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Spectroscopic characterisation of a tetrameric subunit form of the core antenna protein from *Rhodospirillum rubrum*

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Abstract The core light-harvesting complex (LH1) of *Rhodospirillum rubrum* is constituted of multiple heterodimeric subunits, each containing two transmembrane polypeptides, α and β. The detergent octylglucoside induces the stepwise dissociation of LH1 into B820 (an αβ dimer) and B777 (monomeric polypeptides), both of which still retain their bound bacteriochlorophyll molecules. We have investigated the absorption properties of B820 as a function of temperature, whereby a spectral population called 'B851' has been characterised. We show evidence that it is a dimer of the B820 complex. This may represent an intermediate oligomeric form in the process of the LH1 ring formation, as its existence was predicted from global analysis of the absorption spectra of the LH1/B820 equilibrium [Pandit et al. (2001) Biochemistry 40, 12913–12924]. Stabilisation of this dissociated form of LH1 may help in understanding both the electronic properties and the association process of these integral membrane proteins. © 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Membrane protein; Light-harvesting complex; Membrane polypeptide oligomerization; Transmembrane α-helix

1. Introduction

In photosynthetic organisms, light energy is absorbed by light-harvesting (LH) pigment–protein complexes and the excitation energy is rapidly transferred to photochemical reaction centres, where its transduction into chemical potential energy takes place. Purple photosynthetic bacteria often synthesise two types of light-harvesting proteins, namely the core and peripheral antennae, also called LH1 and LH2, respectively [1]. These proteins have similar structural arrangements, comprising repetitions of a basic unit of two low molecular weight (~5 kDa) transmembrane polypeptides, α and β. The structures of the LH2 complexes of *Rhodopseudomonas* (*Rp.*) *acidophila* [2] and *Rhodospirillum* (*Rs.*) *molischianum* [3] have been resolved at high resolution by X-ray crystallography, revealing an oligomeric annular structure of nine or eight αβ-heterodimers, respectively, where the α-polypeptides form an internal and the β-polypeptides an external ring. For the LH1, to date no atomic resolution structure has been determined. Data obtained from analysis of two-dimensional crystals of LH1 from *Rhodospirillum rubrum* have, however, revealed that this protein forms a ring containing 16 heterodimeric subunits, large enough for the reaction centre to be included centrally within the LH1 ring [4,5]. Indeed, in two-dimensional crystals, the reaction centre is embedded in a ring of LH1 polypeptides [6,7], although the question of whether only such closed rings exist in vivo is still a matter of debate [8,9].

Besides their intrinsic photosynthetic interest, LH1 proteins represent an ideal model system for studying membrane polypeptide association. Each of the 32 polypeptides of LH1 binds, in the membrane phase, a bacteriochlorophyll (BChl) molecule through a highly conserved histidine residue. In the native form of the protein these molecules closely interact with each other, and their electronic properties are highly sensitive to these BChl/BChl interactions [1]. As a consequence, partial or complete dissociation of the quaternary structure of LH1 results in a dramatic blue-shift of the lower energy electronic transition of these molecules. The association state of the LH1 polypeptides may thus be studied by simple electronic (absorption and/or fluorescence) measurements. More than 10 years ago it was shown that treatment with n-octyl-β-D-glucopyranoside (βOG) of LH1 isolated from the carotenoidless strain of *Rs. rubrum* (called G_9^+) results in the formation of a smaller subunit form (B820) exhibiting an absorption maximum at 820 nm in the near infrared (i.e. blue-shifted by 53 nm relative to the native LH1, which absorbs at 873 nm in the same spectral region) [10–12]. Additional treatment of B820 with βOG results in a further dissociation of the protein, inducing a concomitant shift of the lowest energy transition of the BChl molecules to 777 nm [13]. This stepwise formation of B777 is reversible and, upon removal of the detergent B820, then intact LH1 (B873) can be re-formed [13].

Fourier-transform infrared absorption spectroscopic measurements have demonstrated that none of these dissociation steps affects the secondary structure of the polypeptides [14]. B777 consists of isolated α-helical α and β polypeptides which still retain their bound BChl molecule [14,15]. The quaternary structure of B820 depends on the βOG concentration. It is a dimer at low detergent concentration, and a tetramer at βOG concentrations higher than 1.3% [16]. However, the electronic transition of B820 arises from a BChl dimer. In order to

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Abbreviations: BChl, bacteriochlorophyll; B873, B851, B820 and B777, dissociated forms of light-harvesting complex 1 absorbing at 873, 851, 820 and 777 nm, respectively; βOG, n-octyl-β-D-glucopyranoside; CMC, critical micellar concentration; FWHM, full width at half maximum; LH1 and LH2, core and peripheral light-harvesting complexes; ODPS, n-octyl-*rac*-2.3-dipropylsulphoxide; *Rb*, *Rhodobacter*; *Rp*, *Rhodopseudomonas*; *Rs*, *Rhodospirillum*

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account for the absence of interactions between the two BChl dimers, it is likely that the tetramer formed at high βOG concentration possesses a non-native conformation, with the two αβ pairs of polypeptides associated tail to tail.

When B873 is dissociated into B820, no isobestic point can be observed between the two electronic transitions, strongly suggesting the existence of other, intermediate spectral forms. A recent global analysis of this reaction led to the proposal that only one intermediate form is necessary to account for the experimental results, absorbing at ca 850 nm [17]. In a similar analysis of the dissociation of LH1 by n-octyl-*rac*-2.3-dipropylsulphoxide (ODPS), an intermediate form absorbing at 845 nm has also been postulated [18].

In this work, we stabilised and characterised a dissociated form of *Rs. rubrum* LH1, which absorbs at 851 nm. This form likely corresponds to those which have been postulated from global analysis of the absorption spectra during the B820–B873 transition [17]. This 'B851' form is a dimer of B820, and its study may help to understand the formation of the native LH1 complex during the B820–B873 transition.

2. Materials and methods

βOG was obtained from Biomol (Hamburg, Germany); all other chemicals were from Sigma (St. Louis, MO, USA) or Merck-Biochemicals (Darmstadt, Germany). The B820 subunits were isolated and purified from the carotenoidless strain G_9^+ from *Rs. rubrum* as described previously [14]. Protein concentrations were determined from the absorption at 777 or 820 nm (with absorption coefficients $\varepsilon_{777} = 55$ mM^{-1} cm^{-1}; $\varepsilon_{820} = 86$ mM^{-1} cm^{-1} [19]). In order to equilibrate the desired detergent concentration the proteins were dialysed overnight using Celly Sep (San Antonio, TX, USA) regenerated cellular membrane cylinders (molecular weight cut off: 6–8000 Da) against 0.8% (w/v) βOG in 20 mM Tris–HCl buffer (pH = 8.0). Throughout all antennae preparations the material was protected from light and kept at 4°C until the spectroscopic measurements.

Absorption spectra were recorded with a Cary 5 Spectrophotometer (Varian plc, Sidney). During absorption measurements, the temperature of the samples was ensured by a thermostated water/ethanol circulation system. Samples were equilibrated for 15 min at each temperature, which was measured with a thermocouple positioned in the cuvette. Analysis of the absorption spectra was performed with the GRAMS32 v5.2 software (Galactic, Salem, New Hampshire, USA).

3. Results

Fig. 1A displays the absorption spectra of a sample containing B820 subunits in 0.8% (w/v) βOG at different temperatures, between 288 K and 271.5 K. In this series of spectra, a red-shifted electronic transition gradually appears, when temperature is below 288 K, with an absorption maximum at ca 851 nm. In the following, this spectral form will be referred to as B851 [20]. This absorption shift is completely reversible, and the B820/B851 transition exhibits a reasonable isobestic point at 836 nm. Above 288 K the contribution of B851 to the absorption spectra becomes extremely weak. At temperatures higher than 288 K the formation of B777 can be observed, with parallel decrease of the B820 absorption transition (data not shown). It must be noted that ensuring the appearance of large amounts of the B851 form is quite difficult. It tightly depends, in particular, on the final detergent concentration in the sample. At higher detergent concentration (e.g. 0.9%), the amount of B851 becomes negligible, whilst below 0.8% βOG concentration lowering the temperature results in the reconstitution of native LH1, i.e. appearance of B873 (data not shown).

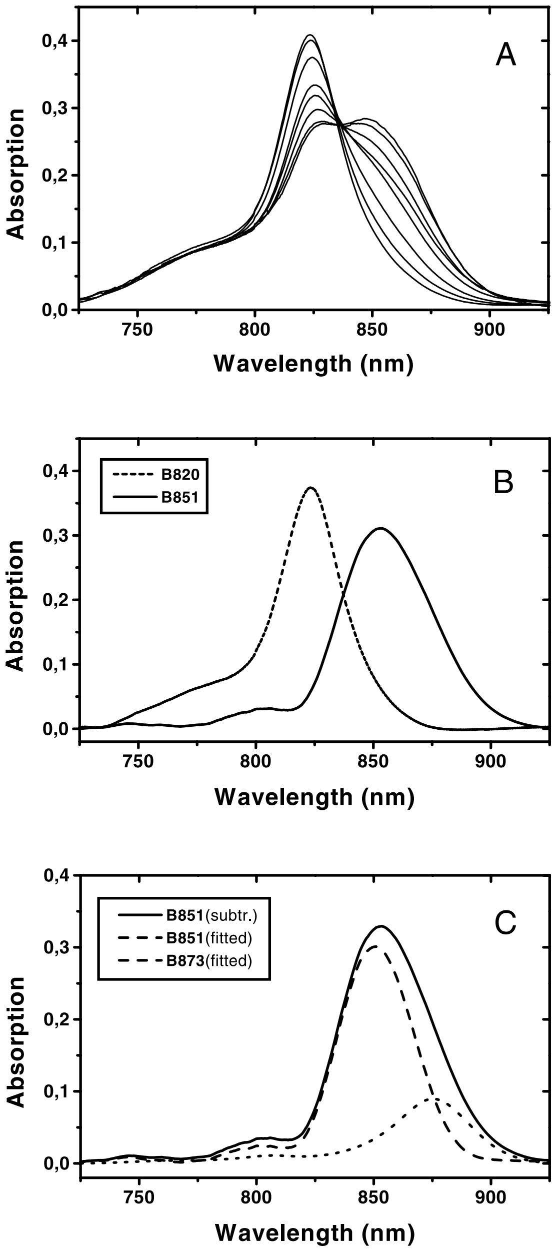

Fig. 1. A: Near infrared absorption spectra showing the temperature mediated dissociation reaction of the B851 form into B820. Spectra were recorded during a 271.5–288 K temperature cycle, first increasing the temperature from 271.5 K to 288 K, then lowering it again to 271.5 K. Spectra shown were taken at temperatures 271.5, 273.5, 275.5, 278.0, 280.5, 283.5, 285.5 and 288.0 K, where absorption of B820 is lowest at 271.5 K. By increasing the temperature, absorption of B820 increases and that of B851 decreases. The βOG detergent concentration was 0.8%. B: Absorption spectra of B851 (straight line) and B820 (dashed line); spectral forms calculated as the weighted difference of spectra from A at 271.5 and 273.5 K. C: Curve fitting analysis of B851 from B (straight line), showing spectral contributions of the B873 (dotted line) and B851 (dashed line) oligomeric forms.

Fig. 1B displays absorption spectra in the near infrared of the B851 subunit obtained by computing a weighted difference between two absorption spectra of Fig. 1A taken at the two lowest temperatures (271.5 and 273.5 K), as compared to the spectrum of the B820 form. This electronic transition exhibits a clear asymmetry, with a weak shoulder at ca 870 nm. This asymmetry is temperature-dependent, and it becomes significant only below 275 K (data not shown). This strongly suggests that, at these temperatures, some fully reconstituted LH1 is present in the sample. From curve fitting analysis, it can be deduced that B873 accounts for about 9% of the total absorption in the near infrared range in the spectrum displayed in Fig. 1B (Fig. 1C). At temperatures higher than 275 K, similar analysis shows that less than 5% of the total absorption in the near infrared arises from B873 (data not shown). The Q_y electronic absorption of B851, after removal of B873 contribution, exhibits full width at half maximum (FWHM) of 535 cm^{-1}.

From Fig. 1A, the value of ε_{851}, the extinction coefficient of the B851 form, can be calculated. Taking into account that $\varepsilon_{820} = 86$ $mM^{-1}cm^{-1}$ [21], a value of 98 mM^{-1} cm^{-1} can be deduced from such a calculation for ε_{851}. This is an intermediate value between those of B820 (86 mM^{-1} cm^{-1}) and of B873 (114 mM^{-1} cm^{-1}).

Contributions of the B851 and B820 forms were extracted from spectra of Fig. 1A by curve fitting at each temperature. The B851 concentration, as deduced from the intensity of the absorption at 851 nm at the different temperatures, was plotted according to the B820 concentration on a log–log scale (Fig. 2). If neglecting the very weak concentration of B873, in this plot the gradient of the linear relationship yields directly the reaction order of the dissociation reaction. The value of this gradient (1.93 ± 0.06) implies an association reaction in which two B820 subunits associate to form one B851 subunit. From such spectral analysis of absorption measurements obtained in the temperature range 288–308 K (results not shown), and consistent with previously published data [15–18], we could deduce that, in our measurements conditions, the structure of B820 is a dimer of B777 polypeptides. We may thus conclude that B851 is constituted by $(\alpha\beta)_2$, i.e. a tetramer of α-helical polypeptides. The association equilibrium can thus be written:

$$4\ B777_{\beta OG} \Leftrightarrow 2\ B820_{\beta OG} \Leftrightarrow B851_{\beta OG}$$

or

$$2\ (\alpha\ BChl)_{\beta OG} + 2\ (\beta\ BChl)_{\beta OG} \Leftrightarrow 2\ (\alpha\beta\ BChl_2)_{\beta OG}$$

$$\Leftrightarrow (\alpha_2\beta_2\ BChl_4)_{\beta OG}$$

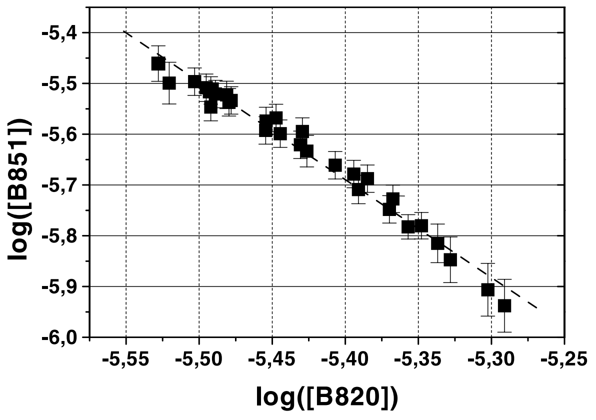

Fig. 2. Reaction order determination of the B820–B851 equilibrium. Logarithm of the concentration of B851 subunits plotted versus the logarithm of the concentration of B820. Dashed line: least squares fit to the experimental data.

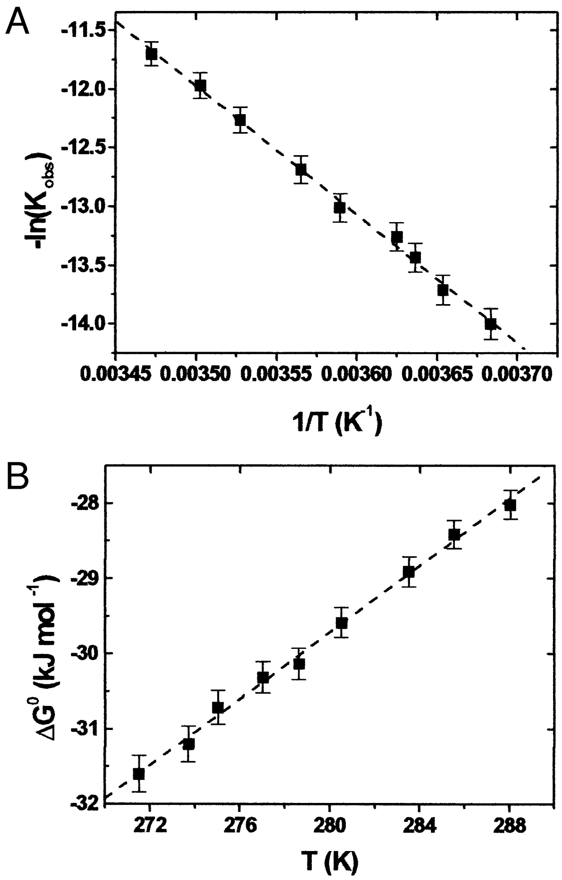

Fig. 3. Determination of $\Delta H°$ and $\Delta S°$ for B851 association. Plots of A: $-\ln(K_{obs})$ versus $1/T$ and B: $\Delta G°$ versus T, derived from the data shown in Fig. 1. K_{obs} was calculated as $[B851]/[B820]^2$, and $\Delta G°$ as $-RT\ln(K_{obs})$.

Fig. 3 shows the plots of ln (K_{eq}) vs. $1/T$ and $\Delta G°$ vs. T used to obtain the thermodynamic parameters $\Delta H°$ and $\Delta S°$ for the association reaction leading to the formation of B851. The values of $\Delta H°$ and $\Delta S°$ calculated from these plots are −91 kJ M^{-1} and −0.22 kJ M^{-1} K^{-1}, respectively.

4. Discussion

In this study we have stabilised a dissociated subunit form of *Rs. rubrum* LH1, absorbing at 851 nm. The existence of such a spectral form has already been suggested by several authors. Ghosh et al. observed a 'discrete transitory state' with a main absorption peak at 850 nm, during reassociation from B777 to B873 using LDAO as detergent [12]. Similarly, the presence of a spectral component with an absorption maximum at 845 nm was necessary for fitting the absorption spectra of partially dissociated LH1 in the presence of 0.4–0.5% ODPS [18]. The bandwidth of this 845 nm absorption transition obtained by these authors (FWHM = 51 nm) is in fair agreement with that of B851 in the present work. More recently, Pandit et al. showed that such a 850 nm transition was necessary to account for the spectral variations occurring during LH1 dissociation, the latter induced by temperature or by detergent addition [17]. However, the ca 850 nm spectral form could not be stabilised in sizeable amounts in any of these previous reports.

As stated above, the stabilisation of the B851 subunit appears only at relatively low temperatures (271.5–288 K) and high protein concentrations ($OD_{820} \geq 0.3$). Large amounts of B851 actually appear in our samples when the temperature is close to the freezing point. As stated above, the amount of B851 subunit present in the sample tightly depends on environmental conditions, such as temperature, protein or detergent concentration. These all appear vital in the competition between formation of B851 and reconstitution of full LH1 (thus giving rise to an absorption transition at 873 nm, data not shown). In many cases, the cooling of B820 to close to the freezing point leads to a mixture of B873 and B851, preventing observation of B851 alone. This may, at least in part, explain why B851 has not been clearly experimentally observed before.

Our results unambiguously show that B851 is a dimer of B820, i.e. an $\alpha_2\beta_2$ $BChl_4$ structure. The existence of the B851 subunit form reveals that the association process of B820 subunits in the path of the ring formation is probably a multiple-state reaction, as previously suggested. It implies that, in the final phase of this process, oligomers of at least 4 α-helices aggregate to form the native structure of this protein. However, the existence of other, less stable, forms cannot be excluded. It must be underlined that the position of the red-most transition of this tetramer is in remarkable agreement with the value predicted by global analysis of the B873–B820 transition [17]. It was recently proposed that $\alpha_2\beta_2$ $BChl_4$ isolated on gels from partially denatured LH1 from *Rhodobacter* (*Rb.*) *sphaeroides* exhibits a main absorption peak in the infrared located at 886 nm [22]. While these absorption experiments were performed at 77 K, it is unlikely that the difference in temperature may fully account for the 33 nm difference between the two sets of measurements. One important factor may be the presence of carotenoid molecules in the dissociated LH1 subunits of *Rb. sphaeroides*. It is known, at least for LH2, that these molecules lie between adjacent αβ pairs. If the organisation of LH1 is, in this respect, similar to that of LH2, their presence should drastically influence the absorption properties of the tetrameric subunit. It should also be noted that the *Rb. sphaeroides* tetramers were isolated on lithium-dodecyl sulphate polyacrylamide gels, from which a series of subunits of different sizes can be isolated. As recently reported, these different-sized subunits do not seem to be in equilibrium with each other on a long time-scale (several minutes) [23]. This suggests that they are in a somewhat locked conformation, which forbids their reassociation and/or dissociation. It is conceivable that this special conformation also has an influence on their absorption properties. Thirdly, the very narrow range of temperature and detergent concentration in which the B851 subunit can be observed suggests that it is stabilised by specific structures formed by the βOG detergent. It is thus possible that the protein–detergent interactions somehow influence the absorption properties of the tetrameric subunit of *Rs. rubrum* LH1. The B820–B851 transition occurs in our experimental conditions at βOG concentrations close to 0.8%. This represents values near to the critical micellar concentration (CMC) of this detergent, where temperature can have significant effect on micelle formation [24]. However, it is now well documented that ionic strength and the presence of lipids and/or proteins tend to decrease the CMC of βOG [24]. The experiments reported here are thus performed above the CMC of the detergent, i.e. in the presence of detergent micelles.

The thermodynamic parameters measured for the formation of a $(\alpha\beta)_2$ tetramer are of particular interest, as they allow the probing of the energy involved in the lateral contacts between dimers, and the comparison of these with the energies involved in the formation of dimers and intact LH1 [14,16,17]. Care must be taken, however, in considering the thermodynamic parameters obtained from our experiments, as these include the loss of the detergent–polypeptide interactions upon tetramer formation. If the B851 subunit requires interaction with a specific detergent structure for its stabilisation, the higher the energy of these specific interactions, the lower will be the observed $\Delta H°$ corresponding to the tetramer formation. It is interesting to note, however, that the $\Delta H°$ associated with the formation of the tetramer is half that attributed to the formation of the αβ dimer (between -175 and -216 kJ M^{-1}) [14,16,17]. It has been reported, for LH2 of *Rp. acidophila*, that a large part of the αβ interdimer interactions were mediated by the carotenoid molecule rhodopsin glucoside [25]. If this is the case in LH1, the absence of carotenoid in LH1 from *Rs. rubrum* might explain this low $\Delta H°$ value. Pandit et al. have determined that LH1 formation from B820 subunits is associated with a $\Delta H°$ of -2391 kJ M^{-1}. From our measurements it can be concluded that the existence of 16 αβ/αβ interfaces in a LH1 closed ring should result in a total enthalpy associated with the dimer/dimer association of 1456 kJ M^{-1}, i.e. about two-thirds of the total enthalpy observed during the B820–B873 transition. Addition of each αβ dimer on the pathway of the complete ring formation should thus induce a partial reorganisation of the whole architecture, resulting in more favourable interactions between the subunits. These interactions should account, upon formation of the complete ring, for a third of the total enthalpy associated with the LH1 formation. This suggests that the latter proceeds through a cooperative process, which may explain the difficulty of stabilising higher oligomeric forms than the αβ dimer.

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