An ultrafast time-resolved anisotropy study of bacteriochlorophyll *a* in pyridine

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Abstract The transient absorption anisotropy spectrum of bacteriochlorophyll *a* (BChl *a*) in pyridine was measured in the wavelength interval 550–850 nm, 1 ps after optical excitation with a 792-nm femtosecond light pulse. In the wavelength region of Q_y absorption and stimulated emission (775–825 nm), the anisotropy was found to be close to the theoretically expected value (0.4) for a two-level system. In the wavelength region 650–750 nm, where the transient absorption signal is dominated by excited state absorption, the anisotropy is reduced to ~0.18. Anisotropy kinetics were measured at several wavelengths and found to be constant within the time window 0–5 ps, showing that no internal dynamics of the BChl *a* molecule change the anisotropy on the time scale of tens of picoseconds.

Key words: Bacteriochlorophyll *a*; Transient absorption anisotropy; Excited state absorption

1. Introduction

Bacteriochlorophyll *a* (BChl *a*) is a pigment found in, amongst other species, photosynthetic purple bacteria. It consists of a tetrapyrrole ring with two of its pyrrole groups saturated (Fig. 1). BChl *a* is the main pigment in the light harvesting antenna and reaction center of photosynthetic purple bacteria. In order to understand the light harvesting and electron transfer properties of the photosynthetic membrane, transient absorption and time-resolved fluorescence experiments have been performed [1–4]. In such experiments, energy transfer between different pigments with different excited state energies is monitored by time dependent spectral changes associated with the processes. One way to study energy transfer between energetically identical molecules is to measure how the anisotropy changes with time. For this method to monitor properly energy or electron transfer processes, the anisotropy of the pigment molecule itself must be well characterized and there should be no intrinsic dynamics of the anisotropy (i.e. no internal dynamics of the BChl *a* molecule that affect the anisotropy) on the time scale of energy/electron transfer.

Previously, anisotropy measurements with picosecond time resolution were performed to measure energy transfer dynamics between different pigment pools in photosynthetic purple bacteria [5,6]. The initial anisotropy was observed to be lower than the theoretically expected value ($r(0) = 0.4$). Due to the limited temporal resolution in these early experiments, this observation could be interpreted as reflecting an unresolved energy transfer process. More recent experiments performed with approximately 100-fs pulses have revealed several cases with initial anisotropies of ~0.4 [7–16]. At the same time it is known that the transient absorption spectrum of BChl *a* [8,17] as well as chlorophyll *a* [18] and chlorophyll *b* [19] carries contributions from several overlapping transitions. These transitions may not have the same directions of their transition dipole moments. If absorption anisotropy is measured at a wavelength where several differently polarized transitions are contributing to the absorption signal, an initial ($t = 0$) anisotropy ($r(0)$) different from the $r(0) = 0.4$ value of a two-level molecule (only one transition) will be obtained. When studying for instance energy transfer in a photosynthetic pigment-protein complex, this can be mistaken for an ultrafast unresolved energy transfer process. In order to provide a solid basis for future studies of energy transfer processes in BChl *a*-containing pigment-protein systems we have undertaken a systematic study of the spectral and temporal dependence of BChl *a* transient absorption anisotropy. In this paper we examine the transient absorption anisotropy of monomeric BChl *a* in pyridine on the picosecond and sub-picosecond time scales and how it varies with wavelength. The results provide direct information as to which wavelengths are best for monitoring energy transfer and at which wavelengths the transient absorption signal, and concomitantly the transient absorption anisotropy, are strongly influenced by excited state absorption. Similar information has previously been reported for chlorophyll *a* [18] and chlorophyll *b* [19] in pyridine.

2. Materials and methods

Crystallized phytol BChl *a* dissolved in pyridine [20] was used in the experiments. It is known that BChl *a* can easily form photoproducts [21–23]. One type is formed when BChl *a* loses its magnesium atom. Another type is formed by (photo)oxidation, which includes allomerization of the isocyclic ring and dehydrogenation of the reduced pyrrole rings to form a chlorin structure. Preparation of the sample was therefore performed in a dark room with only red light; the solution was bubbled with nitrogen and placed in a sealed quartz rotating cell and the purity was checked before and after measurements with an absorption spectrophotometer. Teuchner et al. [24,25] measured a differential optical density spectrum of Pd-Bphe a in diethylether at zero time delay with nanosecond time resolution. They found induced absorption in the wavelength region 550–700 nm and interpreted this as absorption from radicals formed via triplet states. We used a rotating quartz cell and an amplified laser system working at 5 kHz to prevent formation of triplet states. The steady state absorption spectrum was measured with a Jasco V-530 UV/Vis spectrophotometer and the emission spectrum was obtained with a Spex 1681 fluorescence spectrometer.

The femtosecond transient absorption measurements were performed with an amplified Ti:Sapphire laser system operating at 5 kHz and generating ~100-fs pulses at 792 nm with a pulse energy of 0.36 mJ. This laser system and the experimental setup has been

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described in detail elsewhere [13,14]. Briefly, the 792-nm pulses were divided into an excitation pulse and an analyzing pulse by a beamsplitter. A typical excitation pulse energy of ~100 nJ was used with a spot size diameter of 400 μm at the sample position. These parameters correspond to a photon density of $\sim 1\times10^{14}$ photons/pulse/cm^2, and were used to minimize sample degradation. A white light continuum was generated by focussing the analyzing beam into a 5-mm thick sapphire plate, and divided by a beamsplitter into probe and reference beams. The polarization of the analyzing beam was fixed via a Glan polarizer, while a Berek compensator was used to rotate the polarization of the excitation beam. The detection system consisted of a three-photodiode arrangement to detect the intensities of the probe, reference and excitation pulses [18,19]. From these signals, the transient absorption signal was obtained as: $\Delta A = \log(I/I_{ref})_{without\ exc.} - \log(I/I_{ref})_{with\ exc.}$, and only data points within a preset window of excitation pulse intensities were selected (typically 5% intensity variation). The apparatus response function of the measurement system was 150 fs (FWHM) as determined by cross correlation of the excitation and the probe beams. All measurements were performed at room temperature.

3. Results and discussion

In order to examine the intrinsic monomeric BChl *a* anisotropy as a function of wavelength, transient absorption spectra of BChl *a* in pyridine were measured 1 ps after excitation with a 792-nm fs pulse; spectra with pump and probe light polarized parallel or perpendicular to each other were obtained (Fig. 2A). Both spectra exhibit similar features, a strong bleaching/stimulated emission band peaking at ~780 nm and a broad featureless induced absorption band due to excited state absorption in the interval 550–750 nm. At ~625 nm there is a negative dip in this absorption band due to the bleaching of the Q_x absorption band. A similar transient absorption spectrum has been reported earlier [8,17] and was used to calculate the $S_1 \rightarrow S_n$ excited state absorption spectrum of BChl *a* [17]. A very similar ESA spectrum as that reported by Becker et al. [17] was obtained from our data (results not shown). In this report we concentrate on the anisotropy properties of BChl *a*. From the parallel and perpendicularly polarized spectra (Fig. 2A), the transient anisotropy

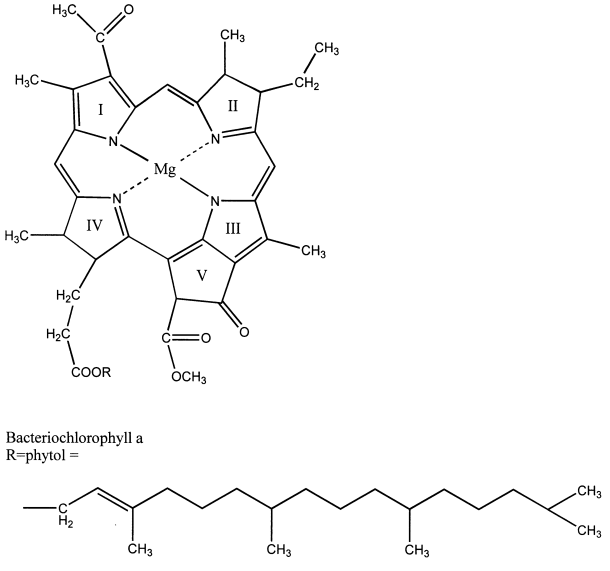

Fig. 1. Structure of BChl *a*.

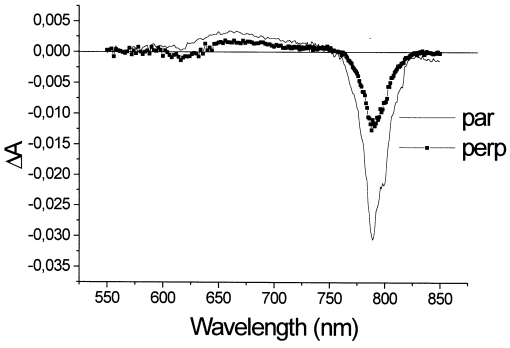

Fig 2A

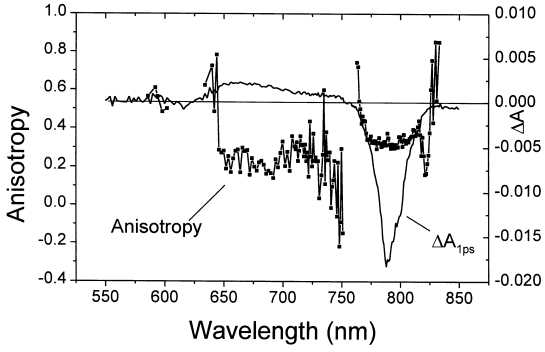

Fig. 2. A: Parallel and perpendicularly polarized transient absorption spectra of BChl *a* in pyridine, measured 1 ps after excitation at 792 nm. B: Time-resolved anisotropy spectrum of BChl *a* in pyridine, 1 ps after excitation, calculated from the spectra in (A).

spectrum at 1 ps was obtained as:

$$r_{1\ ps}(\lambda) = \frac{I_{II}(\lambda) - I_{\perp}(\lambda)}{I_{II}(\lambda) + 2I_{\perp}(\lambda)} \quad (1)$$

where $I_{II}(\lambda)$ and $I_{\perp}(\lambda)$ is the intensity of the transient absorption signal, obtained with parallel (perpendicular) pump and probe pulses (see Fig. 2B). This spectrum shows that the absorption anisotropy varies strongly with wavelength; within the main bleaching/stimulated emission band of the Q_y transition (775–825 nm) the anisotropy is high ($\approx$0.37) and approaching the theoretically expected value 0.4 for a two-level molecule. For wavelengths outside this range ($\lambda < 775$ nm) the anisotropy is low ($\approx$0.18) or high ($\lambda > 825$). The transient absorption signal is a combined result of bleaching, stimulated emission and excited state absorption. At wavelengths where the transient absorption signal is dominated by excited state absorption, anisotropy may attain any value ($-\infty < r < \infty$), depending on the magnitude of the ESA and the polarization of the $S_1 \rightarrow S_n$ transition. This has been discussed in detail in our previous work on Chl *a* and Chl *b* [18,19].

The orientation of the transition dipole moment of the excited state absorption, relative to that of the ground state transition, can be obtained from the expression [26]:

$$r_0(\lambda) = \frac{2(\sigma_{PB}(\lambda) + \sigma_{SE}(\lambda)) + \sigma_{ESA}(\lambda)(1-3\cos_2\theta)}{5(\sigma_{PB}(\lambda) + \sigma_{SE}(\lambda) - \sigma_{ESA}(\lambda))} \quad (2)$$

where σ_{PB}, σ_{SE} and σ_{ESA} are the absorption cross sections of the ground state absorption, stimulated emission, and the ex-

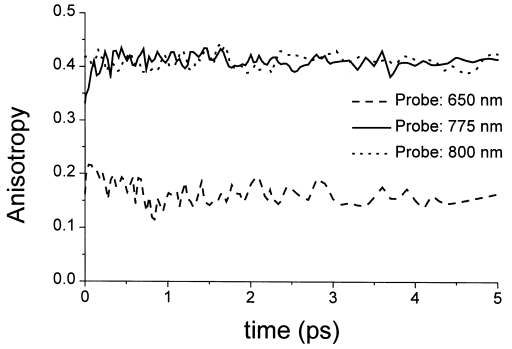

Fig. 3. Transient absorption anisotropy kinetics of BChl *a* in pyridine at 650, 775 and 800 nm with excitation at 792 nm.

cited state absorption. These quantities are obtained from the ground state absorption spectrum, the fluorescence spectrum (following a method of Becker et al. [17]) and the excited state absorption spectrum obtained from the measured transient absorption spectrum (see above). θ is the angle between the transition dipole moments of the ground state and first excited state. From the anisotropy data of Fig. 2B we obtained a value of 46° for the angle θ, correlated to the transition giving rise to the broad absorption in the wavelength interval 650–750 nm. Within the main Q_y bleaching/SE band the anisotropy is close to the theoretically expected value of 0.4 for a two-level molecule, due to the relatively small contribution of ESA to the overall transient absorption signal.

In order to examine whether there is any temporal evolution of the intrinsic BChl *a* anisotropy, due to for instance changing directions of the transition dipole moments, we measured anisotropy kinetics at several wavelengths. Fig. 3 illustrates the results of such measurements at three wavelengths, two at high ($\approx$0.4) anisotropy (775 and 800 nm) and one at low ($\approx$0.18) anisotropy (650 nm). All three measurements show that there is no change of anisotropy within experimental error during the observed time window (0–5 ps). This shows that the absorption anisotropies provided by the anisotropy spectrum of Fig. 2B, measured at 1 ps, are representative of BChl *a* within a time window of tens of picoseconds after excitation.

As mentioned above, transient absorption anisotropy is a powerful tool to monitor energy transfer between similar (or identical) chromophores in for instance photosynthetic light-harvesting complexes [1–4]. For this method to correctly monitor the energy transfer dynamics, the intrinsic anisotropy of the chromophore should meet two requirements, (1) the initial ($t = 0$) anisotropy should be known at the wavelength of measurement, i.e. it should be known whether $r(0)$ is 0.4 (the generally expected value) or something else, and (2) the intrinsic anisotropy should be time independent or its time dependence well characterized. Our results of Fig. 2B show that BChl *a* meets the requirement (1); we see that in the wavelength interval 775–825 nm the anisotropy at early times ($t \sim 0$) is very close to 0.4. The kinetic measurements of Fig. 3, in addition, show that the requirement (2) is also fulfilled. Thus it appears that for BChl *a* the spectral region corresponding to Q_y bleaching and stimulated emission is well suited for monitoring of energy transfer processes. At other wavelengths, the initial anisotropy deviates strongly from 0.4. If such wavelengths are chosen for monitoring of energy transfer this must be kept in mind and not mistaken for an unresolved ultrafast depolarization process.

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References

[1] van Grondelle, R., Dekker, J.P., Gillbro, T. and Sundström, V. (1994) Biochim. Biophys. Acta 1187, 1–65.
[2] Sundström, V. and van Grondelle, R. (1995) in: Anoxygenic Photosynthetic Bacteria (Blankenship R.E., Madigan M.T. and Bauer C.E., Eds.), Chapter 17, pp. 349–372, Kluwer Academic Publishers, Dordrecht.
[3] Sundström, V., Pullerits, T. and van Grondelle, R. (1997) in: Femtochemistry and femtobiology: Ultrafast reaction dynamics at atomic-scale resolution (Sundström V., Ed.), Nobel symposium 101, pp. 319–348, Imperial College Press, London.
[4] Sundström, V., Pullerits, T. and van Grondelle, R. (1999) J. Phys. Chem. B 103, 2327–2346.
[5] Sundström, V., van Grondelle, R., Bergström, H., Åkesson, E. and Gillbro, T. (1986) Biochim. Biophys. Acta 851, 431–446.
[6] van Grondelle, R., Bergström, H., Sundström, V. and Gillbro, T. (1987) Biochim. Biophys. Acta 894, 313–326.
[7] Hess, S., Feldchtein, F., Babin, A., Nurgaleev, I., Pullerits, T., Sergeev, A. and Sundström, V. (1993) Chem. Phys. Lett. 216, 247–257.
[8] Hess, S., Åkesson, E., Cogdell, R.J., Pullerits, T. and Sundström, V. (1995) Biophys. J. 69, 2211–2225.
[9] Pullerits, T., Chachisvilis, M. and Sundström, V. (1996) J. Phys. Chem. 100, 10787–10792.
[10] Jimenez, R., Dikshit, S.N., Bradforth, S.E. and Fleming, G.R. (1996) J. Phys. Chem. 100, 6825–6834.
[11] Bradforth, S.E., Jimenez, R., van Mourik, F., van Grondelle, R. and Fleming, G.R. (1995) J. Phys. Chem. 99, 16179–16191.
[12] Jonas, D.M., Lang, M.J., Nagasawa, Y., Joo, T. and Fleming, G.R. (1996) J. Phys. Chem. 100, 12660–12673.
[13] Monshouwer, R., Baltuška, A., van Mourik, F. and van Grondelle, R. (1998) J. Phys. Chem. A 102, 4360–4371.
[14] Ma, Y.-Z., Cogdell, R.J. and Gillbro, T. (1998) J. Phys. Chem. B 102, 881–887.
[15] Chachisvilis, M., Kühn, O., Pullerits, T. and Sundström, V. (1997) J. Phys. Chem. B 101, 7275–7283.
[16] Diffey, W.M., Homoelle, B.J., Edington, M.D. and Beck, W.F. (1998) J. Phys. Chem. B 102, 2776–2786.
[17] Becker, M., Nagarajan, V. and Parson, W.W. (1991) J. Am. Chem. Soc. 113, 6840–6848.
[18] Martinsson, P., Oksanen, J.A.I., Hilgendorff, M., Hynninen, P.H., Sundström, V. and Åkesson, E. (1999) Chem. Phys. Lett. 309, 386–394.
[19] Oksanen, J.A.I., Martinsson, P., Åkesson, E., Hynninen, P.H. and Sundström, V. (1998) J. Phys. Chem. A 102, 4328–4336.
[20] Oksanen, J.A.I. (1998), in Spectroscopic characterization of some monomeric and aggregated chlorophylls, Thesis, University of Jyväskylä.
[21] Lindsay Smith, J.R. and Calvin, M. (1966) J. Am. Chem. Soc. 88 (19), 4500–4506.
[22] Svec, W.A. (1978) in: The Porphyrins (Dolphin D., Ed.), Vol. 5, pp. 341–399, Academic Press, New York.
[23] Hynninen, P.H. (1991) in: Chlorophylls (Scheer H., Ed.), Chapter 1.7, pp. 145–209, CRC Press, Boca Raton, FL.
[24] Teuchner, K., Stiel, H., Leupold, D., Scherz, A., Noy, D., Simonin, I., Hartwich, G. and Scheer, H. (1997) J. Lumin. 72-74, 612–614.
[25] Teuchner, K., Stiel, H., Leupold, D., Katheder, I. and Scheer, H. (1994) J. Lumin. 60-61, 520–522.
[26] Savikhin, S. and Struve, W.S. (1994) Biophys. J. 67, 2002–2007.