubation in 50 mM HEPES buffer pH 7.7 containing 0.5 mM $ZnCl_2$ or at temperatures higher than 48 °C (not shown).

Hydrocarbon formation always was independent of the chlorophyll content and even continued unchanged after the cells had "completely" bleached. This shows that chlorophyll itself cannot be an important source for the hydrocarbons, although it comprises about 10% of the total lipids [15]. The hydrocarbons therefore must stem from peroxidation of the acyl lipids, which are intrinsic structural components of the membranes. Their degradation could be the reason for the disappearance of the thylakoids in bleached cells as seen in the electron microscope [13]. It is noteworthy that the plasmamembrane of *A. nidulans* is not degraded during the photooxidative process [13], although there are recent hints that the plasmamembrane lipids might contain a higher percentage of unsaturated fatty acids (from which ethane is formed [16, 17]) than those of the thylakoids. Specifically the phase transition temperature of the plasmamembrane was found to be lower (about 15 °C *vs.* 25 °C) both by electron microscopy studies [18] and by investigation of the kinetics of membrane bound electron transport reactions [19]. If it is assumed that the resistance of

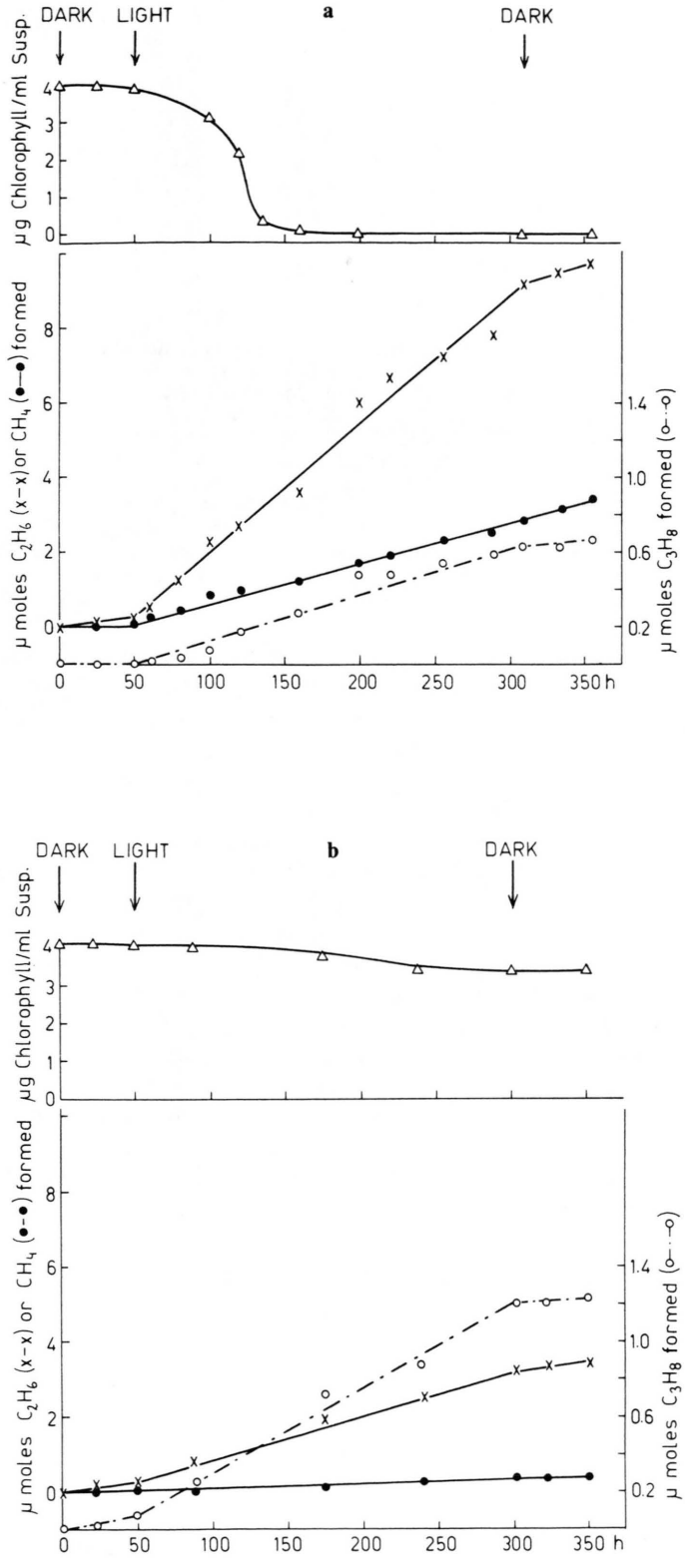

Fig. 2. Production of hydrocarbons by bleaching *A. nidulans*. Cells were suspended in 50 mM potassium phosphate pH 7.7 and gassed with CO_2-free air (Fig. 2a) or N_2 (Fig. 2b). In the latter case the suspension also contained 10^{-6} M DCMU. Further details see Materials and Methods.

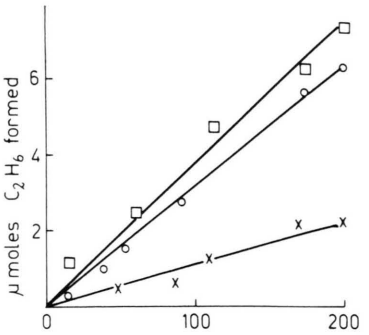

Fig. 3. Effect of suspension medium on ethane production by *A. nidulans*. Cells were suspended either in 50 mM potassium phosphate pH 7.7 (○–○), or in Medium D (without carbonate) of Kratz and Myers [20] (×–×), or in Medium D with 10^{-5} M DCMU added (□–□). In all cases the suspensions were illuminated with 20 W/m² and gassed with CO_2-free air. At the end of the experiment the cells in phosphate buffer and in Medium D + DCMU had bleached completely (compare Fig. 2a), the cells in Medium D had lost only 30% of their original chlorophyll content (20 μg chlorophyll/mg dry weight).

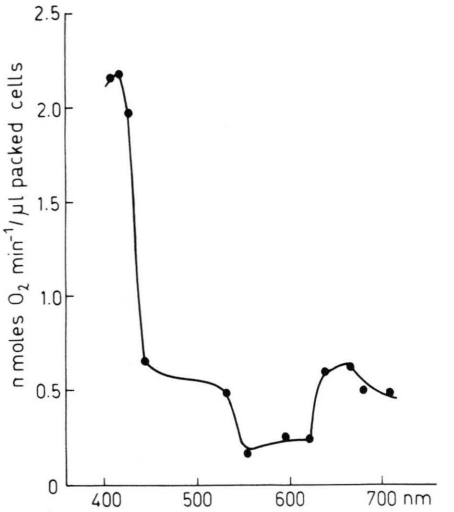

Fig. 4. Action spectrum of photooxidative O_2 consumption in bleached *A. nidulans* cells. The cells were bleached in 50 mM potassium phosphate buffer [12] and kept under photooxidative conditions for 2 more days after chlorophyll had sunk below detection level. The cells were then harvested and resuspended in phosphate buffer saturated with air. The uptake of O_2 was followed with a Clark type electrode. All values are corrected for the small O_2 uptake in the dark [21]. Illumination was with an Oriel 1000 W Xenon lamp equipped with appropriate interference filters of halfbandwidth 11 – 13 nm (Schott, Mainz, FRG). Light intensity was adjusted to 105 W/m² for each wavelength.

the plasmamembrane to photooxidative attack is due to its lacking a photosensitizer [12], the source of the hydrocarbons formed during bleaching of *A. nidulans* must be acyl lipids of the thylakoids.

The fact that ethane production is light-sensitive even in "completely" bleached cells (Fig. 2a) requires that even in these cells some kind of photosensitizer must be present. Absorption spectra and fluorescence spectra of both cell extracts and whole "white" cells did not reveal traces of pigments. As expected for a photooxidative process, however, light-dependent O_2 uptake was found in the bleached cells (Fig. 4). The action spectrum displayed 2 maxima at around 670 nm and 420 nm, characteristic of chlorophyll. This residual chlorophyll would have to be much more resistant to photooxidative bleaching than the bulk chlorophyll, because both the production rate of ethane and the uptake rate of O_2 did not diminish even after the cells had become "white".

The persumed existence of traces of chlorophyll in "completely" bleached. *A. nidulans* also would be imperative to the process of regreening of these cells on transfer to growth conditions [12, 13]. Regreening occurs only in the light and the residual chlorophyll could trigger the production of new pigments.

Acknowledgements

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