

# Energy transfer from carotenoid to bacteriochlorophyll *a* in the B800-820 antenna complexes from *Rhodopseudomonas acidophila* strain 7050

Tomas Gillbro, Richard J. Cogdell\* and Villy Sundström

Department of Physical Chemistry, Umeå University, S-901 87 Umeå, Sweden and \*Department of Botany, University of Glasgow, Glasgow G12 8QQ, Scotland

Received 24 May 1988

We have resolved the excitation energy transfer from carotenoids to bacteriochlorophyll *a* in the B800-820 antenna complex of *Rhodopseudomonas acidophila* and found it to be about 3 ps. This efficient transfer can best be explained by the electron-exchange mechanism with a distance of about 4.5 Å between the molecules.

Energy transfer; B800-820 complex; Picosecond spectroscopy; Carotenoid; (*Rhodopseudomonas acidophila*)

## 1. INTRODUCTION

It is well known that carotenoids play at least two important roles in photosynthesis. Firstly, carotenoid pigments protect the cells against harmful photosensitized oxidation by quenching of (bacterio)chlorophyll triplets and/or singlet oxygen [1]. Secondly, carotenoid pigments absorb light energy in a wavelength region (450–550 nm) where chlorophylls are poor absorbers [2]. The excitation energy can then be transferred from the carotenoids to the chlorophylls with varying efficiency [3]. For the reasons given above light-harvesting pigment-protein complexes normally containing large amounts of carotenoids. In light-harvesting complexes of purple bacteria, for example, the carotenoid to bacteriochlorophyll ratio varies between 1:1 and 1:3 [4]. In spite of the importance of carotenoids as light-harvesting pigments, there has been to our knowledge just one report on the time-resolved energy transfer from carotenoids to bacteriochlorophyll. This is the work of Wasielewski et al. [5] on the B800-850

antenna complex of *Rhodopseudomonas acidophila* strain 7750. They found a  $5.6 \pm 0.9$  ps lifetime for the transfer of energy from the carotenoid to the Bchl 850 chromophore. No transfer of excitation energy to Bchl 800 was observed in that work. We have undertaken a systematic study of carotenoid energy transfer in several light-harvesting complexes of purple bacteria. Here, we report the first results obtained with the B800-820 complex of *Rps. acidophila* strain 7050. One reason for the choice of the B800-820 complex is that it contains a red-shifted carotenoid of the spirilloxanthin family, which has an absorption band which is well-separated from the bacteriochlorophyll Soret and Q<sub>x</sub> bands [2,4]. This fact facilitates selective excitation of the carotenoids.

The antenna complexes of purple bacteria have also been extensively studied by different spectroscopic techniques [6], including picosecond spectroscopy. Furthermore, several of these complexes have been crystallized [10,11]. For a complete understanding of the mechanism of energy transfer between carotenoids and chlorophylls, a detailed knowledge of both chromophore–chromophore distances and the energy transfer kinetics will be required. The present study is focussed on

Correspondence address: T. Gillbro, Department of Physical Chemistry, Umeå University, S-901 87 Umeå, Sweden

the question of whether Förster's long-range dipole-dipole mechanism [12] or the short-range electron-exchange mechanism of Dexter [13] is the best choice for describing the carotenoid-chlorophyll singlet-singlet energy-transfer process [14].

## 2. EXPERIMENTAL

The B800-820 pigment-protein complexes from *Rps. acidophila* strain 7050 were prepared according to Cogdell et al. [4] and stored in a freezer until use. For the picosecond pump-probe measurements the complexes were suspended in 20 mM Tris-HCl buffer solution (pH 8.0), 0.5% LDAO, with an absorbance of about  $0.3 \text{ mm}^{-1}$  at 540 nm.

The picosecond spectrometer used here is based on a cavity-dumped rhodamine 110 dye laser, which is synchronously pumped by a mode-locked argon-ion laser. The pump-probe picosecond technique has been detailed elsewhere [15]. Briefly, pulses of length 5–7 ps (repetition rate 800 kHz) with an energy of about 5 nJ were used to excite the carotenoids at about 540 nm. The time resolution is obtained by delaying the weak probe pulse relative to the pump pulse using a variable delay line in the probe beam. It is important that the polarizations of the pump and probe beams are well-defined. For this purpose polarizers were used in both beams and a Soleil-Babinet compensator in the pump beam, which makes it possible to select freely the angle of polarization between the pump and probe beams. As a result it is possible to record the relaxation of the light-induced anisotropy,  $r(t)$ ,

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)}$$

of the sample or to measure the isotropic kinetics by using the magic-angle ( $54.7^\circ$ ) polarization.

In order to avoid accumulation of photoproducts in the excited volume, we use a rotating sample cell with an optical path length of 1 mm. All experiments were performed at room temperature ( $\sim 23^\circ\text{C}$ ).

## 3. RESULTS AND DISCUSSION

In fig.1 we show the results of two measurements at 543 nm with the analyzing light polarized parallel ( $I_{\parallel}$ ) or perpendicular ( $I_{\perp}$ ) to the excitation light. We first note a weak negative (bleaching) signal, which then relaxes to a positive (absorption) signal. This process is most clearly observed for the parallel trace.

In fig.2 the sum ( $I_{\parallel} + 2I_{\perp}$ ) of the signals in fig.1 is shown (vertical bars) along with an exponential fit. In this fit a Gaussian function with the same width at half maximum as the pulse autocorrelation trace was used. The Gaussian response function was convoluted with the sum of two

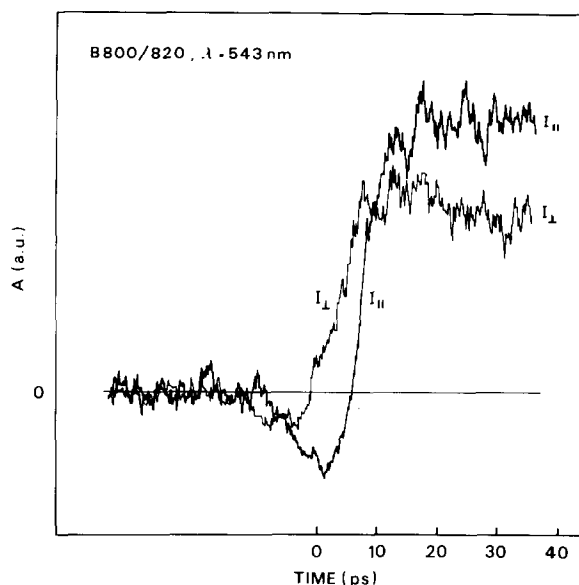


Fig.1. Absorption recovery kinetics at 543 nm of the B800-820 complex of *Rps. acidophila* with the polarization of the excitation light parallel ( $I_{\parallel}$ ) or perpendicular ( $I_{\perp}$ ) to the polarization of the probe light.

exponentials until the best fit to the experimental data points was obtained [9]. From this fit we obtained an amplitude of  $-1150$  (a.u.) and a lifetime of 2.0 ps for the fast component and an amplitude of 290 (a.u.) for the slow (ns) component. Fits of several absorption recovery transients gave values in the range 2–4 ps for the fast process. After a fast initial relaxation the anisotropy,  $r(t)$ , attains a constant value of  $0.12 \pm 0.05$ .

We shall initially discuss the shape of the absorption recovery at 543 nm in figs 1 and 2. The  $\Delta A$  at  $t < 0$  is negative, i.e. we observe a bleaching signal; at  $t > 15$  ps the signal has turned positive and stays constant for several hundred picoseconds. We assign the initial instantaneous bleaching to the formation of the carotenoid's singlet excited state [5,17]. The excitation energy is then transferred within  $3 \pm 1$  ps to the Bchl  $a$  molecules of the B800-820 complex. This is then seen as a change from a negative to a positive  $\Delta A$  signal because the extinction coefficient [18] of the excited singlet state of Bchl  $a$  is greater than that of its ground state at 543 nm. Independent picosecond measurements have shown that the energy transfer from Bchl 800 to Bchl 820 takes place within a few hundred femtoseconds (to be publish-

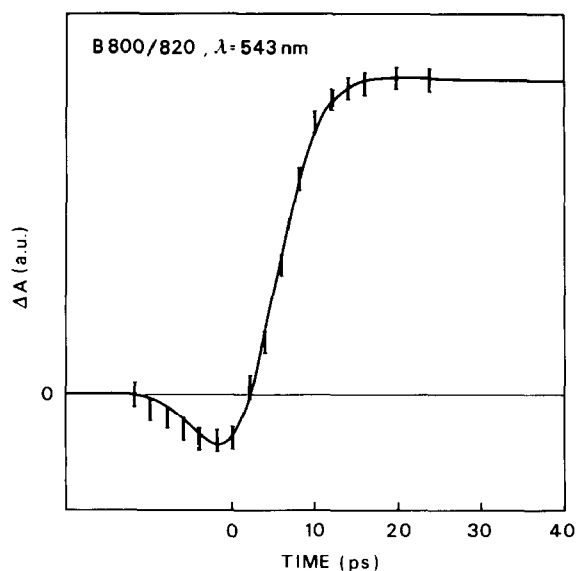


Fig.2. The isotropic signal calculated from the data of fig.1 according to  $I_{\text{iso}} = I_{\parallel} + 2I_{\perp}$  is shown by the vertical bars. The solid line shows the best fit to the experimental data (see text).

ed). Therefore, it can be safely assumed that the positive signal at 543 nm is due to excited Bchl 820 (Bchl 820\*). Since the transfer time from excited carotenoid to Bchl *a* in B800-820 is about 3 ps, it would be difficult to detect any excited Bchl 800 even if Bchl 800 served as a link for the transfer of energy from carotenoid to Bchl 820. At all times during the transfer process the concentration of Bchl 800\* will be too low to be detected. The difference spectrum Bchl 820–Bchl 820\* is not known, but we can estimate  $\Delta\epsilon$  at 543 nm by shifting the B880 difference spectrum recorded for *Rhodospirillum rubrum* chromatophores [16] by 60 nm to shorter wavelengths. Assuming that the maximum  $\epsilon$  is  $150 \text{ mM}^{-1} \cdot \text{cm}^{-1}$  for Bchl 820 (a typical value for other Bchl *a* antenna molecules [19]) we calculate that  $\Delta\epsilon$  (543 nm) for Bchl 820 – Bchl 820\* should be  $16 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ . For carotenoids close to the absorption maximum  $\epsilon$  is typically approx.  $150 \text{ mM}^{-1} \cdot \text{cm}^{-1}$  [17]. If there is a small (say 30%) absorption from the excited state [16] we estimate  $\Delta\epsilon$  (543 nm) to be about  $-105 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ . The ratio  $\Delta A(\text{car.})/\Delta A(\text{Bchl})$  is thus estimated to be about  $-6.5$  at 543 nm in the B800-820 complex (here, no correction for the quantum yield has been made). This should be compared to the value of  $-4$  for the ratio

$\Delta A_1/\Delta A_2$  obtained from the fitted kinetics of fig.2. This supports the interpretation of the transient observed at 543 nm. A further argument in favour of this interpretation is the anisotropy,  $r(t)$ , calculated from the data in fig.1. We find it to be in reasonable agreement with anisotropy measurements of the Bchl 820 bleaching at about 820 nm where we obtained  $r = 0.07$  (to be published). This result suggests that the excited state absorption dipoles are almost cylindrically degenerate [20] in the plane formed by the Bchl 820 molecules.

Having identified the carotenoid  $\rightarrow$  Bchl *a* transfer time to be approx. 3 ps, we shall now discuss our results in relation to existing theories of energy transfer. Two extreme cases have been treated by Förster [12] and Dexter [13]. The Förster energy transfer is a long-range (50–100 Å) dipole-dipole resonance process while Dexter's model describes an electron-exchange process that works only when the donor and acceptor molecules are in close contact ( $>10$  Å). As pointed out by Razi Naqvi [14] the carotenoids should be situated close to the bacteriochlorophylls in photosynthetic systems, since they also act as chlorophyll triplet quenchers. The low fluorescence yield of carotenoids should furthermore diminish the rate of Förster-type energy transfer, since the fluorescence quantum yield of the donor ( $\phi_D$ ) enters directly into the Förster equation. In the Dexter equation, on the other hand, the transfer efficiency is independent of  $\phi_D$ . From several published studies on electron-transfer reactions, it has been found that the transfer rate  $k_{\text{et}} = \nu_1 \exp[-\beta(r - r_0)]$ , where  $\nu_1 \approx 10^{13} \text{ s}^{-1}$  and  $\beta = 1.3 \text{ Å}^{-1}$  [21,22]. Here,  $r_0$  is the contact (van der Waals) distance and  $r$  the center-to-center distance. However, in order to compare  $k_{\text{et}}$  with our data, we also have to take into account that other non-radiative processes ( $k_{\text{nr}}$ ) contribute to the relaxation of the excited state. With  $k_{\text{et}} + k_{\text{nr}} = \tau^{-1}$  and since the quantum yield of energy transfer in B800-820 is about 0.7 [3,24], i.e.  $k_{\text{et}}/(k_{\text{et}} + k_{\text{nr}}) = 0.7$ , we obtain  $k_{\text{et}} = 0.23 \times 10^{12}$ . This also gives  $k_{\text{nr}} = 1.0 \times 10^{11}$ , in good agreement with the non-radiative rates observed for different carotenoids in solution [19]. Using  $r_0 = 1.6 \text{ Å}$  we calculate  $r = 4.5 \text{ Å}$ . Allowing  $\nu_1$  to vary between  $10^{12}$  and  $10^{14} \text{ s}^{-1}$  we obtain  $r = 4.5 \pm 1.7 \text{ Å}$ . In a recent study, Wasielewski et al. [5] resolved the energy

transfer between carotenoids and Bchl *a* in the B800-850 (type I) complex of *Rps. acidophila* strain 7750 and obtained a transfer time of  $5.6 \pm 0.9$  ps, i.e. slightly longer than that observed by us. This difference is, however, not unexpected, since the efficiency of energy transfer is 70–75% in B800-820 as compared to 50–55% in B800-850 [24]. It would be of great interest to follow the corresponding transfer rate in the B880 complex of the same species, since this has a very low (25%) transfer efficiency [23].

Recently, it was also shown by Wasielewski et al. [24] that energy transfer between carotenoid covalently linked to pyrophosphoribide could only occur with high efficiency if the edge-to-edge distance was about 2 Å. This also emphasises that in order to have the high quantum efficiency for energy transfer observed in vivo the distance between the carotenoid and bacteriochlorophyll molecules must be short.

**Acknowledgements:** This investigation was supported by grants from the Swedish National Research Foundation (T.G. and V.S.) and the Science and Engineering Research Council (R.J.C.). R.J.C. also thanks the British Council from a Travel Grant for his visit to Umeå. Expert technical assistance was provided (in Glasgow) by Ms Linda Ferguson.

## REFERENCES

- [1] Krinsky, N.I. (1979) *Pure Appl. Chem.* 51, 649–660.
- [2] Dolphin, D. (1978) *The Porphyrins*, vol.3, Academic Press, New York.
- [3] Cogdell, R.J. (1985) in: *Antennas and Reaction Centers of Photosynthetic Bacteria*. Springer Series in Chemical Physics, vol. 42 (Michel-Beyerle, M.E. ed.) pp.62–66, Springer, Berlin.
- [4] Cogdell, R.J., Durant, I., Valentine, J., Lindsay, J.G. and Schmidt, K. (1983) *Biochim. Biophys. Acta* 722, 427–435.
- [5] Wasielewski, M.R., Tiede, D.M. and Frank, H.A. (1986) in: *Ultrafast Phenomena V*. Springer Series in Chemical Physics, vol. 46 (Fleming, G.R. and Siegman, A.E.) pp.388–392, Springer, Berlin.
- [6] Clayton, R.S. and Sistrom, W.R. (1978) *Photosynthetic Bacteria*, Plenum, New York.
- [7] Sundström, V., Van Grondelle, R., Bergström, H., Åkesson, E. and Gillbro, T. (1986) *Biochim. Biophys. Acta* 851, 431–441.
- [8] Bergström, H., Sundström, V., Van Grondelle, R., Åkesson, E. and Gillbro, T. (1986) *Biochim. Biophys. Acta* 852, 279–287.
- [9] Van Grondelle, R., Bergström, H., Sundström, V. and Gillbro, T. (1987) *Biochim. Biophys. Acta* 894, 313–326.
- [10] Cogdell, R.J., Woolley, K., Mackenzie, R.C., Lindsay, J.G., Michel, H., Dobler, J. and Zinth, W. (1985) in: *Antennas and Reaction Centers of Photosynthetic Bacteria*. Springer Series in Chemical Physics, vol. 42 (Michel-Beyerle, M.E. ed.) pp.85–87, Springer, Berlin.
- [11] Mäntele, W., Steck, K., Wacker, T., Welte, W., Levoir and Breton, J. (1985) in: *Antennas and Reaction Centers of Photosynthetic Bacteria*. Springer Series in Chemical Physics, vol. 42 (Michel-Beyerle, M.E. ed.) pp.88–91, Springer, Berlin.
- [12] Förster, T. (1985) *Disc. Faraday Soc.* 27, 7–17.
- [13] Dexter, D.L. (1953) *J. Chem. Phys.* 21, 836–850.
- [14] Razi Naqvi, K. (1980) *Photochem. Photobiol.* 31, 523–524.
- [15] Åkesson, E., Sundström, V. and Gillbro, T. (1985) *Chem. Phys. Lett.* 121, 513–522.
- [16] Wasielewski, M.R. and Kispert, L.D. (1986) *Chem. Phys. Lett.* 128, 238–243.
- [17] Vetter, W., Englert, G., Rigassi, N. and Schweiter, U. (1971) in: *Carotenoids* (Isler, O. ed.) pp.189–266, Birkhäuser, Basel.
- [18] Nuijs, A.M., Van Grondelle, R., Joppe, H.L.P., Van Bochove, A.C. and Duysens, L.N.M. (1985) *Biochim. Biophys. Acta* 810, 94–105.
- [19] Clayton, R.K. and Clayton, B.J. (1981) *Proc. Natl. Acad. Sci. USA* 78, 5583–5587.
- [20] Kramer, H.J.M., Van Grondelle, R., Hunter, C.N., Westerhuis, W.H.J. and Ames, J. (1984) *Biochim. Biophys. Acta* 765, 156–165.
- [21] Miller, J.R. (1987) *New J. Chem.* 11, 83–89.
- [22] Newton, M.D. and Sutin, N. (1984) *Annu. Rev. Phys. Chem.* 35, 437–480.
- [23] Angerhofer, A., Cogdell, R.J. and Hipkins, M.F. (1986) *Biochim. Biophys. Acta* 848, 333–341.
- [24] Wasielewski, M.R., Liddell, P.A., Barrett, D., Moore, T.A. and Gust, D. (1986) *Nature* 322, 570–572.