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Characterisation of the LH2 spectral variants produced by the photosynthetic purple sulphur bacterium *Allochromatium vinosum*

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ABSTRACT

This study systematically investigated the different types of LH2 produced by *Allochromatium* (*Alc.*) *vinosum*, a photosynthetic purple sulphur bacterium, in response to variations in growth conditions. Three different spectral forms of LH2 were isolated and purified, the B800-820, B800-840 and B800-850 LH2 types, all of which exhibit an unusual split 800 peak in their low temperature absorption spectra. However, it is likely that more forms are also present. Relatively more B800-820 and B800-840 are produced under low light conditions, while relatively more B800-850 is produced under high light conditions. Polypeptide compositions of the three different LH2 types were determined by a combination of HPLC and TOF/MS. The B800-820, B800-840 and B800-850 LH2 types all have a heterogeneous polypeptide composition, containing multiple types of both α and β polypeptides, and differ in their precise polypeptide composition. They all have a mixed carotenoid composition, containing carotenoids of the spirilloxanthin series. In all cases the most abundant carotenoid is rhodopin; however, there is a shift towards carotenoids with a higher conjugation number in LH2 complexes produced under low light conditions. CD spectroscopy, together with the polypeptide analysis, demonstrates that these *Alc. vinosum* LH2 complexes are more closely related to the LH2 complex from Phs. molischianum than they are to the LH2 complexes from Rps. acidophila.

1. Introduction

Allochromatium (*Alc.*) *vinosum* is a purple sulphur bacterium, formerly called *Chromatium vinosum* strain D, which has multiple *puc*BA and *puf*BA genes (the genes that encode the LH2 and LH1 apoproteins, respectively) [1]. It has been well documented that *Alc. vinosum* can produce a variety of spectral forms of light-harvesting proteins that are now known as LH1 and LH2 (see Table 3 in Thornber et al., 1970 [2]). Absorption maxima in the near-infrared (NIR) range of both photosynthetic membranes and isolated light-harvesting complexes have been reported at 800, 820, 840, 850, and 885–895 nm (e.g. [3–12]). The appearance of specific spectral forms of the light-harvesting complexes varies depending on a range of environmental factors, such as light intensity, temperature and type of reduced sulphur compound present in the growth media.

Most of the early work on *Alc. vinosum* was done before the structures of the light-harvesting complexes had been determined and before the *puf*BA and *puc*BA genes had been identified and sequenced. In general, we now have a quite detailed understanding of the structure and function of purple bacterial light-harvesting complexes. This understanding has been obtained through a combination of crystallography and various time-resolved spectroscopic methods. The structural information available for LH2 complexes is much more detailed than that available for the LH1 complexes. Typically, for example, the LH2 from *Rhodopseudomonas* (*Rps.*) *acidophila* is a ring composed of nine repeating heterodimer subunits (nonamer) [13]. Each subunit comprises two hydrophobic proteins, termed α and β, which form a transmembrane heterodimer that non-covalently binds three molecules of bacteriochlorophyll *a* (Bchl *a*) and one molecule of carotenoid. Within the complex, the α and β apoproteins orient the BChl *a* molecules and package them into two distinct groups. The first group consists of nine monomeric BChl *a* (one per αβ pair), which have maximum of their Q_y absorption

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at 800 nm. The second group consists of 18 tightly coupled BChl *a* (two per each αβ pair), which give rise to the NIR absorption band at approximately 850 nm. The position of the NIR absorption band of this second group depends on two factors. The first is the site energy of each individual BChl *a* molecule and the second is how all of the BChl *a* molecules in this group interact excitonically [14]. Changes in either of these factors can result in significant shifts of their absorption bands. For example, in the LH2 from *Rps. acidophila* strain 7050, the absorption bands in the NIR range can shift from 850 to 820 nm under low light conditions [15]. The B800-820 LH2 is more effective at low light conditions than the B800-850 complex since there is a restriction on the back transfer of energy from LH1 to LH2 (larger energy gap) [16]. The shift observed in the absorption spectrum is due to changes in two key amino acid residues of the α apoproteins (αTyr 44 and αTyr 45), which have important roles in H-bond formation with the acetyl groups of the B850 BChl *a* [17–20]. When these change to αPhe 44 and αLeu 45, which do not form H-bonds, the site energies of the tightly coupled BChl *a* molecules are blue shifted. In the LH2 from *Rhodobacter* (*R.*) *sphaeroides*, replacement of αTyr 44 and αTyr 45 with αPhe 44 and αLeu 45, using site directed mutagenesis, led to a blue shift from 850 to 826 nm [19]. The crystal structure of the LH2 from *Rps. acidophila* strain 7050 showed that the residues present at these sites do not form H-bonds, causing the BChl *a* acetyl groups to be rotated out of plane with respect to the macrocycle [20]. In the B800-850 LH2, the BChl *a* acetyl groups are held in plane with the macrocycle; this adds a double bond to the conjugation, that red shifts the Q_y absorption [13,20]. In the B800-820 form, the rotation of the acetyl groups out of plane with the macrocycle reduces the conjugation, causing a relative blue shift of the BChl *a* site energies and thus, the NIR absorption band [20]. The structure of exciton absorption band of the B800-820 form is the same as in the B800-850 form [14]. All that has changed is the starting site energies of the BChl *a* molecules. These changes in the site energies of the BChl *a* molecules do not affect the symmetry of the LH2 complex. The ring is still homogeneous. However, in some species, alterations in the type of α and β apoproteins within a single LH2 ring can occur, leading to heterogeneous rings [21,22]. In heterogeneous rings, the distribution of the BChl *a* site energies is no longer symmetrical and this changes the exciton band structure, which in turn alters the absorption spectra [21,22]. LH2 complexes from *Rhodopseudomonas palustris*, for example, have been shown to have mixed αβ apoprotein compositions under both high light and low light conditions [21,22]. Brotosudarmo et al. [21,22] showed that the tightly coupled BChl *a* s in the low light LH2 rings had both B820 and B850-like site energies. The ring size appears to be the same but now the symmetry has changed [21,22], while nonameric rings appear to be the most common. EM and AFM studies have shown that *R. sphaeroides*, *Rhodovulum sulfidophilum* and *Rubrivivax gelatinosus* all have nonameric LH2 complexes, while octameric LH2 complexes also exist and were found in *Phaeospirillum molischianum* [23].

Recently, a few studies have focussed on the light-harvesting complexes present in *Alc. vinosum* [11,12,24,25]. The one exception is the large body of work that has been carried out on *Alc. minutissimum* (e.g. [26–33]). These studies have focussed largely on the effect of inhibition of carotenoid synthesis on LH2 assembly and on the reconstitution of carotenoids into carotenoid-depleted LH2 complexes. The cultures have been grown under a single set of conditions, specifically under high light at 25 °C, or 30 °C in Krikunova et al. [26], and have thus produced only one LH2 type, with Q_y absorption at 850–855 nm [30,33]. Interestingly, even though this species has yet to have its genome fully sequenced, it is reported to have 98.4% sequence identity with *Alc. vinosum* [1] and comparison of 16S rRNA sequences from *Alc. vinosum* and *Alc. minutissimum* indicates that these may in fact be the same species.

Alc. vinosum has 6 pairs of *puc* BA genes, which, in principle, allows a remarkable degree of plasticity in its LH2 structure and opens the possibility of both homogeneous and heterogeneous rings being present. It is clearly necessary to carefully re-examine the different types of the LH2 produced by *Alc. vinosum* and to try to understand the structural basis for these variants.

In this study, we set out to investigate the different types of LH2 produced by *Alc. vinosum*. Cultures of *Alc. vinosum* were grown under a range of conditions (low light, high light or high temperature with either thiolsulphate or sulphide as the reduced sulphur source) and the LH2 complexes produced were isolated and analysed spectroscopically and characterised by HPLC and tandem MS.

2. Materials and methods

2.1. Culture growth and LH2 complex preparation

Cultures of *Alc. vinosum* strain D were grown anaerobically in the light in the following conditions: high light, HL (70–80 μmol s^{-1} m^{-2}) at 30 °C (HL30), low light, LL (2 μmol s^{-1} m^{-2}) at 30 °C (LL30) or high light at 40 °C (HL40) with either sulphide (S) or (T) thiolsulphate as the electron donor. The composition of the growth media used was as described by Hayashi et al. [9] and Malik [34]. Typically, after 2-day growth, cells were harvested by centrifugation, then broken, in the presence of $MgCl_2$ and DNAase, by three passages through a French press at 15 Kpsi. The unbroken cells and cell wall material were removed by a low speed centrifugation for 10 min at 2700 ×*g*. The photosynthetic membranes were then pelleted by centrifugation for 1 h at 208,000 ×*g* and re-suspended in 20 mM Tris–HCl, pH 8.0. The membranes were adjusted to an optical density (OD) of 25 at the NIR maxima and solubilised at room temperature with dodecyl-β-D-maltoside (DDM) at 2% (w/v), gently agitating, for 90 min. Any unsolubilised material was then pelleted by centrifugation for 20 min at 20,200 ×*g*. The solubilised light-harvesting complexes were loaded onto sucrose density gradients at an OD of 25 at the NIR maxima and centrifuged at 208,000 ×*g* for 12 h to separate RC-LH1 'core' and LH2 complexes. The sucrose gradient was set to 0.6, 0.8, 1.0, and 1.2 M (at 3, 8, 8 and 3 ml, respectively) in Tris–HCl buffer containing 0.02% DDM. The two light-harvesting bands were removed and, where necessary, further purified by ion exchange chromatography on a BioCad 700E perfusion chromatography workstation (Applied Biosystems), and size exclusion chromatography on a Superdex G200 column (GE Healthcare). Once solubilised, the light-harvesting complexes were maintained in 20 mM Tris–HCl, pH 8.0, which contained 0.02% DDM. It was observed that the precise position of the Q_y excitonic band varies with detergent. We attempted to solubilise membranes using a detergent in which the isolated complexes would best mimic their native conditions. DDM was used because it was a sufficiently strong detergent to solubilise the light harvesting complexes; it supported good separation of the RC-LH1 and LH2 complexes during sucrose density gradient centrifugation and it did not induce significant changes in positions of bands in the absorption spectra of separated light harvesting complexes in respect to their in vivo (in membrane) absorption.

2.2. Steady-state absorption and fluorescence spectroscopy

For the room temperature steady-state absorption and fluorescence measurements, membrane and LH2 samples were diluted in 20 mM Tris–HCl buffer, pH 8.0 (and 0.02% DDM for LH2 samples) to sample ODs of 1 and 0.1 at the NIR maximum, respectively. Samples for the low temperature (77 K) absorption measurements were diluted to an OD 1 at with 50% glycerol (v/v) in Tris–HCl buffer, pH 8.0 (containing 0.02% DDM for LH2 samples). Steady-state absorption spectra were recorded in 1 cm path length cuvettes using either Shimadzu 1700 or 1800 spectrophotometers and a Janis 100VNF cryostat (Janis, USA). Room temperature steady-state fluorescence measurements were recorded on a SPEX Fluorolog 2 1681 (Horiba Scientific, UK). The

fluorescence spectra were measured at right angles to the excitation beam, with a bandpass of 4 nm, and corrected for the instrument response.

2.3. Circular dichroism (CD) spectroscopy

CD spectra were recorded at room temperature for the purified 820 and 850 type LH2 complexes from *Alc. vinosum* together with a purified LH2 sample from *Rps. acidophila* 10050 for comparison. Samples were diluted to an OD of 4, at their NIR maximum, in 20 mM Tris–HCl buffer, pH 8.0 (containing 0.02% DDM). Measurements were recorded in 0.2 cm cuvettes using a J-810 spectrophotometer (JASCO) with NIR slit widths of 60 μm.

2.4. Carotenoid composition in purified LH2 complexes

Carotenoids were extracted from purified LH2 complexes with an acetone/methanol solution (7:2, v/v). A 100 μl aliquot of purified LH2 complex (OD 100 at NIR maximum) was added to 10 ml acetone/methanol (7:2) solution and mixed vigorously. Approximately 3 ml of petroleum (PET) ether (40–60 °C b.p.) was added and mixed through the solution with a glass pipette. This was followed by the addition of 10 ml of warm salty water. Carotenoids were preferentially partitioned into the PET ether layer, which was removed, washed with a further 10 ml of warm salty water and then pipetted into a fresh tube and dried under a stream of N_2. The dried carotenoid mixture was redissolved in 210 μl of ethyl acetate, to which 90 μl of acetonitrile/H_2O (9:1) was subsequently added. The mixture was spun in a bench microcentrifuge for 10 min and 200 μl of supernatant was then pippetted into an HPLC vial. Carotenoid extracts were analysed on a Waters Alliance e2695 HPLC system (Waters Ltd., Hertfordshire, UK) employing a reverse phase spherisorb ODS 2 C18 column (4.6 × 250 mm, Waters Ltd., Hertfordshire, UK). The gradient was adapted from Makhneva et al. [30]. Initial conditions were set at 77% solution A (acetonitrile/H_2O (9:1), 0.01% triethylamine) and 23% solution B (ethyl acetate). A linear gradient was begun at 15 min. Solution B was increased to 70% B over 15–30 min, then increased to 100% B at 30–40 min and held at 100% B until 45 min. A flow rate of 1 ml min^{-1} was maintained throughout. Chromatograms were recorded between 350 and 650 nm with a Waters 2998 PDA detector (Waters Ltd., Hertfordshire, UK). Peaks were integrated using Empower 3 software (Waters Ltd., Hertfordshire, UK) and compared with known reference carotenoid standards. The peak area of each carotenoid (measured at a wavelength for which an extinction coefficient was available for that particular carotenoid) was divided by the relevant extinction coefficient to determine the relative proportion of each carotenoid. These wavelengths were 470 nm for rhodopin and lycopene, 475 nm for spirilloxanthin and 482 nm for anhydrorhodovibrin. Extinction coefficients were calculated for each of the detected carotenoids by dissolving equal amounts of dried carotenoid in a solution for which an extinction coefficient had previously been reported and in a solution of HPLC mobile phase (specifically A/B (70:30) as this was the composition of mobile phase at which the carotenoids eluted).

2.5. SDS PAGE

SDS PAGE was used to resolve the polypeptides present in the photosynthetic membranes and isolated LH2 complexes from *Alc. vinosum* cultures grown under each set of growth conditions. Membrane samples (final OD 3 at the NIR maxima) were solubilised in 100 mM dithiothreitol (DTT) and Laemmli buffer (at 2% SDS) and heated for 20 min at 70 °C. Sucrose gradient samples were heated for 5 min at 90 °C in 50 mM DTT and 1× Laemmli sample buffer, and loaded at an OD of 1.5. SDS PAGE was performed on BioRad mini protean Any KD TGX gels (BioRad, UK) with BioRad precision plus dual Xtra protein standards (BioRad, UK), and stained with SimplyBlue SafeStain (Life Technologies).

2.6. Polypeptide composition in purified LH2 complexes

The α and β polypeptides were extracted from purified LH2 complexes, followed by separation with reverse-phase HPLC and MALDI TOF/MS measurement using the same instruments and method as described elsewhere [35]. For HPLC, the detection wavelength was set at 280 nm. In order to quantify the polypeptide composition from HPLC, we used four LH complexes with relatively simple compositions and well-separated peaks on the chromatogram as references for the calibration to test efficiency of the extraction and reliability of the calculation. These were the LH1 (single αβ pair) from *Rhodospirillum rubrum*, LH1 (single αβ pair) and LH2 (three αβ pairs) from *Thermochromatium (Tch.) tepidum* and an LH1 (three αβ pairs) from *Alc. vinosum*. The compositions were calculated from the peak areas using the base-line subtracted chromatogram, and subsequently corrected for the numbers and extinction coefficients of Trp ($\varepsilon_{280} = 5690\ M^{-1}\ cm^{-1}$) and Tyr ($\varepsilon_{280} = 1{,}280\ M^{-1}\ cm^{-1}$) residues in each polypeptide [36]. For the polypeptides that have same elution time and cannot be separated by HPLC, spectral counts of the TOF/MS were used for estimating the relative abundance, which was also calibrated by the LH reference samples.

3. Results

3.1. The effect of growth conditions on steady-state absorption spectra

Cells of *Alc. vinosum* were grown under the following conditions: SLL30, TLL30, SHL30, THL30, SHL40 and THL40, as described in Materials and methods. Room temperature absorption spectra of the photosynthetic membranes isolated from cells grown under these different conditions are shown in Fig. 1a. Taking absorption spectra of the membranes from SHL40 cultures as an example, Fig. 1a shows peaks at 370 (not shown) and 590 nm, which represent the Soret and Q_x bands BChl *a*, respectively, and peaks from 425 to 550 nm, representing carotenoid absorption band. In the NIR region, there are peaks at 806 and 855 nm, associated with the Q_y transition monomeric and excitonically coupled BChl *a* and at ~888 nm, associated with the Q_y transition of excitonically coupled BChl *a* bound in LH1 [14]. Photosynthetic membranes from THL40 show very similar absorption peaks to those produced under SHL40 growth conditions; however, membranes produced under the other growth conditions show remarkable differences in absorption in the NIR region. The SLL30 and TLL30 conditions produced photosynthetic membranes with NIR absorption bands having maxima at 806 nm and 823 nm (which, in the case of TLL30, is really just a shoulder) and ~890 nm, indicating the same spectral form of the LH1 is present but different LH2 types are being produced. THL30 conditions led to the membranes with NIR absorption bands at 806, 850 and ~889 nm, although the relatively low dip in the spectral line between the '800' and 850 nm peaks suggests significant absorption around 820 nm, as observed under low light growth conditions. SHL30 conditions produced membranes similar to those from THL30 conditions NIR absorption maxima at 806, 852 and ~889 nm and a shallow dip between the 806 and 852 nm peaks. It should be noted that although in most cases room temperature absorption spectra of the membranes show bands with maxima characteristic for LH1 Q_y (~890 nm), it was observed that the long wavelength absorption band of the LH1 can vary between 888 and 892 nm in some conditions and this warrants further investigation. For the SLL30 and TLL30 membranes, the absorption peak at 806 nm is substantially higher than that at 889 nm, while for SHL40 and THL40 membranes, the converse is true and the 806 nm peak is lower. This suggests that there is a higher ratio of LH2 to LH1 in photosynthetic membranes produced under low light conditions and demonstrates that relatively more LH2s are being produced under low light conditions. Dependence of the LH1:LH2

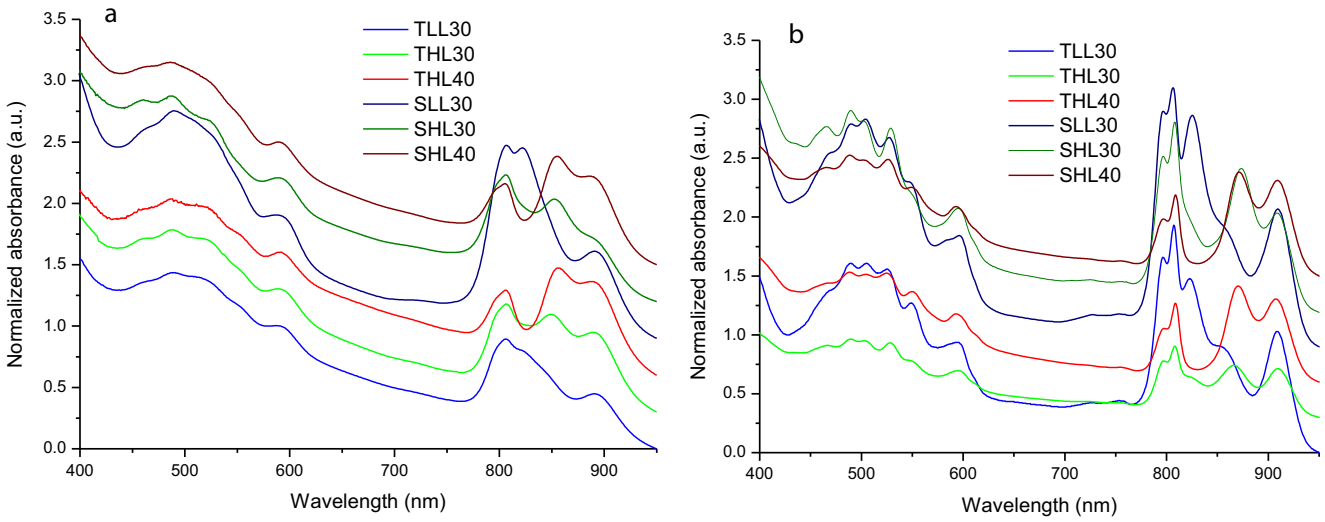

Fig. 1. Steady-state absorption spectra of photosynthetic membranes at (a) room and (b) low (77 K) temperature, from *Alc. vinosum* cultures grown under different conditions. Cultures were grown under low light at 30 °C (LL30), high light at 30 °C (HL30), or high light at 40 °C (HL40) with either thiosulphate (T) or sulphide (S). Spectra were normalised at the BChla Q_x band and then offset by 0.3 on the absorption axis for increased clarity. Actual absorbance values at the NIR absorption maximum ranged between 0.4 and 1.

ratio on light intensity is a common feature in many species of purple bacteria. In the case of HL30 membranes the absorption peak at 806 nm is higher than that at 889 nm, much more so for the SHL30 conditions than for THL30, but this difference is not as large as that observed for LL30 membranes.

Overall, these observations suggest that both light intensity and temperature can influence the LH2: LH1 ratio in photosynthetic membranes from *Alc. vinosum*. Since the room temperature absorption spectra shown in Fig. 1a are so congested in the NIR region, measurements were also performed at low (77 K) temperature (Fig. 1b). At 77 K the B800 band narrows and the B850 bands both narrow and red-shift. In general, 77 K absorption spectra demonstrate increased resolution and individual bands are clearly separated. The broad ~800 nm peak, observed for all growth conditions at room temperature, splits into two separate bands peaking at about 797 and 807 nm (808 nm for HL30 and HL40) at 77 K. The relative amplitudes of these two peaks vary with the growth conditions. The HL40 growth conditions lead to a higher 807:797 ratio than other growth conditions.

At 77 K the Q_y band of excitonically coupled BChl *a* BChl *a* is always shifted to longer wavelengths (red-shift). At 77 K the BChl *a* Q_y band in LH1 is red-shifted by about 20 nm and peaks at ~909 nm in all membrane samples. In SHL40, THL40 and SHL30 photosynthetic membranes there is a shift of B850 band (855 nm to 870 nm) upon lowering temperature to 77 K. In THL30 membranes the LH2 Q_y band observed at 850 nm in room temperature spectrum is shifted to red by 16–866 nm. In this case, an additional peak at 823 nm is revealed, which suggests the presence of an additional type of LH2 that was masked in the room temperature spectrum by the 850 nm band. In the low light membranes, there is no significant temperature-induced red-shift of the 823 nm band.

In order to investigate more precisely which types of LH2 complexes were present under the different growth conditions, the membranes were solubilised in detergent and fractionated into distinct LH1 and LH2 bands using sucrose density gradient centrifugation (Fig. 2). Previous studies had used a variety of different detergents to solubilise and purify the LH2 complexes from *Alc. vinosum*. In some cases, these detergents cause significant spectral shifts of absorption bands in the NIR region. In the present study, a range of detergents were tested for their suitability to both solubilise the light-harvesting complexes and to stabilise the resulting purified preparations. The detergent DDM was found to be the most suitable and in its presence the solubilised LH2 complexes show absorption spectra with peaks closest to those observed in the intact membranes. Fig. 2 shows results of typical sucrose gradient centrifugation of solubilised photosynthetic membrane where the LH1 and LH2 light-harvesting types have been well separated. The LH2 complex fraction was removed from these gradients and used in all of the subsequent experiments.

The Q_y absorption band of BChl *a* in the LH2 complex fractions is shown in Fig. 3, where room temperature and low temperature spectra are compared and details about positions of BChl *a* Q_y bands in respect to their counterparts in the membrane samples are listed in Table 1. At 77 K (Fig. 3b), the split 800 nm peak described above becomes even clearer with noticeable differences in the ratios of the individual peaks within the 800 nm band of the LH2 complexes between the different growth conditions. The ratio of 804 to 792 nm is higher for the LH2 complex fraction produced under HL40 conditions than for those produced under LL30 and THL30 conditions, but is highest for the LH2 fraction produced under SHL30 conditions. A split 800 nm peak has previously been observed in *Alc. vinosum* LH2 complexes [2,3,12,25,37] and, for a B800-850 type LH2 complex from *Alc. vinosum*, was recently

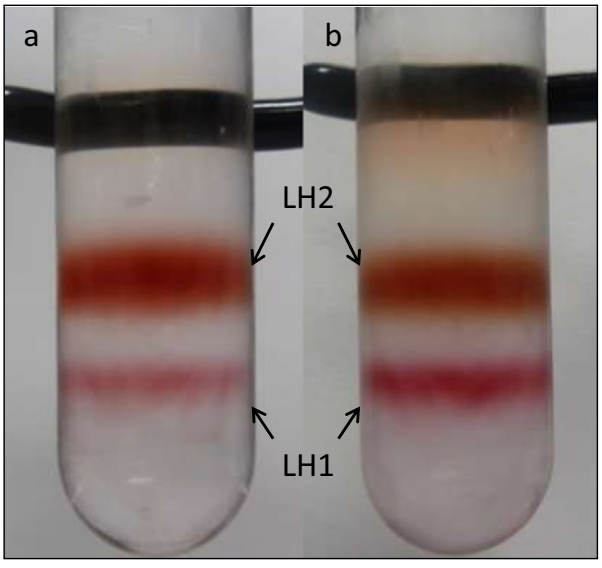

Fig. 2. The results of centrifuging solubilised photosynthetic membranes, using sucrose density gradients, from *Alc. vinosum* cultures grown under (a) low and (b) high light.

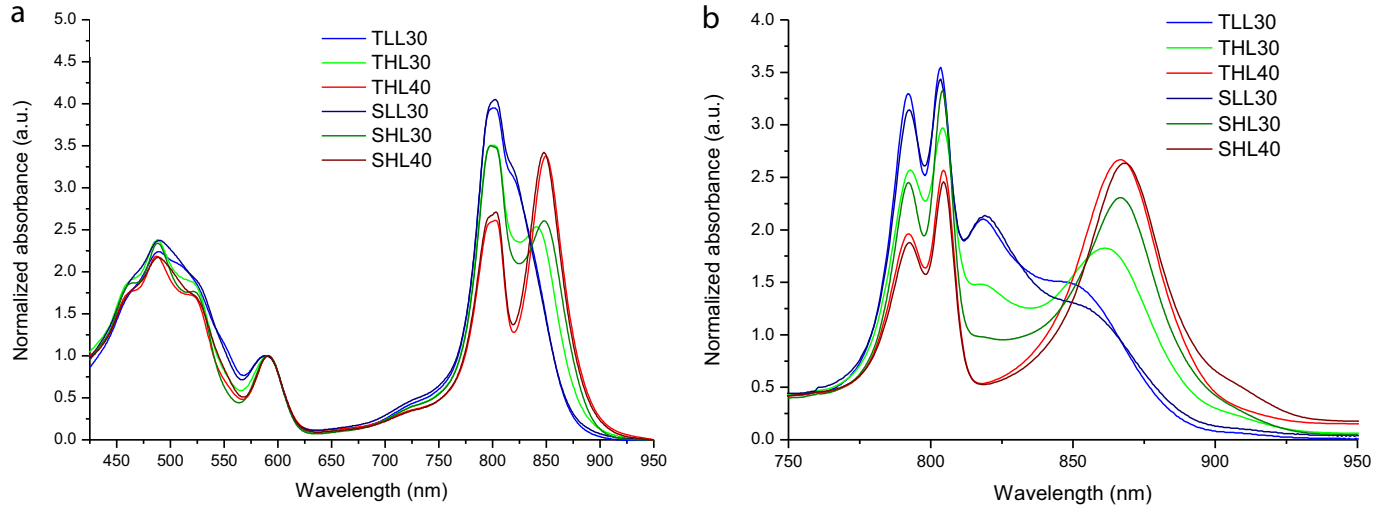

Fig. 3. Steady-state absorption spectra for the LH2 fractions from *Alc. vinosum* cultures grown under different conditions obtained by centrifuging of the solubilised membranes in sucrose gradients and recorded at (a) room and (b) low (77 K) temperature. Cultures were grown under low light at 30 °C (LL30), high light at 30 °C (HL30), or high light at 40 °C (HL40) with either thiolsulphate (T) or sulphide (S). Spectra are normalised at the BChl *a* Q_x band. Actual absorbance values at the NIR absorption maximum ranged between 0.4 and 1.

attributed to heterogeneity among the B800 BChl *a* within a single LH2 ring [12].

The narrower peaks in the low temperature spectra of LH2 complex fractions (Fig. 3b) indicate that under the LL30 and HL30 growth conditions at least two types of LH2 complexes are produced, while under HL40 growth conditions probably only one type of LH2 complex is present, the well-known 'B800-850' type. The LH2 complex fractions produced under SLL30 and TLL30 conditions have similar absorption spectra in the NIR, with a band at ~820 nm and a shoulder at ~850 nm (77 K), in addition to the ~800 nm peaks. Under the SHL30 and THL30 conditions, the LH2 complex fractions produced both have a small absorption at ~820 nm but differ significantly in the position of the main NIR absorption band, which is at 860 nm for THL30 conditions and at ~867 nm for SHL30 conditions. SHL40 and THL40 conditions produce LH2 complex fractions with similar NIR absorption at 867–868 nm (77 K) in addition to the ~800 nm peaks, and no absorption peak at 820 nm.

Since the situation is clearly complicated, we attempted to purify and identify the individual LH2 types present within each LH2 fraction using a combination of anion exchange and molecular sieve chromatography. These purification steps produced B800-820, B800-840 and B800-850 LH2 types (Fig. 4). It is not yet clear, however, whether all the individual LH2 complexes purified in this study represent distinct well-defined types of LH2 complex. If we assume that they do, then *Alc. vinosum* appears to produce B800-820, B800-840 and B800-850 type LH2 complexes, in different relative amounts depending on the precise growth conditions. These results are summarised in Table 2. For SHL30 cultures anion exchange chromatography of the LH2 complex mixture yields a set of fractions having a peak at 820 nm followed by a set of fractions having a peak at 850 nm, with a gradual shift from 820 to 850 nm across the fractions. Overlaying the NIR absorption spectra of these elution fractions shows a clear isosbestic point at 830 nm, suggesting the presence of only two distinct types of LH2, the B800-820 and the B800-850 type.

3.2. Steady-state fluorescence spectroscopy

Steady-state fluorescence emission and excitation spectra were collected at room temperature for the purified LH2 complexes produced by *Alc. vinosum* under the various growth conditions. Fluorescence emission spectra were recorded for purified LH2 complexes upon exciting into the carotenoid absorption band (Fig. 5). The B800-850 type LH2 complexes have a fluorescence maximum at 855 nm and a small rounded peak at 808 nm, while the B800-820 type LH2 complexes only have a single fluorescence maximum at ~ 830 nm. The absence of an observable emission from B800, for the B800-820 LH2, probably reflects an overlap of the emission from the B820. The B800-840 type LH2 complex purified from THL30 LH2 complex fraction had an emission maximum at 850 nm, with a small peak at 806 nm.

In order to compare the efficiency of excitation energy transfer (EET) from carotenoids to BChl *a* within purified B800-850 and B800-820 type LH2 complexes, transmission, %*T*, and fluorescence excitation measurements were conducted at room temperature and normalised at the Q_x band (as 100% EET is expected here). EET was calculated by division of excitation over 1 − *T* (where *T* stands for transmittance) at the second vibronic peak of the carotenoid absorption band. The carotenoid to BChl

Table 1
Positions of BChl *a* Q_y transitions in the LH2 complex fractions from solubilised membranes from *Alc. vinosum* under the growth conditions listed. 'S' and 'T' refer to sulphide and thiolsulphate media, respectively; 'LL' refers to low light conditions (2 μmol s^{-1} m^{-2}) and 'HL' refers to high light conditions (70–80 μmol s^{-1} m^{-2}); '30' and '40' refer to the temperature (in °C) of growth; (sh) indicates that the absorbance maximum is a shoulder rather than a distinct peak.

Growth condition	Positions of BChl *a* Q_y bands in the LH2 (nm)				Spectral shift toward shorter λs (for LH2 complexes in photosynthetic membranes) (nm)			
	RT		LT		RT		LT	
SLL30	802	818–819 (sh)	792, 804	819, 850 (sh)	6–7	2–5	4, 3	5, NA
SHL30	802	848–849	792, 804	866	2–5	3–4	4,4	5
SHL40	802	848	792, 804	868	3–4	7	4, 4	4
TLL30	802	818–819 (sh)	792, 804	819, 850 (sh)	6–7	3–4	4,3	5, NA
THL30	802	840–841	792, 804	818, 861–862	2–5	9	4, 3	NA, 4–5
THL40	802	849–851	792, 804	868	3–4	6	4,3	4

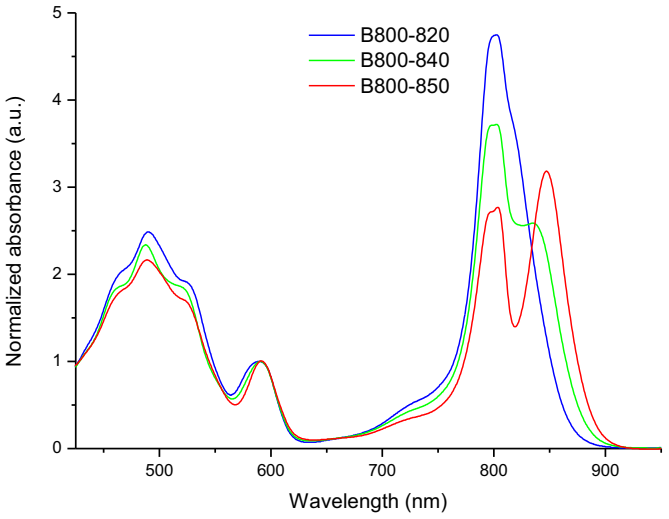

Fig. 4. Steady-state absorption spectra of purified LH2 complexes from *Alc. vinosum* cultures grown under different conditions. Cultures were grown under low light at 30 °C (LL30), high light at 30 °C (HL30), or high light at 40 °C (HL40) with either thiolsulphate (T) or sulphide (S). Spectra are normalised at the BChl *a* Q_x band. Actual absorbance values at the NIR absorption maximum ranged between 0.4 and 1.

a EET was very similar in all different types of LH2 complex: 50 and 53% for the TLL30 B800-820 and THL40 B800-850, respectively, and 47% for the THL30 B800-840.

3.3. Circular dichroism (CD) spectroscopy

The CD spectra recorded for the purified B800-820 and B800-850 type LH2 complexes from *Alc. vinosum* cultures are shown in Fig. 6, together, for comparison, with that from the purified LH2 from *Rps. acidophila* 10050. The relative directions of the transition dipole moments and the excitonic interactions of the BChl *a* within the LH complexes affect the magnitude and the sign (positive or negative) of the NIR CD and CD spectra thus provide information about the structure of these complexes [38,39]. The CD spectra of the *Alc. vinosum* LH2 complexes all show a similar S-shaped band with a pronounced positive peak at ~786 nm (the amplitude of which is lower for the B800-850 LH2 types than for the B800-820 LH2 types), followed by a zero crossing at 796 nm and a pronounced negative band. This negative band has a sharp minimum at ~807 nm for B800-850 and B800-840 type LH2 complexes, while for the B800-820 type LH2 complexes the negative band is significantly broader and centres around 820 nm. For the B800-850 type LH2 complexes from *Alc. vinosum* this S-shape band reaches close to zero at 838 nm (slightly crossing zero in the case of the SHL40 B800-850 LH2) and is followed by a second negative band with a minimum at ~ 864 nm. The magnitude of this red-most CD band is slightly greater for the SHL40 B800-850 than for the corresponding THL40 B800-850

Table 2
LH2 types present in *Alc. vinosum* cultures under the different growth conditions. NIR absorption maxima associated with Q_y transitions of BChl *a* in the LH2 complex fractions solubilised from membranes from cells of *Alc. vinosum* under the growth conditions listed. 'S' and 'T' refer to sulphide and thiolsulphate media, respectively; 'LL' refers to low light conditions (2 μmol s^{-1} m^{-2}) and 'HL' refers to high light conditions (70–80 μmol s^{-1} m^{-2}); '30' and '40' refer to the temperature (in °C) of growth.

Growth condition	LH2 type		
	B800-820	B800-840	B800-850
SLL30	*	*	
SHL30	*		*
SHL40			*
TLL30	*	*	
THL30	*	*	
THL40			*

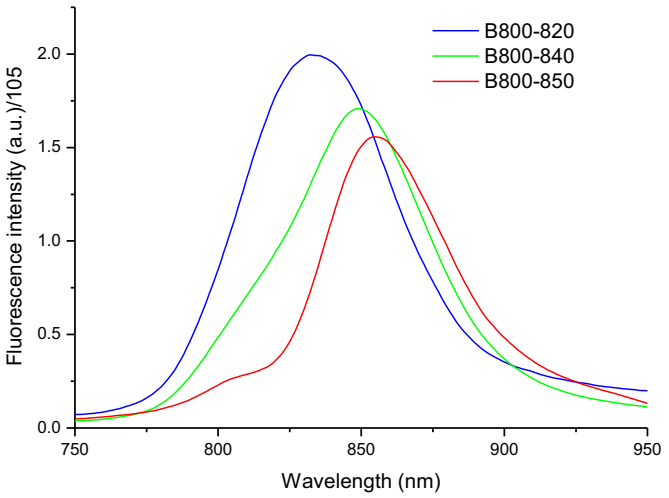

Fig. 5. Fluorescence emission spectra of the purified B800-820, B800-840 and B800-850 type LH2 complexes isolated from *Alc. vinosum* cultures grown with thiosulphate media.

LH2 type. For the B800-840 LH2 the red-most minimum occurs at ~855 nm. These CD spectra are markedly different to the CD profile that was simultaneously collected as a control for the well studied *Rps. acidophila* 10050 LH2, which has no positive band at 786 nm but has a pronounced negative band at 795 nm followed by a S-shape band at 860 nm (with a positive maximum at 850 nm and a negative minimum at 872 nm). The *Rps. acidophila* CD spectrum determined in this study agrees well with previously reported CD spectra for *Rps. acidophila* 10050 LH2 (e.g. [38,39]) and is similar to CD spectra recorded for HL B800-850 type LH2 complexes from *Rps. acidophila* 7750, *Rps. palustris* and *Rps. cryptolactis* [39]. The negative band at 796 nm has previously been attributed to the B800 BChls *a* and the S-shape band to the tightly coupled B850 BChls *a* [38,40,41]. The results of the present study suggest that the organisation of the BChls *a* in *Alc. vinosum* LH2 complexes differs from that in the LH2 complex from *Rps. acidophila* 10050.

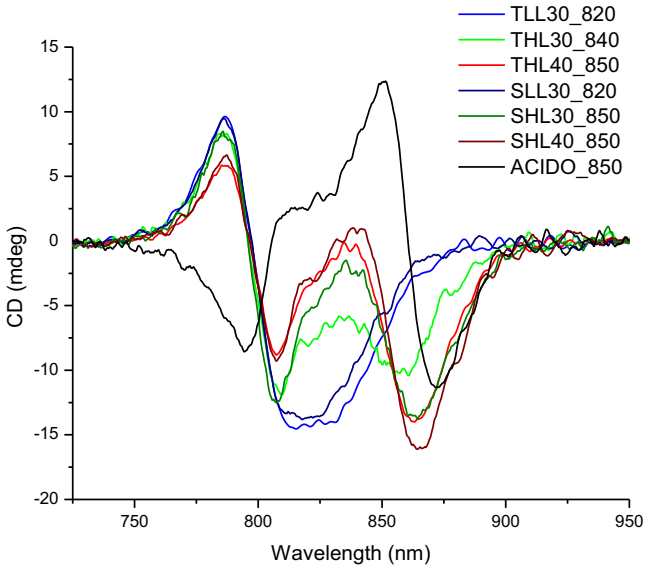

Fig. 6. Circular dichroism (CD) spectra of the B800-820, B800-840 and B800-850 type LH2 complexes isolated from *Alc. vinosum* cultures, together with typical B800-850 LH2 isolated from *Rps. acidophila* 10050.

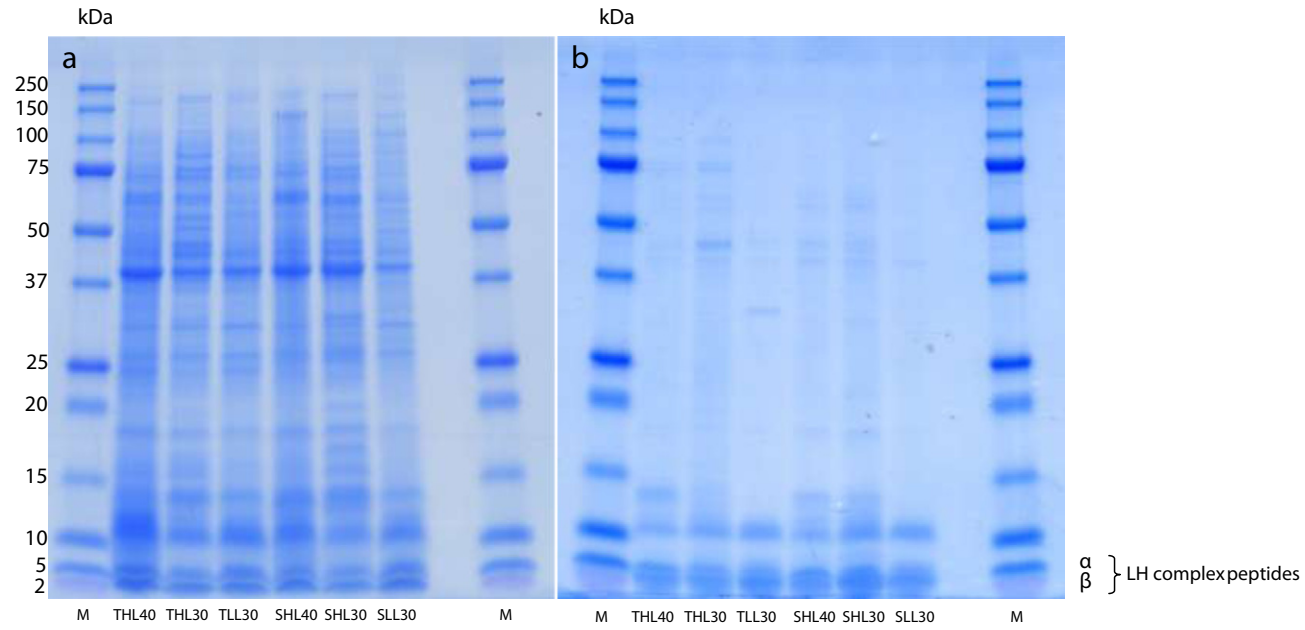

Fig. 7. SDS polyacrylamide gels of (a) membranes and (b) LH2 fractions isolated from sucrose gradients of the solubilised membranes, from *Alc. vinosum* cultures grown under different conditions. Cultures were grown under low light at 30 °C (LL30), high light at 30 °C (HL30), or high light at 40 °C (HL40) with either thiolsulphate (T) or sulphide (S). Samples were loaded at concentrations of either OD 3 at the NIR absorption maxima (membranes) and at OD 1.5 (sucrose gradient fractions). 'M' refers to the protein marker, specifically BioRad precision plus dual Xtra protein standards (BioRad, UK).

3.4. Carotenoid compositions

A reverse phase HPLC analysis of crude carotenoid extracts from purified LH2 complexes indicates that under all growth conditions the LH2 complexes produced by *Alc. vinosum* have a mixed carotenoid composition and contain carotenoids of the spirilloxanthin series, specifically spirilloxanthin, rhodopin, anhydrorhodovibrin and lycopene. HPLC chromatograms for carotenoid extracts from the B800-820, B800-840 and B800-850 LH2 types are shown in Fig. 8. This finding is in agreement with previous reports [1,12,42–45]. The relative proportions of these carotenoids vary among the different LH2 types (Table 3). In all case the most abundant carotenoid is rhodopin; however, the relative amount of rhodopin is higher in the B800-850 than in the B800-820 and the proportion of the longer chained spirilloxanthin is lower in the B800-850 than in the B800-820. The carotenoid mixture in the B800-840 LH2 type represents something of a midway point between that of the B800-850 and that of the B800-820 LH2 types. The differences observed in the carotenoid region of the absorption spectra of purified LH2 complexes are therefore result of a change in carotenoid composition to carotenoids with different numbers of conjugated double bonds. There is a shift towards carotenoids with higher conjugation number under low light.

3.5. SDS PAGE gels

SDS PAGE gels of membranes and of the LH2 fractions isolated from sucrose gradient centrifugation of the solubilised membranes are shown in Fig. 7a and b. Denaturation of the LH2 complexes yields multiple bands in the region of alpha/beta apoproteins, indicating that multiple α and β peptides are present for each growth condition. There appears to be no real difference in these bands between the different growth conditions; however, the resolution on these gels is insufficient to clearly separate the individual α and β polypeptide bands. Therefore we extracted the polypeptides from the purified LH2 complexes and analysed them using HPLC and TOF/MS.

3.6. Polypeptide analysis

There are six *puc*BA genes, each of which code for distinct α and β polypeptides, in *Alc. vinosum* (Fig. 9). The HPLC elution profiles for the peptide extracts obtained from the purified *Alc. vinosum* LH2 complexes are shown in Fig. 10. The elution patterns are similar to those of the LH1 and LH2 polypeptides from other purple bacteria, where the β polypeptides are eluted first and followed by the α polypeptides [35,46–50]. Peptide mass assignments based on the TOF/MS result for each of the HPLC peaks are shown in Table 4. The error range in the TOF/MS detected masses is ±2.0. The B800-850 sample shows six major polypeptide components. These are α1, α2, α3 and β1, β2, and β3. The α1 polypeptide eluted much earlier than other α polypeptides due to its relatively lower hydrophobicity and the large difference in the amino acid sequence from other α polypeptides (see Table 4). The β2 and β3 polypeptides cannot be separated under the current experimental conditions. The B800-820 type LH2 complex shows 4 major peaks in the HPLC trace, which correspond to α1, α2, α3, α5 and α6, and to β1, β2, β3, β5 and β6. In comparison to the B800-850 sample, the relative amounts of α1, α2 and α3 are reduced. The α5 polypeptide eluted at a similar time to the α1 polypeptide due to their high sequence identity (Fig. S1 and S3, Supplementary Information). The B800-840 type LH2 complex contains six major peaks in the HPLC trace. These correspond to α1, α2, α3 and α6, and β1, β2, β3 and β5. Interestingly, the B800-840 lacks α5 and β6. Five pairs of the gene products were identified from the LH2 complexes. Polypeptides corresponding to the *puc*B4A4 genes were not observed. The TOF/MS data clearly shows that most of the LH2 polypeptides are translationally modified when compared with their gene sequences (Table 4). The most striking feature in the *Alc. vinosum* LH2 polypeptides is that the major components, α1 in B800-850 and α5 in B800-820 (see below), undergo progressive and extensive C-terminal processing, resulting in many intermediate products of the degradation. All α polypeptides except for α5 have their N-terminal Met residues removed, and all β polypeptides except for β3 and β6 are N-terminally methylated. In order to be able to make any conclusions about which polypeptides give rise to which spectroscopic

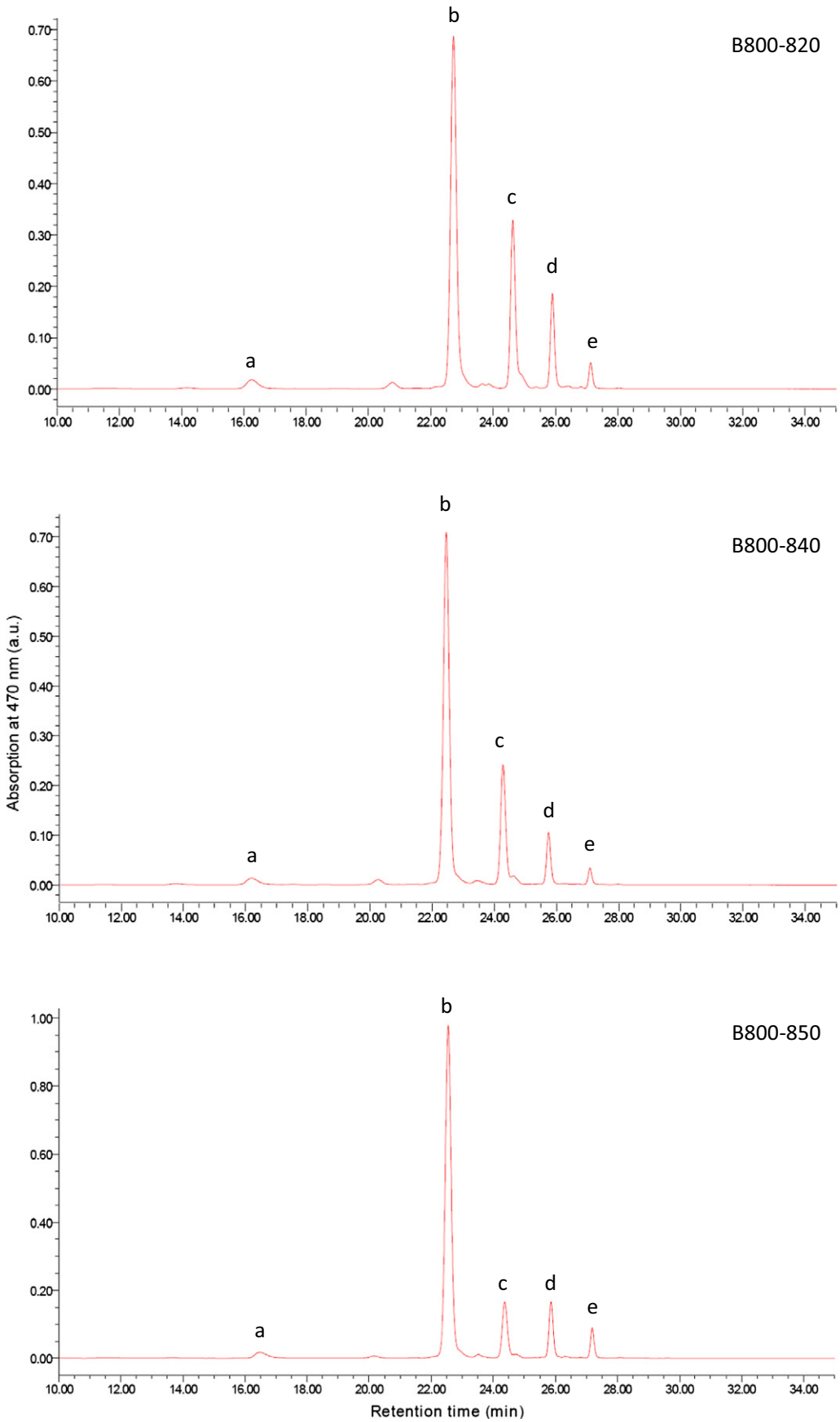

Fig. 8. HPLC chromatograms showing the mixed carotenoid composition present in the carotenoid extracts from the purified LH2 complexes of *Allochromatium vinosum*. The peak at around 16 min (a) represents Bchl *a*. The primary carotenoids detected were rhodopin (b), spirilloxanthin (c), anhydrorhodovibrin (d) and lycopene (e).

Table 3
Percentage composition of the primary carotenoids detected, by HPLC analysis, in the LH2 complexes of *Allochromatium vinosum* (mean ± SE, $n = 3$). To determine the percentage composition the peak area of each carotenoid (measured at a wavelength for which an extinction coefficient was available for that particular carotenoid) was divided by the relevant extinction coefficient. These wavelengths were 470 nm for rhodopin and lycopene, 475 nm for spirilloxanthin and 482 nm for anhydrorhodovibrin.

Carotenoid	*n*	B800-820	B800-840	B800-850
Rhodopin	11	47 ± 1	66 ± 4	70 ± 2
Spirilloxanthin	13	40 ± 2	22 ± 4	19 ± 1
Anhydrorhodovibrin	12	12 ± 1	8 ± 1	7 ± 3
Lycopene	11	2 ± 0.1	4 ± 1	4 ± 0.4
Total		100	100	100

form seen, it is important to quantify the polypeptide data. This can be calculated from the HPLC data using the extinction coefficients based on the number of tryptophan, tyrosine and phenylalanine residues present in the relevant polypeptide sequences [36]. This method was tested using purple bacterial LH complexes with known polypeptide compositions. This calibration as described in Materials and methods showed that the molar compositions calculated from the HPLC chromatograms can be used to quantify the amounts of the different polypeptides present with an error ± 5% error (Fig. S2, Supplementary Information). The polypeptide compositions for different types of the *Alc. vinosum* LH2 determined in this way are shown in Table 5. In B800-850, the α1 accounts for about half of the α polypeptides present, with α2 and α3 making up the rest. The composition of the β polypeptides is more uniform. In B800-820, the α5 and α6 are the major components and are present in similar proportions, whereas the β5 accounts for about half of the β polypeptides. The relative abundance of the other β polypeptides (β1, β2, β3) is similar across the different LH2 types. In the B800-840 type LH2 complex, the major α polypeptides are α1 and α2 and the major β polypeptide is β5. The extensive C-terminal modifications of α1 and α5 may lead to errors in the estimation of the α polypeptide composition. For this reason, the actual proportions of the total α1 and α5 polypeptides should probably be higher than those we were able to measure in this study.

4. Discussion

The multiple LH2 polypeptides of *Alc. vinosum* exhibit diverse expression patterns in response to the changes in their growth conditions such as light intensity and the source of reduced sulphur. In this study we have been able to isolate and purify three different spectroscopic forms of LH2 from *Alc. vinosum*. The polypeptide composition of each of these forms has been determined by a combination of HPLC and

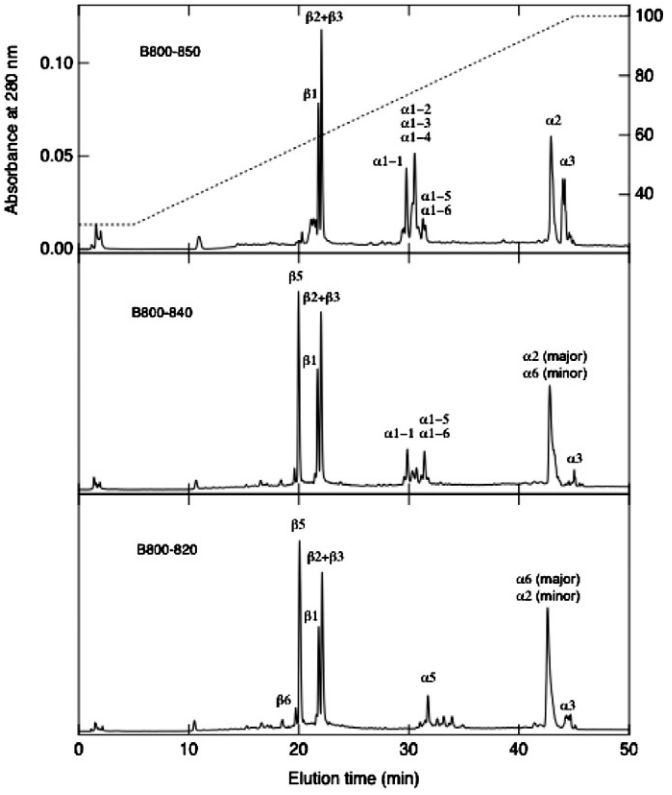

Fig. 10. HPLC chromatograms for the peptide extracts from purified B800-850 (top), B800-840 (middle) and B800-820 (bottom) type LH2 complexes from *Alc. vinosum*.

TOF/MS. In all cases, these different forms of LH2 contain multiple types of both α and β polypeptides and they all have, within the limits of error, a 1:1 ratio of α to β polypeptides. The α apoproteins are all post translationally modified. All but α5 have their N-terminal αMet removed, and α1 and α5 have various degrees of C-terminal truncation. The lack of the N-terminal αMet residue suggests that the B800 BChls *a* probably have their central Mg atom coordinated to the αAsp -28 residue as in the LH2 complex from *Phs. molischianum*. It is not clear that the variable C-terminal truncations are taking place in vivo or in vitro during their isolation and purification although similar behaviour has been reported for the LH2 α polypeptides in *Rubrivivax gelatinosus* [51,52] and *Tch. tepidum* [35]. Previous studies on species of purple bacteria that show B800-850 and B800-820 spectroscopic forms of LH2 have identified a pair of H-bonding residues in the sequence of the α

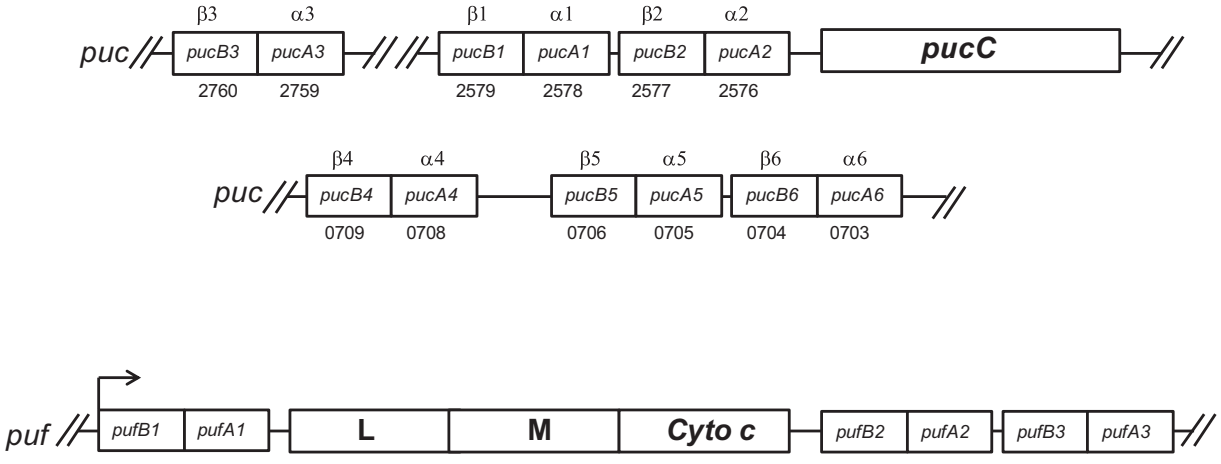

Fig. 9. A schematic of the placement of *puc*BA gene clusters in *Alc. vinosum*. The genes are designated as shown in the boxes. The corresponding polypeptides and the locus tag numbers starting with Alvin_ in the genome are shown above and below the boxes, respectively.

Table 4
The α and β polypeptide assignments for *Alc. vinosum* LH2 complexes based on the TOF/MS result for each HPLC peak. The error range in the TOF/MS detected masses is ±2.0. Most of the LH2 polypeptides experienced post-translational modification.

Designation	Sequence	M_w (cal.)	M_w (measured)	Distribution
Alvin_2578	MAIEFMGYKPLENDYKFWLVVNPATWLIPTLIAVALTAILIHVVAFDLEGQGWHAPAAEAVEAAPAAQ	7405.5		
α1-1	AIEFMGYKPLENDYKFWLVVNPATWLIPTLIAVALTAILIHVVAFDLEGQGWHAPAAEAVEAAPAAQ	7274.3	7274.2	B850
α1-2	AIEFMGYKPLENDYKFWLVVNPATWLIPTLIAVALTAILIHVVAFDLEGQGWHAPAAEAVEAAP	7004.0	7008.0	B850
α1-3	AIEFMGYKPLENDYKFWLVVNPATWLIPTLIAVALTAILIHVVAFDLEGQGWHAPAAEAVEA	6835.8	6838.0	B850
α1-4	AIEFMGYKPLENDYKFWLVVNPATWLIPTLIAVALTAILIHVVAFDLEGQGWHAPAAEAVE	6764.7	6765.1	B850
α1-5	AIEFMGYKPLENDYKFWLVVNPATWLIPTLIAVALTAILIHVVAFDLEGQGWHAPAA	6336.3	6337.4	B840,B850
α1-6	AIEFMGYKPLENDYKFWLVVNPATWLIPTLIAVALTAILIHVVAFDLEGQGWHAPA	6265.2	6264.0	B840,B850
Alvin_2576	MSDVAKPKNPEDDWKIWLVVNPATWLMPIFYALLVLAIAVHAVVFSVGLGWQ	5850.9		
α2	SDVAKPKNPEDDWKIWLVVNPATWLMPIFYALLVLAIAVHAVVFSVGLGWQ	5719.7	5719.6	B850,B840,B820
Alvin_2759	MSNVAKPRNPEDDWKIWLVVNPATWLMPIFYALLLLAIAVHAVVFSVGLGWQ	5891.9		
α3	SNVAKPRNPEDDWKIWLVVNPATWLMPIFYALLLLAIAVHAVVFSVGLGWQ	5760.7	5759.7	B850,B840,B820
Alvin_0708	MEVMGYKPQEQDYRFWLVVNPATWLMPILFAVLLIVLTVHVAVLSLGWFTWG	6080.2		
α4				(not observed)
Alvin_0705	MNIEFMGYKPLEQDHRFWMVVNPATWLMPILIAVALVAVLVHFYAFSLPGQGFSAAPAEAAPAAAAPAE	7457.6		
α5-1	MNIEFMGYKPLEQDHRFWMVVNPATWLMPILIAVALVAVLVHFYAFSLPGQGFSAAPAEAAPA	6947.1	6950.3	B820
α5-2	MNIEFMGYKPLEQDHRFWMVVNPATWLMPILIAVALVAVLVHFYAFSLPGQGFSAAPA	6507.6	6509.9	B820
Alvin_0703	MSDINKPSNPADDWKIWLVVNPSTWLMPILFAVLAIVLTVHITVLQLGWFTWG	6049.1		
α6	SDINKPSNPADDWKIWLVVNPSTWLMPILFAVLAIVLTVHITVLQLGWFTWG	5917.6	5918.7	B820,B840
Alvin_2579	MADMKSLSGLTEQQAKEFHEQFKVTYTAFVGLAALAHLFVIAANPWW	5298.0		
β1	N-(CH3)ADMKSLSGLTEQQAKEFHEQFKVTYTAFVGLAALAHLFVIAANPWW	5180.8	5178.5	B850,B840,B820
Alvin_2577	MAEQSLSGLTEQQAKEFHEQFKVTYTAFVGLAALAHLFVIAANPWW	5180.8		
β2	N-(CH3)AEQSLSGLTEQQAKEFHEQFKVTYTAFVGLAALAHLFVIAANPWW	5063.6	5064.0	B850,B840,B820
Alvin_2760	MASLSGLTDQQAKEFHEQFKVTYTAFVGLAALAHLFVIAANPWW	4909.5		
β3	ASLSGLTDQQAKEFHEQFKVTYTAFVGLAALAHLFVIAANPWW	4778.4	4778.1	B850,B840,B820
Alvin_0709	MAELKNISGLTDQQAQEFHAQFKVTYTAFVGLAAVVHLFVLATNPWF	5254.0		
β4				(not observed)
Alvin_0706	MAEQQKSLSGLTEQQAKEFHEQFKVTYTAFVGLAALAHMMVIAANPWW	5439.2		
β5	N-(CH3)AEQQKSLSGLTEQQAKEFHEQFKVTYTAFVGLAALAHMMVIAANPWW	5322.0	5322.1	B820,B840
Alvin_0704	MNGLTEQQAKEFHAQFKVTYTAFVGLAALAHLMVLANNPWF	4609.3		
β6	MNGLTEQQAKEFHAQFKVTYTAFVGLAALAHLMVLANNPWF	4609.3	4607.2	B820

apoproteins that control the site energies of the strongly excitonically coupled BChl *a* molecules [53]. These two amino acids essentially form a 'fingerprint' region that can be used to predict the BChl *a* site energies. If this logic can be used with the B800-850 and B800-820 complexes from *Alc. vinosum* then one would predict that the α apoproteins found in the B850 form should have H-bonding residues in the fingerprint region and these should be changed to non H-bonding residues in the B820 form. This prediction only appears to be partially satisfied in *Alc. vinosum*. The major α apoprotein found in the B850 form is α1 and this polypeptide has only one of the two fingerprint amino acids, αTrp, that could account for the important H-bond with the bacteriochlorin ring, which in this case is at position +11 relative to the conserved αHis residue. The α2 and α3 apoproteins also have this αTrp, although in these apoproteins this αTrp is present at position +10, but contain a second H-bonding residue, αGln, at position +11. In the B820 form the major alpha apoproteins are α5 and α6. The α5 polypeptide has no H-bonding residues in the fingerprint region; the αTrp found at position +11 in α1 is replaced by a αPhe in the α5 apoprotein. Conversely, the α6 apoprotein does contain the conserved αTrp residue at position +11 and, additionally, has another two potential H-bonding residues in the fingerprint region; these may compete for H-bonding of BChl *a* molecules. This suggests that the origin of the B850 and B820 spectroscopic forms in these two LH2 complexes from *Alc. vinosum* is more complicated than that originally proposed to explain the two equivalent forms of LH2 found in *Rps. acidophila*. However, as already mentioned the N-terminal deletions of the αMet residues from most of the α apoproteins suggest that the LH2 complexes from *Alc. vinosum* are more closely related to the LH2 complex from *Phs. molischianum* than they are to the LH2 complexes from *Rps. acidophila*. In *Phs. molischianum* the position of the αTrp is +11 from the conserved αHis, rather than +14 as in *Rps. acidophila*. One other major difference between the *acidophila* and *molischianum* structures is that a key Trp residue that H-bonds to one of the two strongly excitonically coupled BChls *a* is provided by the β-apoprotein (see Fig. S1, Supplementary Information). All of the β apoproteins present in both the B850 and B820 spectroscopic forms studied here contain this key βTrp residue. This, again, means that the origin of differences in the absorption spectra are unclear and will probably only be resolved when a high resolution X-ray crystal structure of these two types of LH2 can be obtained. The polypeptide composition of the B840 form is also interesting. It has some common polypeptides when compared with the B850 and B820 forms but also completely lacks the α5 but has a large amount of the β5. This gives it a unique polypeptide composition and so it cannot be explained simply as mixture of the B850 and B820 forms. It remains unclear, therefore, whether the B800-820, B800-840 and B800-850 type LH2 complexes are indeed well-defined discrete LH2 complexes or whether there are a wider range of possible polypeptide compositions for LH2 complexes from *Alc. vinosum* than we have been able to prepare so far.

Table 5
Quantified polypeptide compositions for the B800-850, B800-840 and B800-820 LH2 types of *Alc. vinosum*.

	α1	α2	α3		β1	β2	β3		α:β		
B800-850	**56**	27	17		37	39	24		45:55		
	α1	α2	α3	α6			β1	β2	β3	β5	
B800-840	35	32	10	23			18	21	11	**50**	
	α2	α3	α5	α6			β1	β2	β3	β5	β6
B800-820	15	16	**30**	**39**			12	12	11	**49**	16

The progressive C-terminal processing confirmed for the LH2 α1 and α5 polypeptides is intriguing. A phylogenetic analysis reveals that the LH2 α polypeptides of *Alc. vinosum* can be classified into two groups (Fig. S3, Supplementary Information). The major components, α1 in B800-850 and α5 in B800-820, have high sequence homology and belong to the same group. They are subjected to progressive C-terminal truncations. The C-terminal processing itself is not unusual for the LH polypeptides. It is well-known that the LH1 α and β polypeptides of *Rhodospirillum rubrum* have 13 and 10 C-terminal residues removed

respectively in their mature forms by post-translational modification. In this case, both the numbers of the residues cleaved are fixed, and no other truncated products have been identified. The C-terminal extensions have been suggested to play a role in the LH synthesis or assembly and are required for the expression levels of wild-type LH complexes [54].

Based on CD spectral behaviour, the LH2 complexes of purple bacteria can be divided into two groups [39]. The *Alc. vinosum* LH2 complexes of all types in this study again show a '*molischianum*-like' characteristic in their CD spectra (Fig. 4). All of the LH2 complexes produced by *Alc. vinosum* exhibit an unusual split B800 peak in their low temperature absorption spectra. A split B800 peak has previously been observed in the LH2 complexes from *Alc. vinosum* [9,12,25] and from *Thermochromatium tepidum* [37,55,56]. For the 850 nm type LH2 complex produced by *Alc. vinosum* (on thiosulphate media), this band splitting was recently attributed to heterogeneity among the B800 BChls *a* within a single LH2 ring [12]. Subtle differences in the amino acid residues present in the B800 binding region of the α polypeptide sequences could explain the origin of the split 800 nm peak observed in the low temperature absorption spectra for all LH2 types in *Alc. vinosum*. The α polypeptides identified within the *Alc. vinosum* LH2 complexes all share the Asp residue to which the B800 Bchl *a* is liganded in the B800-850 LH2 complex from *Phs. molischianum* [23]. However, while the α polypeptides from pucA2, pucA3 and pucA6 all contain two adjacent Asp residues here (as in *Phs. molischianum*), the α-polypeptides from pucA1 and pucA5 have just a single Asp residue in the B800 binding pocket. Additionally, these α-polypeptides have differential H bonding capabilities in this region, with the α-polypeptides from pucA1 and pucA5 lacking the Asn residue that, in *Phs. molischianum*, locks the B800 molecule into place via an H bond with the Asp residue [23]. In the pucA1 and pucA5 α polypeptides this Asn residue is replaced by a Pro residue, which cannot form H bonds. A B800 molecule bound to one of these α polypeptides will, therefore, be more flexible and potentially orientated differently leading to a relative change in site energy and thus in the Q_y maxima. This explanation of the split B800 absorption peak, which the CD spectra indicates may have an exciton character, can only be sustained if the LH2 rings contain different α apoproteins so that the precise organisation of the B800 BChl *a* binding sites alternates going from one α-/β dimer to the next within a single LH2 ring. It is interesting therefore that all the LH2 types characterised here do not contain any one α apoprotein type that is present in an amount significantly greater than 50%.

In conclusion, the LH2 complexes from *Alc. vinosum* all have a heterogeneous polypeptide composition and it is not yet fully clear how the various polypeptide compositions are controlled or how they lead to the different spectral forms that have been seen. The presence of such a variety of different LH2 types raises the question of what advantage this confers upon *Alc. vinosum*, especially at low light intensities. It has previously been shown in *Rps. palustris* [57] that the presence of the B800-820 type LH2 complex suppresses back transfer from the LH1 back to the LH2 under low light conditions. Under high light conditions back transfer from the LH1 to the B800-850 LH2 complex is clearly observed and serves to allow an exciton arriving at a busy reaction centre to be transferred to a free reaction centre. This function is not necessary under low light conditions, where the abundance of excitons will be relatively low. The production of the B800-820 type complex, and resulting suppression of back transfer, reduces losses from the LH1 back into the pool of LH2 and therefore increases the overall trapping efficiency of the photosynthetic unit under lowlight conditions. We have recently established that this is also the case in *Alc. vinosum* (unpublished data).

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Appendix A. Supplementary data

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