Photosynthesis Research **61:** 157–167, 1999.

Regular paper

Characterisation of hydrophobic peptides by RP-HPLC from different spectral forms of LH2 isolated from *Rps. palustris*

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Received 12 January 1999; accepted in revised form 23 June 1999

Key words: high performance liquid chromatography, low-light, photosynthetic bacteria, reversed-phase

Abstract

The separation of light-harvesting peptides by RP-HPLC is notoriously difficult due to the typically strong interaction of peptides with the column matrix, their relatively low solubility in the mobile phase and the tendency for non-specific aggregation during sample preparation. This paper illustrates a reproducible method for investigating the composition of four spectrally different forms of LH2 isolated from *Rps. palustris*. The method contrasts with previous attempts to isolate peptides from these multi-LH2 complexes and uses the well characterised B800–850 complex from *Rps. acidophila* as a test of reliability. Three pairs of LH2 peptides, $\alpha\beta_a$, $\alpha\beta_b$ and $\alpha\beta_d$, were identified from *Rps. palustris* grown under high- (7000 lux) or intermediate- (1000 lux) light conditions. At lower light (300 and 90 lux), $\alpha\beta_b$ was absent, and the level of $\alpha\beta_a$ was significantly reduced. Results show that $\alpha\beta_a$ and $\alpha\beta_b$ peptides form the high light B800-850 complex, whereas the low light LH2 complex is only composed of $\alpha\beta_d$ peptides and resembles the B800–820 complex from *Rps. acidophila* by sequence homology. The absorption spectrum of this complex has a single peak centred on 800 nm and appears to be a novel LH2 complex. At low light growth conditions, this B800 species is the predominant LH2 complex in *Rps. palustris* and indicates that peptide expression is a crucial factor in adapting to different light intensities.

Abbreviations: B800 – bacteriochlorophyll absorbance at 800nm; FPLC – fast protein liquid chromatography; HPLC – high performance liquid chromatography; HL – high light; IL – intermediate light; LL – low-light; LH2 – light-harvesting complex 2; RP – reversed-phase; RC – reaction centre; TFA – trifluroacetic acid

Introduction

Most purple bacterial LH2 complexes are composed of pairs of small hydrophobic peptides associated with carotenoid and bacteriochlorophyll *a* molecules. These protein–pigment complexes are responsible for harvesting incident solar radiation before passing energy down via LH1 to the photosynthetic reaction centre (RC). In most bacterial LH2 complexes, the repeating cyclic unit has two single polypeptides, α and β, associated with 3 BChl *a* molecules – absorbing in the near infra-red between 800 nm and 850 nm. The cyclical design of the system allows for highly efficient transfer of trapped light energy across the whole of the photosynthetic unit (McDermott et al. 1995; Koepke et al. 1996). However, not all bacterial LH2 complexes appear to have this simple arrangement and there is evidence that some species of purple bacteria have additional peptides and BChls (Tadros et al. 1993; Kerfeld et al. 1994). The synthesis of LH2 complexes is regulated by a variety of external factors such as oxygen concentration and light-intensity and many species are characterised by their ability to synthesis more than one spectral type (Drews 1991; Kramer et al. 1995; Fowler and Hunter 1996). The exact nature of these complexes has not been fully explored because of the difficulty in expressing and isolating such small, intrinsically hydrophobic peptides. Separation is routinely achieved using reverse-phase FPLC or HPLC but the reliab-

ility of these methods very much depends on the extraction procedure, column matrix and choice of organic solvent, many of which are based on acetonitrile (Parkes-Loach et al. 1988; Zuber and Brunisholz 1991; Germeroth et al. 1993). Other methods for the separation of hydrophobic peptides have used formic acid (Bollhagen et al. 1995; Lee et al. 1996), including that used for the separation of peptides from *Rps. palustris* (Tadros et al. 1989). However, such harsh denaturing conditions and low pH can destroy the column matrix over time and produce anomalies with respect to elution profile and peak retention time. This paper reports a reproducible method for the extraction and isolation of LH2 peptides that can be applied to different types of LH2 complexes. The method has been applied to the LH2 complex from *Rhodopseudomonas palustris* which synthesises different spectral forms according to the light-intensity of the growth conditions. Previous work shows that several peptides in this species are expressed under different light intensities (Tadros et al. 1989, 1993), but the work was unable to precisely define which peptides were associated with each particular spectral form. In addition, these methods were found to give very variable results in our laboratory, necessitating the development of better peptide isolation procedures. We show that we can achieve reproducible separation of peptides from four different spectral forms of LH2 from *Rps. palustris*. We discuss the differences in LH2 composition of high- and low-light grown cells and compare it to that of the structurally determined LH2 complex from *Rhodopseudomonas acidophila*.

Materials and methods

Organisms and culture conditions

Rps. palustris strain 2.1.6 was grown anaerobically at 30 °C under high- (7000 lux), intermediate- (1000 lux) or low- (300–90 lux) light. *Rps. acidophila* 10050 was grown at 7000 lux. Bacteria were cultured in an aluminium-lined incubator in which light intensity and temperature was controlled. Light intensity was measured using a RS Components digital light meter.

Isolation and purification of LH2 complexes

LH2 was purified according to Evans et al. (1990) using 1.0% (w/v) dodecyl-maltoside as the solubilising detergent. An additional FPLC gel filtration step (Pharmacia Superdex 200) was introduced following sucrose density gradient centrifugation to further purify LH2 from LH1-RC. Absorption spectra were recorded at room temperature in a Perkin Elmer Lambda 16 spectrophotometer with an optical bandwidth of 2 nm. LH2 complexes were identified as high- (HL), intermediate- (IL) or low-light (LL) depending on the absorbance ratio taken at the NIR absorption maxima around 800 and 850 nm.

HPLC

Samples were analysed on a Hewlett-Packard 1100 HPLC fitted with a diode array detector, a temperature controlled column oven and autosampler and using HPLC grade solvents. A RP-C8 column was equilibrated at 1.0 cm^3 min^{-1} in the starting solvent, for at least 30 min, before a test gradient was run. The column was then re-equilibrated until a flat baseline was obtained. Samples were filtered (0.2 μm PTFE) immediately prior to injection. The injection volume was kept constant at 90 mm^3. Absorbance was monitored at 280, 375, 800 and 850 nm, with a bandwidth of 16 nm, to include protein and pigment contributions. A cycle of column equilibration, test gradient and column re-equilibration was carried out between each injection.

Method 1

Freeze dried LH2 complexes were incubated for 10 min in a buffer containing 1:1 (v/v) chloroform/methanol, 100 mM ammonium acetate and 10 mM DTT. Particulate material was removed by centrifugation at 14 000 rpm for 5 min. The supernatant was filtered and loaded onto a Sephadex LH60 column (Pharmacia) and eluted in the same buffer minus DTT. Protein containing fractions were pooled and dried to completeness under vacuum. Then, 2.0 mg of dried pellet was resuspended in 100 mm^3 of 50% formic acid and immediately injected onto a RP-C8 column. The gradient was based on that described by Tadros et al. (1989) for the RP-HPLC of intact LH2 from *Rps. palustris*. The starting solvent was 50% formic acid in water and the gradient was developed by increasing the concentration to 50% formic acid in propan-2-ol over 40 min.

Method 2

LH2 peptides were prepared as described in ‘Method 1’, except that the RP-C8 column was equilibrated in 30% (v/v) acetonitrile in water with the addition

of 0.1% (v/v) TFA. Ten min after sample injection a gradient of 0–100% acetonitrile was developed over 40 min.

Method 3

LH2 peptides were prepared as described in 'Method 1' but were not dried to completeness after the LH60 gel filtration step. Instead, protein fractions were pooled and the volume reduced to approximately 2 cm^3 by rotary evaporation at 60 °C. The concentration of the sample at this stage was typically 3–4 mg cm^{-3}. Concentrated samples were maintained at 40 °C and used for HPLC analysis within 6 h. Care was taken to prevent further evaporation of the sample, thus avoiding protein aggregation. The gradient profile was identical to that described in 'Method 2' except that the column temperature was maintained at 50 °C and the autosampler thermostat at 40 °C.

SDS-PAGE, Western blotting and protein microsequencing

Fractions were collected directly from the diode array detector, dried under vacuum as before and stored at –20 °C until required. Samples for protein microsequencing were analysed by SDS–PAGE, transferred onto Immobilon P^{SQ} membrane and stained using Coomassie Blue.

5–18% SDS gradient gels were prepared according to Laemmli (1970). Gels were run in duplicate at 35 mA for 2.5 h. One gel was stained and destained – the other was transferred onto Immobilon-P^{SQ} membrane using a Sartorius semi-dry electroblotter for 2 h at 1.0 mA cm^{-2}. Coomassie Blue stained protein bands were excised and stored at –70 °C until required. N-terminal amino-acid sequencing was carried out by Alta Bioscience (University of Birmingham, UK) using a technique based on the original work by Edman and Begg (1950). Sequences were identified by searching the Swissprot database.

Results

Spectral profiles of LH2 complexes

Rps. palustris cells were grown until an optimal absorbance ratio of 800 to 850 nm was reached. For cells grown at 7000 lux (HL), 1000 lux (IL), 300 lux (LL) and 90 lux (LL) this ratio was defined as 1.1, 1.2, 1.5 and 1.7 respectively. After purification, the B800 to B850 nm absorbance ratios increased following removal of LH1-RC by sucrose density gradient centrifugation and gel filtration. For the different spectral types of LH2, typical B800 to B850 nm absorption ratios were 1.0 (HL), 1.6 (IL), 3.6 (300 lux LL) and 5.0 (90 lux LL). Figure 1 shows typical NIR absorbance spectra of LH2 complexes isolated from *Rps. palustris* grown under different light intensities and the B800–850 LH2 complex from *Rps. acidophila*.

RP-HPLC of high-light LH2 peptides from Rps. palustris

LH2 complexes were first analysed using the protocol described in 'Method 1'. Three chromatograms of HL LH2 prepared in this way, and monitored at 280 ± 8 nm, are shown in Figure 2. The absence of bacteriochlorophyll pigments was shown by negligible absorbance when monitored at 375, 800 and 850 nm.

Using 'Method 1', the peak retention time and relative area of each peak varied considerably between each run despite the fact that each injection was from the same sample (Table 1). The first major peak eluted between 14.8 and 48.7 minutes. The relative area of this peak varied between 28% and 54% of the total area. In addition, the peak profile was inconsistent and the number of identifiable peaks varied between runs. Although large quantities of material, typically 2 mg per injection, were required for each chromatogram, there was no evidence of sample loss through irreversible binding to the column. There was no notable reduction in column performance when standards were applied or increased back-pressure over time. In addition, no peaks were observed in test gradients carried out before and after each injection.

The requirement for better resolution and reproducibility of chromatograms led to the development of an alternative method utilising a standard acetonitrile/water gradient. This method eliminated the use of formic acid in the gradient, although formic acid was still required to solubilise dried peptides. Figure 3 shows HL LH2 monitored at 280 nm ± 8 nm after RP-HPLC using 'Method 2'. A comparison of three consecutive chromatograms revealed that retention times and relative peak heights was less variable than with 'Method 1'. In addition, peak profile, number of peaks, retention times and relative peak areas were more consistent between runs. The relatively poor resolution of peaks is shown by the large standard deviation of relative peak areas (Table 1). Chromatograms of HL LH2 were further improved

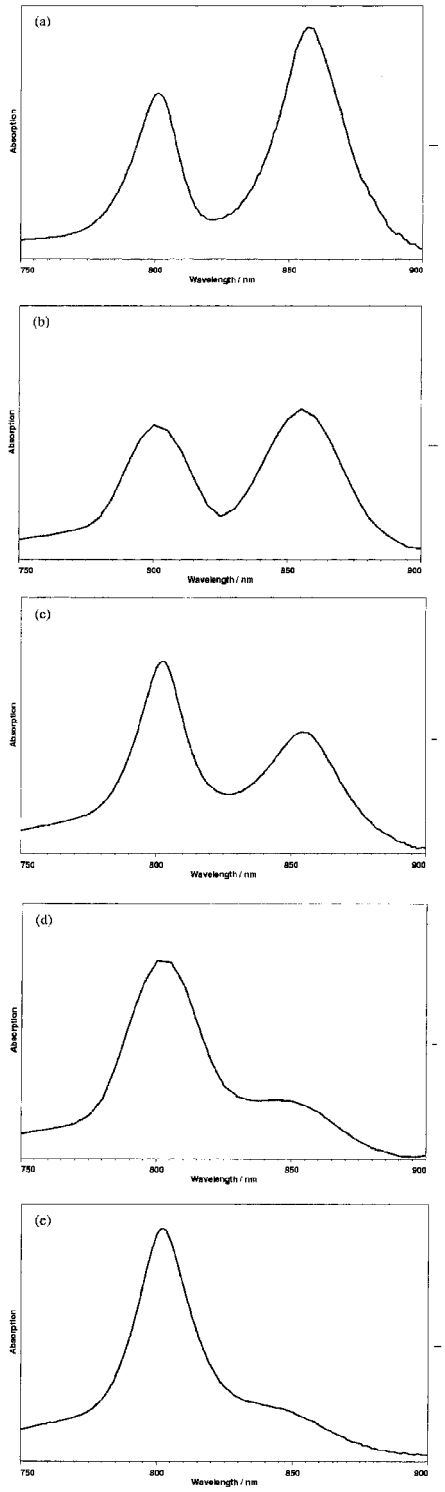

Figure 1. Absorbance spectra of LH2 complexes isolated from cells grown under high-light (HL), intermediate-light (IL) and low-light (LL) intensities. (a) *Rps. acidophila* HL (b) *Rps. palustris* HL (c) *Rps. palustris* IL (d) *Rps. palustris* 300-lux LL (e) *Rps. palustris* 90-lux LL.

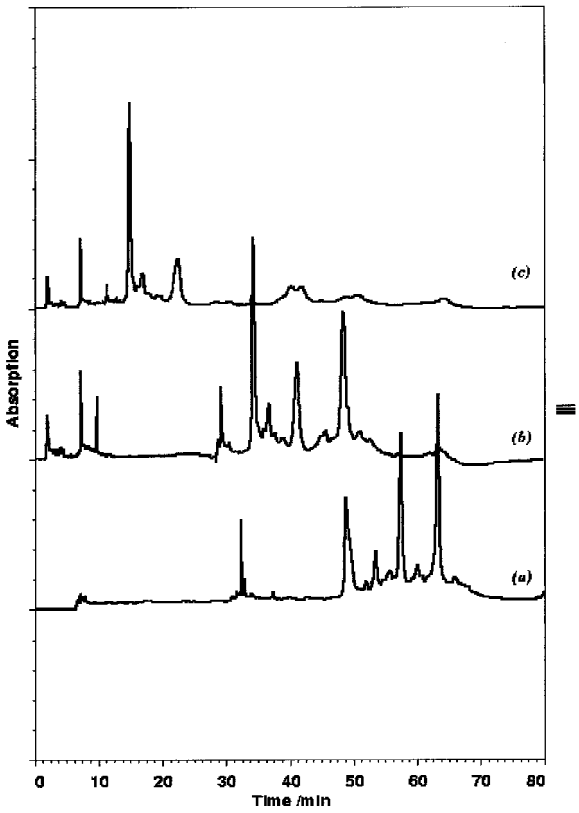

Figure 2. RP-HPLC profile of *Rps. palustris* high-light LH2 peptides prepared using method 1. (a)–(c) Three chromatograms were run consecutively over a 6-hour period from the same sample.

using 'Method 3' which eliminated the use of formic acid in both the gradient and sample preparation. Figure 4 shows consecutive chromatograms of HL LH2 peptides run over a period of 6 h. Unlike 'Methods 1 and 2', consecutive chromatograms were measured over time since long-term stability of the sample in chloroform/methanol was unknown.

Major improvements were seen in the resolution of peaks when compared with 'Methods 1 and 2'. This can be seen visually in Figure 4 and as a reduction in the standard deviation of the relative peak areas (Table 1). The number of peaks resolved, however, did decline from 3 to 2 (at 29 and 29.7 min) over the three scans. This variability was reflected in the large standard deviation for the relative area of peak 6 (Table 1). With this exception, the absolute areas of peaks 1–4 remained constant between consecutive chromatograms over several hours.

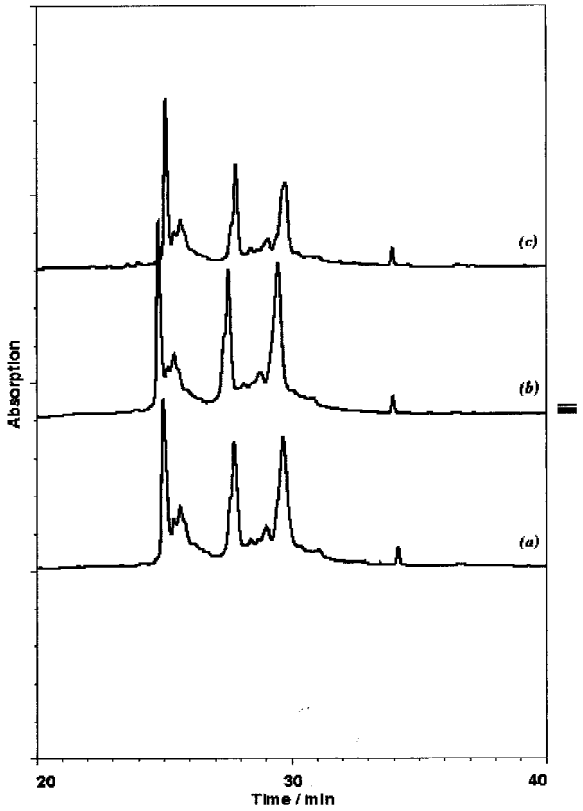

Figure 3. RP-HPLC profile of *Rps. palustris* high-light LH2 peptides prepared using method 2. (a)–(c) Three chromatograms were run consecutively over a 6-hour period from the same sample.

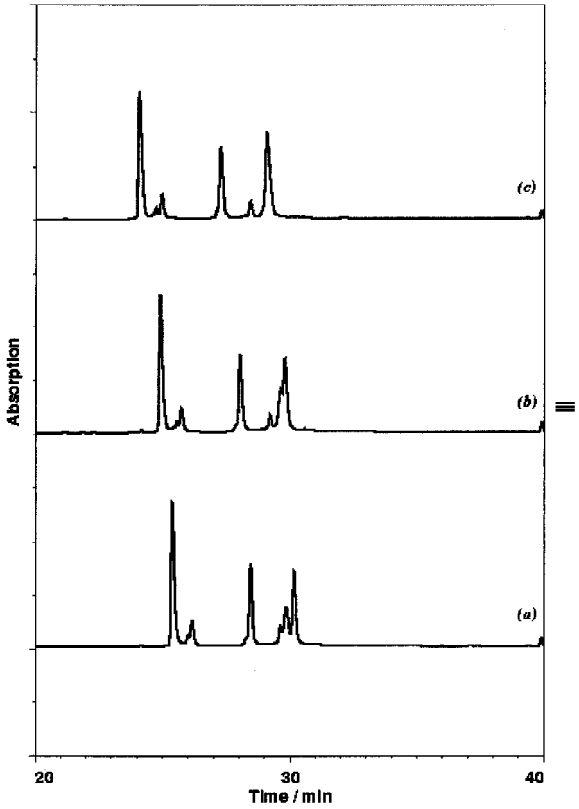

Figure 4. RP-HPLC profile of *Rps. palustris* high-light LH2 peptides prepared using method 3. (a)–(c) Three chromatograms were run consecutively over a 6-hour period from the same sample.

RP-HPLC of LH2 peptides from Rps. acidophila

In order to assess our method, duplicate experiments were performed on *Rps. acidophila* 10050 LH2, which is known to have a single α and β peptide. LH2 peptides from *Rps. acidophila* were prepared as outlined in 'Method 2' and the chromatogram is shown in Figure 5a. Four major peaks were sequenced which were identified solely as the α peptide. However, 'Method 3' gave three additional peaks presumed to be the β peptides. These represented 25% of the total peptide area (Figure 5b). In retrospect, it is possible to see these peaks in Figure 5a although they only represent 3% of the relative total peptide area and were not present in sufficient quantity for sequencing.

HPLC analysis of different spectral forms of LH2 complexes from Rps. palustris

Peptides from HL, IL, 300-lux LL and 90-lux LL LH2 were separated by RP-HPLC. Samples were prepared according to 'Method 3' and eluted using identical chromatography conditions. Figure 6 shows the chromatograms for each of the four LH2 complexes analysed. The relative peak area was determined at 280 $\pm$ 8 nm and expressed as a percentage of the total area (Table 2).

Sequence data

Primary sequence data for the six main peaks for HL and 300-lux LL LH2 are shown in Figure 7. The first 13 N-terminal amino acids were 100% identical to peptides previously identified in the genome of *Rps. palustris* as presented in Table 3. In HL, three peptide pairs were identified as $\alpha\beta_a$, $\alpha\beta_b$ and $\alpha\beta_d$. All peaks, except 1 and 4, were single clean sequences. Despite efforts to improve resolution, these could not be resolved further. Peak 1 contained β_a and β_b in equal amounts, while peak 4 was mainly composed of α_b with α_d as a minor component. Assuming that peak 1 contains approximately equal proportions of β_a and

Table 1. Retention time and relative peak area for *Rps. palustris* high-light LH2 peptides prepared by three different methods The mean value is given for each peak resolved for three consecutive HPLC runs shown in Figures 2–4

Method	Peak no.	Retention time (mins)	Relative area
1	1	32.6 ± 13.9	39.7 ± 10.8
	2	35.6 ± 14.9	9 ± 2.2
	3	40.3 ± 14.3	27 ± 4.1
	4	48.5 ± 11.9	24.3 ± 15.9
2	1	24.9 ± 0.3	20.3 ± 1.7
	2	25.5 ± 0.1	15.7 ± 2.1
	3	27.7 ± 0.1	22.7 ± 0.5
	4	29.0 ± 0.1	8 ± 0.8
	5	29.7 ± 0.1	33.3 ± 2.5
3	1	24.8 ± 0.5	35 ± 0.8
	2	25.5 ± 0.6	7.3 ± 1.2
	3	28.2 ± 0.9	20.6 ± 0.5
	4	29.1 ± 0.5	4.7 ± 0.5
	5	29.7 ± 0.1	12.5 ± 0.5
	6	30.0± 0.2	24 ± 5.7

Table 2. Relative peak area from HPLC profiles of four different LH2 complexes from *Rps. palustris*. The relative area is expressed as a percentage of the total area. LH2 complexes are defined according to their spectral profile when grown under conditions of different light intensity

Peak no.	High-light	Intermediate-light	300-lux low-light	90-lux low-light
1	36	9	7.5	7.5
2	6	8	8	7.5
3	21	27	24	34
4	5	11	9	8
5	13	31	39.5	36
6	19	14	12	7

β_b, the relative amount of each peptide was calculated as a percentage of the total peak area (Table 4).

Sequence data for 300-lux LL showed two peptide pairs, $\alpha\beta_a$ and $\alpha\beta_d$ (Table 3). Peak 1 contained mainly β_a with β_d as a minor component. Analysis of the relative peak area showed the $\alpha\beta_d$ peptides to be the most abundant (Table 4).

Assigning sequence data to IL and 90-lux LL LH2

Although IL and 90-lux LL LH2 samples were not sequenced, it was possible to assign sequences to these peaks by comparing profile and retention times with chromatograms from HL and 300-lux LL LH2 samples.

The retention times of HL and 300-lux LL LH2 β peptides occur within a narrow band between 25.4 and 26.3 min. The α peptides are eluted between 28.4 and 30.4 min and are well separated from the β peptides. IL LH2 shows a very similar profile compared to that of HL (Figure 6) as does 90-lux LL compared to 300-lux LL. It is possible then, based on retention times and profile shapes, to identify peptides and we propose that the same three peptide pairs, $\alpha\beta_a$, $\alpha\beta_b$ and $\alpha\beta_d$, constitute the IL LH2 complex whereas in the 90-lux complex only $\alpha\beta_a$ and $\alpha\beta_d$ are present. A comparison of the total peak area indicates progressively increasing levels of $\alpha\beta_d$ going from HL to IL to LL LH2 (Table 4).

Sequence homology

The sequences identified in our experiments were compared to those in protein sequence databases using the BLASTP algorithm (Altschul et al. 1990). As expected they score highly against purple bacterial light harvesting peptides. In general, the amino acid identity ranges from 78% down to 56%. The α peptides are particularly interesting in that they exhibit specific sequence markers that are associated with certain spectral forms of LH2. The comparison of α_d peptide with the protein database indicates an identity with the B800–820 α peptides from *Rps. acidophila* strains 7050 and 7750, scoring 67% and 71% respectively and the B800–850 α peptides from strains 7050, 7750/10050, scoring 64% and 56%, respectively. The similarity of α_d to the α peptide of the B800–820 complexes of *Rps. acidophila* is further highlighted when the sequences are compared at position +13 and +14 relative to the bacteriochlorophyll liganding histidine residue (Figure 8). At these positions, the B800–850 α of *Rps. acidophila* and the α_a and α_b peptides of *Rps. palustris* have YW, whereas the B800–820 complexes and the α_d peptide have FL, FT or FM. We are assuming that the α_a and α_b sequences identified are true B800–850 α peptides since we have shown that removal of complexes containing these peptides reduces proportionally the absorbance peak at 850 nm. It is also known that successive site-directed mutagenesis of YW to FL in LH2 from *Rb. sphaeroides* causes a

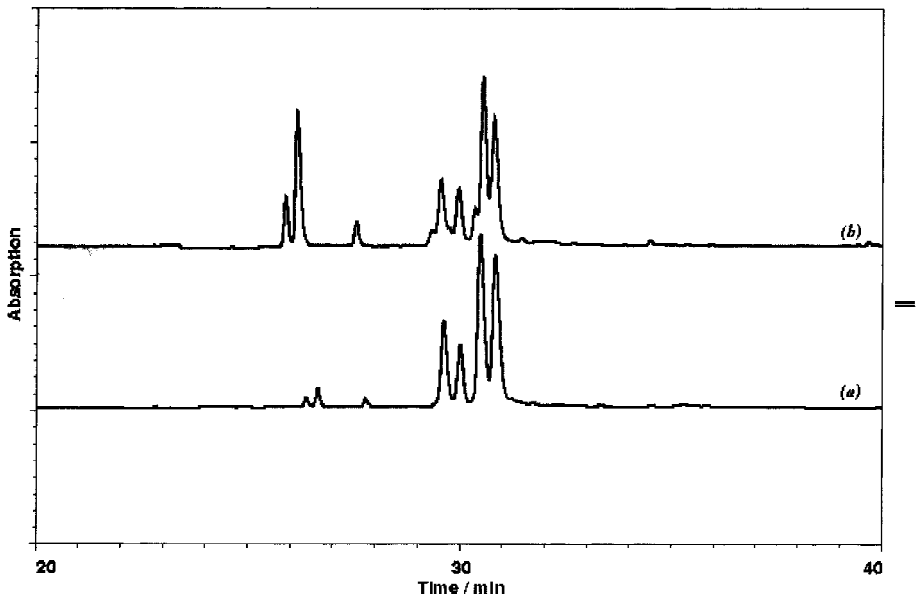

Figure 5. RP-HPLC profile of high-light *Rps. acidophila* LH2 peptides prepared using different methods. (a) Dried peptides were solubilised in 50% formic acid using Method 2. (b) Peptides were prepared using 'Method 3' in which formic acid is eliminated.

blue shift of the 850 nm band by 24 nm. (Fowler et al. 1992). Knowing the structure of *Rps. acidophila*, this can be attributed to the breaking of hydrogen bonds to the C3-acetyl group on ring A of BChl *a* (McDermott et al. 1995; Prince et al. 1997). Another sequence indicator is the presence of IAIL at position –5 to –2 in the B800–850 α of *Rps. acidophila* strains 7050, 7750 and 10050 and the α_a and α_b peptides from *Rps. palustris*. Whereas the B800–820 complexes of *Rps. acidophila* and the α_d of *Rps. palustris* differ at these positions. It seems, at least from the sequence data, that the LH2 complex of which the α_d peptide is a part resembles other B800–820 complexes. Whether it really exists as a true B800–820 complex is questionable, since if there is a component at 820 nm it must be very small and ideally it should be referred to as a B800 complex. Initially the B800/850 nm absorption ratio was used as a measure of low-light LH2 expression. This choice was fortuitous, as there is very little contribution in this region from the LL LH2 since any absorption is small and in any case will occur at 820 nm. To a first approximation then, it can be assumed that absorption at 850 nm is entirely from the high-light species composed predominantly of *a* and *b* peptides and the decrease in absorption seen at 850 nm is directly proportional to the amount of HL LH2 lost.

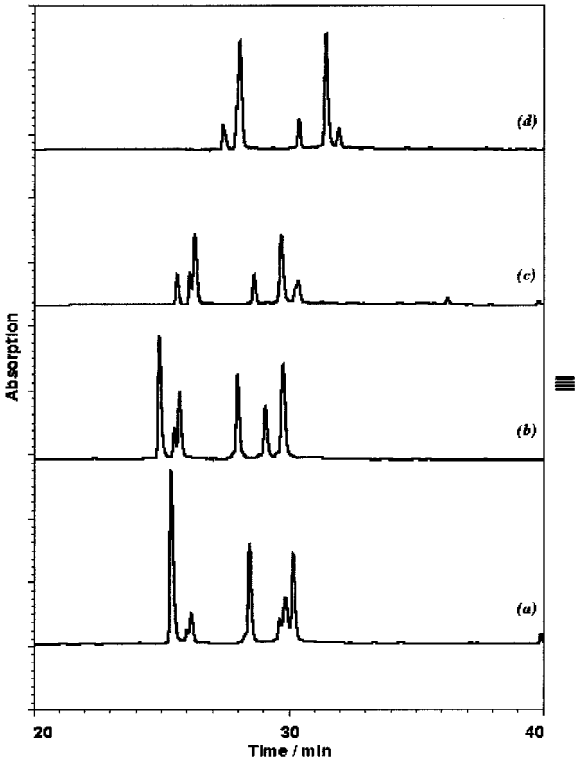

Figure 6. RP-HPLC profile of *Rps. palustris* LH2 peptides isolated from cells grown under (a) high-light (HL), (b) intermediate-light (IL) (c) 300-lux low-light (LL) (d) 90-lux low-light (LL). Samples were prepared using 'Method 3'.

Table 3. Peptide identification and retention time for High-Light and 300-lux Low-Light LH2 HPLC peaks from *Rps. palustris*. Samples were prepared using 'Method 3'. HL peak 1 contains β_a and β_b in equal amounts. HL peak 4 and LL peak 1 both contain minor components (shown in brackets)

Peak no.	High light		300-lux low-light	
	Time (mins)	peptide	time (mins)	peptide
1	25.4	β_a β_b	25.5	β_a (β_d)
2	26.0	β_d	26.1	β_d
3	28.4	α_a	26.3	β_d
4	29.6	α_b (α_d)	28.6	α_a
5	29.8	α_d	29.7	α_d
6	30.2	α_d	30.4	α_d

(a)

Peak 1: **NH2-ADKTLTGLTVEES-, NH2-ADDPNKVWP..**
Peak 2: **.VDDPNKVWPTGL-**
Peak 3: **NH2-MNQARIWTVVKPT-**
Peak 4: **NH2-MNQGRIWTVVNPG-**
Peak 5: **NH2-MNQGRIWTVVLPT-**
Peak 6: **NH2-MNQGRIWTVVKPT-**

(b)

Peak 1: **NH2-ADKTLTGLTVEES-**
Peak 2: **NH2-MVDDPNKVWPTGLT-**
Peak 3: **NH2-MVDDPNKVWPTGL-**
Peak 4: **NH2-MNQARI.TVVKPT-**
Peak 5: **NH2-MNQGRIWTVVKPT-**
Peak 6: **NH2-MNQGRIWTVVKPT-**

Figure 7. N-terminus primary sequence data of peptides isolated from HPLC chromatographs. The peak numbering convention is as in Table 3. (a) HL sequences; (b) 300 lux LL sequences. Sequences are only given for the major component in each peak. Dots represent amino acids that were not identified.

Table 4. Relative areas for the 6 peptides expressed in four different LH2 complexes from *Rps. palustris*. The area is expressed as a percentage of the total area

Peptide	High-light	Intermediate-light	300-lux low-light	90-lux low-light
β_a	18	12.5	9	7.5
β_b	18	12.5	–	–
β_d	6	19	35	41.5
α_a	21	18	11	8
α_b	18	12	–	–
α_d	19	25	45	43

Discussion

The need for a consistently reliable method for the separation of small hydrophobic LH2 peptides by RP-HPLC was demonstrated by our inability to reproduce previous methods in our laboratory. The method presented here enables reproducible RP-HPLC analysis of peptides from *Rps. palustris* and *Rps. acidophila* LH2 complexes. We found that treatment of samples following LH60 gel filtration and the exclusion of formic acid proved particularly crucial for such reproducibility to be achieved.

The exclusion of formic acid in the gradient led to improvements in chromatograms of LH2 peptides. The HPLC profile of *Rps. palustris* HL LH2, prepared according to 'Method 2' showed consistent peak retention times, the resolution was not optimal as shown by the large standard deviation of relative peak areas. In addition, comparable experiments with *Rps. acidophila* showed that 50% formic acid was not effective in solubilising the β peptides known to be present in this complex. Although four peaks were eluted from the RP-C8 column, sequence analysis revealed only the α peptide. Three additional β peptide peaks were identified only when *Rps. acidophila* LH2 was prepared using 'Method 3'. Since β peptides in both HL and LL *Rps. palustris* LH2 are eluted consistently at 26 min, it is safe to assign these peaks as β peptides. The data clearly show that formic acid results in the selective solubilisation of peptides even though it initially proved more useful than other solvents including hexane, heptane, chloroform, propan-2-ol, acetonitrile, ethanol and methanol. Moreover, low pH was shown to irreversibly change the properties of the silica-based column over time by removing a proportion of the C8 ligands and the need for a larger quantity of dried sample per chromatogram (typically 2 mg) was indicative of the low solubility in 50% formic acid for 'Method 1 and Method 2'.

The major consideration for the complete elimination of formic acid led to the development of 'Method 3'. When samples were applied directly to the RP-C8 column, chromatograms were reproducible in that peaks of similar retention time and relative area were eluted in consecutive runs. 'Method 3' also required significantly less sample than 'Method 1 or Method 2' with a typical concentration per injection of 0.3 mg. More importantly, the development of 'Method 3' reduced the problem of selective solubilisation of peptides as clearly demonstrated by the appearance of β peptide in the profile of *Rps. acidophila* LH2.

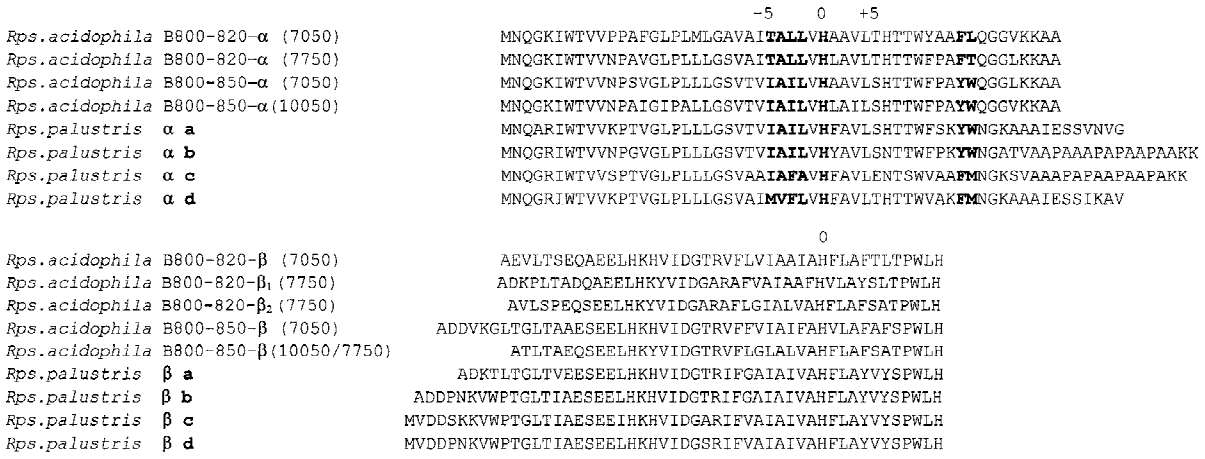

Figure 8. Primary sequence data for α and β peptides from the B800–820 and B800–850 LH2 complexes from *Rps. acidophila* 7070, 7750 and 10 050 (Zuber et al. 1991) and the a, b, c and d peptides from *Rps. palustris* (Tadros et al. 1993). Amino acid residues are numbered relative to the B850- or B820-liganding histidines at position 0.

However, there was still some variation in peptide solubility to an extent that it was not possible to quantify the relative amounts of peptides present within each LH2 complex to better than 5%. Nevertheless, we believe the accuracy is sufficient to see relative trends in peptide expression at different growth conditions, and make some inference on the changes in expression levels for individual peptides. We are confident that all peptides within particular LH2 complexes were detected since there was no visible precipitation in samples prepared using 'Method 3'. Indeed, every effort was made to reduce the potential loss of material by preparing and injecting the sample over a minimum time period and maintaining samples at 40 °C thereafter.

Analysis of the relative peak areas of *Rps. palustris* LH2 complexes showed there were distinct differences in the relative amounts of peptides depending upon the spectral form of the complex from which they originated.

In HL LH2 three peptide pairs identified as $\alpha\beta_{\mathrm{a}}$, $\alpha\beta_{\mathrm{b}}$ and $\alpha\beta_{\mathrm{d}}$ were estimated to be produced in roughly equal amounts. The exact value is not known because the β_{d} peptide is less soluble than its associated α_{d}. These data contrast with the work of Tadros et al. (1993) who proposed that five gene pairs $\alpha\beta_{\mathrm{a}}$, $\alpha\beta_{\mathrm{b}}$, $\alpha\beta_{\mathrm{c}}$, $\alpha\beta_{\mathrm{d}}$ and $\alpha\beta_{\mathrm{e}}$, were expressed in HL LH2 of *Rps. palustris*. In their paper they make the point that the amount of mRNA cannot be directly related to protein content. The work described here presents, for the first time, the proportions of functionally active expressed LH2 complexes at different light growth conditions.

Analysis of IL LH2 shows that the relative proportions of $\alpha\beta_{\mathrm{d}}$ increases significantly compared to the levels present in HL LH2. The 300-lux and 90-lux LL LH2 show a complete absence of $\alpha\beta_{\mathrm{b}}$ peptides with $\alpha\beta_{\mathrm{d}}$ being the predominate peptides. Interestingly, the relative proportions of $\alpha\beta_{\mathrm{a}}$ to $\alpha\beta_{\mathrm{d}}$ decreased from 20% in the 300-lux LL LH2 when compared with 15.5% in the 90-lux LL LH2, although this may not be significant given the variability in solubility from sample to sample. Nevertheless, our results show a clear trend of increasing amounts of $\alpha\beta_{\mathrm{d}}$ and decreasing amounts of $\alpha\beta_{\mathrm{a}}$ and $\alpha\beta_{\mathrm{b}}$ peptides as the light intensity of the cells is reduced. This work indicates that LL LH2 consist only of $\alpha\beta_{\mathrm{d}}$.

The spectral nature of LL LH2 is puzzling as it exhibits sequence homology with B800–820 complexes at key residues – yet unlike true B800–820 complexes, it has significantly reduced absorption in the 820 nm region. Using B800/850 nm absorption ratios obtained from Figure 1 and the relative proportions of HL and LL LH2 determined from HPLC analysis, one can estimate that if the HL component was removed this ratio would be >10 and the 820 nm peak would be lost in the tail of the 800 nm peak. Indeed, we have achieved this level of purity in more recent LL preparations (unpublished data). Earlier calculations estimate that LL LH2 contains 20% more BChl *a* than HL preparations (Evans et al. 1990). Retrospectively, these preparations are more similar to our IL preparations and may infact only contain 50–60% LL complex. In view of this, 30–35% more BChl *a* would be a better estimate. In most HL LH2 complexes, there

are three BChl *a* molecules per $\alpha\beta$ pair – an extra 33% would equate to four BChl *a* molecules per $\alpha\beta$ pair. For one additional BChl *a* molecule to influence the absorption at 820 nm so much – to the extent of removing absorption in that region – would require it to be strongly coupled and close to the B820 BChl *a* molecules. Alternatively, it may be situated at a similar distance to the B820 as are the B800 pigments to B850 in *Rps. acidophila* (ca. 17Å) and be weakly coupled – in this case the B820 pigments would be required to have a very different orientation: For example, the Qy dipoles may be pointing radially rather than tangentially to the ring of B820 pigments, which would cause intense absorption in the blue end of the excitonic band, in the 780–800 nm region, and very little at 820 nm. If in fact the extra BChl *a* molecule is close to the B820 pigments and strongly coupled, it is tempting to suggest the binding site residues are at positions -5 to -3 ie MVF where we find a relatively large departure from the other LH2 sequences found in *Rps. acidophila* and *Rps. palustris*. These residues are only one turn of an α-helix away from the His liganding to B820 and may be able to accommodate a BChl *a* molecule at a distance of 6–10 Å from this pigment. Since the spectral nature of LL LH2 is very different to the HL complex, it is reasonable to suggest that in a natural environment, the HL complex is never completely lost. Given that the LL complex is so blue-shifted, it may be that a small amount of HL LH2 is needed to provide a link in the spectral gradient from LL LH2 to the LH1/RC core complex.

We did not observe $\alpha\beta_c$ sequences in any of the HL or LL samples. Even those peaks with minor components could only be assigned to $\alpha\beta_a$, $\alpha\beta_b$ or $\alpha\beta_d$. If the $\alpha\beta_c$ peptide is a component of any of the tiny unidentified peaks, it must constitute $<3\%$ of the total peptides. Interestingly, the α_c peptide resembles α_d in that it has the sequence signature of a B800–820 complex. It may be that its expression is more sensitive to other stimuli such as temperature, and is not expressed under our growth conditions.

Conclusion

We have shown that formic acid is unsuitable for RP-HPLC of LH2 complexes. At best, it has severe effects on the reproducibility of chromatograms; affecting retention times, relative areas and peak resolution. At worst, formic acid does not fully solubilise all LH2 peptides and degrades the silicon matrix. This is clearly demonstrated by our studies with*Rps. acidophila* LH2 in which the β peptides are found only in trace amounts. Using this improved method, we have characterised the relative peptide composition of LH2 complexes isolated from *Rps. palustris* grown under different light intensities. We propose that in *Rps. palustris* strain 2.1.6 there are three different LH2 complexes each consisting of a pair of α and β peptides. At high-light (7000 lux), B800–850 complexes, composed of $\alpha\beta_a$ and $\alpha\beta_b$ peptides, and B800 complexes composed of $\alpha\beta_d$ peptides are expressed in roughly equal amounts. As the light intensity decreases to 1000 lux, the relative levels of $\alpha\beta_d$ increase. At 300 lux, the $\alpha\beta_b$ peptides are no longer expressed leaving two LH2 complexes – $\alpha\beta_a$ and $\alpha\beta_d$. At 90 lux, the $\alpha\beta_d$ peptides continue to increase and form the major LH2 complex in this organism at low-light. The relative amount of expressed peptides appears to be consistent with the absorption spectra shown in Figure 1, assuming that a true *Rps. palustris* high-light spectrum resembles that of *Rps. acidophila* (Figure 1a). The change in the absorption spectra going from high-light to low-light can therefore be seen to mirror our observations of the HPLC analysis. The LL LH2 complex seems to be, as far as the absorption spectrum is concerned, a unique blue shifted B800 species with little similarity to the B800–820 complexes. We conclude that peptide expression is a crucial factor in response to changes in light intensity.

Acknowledgement

H.T. was supported by the BBSRC of the UK (grant no: 719/B04960).

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