The effect of chemical oxidation on the fluorescence of the LH1 (B880) complex from the purple bacterium *Rhodobium marinum*

Christopher J. Law, Richard J. Cogdell*

Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, UK

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Abstract The effect of chemical oxidation on the absorption and fluorescence emission spectra of the LH1 complex from *Rhodobium marinum* was investigated. Mild chemical oxidation of the LH1 complex, by addition of 10 mM potassium ferricyanide, caused a 2–3% bleaching of the 880-nm Q_y absorption band. In contrast, at the same ferricyanide concentration, fluorescence emission intensity of the LH1 complex was quenched by about 50%. This result demonstrates that oxidation of very few bacteriochlorophyll (BChl) molecules in the LH1 ring is enough to completely quench its fluorescence.

Key words: Light-harvesting complex 1; Fluorescence quenching; Bacteriochlorophyll *a*; Chemical oxidation; Purple photosynthetic bacterium; *Rhodobium marinum*

1. Introduction

The primary events of photosynthesis involve the absorption of light energy by antenna complexes and its transfer to the photochemical reaction centres (RCs) where it is used to drive the primary redox reactions [1].

Most purple bacteria contain two major types of antenna or light-harvesting (LH) complex which are housed in the intracytoplasmic membrane [2]. The LH1 or B880 complex (named according to the λ_{max} of the near-infrared absorption band) is intimately associated with the RC to form the so-called LH1-RC core complex. The LH2 or B800–850 (820) complex, which can be present in variable amounts, forms a peripheral network of pigment-protein complexes which surround and connect the core complexes [2]. However, some species of purple bacteria, such as *Rhodospirillum* (*Rs.*) *rubrum*, *Rhodopseudomonas* (*Rps.*) *viridis* and *Rhodobium* (*Rh.*) *marinum* possess LH1 as their only antenna complex [3].

The study of the primary events of photosynthesis has been revolutionised in recent years with the elucidation, by X-ray crystallography, of the three-dimensional structures of the bacterial RC [4,5] and LH2 complex [6,7]. To date, no such structure exists for the LH1 complex. However, image analysis of electron micrographs of *Rps. viridis* and *Rh. marinum* membranes [8,9] and 2-D crystals of LH1-RC from *Rh. marinum* [9], *Rps. viridis* [10] and *Rs. rubrum* [11,12] have clearly indicated that the RC is surrounded by the LH1 'ring'. Electron diffraction of 2-D crystals of the LH1 complex from *Rs. rubrum* suggests that the 'ring' consists of 16 α-β pigment-binding apoproteins [11]. By analogy with the LH2 structure, the LH1 complex is thought to non-covalently bind 32 bacteriochlorophyll *a* (BChl *a*) molecules. The hole in the centre of this 'ring' is large enough to accommodate a RC [11].

Previous reports have shown that it is possible to chemically oxidise the BChl components of the LH1 ring [13–15]. Such treatment results in characteristic changes in the absorption, CD and electron paramagnetic resonance (EPR) spectral signals of the complex [13–15]. Chemical oxidation of LH1 BChl *a* in chromatophores of *Rs. rubrum* resulted in distinctive absorbance changes in the near-infrared (NIR) [14]. A decrease in absorbance around 880 nm was accompanied by the appearance of a new absorption band at 1230 nm. These changes were very similar to those observed upon oxidation of the RC 'special pair' BChls, i.e. a bleaching at 865 nm and an increase in absorbance at 1245 nm [16]. The existence of cationic BChl in the LH1 'ring' was supported by EPR spectroscopy [13,15]. The room temperature EPR signal from oxidised LH1 BChl showed a nearly Gaussian signal with a *g*-value of 2.0025 and a very narrow linewidth [13,15]. The line-narrowing of this signal was interpreted as being due to the extensive delocalisation of the cation over an aggregate of 10–12 BChl molecules in the 'ring' [15].

Rafferty et al. [17] observed similar changes in the absorption and CD spectra of the LH2 complex in chromatophores from a carotenoidless mutant of *Rb. sphaeroides* upon photo-oxidation. In this case the progressive loss and concomitant blue-shift of the NIR absorption band around 860 nm were accompanied by a decrease in the NIR CD signal. These results were attributed to the conversion of BChl dimers to monomers upon photo-oxidation. Very recently, single-molecule spectroscopy suggested that fluorescence emission from the LH2 complex from *Rps. acidophila*, which is structurally similar to the LH1 complex, is very strongly quenched by the presence of photo-oxidised B850 BChl in the 'ring' [18]. However, until now it was not known how extensive the oxidation in the LH1 or LH2 'ring' must be in order to produce the dramatic quenching of the fluorescence emission observed in this study. Here we describe the results of experiments designed to investigate this by studying the effect of chemical oxidation on the fluorescence emission and absorption spectra of the LH1 complex from the purple bacterium *Rhodobium marinum*. This species was chosen for this study because its RC can easily be dissociated from the LH1 ring during the purification process [19]. This results in a homogeneous population of reaction centreless LH1 complexes which are particularly amenable to spectroscopic study.

2. Materials and methods

2.1. Strains and growth conditions

Rhodobium marinum (formerly named *Rhodopseudomonas marina*) DSM 2698 was purchased as a liquid culture from the German Micro-

*Corresponding author.
E-mail: r.cogdell@bio.gla.ac.uk

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organism Collection, Braunschweig. This culture was used to inoculate a 500-ml bottle of modified DSM-27 Rhodospirillaceae medium supplemented with 3% NaCl [20]. The culture was grown anaerobically at 28 ± 2°C at a light intensity of 150 μmol/s/m^2 provided by a bank of 150-W incandescent bulbs. As the cultures grew slowly in small volumes of media, a 20-l flask of medium was heavily inoculated with cells and incubated for 5 days before harvesting. Cells were harvested by centrifugation (2500×*g*, 30 min, 4°C).

2.2. Isolation of the B880 LH1 complex

Isolation of the B880 complex was essentially that described by Meckenstock et al. [19] with the following modifications. Harvested cells were immediately resuspended in a minimum volume of 20 mM Tris-HCl, pH 8.0. A few grains of DNase (bovine deoxyribonuclease I, Sigma Chemical Co.) and $MgCl_2$ were added to the cell suspension and the cells broken by passage through a French pressure cell at 950 p.s.i. Photosynthetic membranes were collected with a high speed spin (240 000×*g*, 1 h, 4°C), resuspended in 20 mM Tris-HCl, pH 8.0, then homogenised. Prior to solubilisation, the optical density of the sample was adjusted to 50 cm^{-1} at the NIR absorption maximum. Photosynthetic membranes were solubilised by addition of 1% (v/v) LDAO (Fluka) and stirring for 15 min at room temperature in the dark. Unsolubilised material was removed by low speed centrifugation (12 000×*g*, 10 min, 4°C). The OD_{880} of the supernatant was adjusted to ∼80 cm^{-1} by ultrafiltration in an Amicon 8200 ultrafiltration cell. To dissociate the RC from the LH1 complex the concentrated sample was mixed in a volume ratio of 1:1 with a 3.5 M $(NH_4)_2SO_4$ solution, which had its pH adjusted to 8.0 immediately before use. The mixture was stirred for 2 h in the dark at 4°C and then centrifuged (197 000×*g*, 1 h, 4°C). The supernatant, which contained the B880 complex, was dialysed overnight against 20 mM Tris-HCl, pH 8.0, and 0.05% LDAO. The reaction centreless LH1 sample was stored in the dark at 4°C until required.

2.3. Absorption and fluorescence emission spectra

Absorption spectra were recorded at room temperature in a Shimadzu UV-2101PC double-beam spectrophotometer. Room temperature fluorescence emission spectra were measured between 800 and 1000 nm with a Spex Fluorolog 1681 spectrofluorimeter using a 1-cm pathlength quartz cuvette. Slit widths were set to 10 nm and 2 nm on the excitation and emission sides, respectively. The excitation wavelength was 590 nm and fluorescence emission was measured at 90° to the excitation beam. For all measurements, the OD_{880} of the LH1 sample was ∼0.17 cm^{-1}.

Potassium ferricyanide was made up as either a 500-mM or 100-mM stock solution in 20 mM Tris-HCl, pH 8.0, containing 0.05% (v/v) LDAO. The effects of different concentrations of ferricyanide, ranging from 1 mM to 100 mM, were investigated. All spectra were recorded immediately, over a 2-min period, upon addition of potassium ferricyanide to the sample. A fresh cell filling was used for each measurement.

3. Results

The absorption spectra of purified LH1-RC 'core' and isolated LH1 complex of *Rh. marinum* are shown in Fig. 1. The spectrum of the 'core' complex shows an NIR absorption maximum at 880 nm with two smaller peaks at 800 nm and 760 nm. The former is attributable to the LH1 BChl *a* Q_y transition and the latter two peaks to the BChl *a* and bacteriopheophytin components of the RC, respectively. The small peak at 590 nm represents the BChl *a* Q_x transition and the large absorption peak at 380 nm the Soret band [19]. The three peaks located between 470 nm and 570 nm are due to the carotenoid component of the complex. The LH1 complex alone also has a NIR absorption maximum at 880 nm. However, the two NIR peaks at 800 nm and 760 nm that are associated with the presence of RC are absent, indicating successful dissociation of the RC from the LH1 complex during the purification process. In the rest of the spectrum the absorption of the LH1 complex is the same as that of the full 'core' complex.

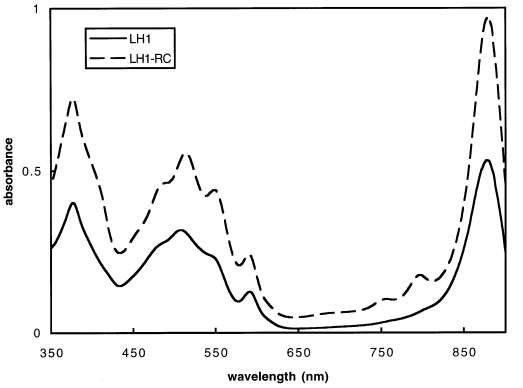

Fig. 1. The absorption spectra of the LH1-RC 'core' (- - -) and LH1 (———) complexes of *Rh. marinum*. For clarity the OD_{880} of the 'core' sample was adjusted to ∼1 cm^{-1} and that of the LH1 complex to ∼0.5 cm^{-1}.

The effect of chemical oxidation on the NIR absorption of the LH1 complex is shown in Fig. 2. Addition of potassium ferricyanide to the LH1 complex causes a bleaching of the 880-nm absorption band. The extent of this bleaching increases as the concentration of potassium ferricyanide increases. These measurements were performed immediately upon addition of ferricyanide and took 2 min to record. There was no observable shift of the λ_{max} associated with the bleaching during the time course of these measurements. Bleaching of the 880-nm absorption band was irreversible and the original absorption could not be recovered by addition of either sodium ascorbate or sodium dithionite. The extent of the fer-

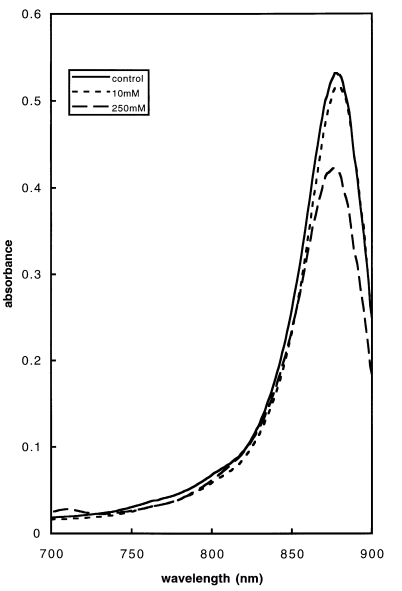

Fig. 2. The effect of chemical oxidation on the absorption spectrum of the LH1 complex. (———) No ferricyanide; (- - -) 10 mM ferricyanide; (—— ——) 250 mM ferricyanide. For illustrative purposes, the OD of the LH1 sample for this figure was adjusted to ∼0.53 cm^{-1} compared to an OD of ∼0.17 cm^{-1} used for the fluorescence measurements. The bleaching effect is very small at low concentrations of ferricyanide. Addition of 250 mM ferricyanide results in ∼20% loss of absorbance at 880 nm.

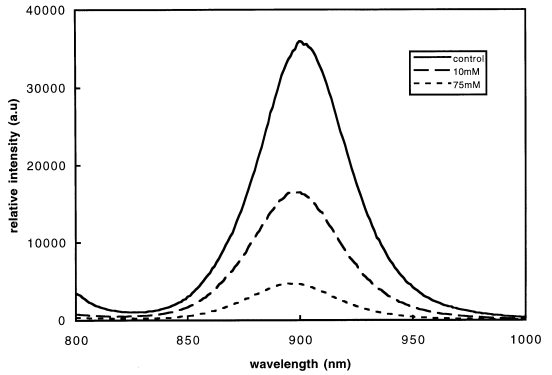

Fig. 3. The effect of chemical oxidation on the fluorescence emission spectrum of the LH1 complex. (———) No ferricyanide; (— — —) 10 mM ferricyanide; (- - -) 75 mM ferricyanide. Fluorescence intensity decreases to ~50% of its original value upon addition of 10 mM ferricyanide. Addition of 75 mM ferricyanide to the sample reduces fluorescence intensity to ~13% of the original.

ricyanide induced bleaching was not very extensive even at 250 mM ferricyanide (~20% loss after 2 min).

The chemical oxidation of the LH1 complex was found to be time dependent and required ~40 min to go to completion (data not shown). For example after 40 min in the presence of 100 mM potassium ferricyanide the 880-nm absorption band of the LH1 complex decreased to 28% of its original value. Additionally, in contrast to measurements performed over a 2-min period, there was a concomitant 22 nm blue-shift of the λ_{max} from 880 nm to 858 nm. We also observed a slow photobleaching of the LH1 complex, in the absence of potassium ferricyanide, caused by the measuring beam of the spectrophotometer (data not shown). After 30 min exposure to this light ~26% of the original absorbance at 880 nm was lost. Therefore, both absorption and fluorescence emission spectra were measured under identical conditions over the same time period of 2 min immediately after addition of potassium ferricyanide.

The effect of chemical oxidation on the fluorescence emission intensity of the LH1 complex is shown in Fig. 3. A significant quenching of fluorescence intensity can be observed even in the presence of relatively low concentrations of potassium ferricyanide. The quenching increases as the concentration of potassium ferricyanide in the sample increases. Unlike the absorption of LH1 its fluorescence is very sensitive to chemical oxidation. Even as little as 10 mM ferricyanide causes the loss of about 50% of the original fluorescence intensity. A progressive blue-shift of the fluorescence λ_{max} accompanies the quenching effect. The λ_{max} shifts by 6 nm from 901 nm in the absence of potassium ferricyanide to 895 nm in the presence of 75 mM potassium ferricyanide.

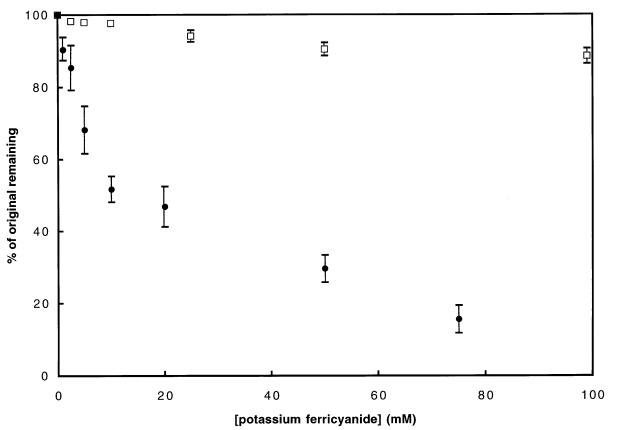

Fig. 4. Comparison of the effect of chemical oxidation on the transmission (open squares) and fluorescence emission intensity (filled circles) of the LH1 complex from *Rh. marinum*. The change in the transmission of the LH1 complex is very small compared to the dramatic effect chemical oxidation has on the fluorescence intensity of the complex. The data represent the arithmetical mean ± S.D. of five separate measurements.

The concentration dependence of chemical oxidation by potassium ferricyanide on the transmission (squares) and fluorescence emission intensity (filled circles) of the LH1 complex is shown in Fig. 4. In order to compare two linear scales the absorbance changes in Fig. 4 have been transformed to 1-transmission. Each data point in this figure is the mean of 5 measurements. 50% of the fluorescence is quenched at a ferricyanide concentration of 10 mM, while at this ferricyanide concentration only about 2–3% of the original transmission is lost.

4. Discussion

Mild chemical oxidation of the LH1 antenna complex dramatically quenches its fluorescence at concentrations of added ferricyanide that only bleach its Q_y absorption band by 2–3%. Assuming that a single LH1 molecule contains 32 BChl *a* molecules [11,12] and that these contribute equally to the 880-nm absorption band, oxidation of about only one BChl *a* molecule of the 'ring' is sufficient to produce about a 50% quenching of the fluorescence intensity. However, due to the very small changes in absorbance which were observed at these low concentrations of ferricyanide a more exact quantitation of how many BChl *a* molecules that must be oxidised per 'ring' to produce complete fluorescence quenching is not possible. Nevertheless, our data clearly show that the fluorescence properties of the LH1 antenna are shut down by the chemical oxidation of a very small fraction of the total number of BChls in the LH1 ring. This observation is consistent with the results of single molecule spectroscopy experiments performed on LH2 complexes from *Rps. acidophila* which suggested that photobleaching of BChl in the LH2 ring caused fluorescence to switch off completely [18]. It appears therefore that the presence of a single oxidised BChl molecule in the LH1 or LH2 ring (a single quencher) can effectively quench the fluorescence of the complete ring. These results demonstrate the possibility of redox control of energy transfer. It is not clear whether this occurs in purple bacteria. However, measurements in chlorosomes of the green sulfur bacterium *Chlorobium tepidum* have shown that their fluorescence lifetime is redox dependent and that this effect may be due to a few oxidised BChl *c* molecules [21]. Certainly, control of the fluorescence lifetime (and therefore energy transfer) by redox potential is a very effective mechanism for switching off and on energy transfer.

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