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The reaction order of the dissociation reaction of the B820 subunit of *Rhodospirillum rubrum* light-harvesting I complex

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Abstract We have studied the equilibrium between the dissociated B777 form (absorbing at 777 nm) of the light-harvesting complex of *Rhodospirillum rubrum* and the oligomeric B820 form. Analysis of the reaction order for the B820 dissociation reaction to form B777 shows that this reaction depends on the concentration of octylglucoside detergent (*n*-octyl-β-D-glucopyranoside (βOG)) present in the sample. At low βOG concentrations (less than 1.2%) this reaction requires two components, presumably one α-B777 and one β-B777, implying that the B820 subunit is a dimer. At higher βOG concentrations this reaction requires four components, implying that B820 is a tetramer. These results partly explain the discrepancies in the literature about the stoichiometry of B820 and open an original way for studying protein–detergent interactions.

Key words: Membrane protein; Purple photosynthetic bacterium; Bacteriochlorophyll; Transmembrane α-helix

1. Introduction

In purple photosynthetic bacteria, the primary steps of the photosynthetic process occur in the intracytoplasmic membrane. This membrane contains two types of light-harvesting (LH) complexes, namely LH1 and LH2, also called core and peripheral antenna, respectively. These pigment–protein LH complexes collect the light energy and ensure its funnelling to the photochemical reaction centre where its transduction into chemical potential energy takes place [1]. Both LH1 and LH2 are oligomers of a minimal unit containing two small transmembrane polypeptides, α and β. These polypeptides bind the pigment cofactors, bacteriochlorophyll (Bchl) and carotenoid molecules. The structures of two related LH2 complexes have been resolved at atomic resolution by X-ray crystallography, that of the peripheral antenna complexes of *Rhodopseudomonas acidophila* [2] and of *Rhodospirillum molischianum* [3]. They consist of an annular structure of 9 or 8 αβ heterodimers, respectively, in which the α polypeptides form the internal ring and the β polypeptides the external one. In the membrane phase, the α and β polypeptides bind each a Bchl molecule through a conserved histidine residue. The latter are sandwiched between the polypeptides and form a ring of 18 (respectively 16) strongly interacting molecules. To date only projection structures and two-dimensional crystals of LH1 complexes were obtained [4]. These are composed of larger rings than LH2 containing 16 αβ subunits and 32 intercalated chromophores large enough to contain the reaction centre [5,6]. Whether these closed rings are the only LH1 form existing in vivo is still under debate, as in an LH2-lacking mutant from *Rhodobacter capsulatus* LH1 complexes were observed as open rings of approximately 12 αβ subunits (3/4 of a turn) [7].

The LH1 protein is a particularly interesting membrane protein as it can be reversibly dissociated by detergent treatment to yield a subunit form [8–12]. In LH1 isolated from the G_9^+ carotenoidless strain of *R. rubrum*, treatment with the detergent *n*-octyl-β-D-glucopyranoside (βOG) results in formation of a small subunit form (B820) and a concomitant shift of the absorption maximum from 873 to 820 nm [12,13]. Additional treatment with βOG results in a further shift in the absorbance to 777 nm [13], associated with a further dissociation of the protein. This step-wise formation of the 777 nm-absorbing form (B777) is reversible, and upon removal of the detergent, the polypeptides reassociate to form the B820, and then intact LH1 (B873) complexes [13]. It has been shown, by FTIR spectroscopy, that neither of these dissociation steps affects the secondary structure of the polypeptides [14]. B777 consists of isolated α-helical, α and β polypeptides still retaining their bound Bchl molecule [14]. The exact nature of B820 is still unclear. Spectroscopic studies have established that its electronic absorption transition at 820 nm arises from a Bchl dimer [15,16]. However, measurements of hydrodynamic size suggest that B820 behaves as a tetramer [9,12]. Similarly, the reaction order of the B820 formation from the association of B777 was measured, and it appeared that four B777 are necessary to build up a B820 [14]. However, in a recent work, this reaction order was revisited, and it was concluded that the B820 formation required only two B777 [17]. In order to clear up these apparent discrepancies, we have determined in this paper the reaction order of the B820 association/dissociation reaction at different detergent concentrations. We show that the quaternary structure of the B820 form tightly depends on the βOG concentration present in the sample.

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Abbreviations: Bchl, bacteriochlorophyll; B873, B820 and B777, dissociated forms of light-harvesting complex 1 absorbing at 873, 820 and 777 nm, respectively; CMC, critical micellar concentration; LH, light-harvesting complex; βOG, *n*-octyl-β-D-glucopyranoside

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2. Material and methods

βOG was from Biomol (Germany). All other chemicals were from Sigma (St. Louis, MO, USA) or Merck-Biochemicals (Darmstadt, Germany). The B820 subunit was purified from the carotenoidless strain G_9^+ of *R. rubrum* as described earlier [14]. Protein concentrations were determined either from the absorption at 280 nm (absorption coefficient at 280 nm calculated from the amino acid composition $\varepsilon_{280}^{1\,mg/ml} = 2.9$) or from the absorption at 777 or 820 nm (absorption coefficients $\varepsilon_{777} = 55\ mM^{-1}\ cm^{-1}$, $\varepsilon_{820} = 86\ mM^{-1}\ cm^{-1}$) [11]. For reaction order determination, B820 was serially diluted, at a constant detergent concentration, with Tris–HCl buffer (20 mM, pH 8) containing 100 mM NaCl and βOG. Absorption spectra of the B820/B777 equilibrium were recorded at 20°C with a Cary 5 Spectrophotometer (Varian plc, Sidney). Absorption of the B777 and B820 forms were extracted from these spectra by curve fitting, using the GRAMS32 software (Galactic, Salem, New Hampshire, UK).

3. Results and discussion

Fig. 1A shows the effects of dilution on the B820/B777 equilibrium in the presence of 1.4% βOG. Diluting the sample at a constant βOG concentration induces the dissociation of the B820 form of the LH1 into B777, as previously reported [14,17]. The same measurements were also performed in the presence of 0.8, 0.95, 1.1, 1.25, 1.4, 2 and 3% βOG. The B820 concentration was plotted according to the B777 concentration on a log–log scale. Fig. 1B displays such plots for βOG concentrations of 0.8 and 1.4% as examples. It clearly shows that there is a linear relationship between the logarithms of these two concentrations, over the whole range of concentrations studied. In such a plot, the gradient of the linear relationship yields directly the reaction order of the dissociation reaction. These gradients were plotted as a function of total βOG concentration (Fig. 2). The reaction orders as deduced from these gradients are close to 2 for βOG concentrations ranging from 0.8 to 0.95%, in good agreement with the results of Pandit et al. [17]. At similar βOG concentrations (0.8%), these authors determined that the reaction order of the reaction is 2 in buffer potassium phosphate 50 mM, pH 7.5. This indicates that, in this detergent concentration range, B820 is formed by a polypeptides dimer. The ΔH^0 and ΔS^0 values, deduced from the analysis of the B820/B777 equilibrium as a function of temperature, were nearly identical to those determined previously [17]. For concentrations ranging from 1.4 to 2% βOG, our study indicates a reaction order of 4, in agreement with the results obtained by Sturgis and Robert [14]. In the latter paper, care was taken to maintain the samples at detergent concentrations above the critical micellar concentration (CMC), and all the experiments were performed above 2% βOG (Sturgis, personal communication). As documented in [14], this fourth order reaction does not involve the Bchl pigments, and it must be concluded that B820, in these experimental conditions, is composed of a polypeptides tetramer. For intermediate concentrations, between 1.1 and 1.3, an intermediate value of 3 is found. It is likely that, in this βOG concentration range, the dimeric and tetrameric forms of B820 coexist in equilibrium.

These results clearly put to evidence that the dominant association form of the LH polypeptides may vary according to the experimental condition, i.e. that the nature of the B820 actually depends on the detergent concentration present in the sample. This may, at least in part, explain the many discrepancies between the results reported in the literature (e.g. between [14] and [18]). It is worth noting that Ghosh et al. [12,18], who performed analytical centrifugation experiments on B820 in the presence of *n*-octylpolyoxyethylene, found that the B820 subunit was mainly a tetramer. The latter result suggests that the oligomeric state of B820 also depends on the chemical nature of the detergent used. From spectroscopic studies it was concluded that after excitation of light by B820, there was no energy transfer between 820 nm-absorbing species [15]. From this result, it has been deduced that the 820 nm-absorbing species was constituted from an isolated Bchl dimer [15,16]. The absorption properties of B820 at various βOG concentrations were compared (data not shown). At room temperature, there is no difference between the position and width of the 820 nm electronic transition. This suggests that the two Bchl dimers do not strongly interact in the tetramer, if at all. This is in good agreement with the hypothesis drawn in Sturgis et al. [19] who proposed that the tetramer formation might occur through contacts between the amino-terminal ends of the αβ polypeptides. In this configuration, each of the 820 nm-absorbing dimers would be maintained too far apart for excitation transfer to occur with a high efficiency. It is worth noting that, in the frame of this model, this 'tail to tail' association of two B820 dimers does not represent a step towards the LH1 formation, as in the latter each B820 dimer finally interacts with its closest neighbours. Recently, a 850 nm-absorbing form was spectroscopically characterised, which is likely to constitute such an intermedi-

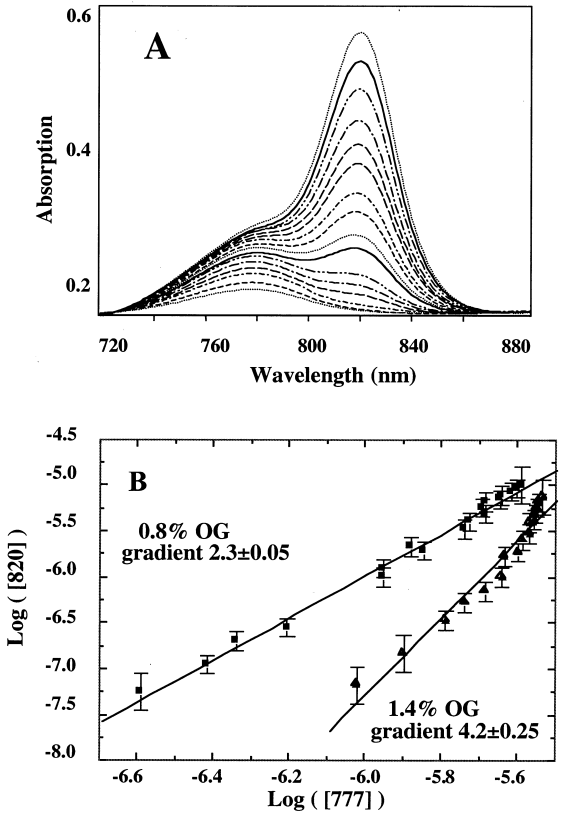

Fig. 1. Reaction order determination. A: Effect of serial dilutions on B820/B777 equilibrium in buffer containing 1.4% βOG. B: Relationship between the concentration of B820 and B777, plotted on a log–log scale for 1.4 and 0.8% βOG as examples. Lines: Least square fits to the experimental data. Same measurements were also performed in buffer containing 0.95, 1.1, 1.2, 2 and 3% βOG (results not shown).

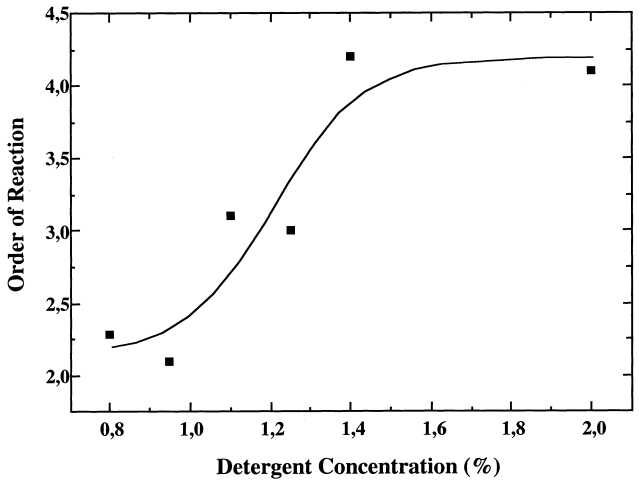

Fig. 2. Reaction order of the B820 dissociation reactions into B777, plotted as a function of βOG concentration (squares). Black curve: sigmoidal fit of the data.

ate association form of B820 dimers on the path of LH1 formation [17].

An obvious question raised by the results of this work concerns the mechanisms, which lead, depending of the βOG concentration, the isolated polypeptides towards a dimeric or a tetrameric association form. The dimer-to-tetramer transition occurs, in our experimental conditions, at a βOG concentration around 1.2%, i.e. apparently higher than, but close to, the CMC (ca. 0.8%). It must be noted than the CMC of βOG is influenced by most of the experimental parameters (temperature, ionic strength, lipid and protein concentration, etc.) [20]. However, it was reported that ionic strength and presence of lipids tend to decrease the CMC rather than increase it [20]. In our experimental conditions, thus, the CMC of βOG should be lower than 0.8%. However, the amount of βOG molecules involved in solvating the B777 and/or B820 subunits should also be taken into account. This number is not precisely known, but it must be underlined that the phase transition of βOG in our experimental conditions is likely to be very complex. A complete characterisation of the dimer-to-tetramer transition of the B820 will require a careful characterisation of the structures formed by the βOG between 0.8 and 1.2%, in the presence of different concentrations of antenna polypeptides. In the absence of this information, a precise interpretation of the change observed in the reaction order of B820 formation is not possible. It must be noted however that the enthalpy of formation of the dimeric and tetrameric forms of B820 are very similar. This was already underlined in the paper of Pandit et al. [17], where the authors noted that the thermodynamic values (both enthalpy and entropy) observed for the dimer formation were altogether close to those determined by Sturgis et al. for the tetramer formation. In the absence of additional information about the detergent structures in the presence of protein in the 0.8–1.5% range, the precise meaning of this observation is still unclear. Indeed, we are currently trying to characterise these structures, which are likely to be relevant for our understanding of the membrane protein–detergent interactions.

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