Light-harvesting in *Acaryochloris marina* – spectroscopic characterization of a chlorophyll *d*-dominated photosynthetic antenna system

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Abstract Oxygenic photosynthesis of the prokaryote *Acaryochloris marina* involves chlorophyll *d* (Chl *d*) as the major pigment [Miyashita et al. (1996) Nature 383, 402]. Four spectral forms of Chl *d* (peak wavelengths: 694, 714, 726 and 740 nm) are resolvable by low-temperature absorption spectroscopy on intact cells. Based on fluorescence spectra (at 290 K and 77 K) and on analysis of fluorescence induction curves we conclude: (1) excitation energy is efficiently transferred between the various spectral forms of Chl *d* and the PS II reaction center; (2) Chl *d* serves as a light-harvesting pigment for both, Photosystem II (PS II) and PS I; (3) excitation energy transfer between PS II units occurs.

Key words: Chlorophyll *d*; Chlorophyll fluorescence; Excitation energy transfer; Light-harvesting complex; Photosynthesis

1. Introduction

Recently *Acaryochloris marina*, a prokaryote performing oxygenic photosynthesis, has been isolated from a suspension of algae squeezed out of an ascidian collected at the marine coast of the Palau island [1]. Surprisingly, the dominating pigment of *Acaryochloris* is Chl *d*. The Chl *a* content of *Acaryochloris* cells is low (depending on culture conditions 3–10% of the Chl *d* content, [1]); phycobiliproteins are present in relatively small amounts only [2].

Chl *d* differs from Chl *a* by the presence of a 3-formyl group. Consequently its Q_y-absorption peak (at 688 nm in diethyl ether, 710 nm in *Acaryochloris*, see Fig. 1) is red-shifted in comparison to Chl *a* (at 662 nm in diethyl ether, around 680 nm in photosynthetic pigment-protein complexes). In all previously known organisms capable of oxygenic photosynthesis the dominating chlorophyll is Chl *a*. The high Chl *d* content in *Acaryochloris* is suggestive of the existence of a unique and as yet unknown light-harvesting system.

Upon excitation of special Chl *a* molecules (P680 in PS II, P700 in PS I) the primary charge separation reactions of oxygenic photosynthesis are initiated [3]. The excited state energy needed to drive the primary PS II charge separation is often assumed to be ~1.82 eV which corresponds to the bleaching maximum at 680 nm observed upon formation of the $P680^+$ radical [3,4]. The question arises whether the 'low-energy' Chl *d* (the absorption maximum at 710 nm corresponds to an excited state energy of only ~1.75 eV) can serve as a light-harvesting pigment for the PS II reaction center.

PS II and PS I preparations of *Acaryochloris* are presently not available. Therefore, we decided to use established spectroscopic methods for an investigation on intact cells. This approach avoids preparation artefacts and allows some first conclusions concerning the role of Chl *d* as a light-harvesting pigment in oxygenic photosynthesis.

2. Materials and methods

Culture conditions are described elsewhere [2]. Some 'stress' conditions (suboptimal media, higher light intensities, transport from Japan to Germany) stimulated the development of a phenotype characterized by an increased Chl *a*/Chl *d* ratio (as judged by absorption spectroscopy, see Fig. 2).

Absorption and fluorescence emission spectra were collected using a UV 3000 spectrophotometer and a RF 540 fluorometer, respectively (both manufactured by Shimadzu, Kyoto, Japan). Emission spectra were corrected for the spectral sensitivity of the detection system; fluorescence reabsorption artefacts were minimized by using cell suspensions with a maximal absorbance of less than 0.05 absorbance units. Low temperature fluorescence spectra were recorded using light-guides fitted into a cuvette, which was plunged into liquid nitrogen. The 77 K absorption spectra were measured using a cuvette (optical path of 1 mm) immersed in liquid nitrogen; the spectra were corrected by subtraction of a nonlinear background.

For measurement of fluorescence induction curves the absorbance of the sample was below 0.1 absorbance units at the Chl *d* absorption peaks. A laboratory-built set-up was used consisting of a slide projector equipped with an interference filter for selection of the excitation wavelength, a shutter and a 1 cm×1 cm sample cuvette. The fluorescence emission was measured at right angle by a photodiode detector; the emission wavelength was selected by interference filters. In the absence of DCMU, the ratio between the maximal fluorescence (F_M) and the minimal fluorescence of dark-adapted algae (F_0) was determined by the saturation pulse method [5] using a PAM fluorometer (Walz, Effeltrich, Germany).

3. Results and discussion

In Fig. 1, the room temperature (RT) and low temperature (LT, detected at 77 K) absorption spectra are shown. Due to decreased homogeneous linewidths, peaks and shoulders are better resolved in the LT-spectrum. The extent of LT line-narrowing is of the same order of magnitude as that observed for organisms with a Chl-*a*/*b* antenna system [6]. Seemingly, the Chl-*d* antenna system and the Chl-*a*/*b* antenna systems are roughly similar with respect to the relative contributions of homogeneous and inhomogeneous broadening of absorption bands.

Below 540 nm the absorption spectra are determined by Chl

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_0, dark-level fluorescence yield; F_M, maximal fluorescence yield; F_V, equals ($F_M - F_0$); *p*, connectivity parameter; PS, photosystem

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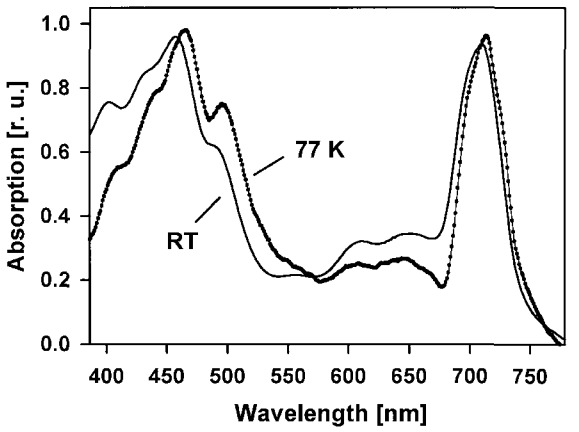

Fig. 1. Room temperature and low-temperature (77 K) absorption spectrum of *Acarychloris marina*.

Soret bands and carotenoid absorption; above 660 nm Chl Q_y-bands prevail. We assign the absorption bands between 580 and 660 nm to phycobilins, which have been shown to be present in *Acaryochloris* [2]. In the LT-spectrum the broad Chl Q_y band (660–750 nm) clearly exhibits a substructure indicating that various spectral forms of Chl are present. A fourth derivative analysis (Fig. 2) reveals that at least five spectral forms are resolvable: Chl_{670}, Chl_{694}, Chl_{714}, Chl_{726} and Chl_{740} (the subscripts give the estimated positions of the absorption peaks which correspond to maxima of the fourth derivative). In Fig. 2, LT absorption spectra of a low-Chl *a* and a high-Chl *a* phenotype are displayed (see Materials and Methods). There are clear differences between the two absorption spectra shown in Fig. 2. Nonetheless, the maxima of the fourth derivatives are found at approximately the same wavelengths (differences of 1–2 nm).

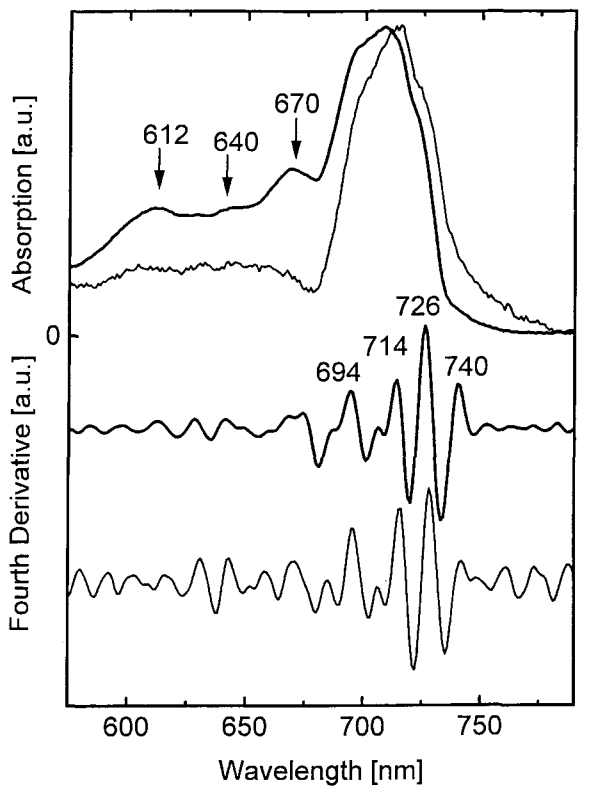

Fig. 2. 77 K-absorption spectrum and fourth derivative spectra of cells with a relatively high Chl *a* content (thick lines) or a relatively low Chl *a* (thin lines). Maxima of the fourth derivative are found at 694, 714, 726 and 740 nm for high-Chl *a* cells and at 695, 715, 728, 741 nm for low-Chl *a* cells. The maxima at 612 and 640 nm can be assigned to biliproteins. Five scans have been averaged.

The Q_y absorption peaks of Chl *a* and Chl *d* in diethyl ether are at 662 and 688 nm, respectively. The absorption maxima of chlorophylls in photosynthetic antenna systems are typically red-shifted with respect to the chlorophyll absorption maxima in organic solvents. Therefore we assume that Chl_{670} is not a Chl *d*, but a Chl *a*. Considering that only a minor fraction of all *Acaryochloris* chlorophylls is Chl *a* (~4% [1]), we believe that the Chl_{694}, Chl_{714}, Chl_{726} and Chl_{740} are spectral forms of Chl *d*. It should be noted that also in Chl *a/b* antenna systems various spectral forms of the major pigment, Chl *a*, are found [6,7]. The underlying reasons for the heterogeneity in the spectral characteristics of Chl are not fully understood. Presumably, distinct types of protein binding sites result in distinct optical properties. In conclusion, with respect to homogeneous broadening, inhomogeneous broadening and occurrence of distinct spectral forms the Chl-*d*-dominated antenna system of *Acaryochloris* exhibits qualitatively the same characteristics as the Chl *a*-dominated antenna systems of plants.

At room temperature the fluorescence emission peak of *Acaryochloris* cells is at ~720 nm; at liquid nitrogen temperature it is at ~730 nm (Fig. 3). Assuming the absorption peak to be at ~710 nm, we find an increase in the Stokes shift from 10 nm to 20 nm upon cooling. At room temperature various excitation wavelengths result in virtually identical emission spectra (data shown only for excitation at 461 nm). Also at 77 K the position of the main emission peak is almost independent of the excitation wavelength; however, upon predominant excitation of Chl *a* at 430 nm, we observe an increase in the fluorescence emission around 760 nm (Fig. 3).

It has been shown that the PS-II antenna system of higher plants is characterized by rapid exciton equilibration [8]; the spectroscopic consequences of rapid exciton equilibration are discussed elsewhere [9,10]. Briefly, in case the excitation energy transfer between pigments is fast in comparison to the

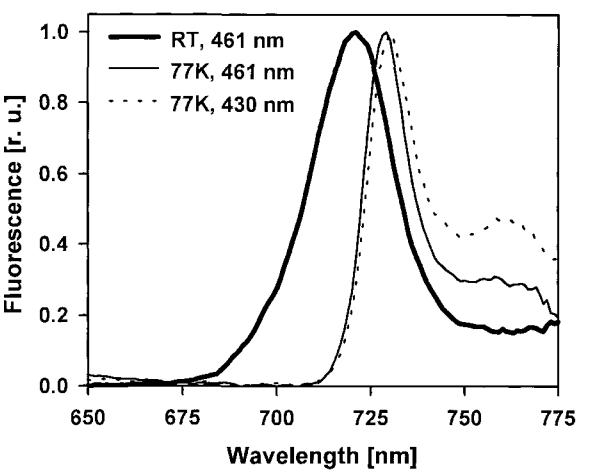

Fig. 3. Chl-*d* fluorescence emission spectra of *Acaryochloris* cells. 461 nm excitation at RT (thick solid line); 461 nm excitation at 77 K (thin solid line); 430 nm excitation at 77 K (dotted line).

excited state decay (rapid exciton equilibration), a Boltzmann-type excited-state equilibrium distribution, the exciton equilibrium, determines the emission spectrum. Thus, the emission spectrum is independent of the excitation wavelength. At low temperatures the low-lying excited-state energy levels, which are related to the long-wavelength Chl *d* absorption bands, are predominantly populated, whereas at RT also higher energy levels are populated to a significant extent. Consequently, at low temperature low-energy fluorescence decays prevail, and the emission spectrum is red-shifted in comparison to the RT emission spectrum. We consider the excitation wavelength independence and the observed changes in the *Acaryochloris* emission spectra upon cooling (Fig. 3) to be clear indications that the emission spectra are determined, at least to some extent, by the excited-state equilibrium distribution. This finding necessarily implies that efficient excitation energy transfer between various spectral forms of Chl *d* occurs.

The Chl *d* fluorescence of *Acaryochloris* cells is variable. Using the saturation pulse method [5], we find a ratio between the F_M-level (maximal fluorescence level) and the F_0-level (level in dark-adapted algae) of up to 5. This relatively high value is suggestive of a high quantum yield for the PS II charge separation reactions and efficient excitation energy transfer between antenna chlorophylls [9].

The fluorescence induction curve of *Acaryochloris* cells exhibits the typical O-I-D-P transient [11,12]. Unbalanced excitation of PS II and PS I affects the I-D-P kinetics: predominant excitation of PS II results in the disappearance of the I-D lag phase, whereas predominant excitation of PS I results in a pronounced minimum at D [13,14]. Thus, the fluorescence induction curve is indicative that upon excitation of Chl *d* (at 461 nm) PS II and PS I are excited to approximately the same extent. Taking into consideration that in *Acaryochloris* roughly 95% of the antenna chlorophylls are Chl *d*, the above finding implies that Chl *d* serves as an antenna pigment for both photosystems.

The electron transport inhibitor DCMU affects the fluorescence induction curves of *Acaryochloris* cells in exactly the same way, as it affects the induction curves of plant cells or thylakoid preparations. The sigmoidicity of the DCMU inductions curves is indicative of efficient excitation energy transfer between PS II units, a phenomenon often denoted as PS II connectivity or cooperativity [9,15]. The measured

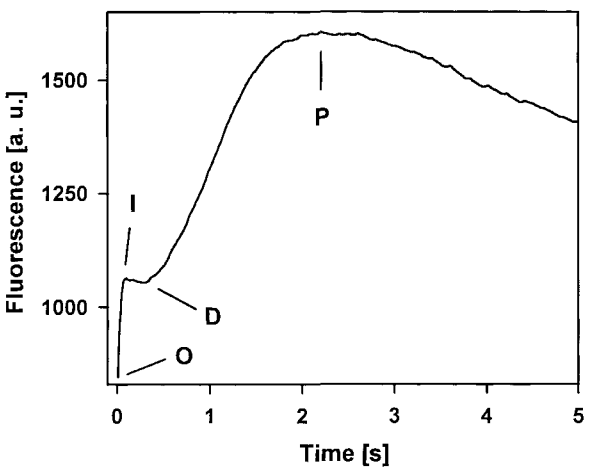

Fig. 4. Fluorescence induction curves for Chl *d* excitation at 461 nm (28 μE m^{-2} s^{-1}) and fluorescence detection at 716 nm.

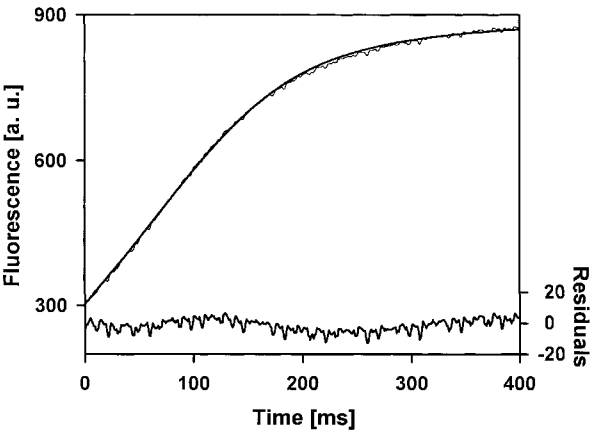

Fig. 5. DCMU-induction curve for Chl *d* excitation at 461 nm (8 μE m^{-2} s^{-1}) and fluorescence detection at 716 nm. The measured (thin line) and simulated (thick line) induction curves are shown. The right axis applies to the lower line, the residual plot (differences between simulated and measured values). Good agreement between data and simulation has been obtained for: $p = 0.5$ (connectivity parameter for the α-phase; $F_V^{\alpha}/F_V^{\beta} = 4$ (ratio between the variable fluorescence of the α-phase and the β-phase); $\phi^{\alpha}/\phi^{\beta} = 4.4$ (ratio of absorption cross sections). The quantum yield for Q_A-reduction of α- and β-units has been assumed to be equal to F_V/F_M (= 0.67).

DCMU induction curves were simulated on basis of the rationale originally proposed by Melis and Homann ([16]; see also [9,17]); the corresponding differential equations were solved by numerical integration. Good agreement between data and simulation is obtained for a value of the connectivity parameter, *p*, of 0.5, which is similar to values found for PS II with a Chl *a/b* antenna system. (Good agreement is obtained only, if the existence of PS II β-units is assumed. The meaning of the slow phase of DCMU induction curves, the β-phase, is a matter of debate [18]; the existence of the β-phase does not necessarily indicate that two PS II populations with distinct antenna sizes, the α-units and β-units, are present.)

In summary, apart from the red-shifted absorption and emission bands similar characteristics are found in Chl *a*-dominated and Chl *d*-dominated antenna systems: (1) existence of several distinct spectral forms of the predominant Chl; (2) efficient excitation energy transfer between Chl of these spectral forms; (3) the dominating Chl serves as a light-harvesting pigment for both, PS II and PS I; (4) excitation energy transfer between PS II units occurs (Figs. 4 and 5).

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