Characterisation of uniformly C-13, N-15 labelled bacteriochlorophyll a and bacteriopheophytin a in solution and in solid state: complete assignment of the C-13, H-1 and N-15 chemical shifts
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Characterisation of uniformly $^{13}$C, $^{15}$N labelled bacteriochlorophyll $a$ and bacteriopheophytin $a$ in solution and in solid state: complete assignment of the $^{13}$C, $^1$H and $^{15}$N chemical shifts

Tatiana Egorova-Zachernyuk,a,b* Barth van Rossum,a† Cees Erkelensa and Huub de Groota

In this investigation we report a complete assignment of $^{13}$C, $^1$H and $^{15}$N solution and solid state chemical shifts of two bacterial photosynthetic pigments, bacteriochlorophyll ($BChl$) $a$ and bacteriopheophytin ($BPheo$) $a$. Uniform stable-isotope labelling strategies were developed and applied to biosynthetic preparation of photosynthetic pigments, namely uniformly $^{13}$C, $^{15}$N labelled $BChl$ $a$ and $BPheo$ $a$. Uniform stable-isotope labelling with $^{13}$C, $^{15}$N allowed performing the assignment of the $^{13}$C, $^{15}$N and $^1$H resonances. The photosynthetic pigments were isolated from the biomass of photosynthetic bacteria $Rhodopseudomonas palustris$ 17001 grown in uniformly $^{13}$C (99%) and $^{15}$N (98%) enriched medium. Both pigments were characterised by NMR in solution ($acetone-d_6$) and by MAS NMR in solid state and their NMR resonances were recorded and assigned through standard liquid 2D $^{13}$C–$^{13}$C COSY, $^1$H–$^{13}$C HMOC, $^1$H–$^{15}$N HMBC and solid 2D $^{13}$C–$^{13}$C RFD, $^1$H–$^{13}$C HSQC HETCOR and $^1$H–$^{15}$N HETCOR correlation techniques at 600 MHz and 750 MHz. The characterisation of pigments is of interest from biochemical to pharmaceutical industries, photosynthesis and food research. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: $^{13}$C; $^1$H; $^{15}$N; magic angle spinning NMR; multinuclear magnetic resonance; uniform labelling; stable isotopes; bacteriochlorophyll; bacteriopheophytin; photosynthesis; ligand

Introduction

Bacteriochlorophylls are the main pigment species taking part in the primary processes of photosynthesis in anoxygenic photosynthetic bacteria. The characterisation of pigments is important for fundamental research, for the study ligand–protein interactions[1] and functional diversity,[2] for medical applications of porphyrin derivatives as drugs[3] and for food industry.[4] It demonstrates characterisation of natural products in the context of the growing importance of natural products in new medical technologies, in food and feed ingredients and as biofuels.[5]

The chemical structure of $BChl$ is depicted in Fig. 1(a). The basic structure of bacteriochlorophyll $a$ ($BChl$ $a$) is a porphyrin ring that can be distinguished from other porphyrins like haem by the presence of a fifth cyclopentanone ring and a coordinated $Mg^{2+}$ ion. Chlorophylls function both as light-harvesting pigments in the antenna and as primary donors in the reaction centres of photosynthetic systems. Another important pigment in the photosynthetic energy conversion process is bacteriopheophytin ($BPheo$), since the primary electron acceptor in photosynthesis can be either $BChl$ $a$ or $BPheo$ $a$. It differs from the $BChl$ $a$ by lacking a coordinated $Mg^{2+}$ ion and having two protons (Fig. 1(a)). $BPheo$ $a$ can be easily obtained under acidic conditions from $BChl$ $a$.

To pave the way for magic angle spinning nuclear magnetic resonance (MAS NMR) studies of the chemical and electronic structures of pigments in membrane protein complexes it is important to characterise pure pigments or cofactors. Some studies on plant pigments have been reported already. Solution assignment and re-assignments of $^{13}$C and $^1$H resonances of the plant pigment chlorophyll have been reported in the past in a number of investigations[6,7] that are summarised in Ref. [8] using samples which were biosynthetically $^{13}$C enriched to 90% and 15% levels. The difficulty in enriching the bacteriochlorophylls had restricted previous research in their $^{13}$C spectra.[8] A natural abundance $^{13}$C NMR spectrum of $BChl$ $a$ in solution was reported in Ref. [9], using a mixed-solvent system, methanol–pyridine (1:4), in order to stabilise the concentrated solution. The $^{13}$C chemical shifts of $BChl$ $a$ were only assigned using the magnitude spectrum and the assignment was based partly on the assignment of Chl $a$ reported in Ref. [7]. Further corrections of the spectral
Spectral assignments and reference data

Figure 1. Chemical structures of BChl a (a) and BPheo a (b) with the International Union of Pure and Applied Chemistry (IUPAC) numbering scheme.

Assignment of BChl a were described in the literature\textsuperscript{10,11} and within the context of study of the pathway of the BChl biosynthesis, selective stable-isotope labelling of BChl a of Rhodopseudomonas spheroides\textsuperscript{10} and of Chromatium vinosum\textsuperscript{11} was performed in the presence of metabolic precursors such as L-[1-\textsuperscript{13}C] glutamate and [2-\textsuperscript{13}C] glycine. The density functional theory calculations of the structure and \textsuperscript{15}N and \textsuperscript{13}C chemical shifts of BChl a strongly suggested that several assignments of the \textsuperscript{13}C resonances of the pyrrolic ring carbons should be revised.\textsuperscript{12} The \textsuperscript{1}H NMR spectrum of BChl a was reported in Refs \textsuperscript{13–15} and the observed chemical shifts were in agreement with the values reported for acetone but differed markedly from those recorded in pyridine-D$_{5}$ solution.\textsuperscript{7} The \textsuperscript{15}N NMR data for BChl a in solution were reported in Refs \textsuperscript{16,17}. Thus controversial assignments, mis-assignments and re-assignments have been reported for these bacterial pigments either using natural abundance molecules or applying selective stable-isotope labelling.

The extensive role of various isotopic labelling techniques in elucidating the pathway of tetrapyrrole-pigment biosynthesis including the research of David Shemin who introduced isotopic tracer technique is discussed in the literature.\textsuperscript{18} Uniform stable-isotope labelling of biomolecules is a powerful tool for structural studies since it allows performing NMR studies on a single uniformly labelled sample and could be achieved biosynthetically by culturing a chosen micro-organism on a medium containing uniformly \textsuperscript{13}C, \textsuperscript{15}N labelled substrates as metabolic precursors. Issues related with cost-effective preparation of uniformly labelled biomass of photosynthetic organisms are discussed in the literature.\textsuperscript{23–25} Uniformly \textsuperscript{13}C, \textsuperscript{15}N enriched BChl a was isolated from the biomass of photosynthetic bacteria Rhodopseudomonas palustris 17 001 grown on uniformly labelled medium as described in Ref. \textsuperscript{21}. Uniformly \textsuperscript{13}C, \textsuperscript{15}N enriched BPheo a under acidic conditions.\textsuperscript{26} The NMR results from three independent preparations of the pigments that were reproducible.

From the solution and solid state NMR data collected with the homonuclear (\textsuperscript{13}C--\textsuperscript{13}C) correlation technique the assignment of both B800 and B850 has been performed in LH2 of Rhodopseudomonas acidophila 10 050.\textsuperscript{22}

In this investigation we report a complete assignment of \textsuperscript{13}C, \textsuperscript{1}H and \textsuperscript{15}N solution and solid state chemical shifts of two bacterial photosynthetic pigments, BChl a and BPheo a. This became possible via applying uniform stable-isotope labelling strategies and isolating both pigments [U–\textsuperscript{13}C, \textsuperscript{15}N] BChl a and [U–\textsuperscript{13}C, \textsuperscript{15}N] BPheo a from the biomass of the photosynthetic bacteria. The isotopic enrichment for both pigments for \textsuperscript{13}C and \textsuperscript{15}N nuclei is > 99% and 98% respectively.

Results and Discussion

Uniform stable-isotope labelling of biomolecules is a powerful tool for structural studies since it allows performing NMR studies on a single uniformly labelled sample and could be achieved biosynthetically by culturing a chosen micro-organism on a medium containing uniformly \textsuperscript{13}C, \textsuperscript{15}N labelled substrates as metabolic precursors. Issues related with cost-effective preparation of uniformly labelled biomass of photosynthetic organisms are discussed in the literature.\textsuperscript{23–25} Uniformly \textsuperscript{13}C, \textsuperscript{15}N enriched BChl a (Fig. 1(a)) was isolated from the biomass of photosynthetic bacteria Rhodopseudomonas palustris 17 001 grown on uniformly labelled medium as described in Ref. \textsuperscript{21}. Uniformly \textsuperscript{13}C, \textsuperscript{15}N enriched BPheo a (Fig. 1(b)) was obtained from the \textsuperscript{13}C, \textsuperscript{15}N uniformly enriched BChl a under acidic conditions.\textsuperscript{26} The NMR results from three independent preparations of the pigments that were reproducible.

From the solution and solid state NMR data collected with the homonuclear (\textsuperscript{13}C--\textsuperscript{13}C) correlation technique the assignment of the carbon resonances was first obtained. The carbon assignment was used to assign the proton chemical shifts in 2D (\textsuperscript{1}H--\textsuperscript{13}C) heteronuclear correlation spectra. Since \textsuperscript{13}C shifts are generally
Figure 2. Contour plot of a 2D $^{13}$C–$^{13}$C COSY (a), 2D $^1$H–$^{13}$C HMQC (b) and $^{1}$H–$^{15}$N HMBC (c) NMR dipolar correlation spectra of [U–$^{13}$C, $^{15}$N] BChl $\alpha$ in acetone-$d_6$ (left panel) and of [U–$^{13}$C, $^{15}$N] BPheo $\alpha$ in acetone-$d_6$ (right panel) recorded in a magnetic field of 14.1 T at room temperature. The assignments of correlations (x/y) on the plot correspond with the numbering of pigments in Fig. 1.

quite sensitive to atomic charge density variations, the shifts provided information about the electronic structure of the molecule at the atomic level. The proton assignment was used to assign nitrogen chemical shifts in 2D ($^{15}$N–$^1$H) heteronuclear correlation spectra. Characterisation of U–$^{13}$C, $^{15}$N BChl $\alpha$ and of U–$^{13}$C, $^{15}$N BPheo $\alpha$ in solution is presented in Fig. 2 and in solid state in Figs 3 and 4 on the left and right panels respectively. Figure 5 shows $^{13}$C and $^1$H chemical shift correlation plots of BChl $\alpha$ and BPheo $\alpha$.

The 2D homonuclear $^{13}$C–$^{13}$C correlation spectrum of the [U–$^{13}$C, $^{15}$N] BChl $\alpha$ in solution is presented in Fig. 2(a) (left panel). From the high-field 2D $^{13}$C–$^{13}$C homonuclear correlation spectrum of [U–$^{13}$C, $^{15}$N] BChl $\alpha$, the following nearest-neighbour $^{13}$C–$^{13}$C correlation networks in the molecule of [U–$^{13}$C, $^{15}$N] BChl $\alpha$ are clearly seen: C4–C5–C7–C9–C10–C11–C12; C13–C131–C132; C15–C16–C17–C18–C19–C20–C21. In addition there are correlations between C3–C3′–C3″, C7–C7′, C8–C8′–C8″, C12–C12′, C13′–C13″ and C17–C17′–C17″.

Although no correlations are observed between C2–C3, C3–C4, C12–C13, C13–C14, C14–C15 and C13′–C15, these signals are clearly present on the diagonal and the correlations with the other neighbouring atoms are clearly seen. For example, C2 has been assigned from C2–C2′, C3 from C3–C3′, C4 from C4–C5, C12 from C12–C11 and C12–C12′, C13 from C13–C13′, and C15 from C15–C16. Finally the C14 corresponds to the data from the literature.\[10\]

In the phytyl chain there are the correlations between p1–p2–p3–p4–p5–p6–p7–p8–p9–p10. There are also the correlations observed between p3 and p17, p7 and p18, p11 and p19, and p16 and
Figure 2. (Continued).

p20. Definite assignments were found for C81, C132, C171, C172 and C19.

The 2D homonuclear $^{13}$C--$^{13}$C correlation spectrum of the $[\text{U}^{13}$C, $^{15}$N] BPheo $a$ in solution is presented in Fig. 2(a) (right panel). Following nearest-neighbour $^{13}$C--$^{13}$C correlation networks in the molecule of $[\text{U}^{13}$C, $^{15}$N] BPheo $a$ are clearly seen: C13-C14-C15-C16-C17-C18-C19-C20-C1-C2-C3-C4-C5-C6-C7-C8-C9-C10-C11-C12. In addition there are correlations between C3-C31–C32, C12–C121–C132–C133, C8–C81, C17–C171 in the ring IV, and C132–C133 in the ring V. The following nearest-neighbour $^{13}$C--$^{13}$C correlation networks in the molecule such as C1-C2-C3-C4-C5-C6-C7-C8-C9-C10-C11-C12, C13-C14-C15-C16-C17 and C18-C19-C20-C1. In addition there are the correlations C3–C31–C32, C12–C121, C13–C131–C132–C133, and C2–C21. In the phytyl chain of BChl $a$ there is the correlation between p1-p2-p3-p4-p5-p6-p7-p8-p9-p10. Finally, there is a correlation between p3 and p17. Doubling of peaks is observed for p3–p4 and p3–p17. Some of these correlation networks, involving the strongest cross-peaks, are depicted in Fig. 3(a) with the dashed lines. Although all peaks are present on a diagonal there are no strong correlations between C7–C8, C7–C18, C7–C171, C18–C181, C17–C171 and C17–C172.

Characterisation of $[\text{U}^{13}$C, $^{15}$N] BPheo $a$ has been performed as described above for $[\text{U}^{13}$C, $^{15}$N] BChl $a$. From the Fig. 3(a) right panel the assignment procedure led to the identification of several extended nearest-neighbour solid state $^{13}$C correlation networks in the molecule such as C1-C2-C3-C4-C5-C6-C7-C8-C9-C10-C11-C12, C13-C14-C15-C16-C17 and C18-C19-C20-C1. In addition there are the correlations C3–C31–C32, C12–C121, C13–C131–C132–C133, and C2–C21. In the phytyl chain of BChl $a$ there is the correlation between p1-p2-p3-p4-p5-p6-p7-p8-p9-p10. Finally, there is a correlation between p3 and p17. Doubling of peaks is observed for p3–p4 and p3–p17. Some of these correlation networks, involving the strongest cross-peaks, are depicted in Fig. 3(a) with the dashed lines. Although all peaks are present on a diagonal there are no strong correlations between C7–C8, C7–C18, C7–C171, C18–C181, C17–C171 and C17–C172.

For a short mixing time, $\sim$1 ms, the correlations in the MAS NMR are predominantly associated with nearest-neighbour carbon–carbon connectivities. Assignment of BChl $a$ resonances was based on a detailed analysis of the two spectra collected with spinning frequency of 9000 and 13 000 Hz. The assignment procedure of $[\text{U}^{13}$C, $^{15}$N] BChl $a$ led to the identification of several extended nearest-neighbour solid state $^{13}$C correlation networks in the molecule such as C1-C2-C3-C4-C5-C6-C7-C8-C9-C10-C11-C12, C13-C14-C15-C16-C17 and C18-C19-C20-C1. In addition there are the correlations C3–C31–C32, C12–C121, C13–C131–C132–C133, and C2–C21.

Although no correlations are observed between C7–C71, C8–C81, C17–C171 and C18/C181, these signals are clearly seen on the diagonal. Some of these correlation networks are depicted in Fig. 3(a) with the dashed lines. It should be mentioned that C4–C5 and C10–C11, C3–C4 and C12–C13 are strongly overlapping and the chemical shifts of C1, C4, C11, and C2 are very similar; the shifts for C32, C81, C171 and C172 are also very similar.

In the phytyl chain of BPheo $a$ the following correlation network is observed: p1-p2-p3-p4-p5-p6-p7-p8-p9-p10. Finally, there is a very clear correlation between p3 and p17. There is overlap observed for p8-p6-p10-p12, p7-p11, p5-p9-p13 and p19-p20.

In Table 1 the chemical shifts of $[\text{U}^{13}$C, $^{15}$N] BChl $a$ and $[\text{U}^{13}$C, $^{15}$N] BPheo $a$ in solution and in the solid state are summarised. The proton assignment for BChl $a$ in solution is in agreement with the assignment published earlier with the exception for the protons of C132 (difference is 1.59 ppm) (see
Table 1. A good resolution can be obtained by $^{13}$C detection and by exploiting the large $^{13}$C chemical shift dispersion in heteronuclear ($^{1}$H–$^{13}$C) correlation spectroscopy. For fast MAS heteronuclear ($^{1}$H–$^{13}$C) correlation spectroscopy, the resolution on the proton side can be improved dramatically through the application of frequency-switched Lee–Goldburg (FSLG) irradiation during proton evolution.[28]

In Fig. 3(b) we present a high-field and high-spinning speed 2D $^{13}$C–$^{1}$H heteronuclear FSLG decoupled correlation data collected from [U–$^{13}$C] BChl a and [U–$^{13}$C] BPheo a. The data were recorded using a modified version of the pulse sequence discussed in Ref. [28]. The assignment of the proton chemical shifts was performed after the assignment for carbon was completed. The proton–carbon correlations are well resolved, also in the aliphatic region of the spectrum. The overall sensitivity is good, owing to the high-spinning frequency used for the experiment. The proton lines are also partially resolved in the F1 projection, which underlines the good overall performance of the FSLG decoupling.

On the basis of proton chemical shifts assignments obtained in solution and in solids, $^{15}$N chemical shifts of [U–$^{13}$C, $^{15}$N] BChl a and [U–$^{13}$C, $^{15}$N] BPheo a were assigned and are summarised in Table 2 (see also Figs 2(c), 3(b) and 4). Uniformly labelled samples allow complete assignment of all resonances and in Table 3 the chemical shifts for the BChl a in acetone-d$_6$ are compared with the chemical shifts published earlier in acetone-d$_6$ methanol-d$_4$ (4 : 1).[10] For several atoms very different assignments have been found, compared with the...
assignments published in Ref. [10]; the comparisons are as follows for the C2, C3 in ring I, C6, C9 in ring II, C12, C13 in ring III, C16 in ring IV: C2 (142.0 vs 127.6), C3 (137.7 vs 122.3); C6 (168.9 vs 140.6), C9 (158.5 vs 151.7); C12 (124.0 vs 135.1), C13 (130.6 vs 156.6); C16 (152.0 vs 160.2). C13 and C32 were assigned in this work. This demonstrates the utility of the uniform labelling strategies for NMR applications.

For the other 13C responses listed in Table 3, the shift variations due to effects associated with the two different solvents are less than 2.5 ppm. Our assignment of C5, C10, C15, C20, C4 and C14 is in agreement with the corrected assignments for the carbons published in Ref. [12].

The 1H and 13C shifts in the solid and solution samples are plotted against monomer shifts in acetone-6 in Fig. 5(a,b) respectively. When 13C solid state chemical shifts are compared with solution chemical shifts for [13C,15N] Bchl a obtained in this work, the largest differences are observed for C21 (−2.6), C31 (−4.7), C32 (−2.9), C121 (−3.2), C134 (−2.3); C13 (−6.5), C15 (−3.9), C8 (−3.6), C10 (−2.7) and C20 (−2.4), which are upfield shifted by 3–5 ppm while downfield shifts are detected for C1 (2.3), and C172 (3.1) by about 2–3 ppm (see Fig. 5a). When the 1H solid state chemical shifts are compared with the solution data, large differences are seen for C21−CH3 and C121−CH3 providing evidence for ring current shifts due to aggregation in the solid state.

By comparison of solid state chemical shifts with solution chemical shifts for [13C,15N] Bpheo a (Table 4), the biggest differences are observed for C1 (−2.8 ppm), C21 (−6.6 ppm), C3 (7.9 ppm), C31 (−7.0 ppm), C32 (−4.7); C6 (−3.5); C10 (−3.5 ppm), C11 (−3.4), C12 (−4 ppm), C121 (−6.6 ppm); C13 (−4.5), C132 (−3.1), C14 (−2.8) and C18 (−3.3 ppm) which are upfield shifted by 3–7 ppm.

For the carbon assignment for [13C,15N] Bpheo a versus [13C,15N] Bchl a in solution the largest differences are found in ring I for C1 (−13.6 ppm), C2 (−6.1 ppm), C3 (−10.6 ppm) and C4 (−14.1 ppm); in ring II for C9 (12.6 ppm); in ring III for C11 (−13.6 ppm) and C14 (−14.9 ppm) and in ring IV for C16 (6.7 ppm) and C19 (3.8 ppm).

For the solid state carbon assignment of the two pigments, the following differences in the values of chemical shifts are observed: in ring I for C1 (−16.6 ppm), C2 (−5 ppm), C21 (−3.6 ppm), C3 (−8.6 ppm) and C4 (−16.0 ppm); in ring II for C9 (4.7 ppm); in ring III for C11 (−11.2 ppm) and for C14 (−14.7 ppm); in ring IV for C16 (−9 ppm); in ring V for C13 (−3.6 ppm). For methine carbons, the differences are C10 (−3.0 ppm), C5 (−2.4 ppm) and C20 (−2.2 ppm). The result implies that the structure and electronic states are significantly influenced by the presence of the central magnesium ion in the molecule of Bchl a.

### Experimental

#### Sample preparation

To obtain uniformly enriched pigments, photosynthetic bacteria *R. palustris* 17001 were grown on a medium containing:
Figure 5. ¹³C and ¹H chemical shift correlation plots of [U–₁³C, ₁⁵N] BChl a (left panel) and of [U–₁³C, ₁⁵N] BPheo a (right panel). The ¹H and ¹³C shifts in the solid sample are plotted against the monomer shifts in acetone-d₆ solution. The solid lines represent the diagonals. Several ¹H signals are indicated that show a large up field shift in the solid compared with the monomer.

Table 2. ¹⁵N chemical shift of [U–₁³C–₁⁵N] BChl a and [U–₁³C–₁⁵N] BPheo a. δ* (ppm) – values of nitrogen chemical shift in acetone as published in Ref. [16], δ (ppm) solution chemical shift (this work); δc (ppm) – solid state chemical shift (this work).

<table>
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<th>Position</th>
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<th>BChl a</th>
<th>BChl a</th>
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<th>BPheo a</th>
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[U–₁³C, ₁⁵N] algae hydrolysate (Heidelberg, EMBL) – 2.5 g l⁻¹, KH₂PO₄ (Darmstadt, Germany) – 1 g l⁻¹, MgSO₄ × 7H₂O (Darmstadt, Germany) – 0.4 g l⁻¹, NaCl (Darmstadt, Germany) – 0.4 g l⁻¹, CaCl₂ × 2H₂O (Darmstadt, Germany) – 0.05 g l⁻¹, and trace metals – 10 ml l⁻¹. NaOH (1 N) was used for the adjustment to pH 7. The solution of trace elements was prepared as described for M 550 in Ref. [21], but Na₂MoO₄ × 2H₂O was used instead of (NH₄)₆Mo₇O₂₄ × 4H₂O and ethylenediaminetetraacetic acid (EDTA) was excluded. The light intensity was 2 klux obtained from incandescent lamps.

After 20 days of growing, the cells were harvested by centrifugation (15 min at 10 000 g) and the pigments were extracted by incubating under mild sonication (Standardgraph, Ultrasonic cleaner) at 4 °C for 2 min in a 20-fold volume of acetone and centrifuged at 5400 g for 10 min. The supernatant consisted mostly of carotenoid pigments. The precipitate was extracted with MeOH and centrifuged at 5400 g for 20 min, releasing the BChls in the supernatant. The acetone and methanol extracts were filtered over a 0.45 μm teflon (TOSOH H-25-5) membrane filter separately, dried at reduced pressure with a rotary evaporator and subsequently dissolved in a mixture of n-hexane, 2-propanol, and methanol.

Table 3. Assignment of ¹³C chemical shifts of [U–₁³C–₁⁵N] BChl a in solution and in solid state). Assignments of chemical shifts: δ* (ppm) – in acetone-d₆–methanol-d₄ as described earlier in Ref. [9] and further corrected in Ref. [10]; δ (ppm) – in acetone-d₆ and δc (ppm) in solid state, both obtained in this work based on a uniformly ¹³C–₁⁵N labelled sample. The accuracy of the solid state shifts is ∼0.5 ppm. The numbering is according to the chemical structure in Fig. 1(a).

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Table 3. (Continued)

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Note: For the solid state correlations C7–C7b and C17–C17b are not observed and p3–p4 and p3–p17 appeared as double peaks in the MAS NMR spectra.

*a* Unknown

*b* Corrected assignment in Ref. [10]

(100/3/3 v/v/v) used as an eluent for high performance liquid chromatography (HPLC). The pigments were separated on a normal phase silica HPLC column (Senshu Pak, 1251–N, 250 × 4.6 mm i.d.), cooled to 4 °C, using a mixture of n-hexane, 2-propanol and methanol (100/3/3 v/v/v) as an eluent. For at least 99% of the BChl in *R. palustris*, phytyl was found to be the esterifying alcohol. The [U-13C, 15N] BPheo a fraction was collected and an additional amount of [U-13C, 15N] BPheo a was obtained by phoeythynisation of [U-13C, 15N] BChl a by bubbling a stream of nitrogen gas containing gaseous HCl into the ether solution of [U-13C, 15N] BChl a as described in Ref. [26]. More than 80 mg of stable-isotope-labelled pigments were isolated and used for various applications such as development of pulse sequences for MAS NMR, or fundamental research, in particular, characterisation of pigments and

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study of ligand–protein interactions as well as for other studies. Both pigments, [U–13C, 15N] BChl a and [U–13C, 15N] BPheo a, were characterised by optical, NMR and FTIR spectroscopy.

For the solution and CP/MAS NMR experiments, a Bruker 600 MHz DMX spectrometer was used. Solid state MAS NMR experiments were performed on DMX-600 and DSX-750 spectrometers equipped with a double resonance 4 mm CP/MAS probe (Bruker, Karlsruhe, Germany).

For the solution NMR measurements, 3 mg of micro (nano)–crystalline precipitates of [U–13C, 15N] BChl a or [U–13C, 15N] BPheo a was dissolved in 0.7 ml 2H2O-d-acetone and placed in a 5-mm NMR tube, followed by the degassing of the solution with nitrogen gas. For MAS NMR measurements two samples of micro(nano)–crystalline precipitates were measured. They were prepared by placing a solution of the pigment in a 4 mm CRAMPS rotor (Bruker) and evaporating the solvent with nitrogen gas. The sample volume was restricted to the centre of the rotor to improve the RF homogeneity. In a similar way a sample of [U–13C, 15N] BPheo a was prepared. For MAS NMR studies an amount of 10 mg of each of the pigments was used. Solution spectra were obtained with a 600 MHz DMX spectrometer and MAS NMR ([13C–13C, 13C–1H, 15N–1H]) spectra were obtained using 600 MHz DMX and 750 MHz Bruker spectrometers. 13C NMR spectra of pigments in liquid were referenced using an external standard of tetramethylsilane (TMS) with the methyl shift of 0 ppm. The 13C MAS NMR spectra of pigments in the solid state were referenced using Gly with the carbonyl chemical shift of 176.04 ppm. The 1H NMR spectra of pigments in liquid were referenced using an external standard of TMS with the methyl chemical shift of 0 ppm. The 1H MAS NMR spectra of pigments in solid state were referenced using TMS with the methyl chemical shift of 0 ppm. The 15N NMR spectra of pigments in solution were referenced using an external standard of saturated 15NH4NO3 with a 15N chemical shift of 22.3 ppm towards liquid ammonia. The 15N MAS NMR spectra of both pigments in solid state were referenced to solid 15NH4NO3 with a corresponding chemical shift of 23.5 ppm towards liquid ammonia.

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Note: For solid state the correlations C7–C7, C17–C17 and C18–C18 are not observed. The values of chemical shifts of (C1, C4, C11, C2) and (C32, C81, C171, C172) are very similar to each other. In the phytyl chain the following chemical shifts have similar values: p5, p9, p13; p6, p8, p10; p7, p11 and p19, p20.

NMR spectroscopy

13C NMR in solution and in the solid state. For recording 13C–13C NMR spectra of the pigments in solution COSY (correlation spectroscopy) technique with the proton decoupling was used. For 13C MAS RFDR NMR measurements the following acquisition parameters were applied: 90° 1H pulse – 4 µs, 180° 13C pulse – 23 µs, 90° 13C pulse – 12 µs, 13C–13C mixing time – 2.5 ms, 13C–1H mixing time 2 ms at a 13C–H Hartmann–Hahn matching condition, [29] repetition time 1 s, number of scans – 288, number of slices – 512 and number of data points in F2 dimension in one slice – 2048. Spinning frequencies of 8, 9, 10 and 13 kHz were used to assign side-bands and collect spectra of BChl a, and BPheo a. The resolution of the set of 2D spectra is sufficient to allow the identification of all 55 individual correlations and to arrive at a complete assignment of the 13C response. The relatively short mixing time ensures that the observed cross-peaks in these spectra are predominantly associated with the nearest-neighbour carbon–carbon correlations.

1H NMR in solution and in the solid state. For 1H NMR in solution, the pulsed field gradient (PFG) heteronuclear multiple quantum coherence (HMQC) technique was used. The 1H NMR in solids is more difficult due to the combination of the very strong homonuclear dipolar interactions between the abundant protons and of the small chemical shift dispersion of protons. Adequate resolution can be obtained by exploiting the large 13C chemical shift dispersion in 1H–13C Heteronuclear correlation (HETCOR) correlation spectroscopy. It has been demonstrated that high magnetic fields attenuate the 1H homonuclear dipolar line broadening which is of help in 1H response. In high-field MAS experiments high-spinning speeds are required to obtain sufficient resolution in the multidimensional spectra. To determine 1H solid state shifts in [U–13C, 15N] BChl a and [U–13C, 15N] BPheo a, solid state CP/MAS with FSLG irradiation during the proton evolution was employed. The mixing time for the CP was 0.3 ms, and the Lee–Goldberg offset was 4 or 5 kHz, with a repetition time of 1 s. For the 2D data sets the number of scans was 256, in 121 slices with 1494 data points in the F2 dimension. Since the Hartmann–Hahn matching and corresponding efficiency of CP magnetisation transfer is very sensitive to RF power instabilities at high MAS frequencies, a ramped-amplitude cross-polarisation sequence (RAMP-CP) was used to restore a broader matching profile. During 13C acquisition the protons were decoupled from the carbons by using a TPPM decoupling scheme. This improves the high-field 13C resolution considerably compared to Continuous wave (CW) decoupling and is essential for the assignment of the heteronuclear correlation signals. The phase modulation angle for the TPPM decoupling was set to 15°, and the flip-pulse length was optimised to 8.8 µs in order to yield optimal 13C resolution. The magic angle pulse length was 2.44 µs. The 1H shifts in the 1H–13C FSLG spectra of the pigments were referenced using the 1H–13C FSLG dataset of [U–13C] Tyr as an external reference. The 1H chemical shift scale was calibrated from the phase modulated LG spectrum of solid tyrosine hydrochloric salt.

15N NMR in solution and in solid state. The 15N NMR spectra of [U–13C, 15N] BChl a and [U–13C, 15N] BPheo a were referenced to an external standard, nitromethane at 380.23 ppm with respect to reference to liquid ammonia. The 1H–15N correlations were determined by measuring PFG heteronuclear multiple-bond...
correlation (HMBC) spectra in acetone-d$_6$ solution or by measuring correlation by long-range coupling (COLOC) spectra.$^{[16]}$ The 1D $^{15}$N CP MAS NMR spectra of pigments were recorded using the TPPM technique. The assignment of N-I and N-III in $[^{13}$C, $^{15}$N] Bpheo was confirmed from the $^{13}$C--$^{15}$N correlation spectrum. For obtaining $^{13}$C--$^{15}$N MASNMR spectra a double CP pulse sequence was used.$^{[32]}$ CP from $^1$H to $^{15}$N was 6 ms, evolution of $^{15}$N was 20 ms and CP from $^{15}$N to $^{13}$C was 2 ms. Data were collected in 98 slices, zero filled to 128 with 1794 data points in the F2 dimension.

Conclusion

Uniformly labelled ($^{13}$C, $^{15}$N) photosynthetic pigments, BChl $a$ and BPheo $a$ were obtained biosynthetically and characterised with solution and solid state NMR. A complete assignment of all $^{13}$C solid state resonances of ($^{13}$C, $^{15}$N) BChl $a$ and $^{13}$C resonances of ($^{13}$C, $^{15}$N) BPheo $a$ was obtained in solution and in solid state from the analysis of 2D and $^{13}$C--$^{13}$C COSY and CP MAS RFDR correlation spectra accordingly. The $^1$H solution resonances of both pigments were assigned from the high-field $^1$H--$^{13}$C HMQC spectra and $^1$H solid state resonances were assigned from high-field 2D $(^1$H--$^{13}$C). Frequency-switched Lee–Goldburg (FSLG) dipolar correlation spectra $^{15}$N solution resonances for both pigments were assigned from $^1$H--$^{15}$N HMBC spectra, and $^{15}$N solid state resonances of pigments were assigned from CP MAS NMR spectra. Subsequent NMR studies on a single sample allow complete assignment of $^{13}$C, $^{15}$N and $^1$H resonances of the $^{13}$C, $^{15}$N labelled pigments and provide details of a structure, which is considered as a fundamental basis for all follow-up ligand–protein interaction studies in photosynthesis research.

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References


