Quantum Requirement of Photosynthesis in the Primarily Chlorophyll *d* Containing Prokaryote *Acaryochloris marina*

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The recently isolated and characterized unicellular photosynthetic prokaryote *Acaryochloris marina* (Miyashita *et al*., 1996) contains chlorophylls *a*, *d*, and traces of a chlorophyll *c*-like pigment as well as phycocyanin, a type of allophycocyanin, zeaxanthin and α-carotene, chlorophyll *d* being the predominant chlorophyll component. Quantum requirement measurements of the photosynthetic oxygen evolution resulted in about 12 quanta for excitation of chlorophylls *a* and *d* and 18 for phycocyanin. The data also revealed that these pigments are involved in energy absorption for photosynthetic oxygen evolution. Energy is transferred efficiently and equally well between the chlorophylls. Light absorbed by phycocyanin which is organized in phycobiliprotein aggregates (Marquardt *et al*., 1997), results in a less efficient energy transfer to the reaction center chlorophylls.

Introduction

Recently an unique new prokaryotic organism with oxygenic photosynthesis has been isolated and described as *Acaryochloris marina* (Miyashita *et al*., 1996). Surprisingly, the predominant photosynthetic pigment of *Acaryochloris* is chlorophyll *d*. Only minor concentrations of chlorophyll *a*, a chlorophyll *c* like pigment, phycocyanin, a type of allophycocyanin, zeaxanthin and α-carotene (Miyachita *et al*., 1996; Marquardt *et al*., 1997) complete the pigment pattern. The ratio of chlorophylls *a/d* is about 0.1–0.03, depending on the culture conditions. The pigments are organized in thylakoid-like lamellae without detectable phycobilisomes (Miyashita *et al*., 1996). Recently, biliprotein aggregates containing phycocyanin and a type of allophycocyanin could be isolated (Marquardt *et al*., 1997). The organism develops normal amounts of oxygen upon illumination (Miyashita *et al*., 1996).

With respect to the unusual pigment composition and structure of the photosynthetic organelle we investigated the quantum requirement for photosynthetic oxygen evolution upon irradiation into the main absorption peaks of chlorophyll *a* (683 nm) and *d* (712 nm) as well as that of phycocyanin (609 nm). A comparison of these quantum requirements should also allow some conclusions about the energy transfer between these three pigments.

Materials and methods

Organism and growth

The prokaryotic organism with oxygenic photosynthesis, named *Acarychloris marina*, was isolated from the interior of the colonial ascidian *Lissoclinum patella* (Miyashita *et al*., 1996). It is of elliptical shape with axes of 1.5–2.0 to 1.5–3.0 μm. Up to 12 layers of stacked thylakoid-like membranes are appressed in a peripheral continuum against the cell wall. These membranes contain the above mentioned pigments. The phycobiliprotein aggregates are also part of these photosynthetic membranes, however, they are not yet localized exactly (Miyashita *et al*., 1996; Marquardt *et al*., 1997). Stock cultures of *Acaryochloris* are kept at the Marine Biotechnology Institute in Kamaishi, Iwate 026, Japan.

Acaryochloris marina was grown in axenic batch cultures in K+ESM medium (Keller *et al*., 1987) at 28 °C, bubbled gently with air under a light

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(80 μE)-dark cycle of 12:12 hours. *Acaryochloris* is growing very slowly under these conditions with a doubling rate of more than 48 hrs. Cells were harvested from the same culture for each set of experiments and concentrated by centrifugation to a density of 10 μl packed cell volume per ml.

Cell mass determination

The cell mass was determined as packed cell volume (PCV) in hematocrite tubes by centrifugation at 1,400×*g* for 10 min as described earlier (Senger, 1970).

Determination of the quantum requirement

Quantum requirement was determined with a custom-made apparatus described in detail before (Senger, 1971). Oxygen evolution was measured with a Gilson-allglas manometer (Gilson medical electronics, Madison, Wisc., USA). The rectangular Warburg vessel was shaken identically positioned in the light beam during gas exchange and absorption measurements.

Monochromatic light was provided by a tungsten halogen lamp (Sylvania DXN, 1000 W, 120 V) combined with interference filters (DAL, Schott, Mainz, Germany) with maximum transmissions at 609, 683 and 712 nm. Quantum flux was measured with a thermistor (YSI-Kettering Radiometer, Yellow Springs, Ohio, USA) calibrated against a chlorophyll/thiourea actinometer (Gaffron, 1927; Warburg and Schocken, 1949).

The samples contained 30 μl PCV in 3 ml of Warburg's carbonate/bicarbonate buffer #9 (pH 9.5). No difference in oxygen evolution between cells suspended in the Warburg- or phosphate-buffer was observed. Oxygen exchange was measured for 2x5 min in monochromatic light of various intensities, corrected by the dark respiration, measured before and after irradiation. Light absorptions by the algal samples were measured as percent of the control in an integrating Ulbricht sphere. The integrating sphere contained 8 symmetrically arranged photovoltaic cells (Type Si 09, Bruno Lange, Berlin, Germany) which were wired in parallel. The photocurrent generated by the light beams was measured with a null detector micro-voltmeter (Type 155, Keithly Instruments, Yellow Springs, Ohio, USA). The absorption of the algal samples was measured against water, set as 0%, and chinese black ink, set as 100% absorption.

Results and Discussion

The photosynthetic prokaryote *Acaryochloris marina* reveals an exceptional pigment pattern. It contains chlorophyll *d* as major and chlorophyll *a*, phycocyanin, zeaxanthin and α-carotene as minor pigments. Chlorophyll *c* and allophycocyanin occur only as traces (Miyachita *et al.*, 1996; Marquardt *et al.*, 1997). The *in vivo* spectrum demonstrates peaks at 713 and 608 nm attributed to chlorophyll *d* and phycocyanin, respectively (Fig. 1). Chlorophyll *a* was not detected in the *in vivo* spectrum due to its low concentration. However, its absorption *in vivo* has to be assumed to be located around 685 nm. In order to determine the participation of these 3 pigments in the process of photosynthetic oxygen evolution and the presumable energy transfer among them, quantum requirement was measured in monochromatic light of 609, 682 and 712 nm using interference filters with transmission maxima as close to the pigment absorption maxima of the organism as available. In accordance with the theory of quantum yield measurements (Warburg, 1919) the photosynthetic oxygen evolution was determined in the linear part of the intensity curve, just above the compensation point. Three values were determined for each wavelength in two separate experi-

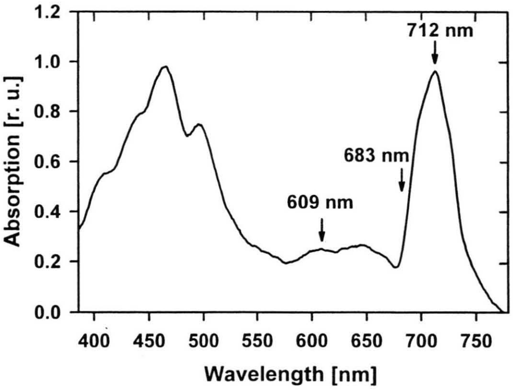

Fig. 1. Low temperature (77K) *in vivo* absorption spectrum of *Acaryochloris marina.* The sample contained 20 μl of PCV (packed cell volume) in a 1 mm-light path plexiglas cuvette. The cells were frozen in culture medium. Spectra were measured with a UV-3000 Shimadzu spectrophotometer.

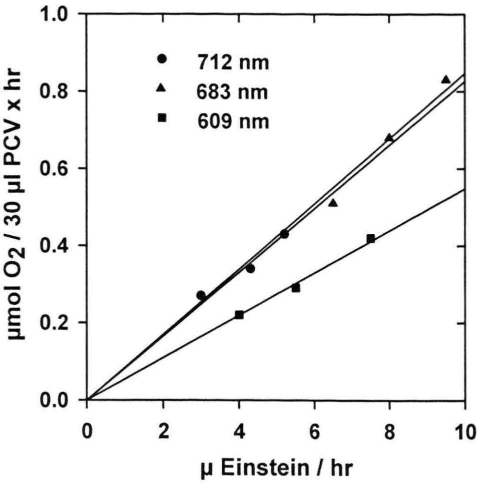

Fig. 2. Photosynthetic oxygen evolution (µmol 0_2/30 µl PCV x hr) per reaction vessel was determined manometrically for *Acaryochloris marina* at 28 °C, at various doses of absorbed quanta (µEinstein per hr and reaction vessel) of different wavelengths.

ments with cells deriving from the same culture (Fig. 2). With a slight deviation the determined values could be extrapolated through zero. During an experiment with samples of a different culture we could confirm the value of 18 quanta needed at 608 nm. However, 12 quanta were needed at 683 and 712 nm for the formation of one molecule of oxygen.

From the results reported here we concluded that the energy transfer between the three pigments is efficiently established and that they are all actively participating in the photosynthetic process. The quantum requirement measured is in the same range as measured during the 16th hour of the synchronized cell cycle of the green alga *Scenedesmus obliquus* (Senger and Bishop, 1967) and some pigment mutants of the same organism (Bishop and Senger, unpublished). In accordance with earlier reports, we draw the conclusion that the phycocyanin acts as light harvesting pigment together with the bulk of chlorophyll *d*. However, it is not possible at the current stage to predict whether chlorophyll *a* or some fraction of *d* acts as the energy sink, i.e. the reaction center pigment. If chlorophyll *a* were the traditional reaction center chlorophyll, the chlorophyll *d* containing antenna would have to transfer energy uphill. Although such a transfer from longer wavelengths pigments to shorter wavelength reaction centers is known, this mechanism would not be very economic for the photosynthesis of *Acaryochloris*. However, this question has to be solved by future experiments.

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