PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

How carotenoids protect bacterial photosynthesis

Richard J. Cogdell

Phil. Trans. R. Soc. Lond. B 2000 **355**, 1345-1349
doi: 10.1098/rstb.2000.0696

THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0696

How carotenoids protect bacterial photosynthesis

Richard J. Cogdell[1*], Tina D. Howard[1], Robert Bittl[2], Erberhard Schlodder[2], Irene Geisenheimer[2] and Wolfgang Lubitz[2]

[1]*Division of Biochemistry and Molecular Biology, IBLS, Davidson Building, University of Glasgow, Glasgow G12 8QQ, UK*
[2]*Max Volmer Institute for Biophysical Chemistry and Biochemistry, Technical University of Berlin, Strasse des 17 Juni 135, 10623 Berlin, Germany*

The essential function of carotenoids in photosynthesis is to act as photoprotective agents, preventing chlorophylls and bacteriochlorophylls from sensitizing harmful photodestructive reactions in the presence of oxygen. Based upon recent structural studies on reaction centres and antenna complexes from purple photosynthetic bacteria, the detailed organization of the carotenoids is described. Then with specific reference to bacterial antenna complexes the details of the photoprotective role, triplet–triplet energy transfer, are presented.

Keywords: bacteriochlorophyll; carotenoids; reaction centres; antenna complexes; bacterial photosynthesis; membrane proteins

1. INTRODUCTION

The essential photoprotective role of carotenoids was first demonstrated almost 50 years ago (Griffiths *et al.* 1955). These workers showed that carotenoidless mutants of the purple photosynthetic bacterium *Rhodobacter sphaeroides*, such as R26, are rapidly killed by the combination of light and oxygen. This harmful photodynamic reaction is prevented, in wild-type strains, by the presence of carotenoids. It has now been shown by many workers (for a review, see Krinsky 1978; Cogdell & Frank 1987) that the photochemical reactions that give rise to the photodynamic effect proceed through a metastable state of an excited sensitizer, in this case triplet excited bacteriochlorophyll. The triplet excited bacteriochlorophyll *a* (Bchl*a*) molecule lasts long enough to react with molecular oxygen to generate singlet oxygen. The sensitized production of singlet oxygen in this reaction has been directly demonstrated by monitoring the luminescence produced by singlet oxygen at 1270 nm (e.g. Borland *et al.* 1987). Singlet oxygen is a very powerful oxidizing agent and rapidly kills cells that are exposed to it (Krinsky 1978; Foote 1976). These reactions can be summarized as follows

$$\text{Bchl}a + h\nu \rightarrow {}^1\text{Bchl}a^* \text{ (singlet excited Bchl}a\text{)}, \quad (1)$$

$${}^1\text{Bchl}a^* \rightarrow {}^3\text{Bchl}a^* \text{(triplet excited Bchl}a\text{)}, \quad (2)$$

$${}^3\text{Bchl}a^* + h\nu \rightarrow {}^3\text{Bchl}a + {}^1\Delta_g O_2^* \text{ (singlet oxygen)}. \quad (3)$$

In principle carotenoids can prevent the harmful effects of singlet oxygen in two ways: they can quench singlet oxygen directly (Foote & Denny 1968) and they can quench the ^{3}Bchl*a** sensitizer, preventing the production of singlet oxygen (Borland *et al.* 1988). *In vivo*, the major protective effect is the rapid quenching of ^{3}Bchl*a** so that no detectable singlet oxygen is produced (Cogdell & Frank 1987).

$${}^3\text{Bchl}a + \text{Car} \rightarrow + \text{Bchl}a + {}^3\text{Car}^* \text{ (triplet excited carotenoid)}, \quad (4)$$

$${}^3\text{Car}^* \rightarrow \text{Car} + \text{heat}. \quad (5)$$

All wild-type photosynthetic organisms have carotenoids whose first excited triplet energy level lies below that of singlet oxygen, i.e. below 94 kJ mol^{-1}, so that ^{3}Car* decays harmlessly to the ground state, releasing its excess energy as heat (Frank *et al.* 1999). The quenching of the ^{3}Bchl*a** by the carotenoid is a triplet–triplet energy transfer reaction and is generally assumed to occur by an electron exchange mechanism (Dexter 1953). In solution this occurs in collisional process (Borland *et al.* 1989) but *in vivo*, where the photosynthetic pigments are non-covalently attached to proteins, it requires the reacting molecules (Bchl*a* and Car) to be in Van der Waals' contact.

The photosynthetic apparatus of purple bacteria consists of two types of integral membrane pigment–protein complexes, reaction centres and light-harvesting complexes (Cogdell *et al.* 1999). Both types of complex contain Bchl*a* and carotenoid molecules. There are now high-resolution three-dimensional crystal structures for reaction centres from both *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* (see, for example, Deisenhofer *et al.* 1985; Ermler *et al.* 1994) and for light-harvesting

*Author for correspondence (r.cogdell@bio.gla.ac.uk).

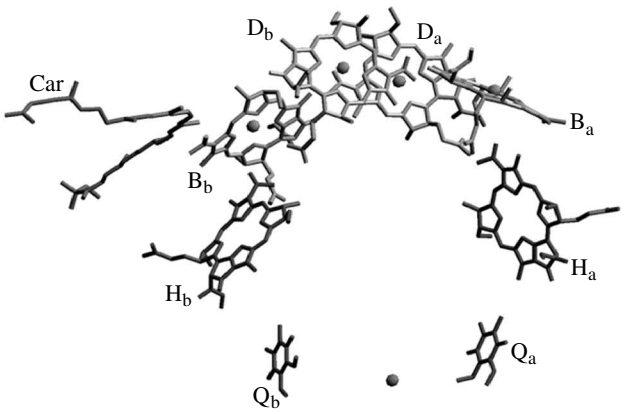

Figure 1. The organization of the pigments in the reaction centre from *Rhodobacter sphaeroides*. D, primary donor Bchl*a*; B, monomeric Bchl*a*; H, bacteriopheophytin *a*; Car, carotenoid; Q, ubiquinone. The subscripts 'a' and 'b' denote the active and inactive branches of the electron transport 'arms', respectively. The 'dot' represents the Fe^{2+}.

complexes (LHC II) from *Rhodopseudomonas acidophila* and *Rhodospirillum molischianum* (McDermott *et al.* 1995; Koepke *et al.* 1996). In this paper, the organization of the carotenoids is described in both reaction centres and antenna complexes and, with special reference to antenna complexes, some new experimental data that reveal the details of the Bchl*a*/carotenoid triplet–triplet exchange reaction are reviewed.

2. ORGANIZATION OF THE CAROTENOIDS IN REACTION CENTRES FROM *RHODOBACTER SPHAEROIDES* AND THE LHC II COMPLEX FROM *RHODOPSEUDOMONAS ACIDOPHILA*

The reaction centre form *R. sphaeroides* consists of four molecules of Bchl*a*, two molecules of bacteriopheophytin *a*, two molecules of ubiquinone and one carotenoid molecule, all of which are non-covalently bound to three proteins (H, M and L) (Feher *et al.* 1989). The overall organization of the pigments is presented in figure 1. The primary electron transport reactions proceed down the 'A' branch. The carotenoid is located on the so-called 'inactive' 'B' branch, in Van der Waals' contact with the monomeric Bchl*a* (B_b). The carotenoid adopts a 15-15′ *cis* configuration, which can now be clearly seen in a recent structural determination of a site-directed mutant reaction centre from *R. sphaeroides* where the resolution was improved to 0.21 nm (figure 2; McAuley *et al.* 2000). The closest approach of the conjugated region of the carotenoid B_b is *ca.* 0.4 nm.

The LH II antenna complex from *R. acidophila* is a monomeric ring formed from nine αβ-apoprotein pairs, each of which non-covalently bind three molecules of Bchl*a* and one molecule of carotenoid. The organization of the pigments in this circular structure is shown in figure 3. The Bchl*a* molecules are arranged into two groups. Nine monomeric ones form the B_{800} Bchl*a* molecules. These have their Q_y absorption band located at 800 nm. Eighteen tightly coupled ones form the B_{850} Bchl*a* molecules and have their Q_y absorption band at *ca.* 850 nm. The carotenoids run between these two groups of Bchl*a* molecules.

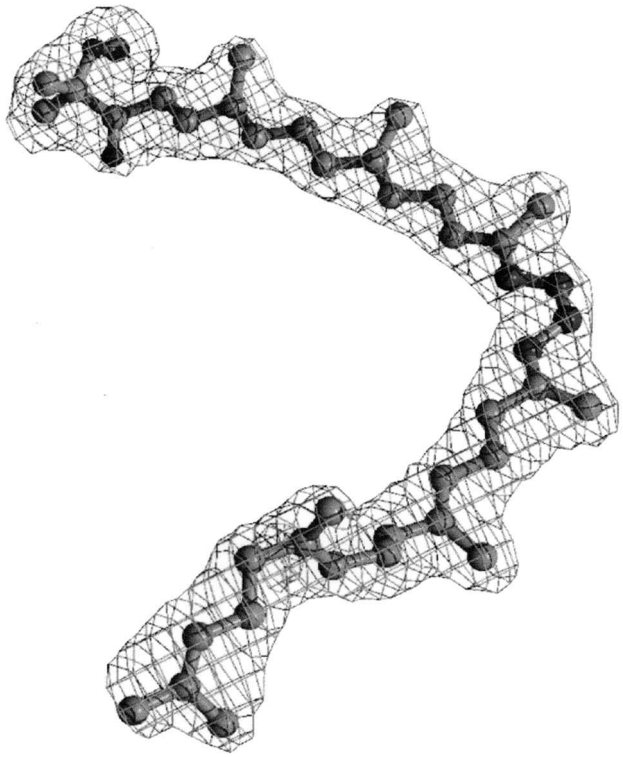

Figure 2. The detailed structure of the carotenoid spheroidenone in the reaction centre from *Rhodobacter sphaeroides*. The structure of spheroidenone in its electron density is shown. The data for this were taken from McAuley *et al.* (2000). The excellent quality of the electron density clearly shows that the *cis* bond is in the 15-15′ position.

They are in an all-*trans* configuration and are slightly twisted along their long axis to form about one-half of a helix (figure 4). These carotenoids come into Van der Waals' contact with the edge of the B_{800} Bchl*a* bacteriochlorin rings (closest approach 0.34 nm) and pass over the face of the α-apoprotein and the bound B_{850} Bchl*a* bacteriochlorin rings (closest approach 0.368 nm).

In both types of pigment–protein complexes the carotenoids are ideally positioned for fast efficient triplet–triplet energy transfer from Bchl*a* molecules.

3. CAROTENOID TRIPLET FORMATION IN ANTENNA COMPLEXES

It has been well documented that if carotenoidless antenna complexes are excited by light then Bchl*a* triplet states are formed (e.g. Monger *et al.* 1976). Following laser flash excitation these triplet states typically decay over a few tens of microseconds. If, on the other hand, antenna complexes containing carotenoid are excited, then only carotenoid triplet states are seen in a microsecond time-scale (Cogdell *et al.* 1981). The carotenoid triplets are formed in a nanosecond time-scale and decay in a few microseconds. These results were interpreted as demonstrating the triplet–triplet exchange reaction between Bchl*a* molecules and carotenoids. Since there was an approximately 10^3-fold reduction in the lifetime of the donor ^{3}Bchl*a** it was suggested that the major photoprotective effect was a direct quenching of this potentially harmful triplet sensitizer.

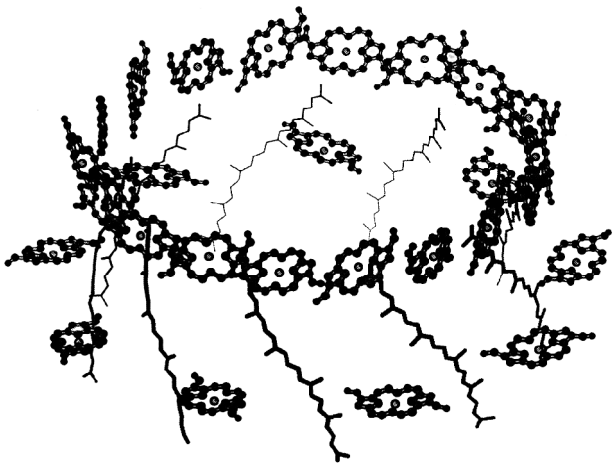

Figure 3. The organization of the pigments in the LH II complex from *Rhodopseudomonas acidophila*. This figure shows a schematic representation of the organization of the chromophores in the LH II complex from *R. acidophila* strain 10050. The Bchl*a* molecules are represented by their bacteriochlorin rings. The B_{800} Bchl*a* molecules lie flat within the structure, separated centre-to-centre by about 2.1 nm (21 Å). The B_{850} Bchl*a* molecules form the tightly coupled ring of 18 chromophores. The carotenoids run between the two groups of Bchl*a* molecules.

Figure 4. The detailed structure of the carotenoid rhodopin glucoside in the LH II complex from *Rhodopseudomonas acidophila*. A space-filling model of the structure of rhodopin glucoside in the LH II complex from *R. acidophila* strain 10050 is shown. (*a*) A side view, which clearly demonstrates that the carotenoid is in an all-*trans* configuration. (*b*) A view looking down the long axis of the carotenoid, which shows that it is twisted about this axis to form about half a helix.

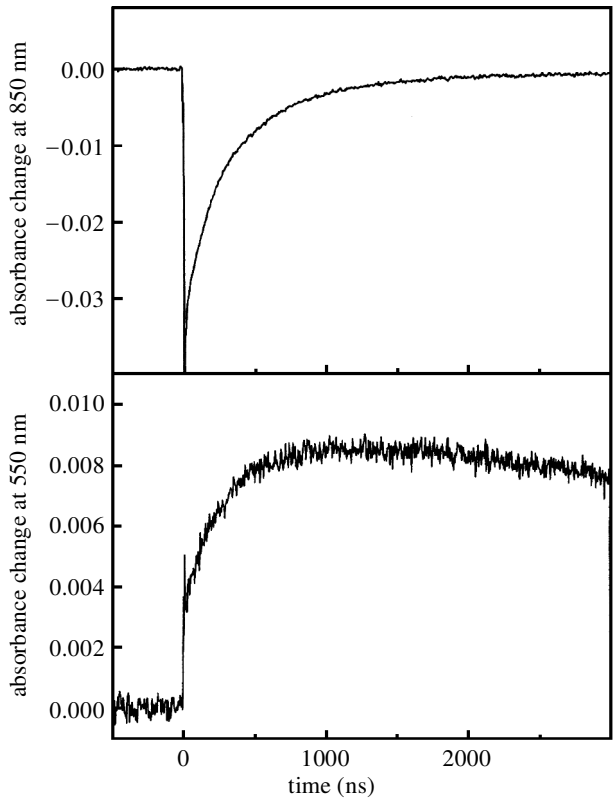

Figure 5. The laser flash-induced kinetics of the decay of the ^{3}Bchl*a** and the formation of the ^{3}Car* in the LH II complex from *Rhodobacter sphaeroides* strain 2.4.1 at 25 K. The upper trace shows the kinetics of the absorption changes at 850 nm, which are due to the formation and decay of ^{3}Bchl*a**. The lower trace shows the kinetics of the absorption changes of 550 nm, which are due to the formation ^{3}Car*. These changes were induced by a 3 ns laser flash at 532 nm as described in Bittl *et al.* (2000).

However, due to the lack of time resolution when most of these early studies were done, there was never a detailed analysis of this reaction over the time-scale in which it occurred, i.e. the first few nanoseconds after the laser flash. Strangely, even though the time resolution of laser flash photolysis is now in the femtosecond region, these experiments had not been revisited until quite recently (Bittl *et al.* 2000). Figure 5 shows the result of a laser flash photolysis experiment carried out at 25 K on an isolated LH II antenna complex sample prepared from *R. sphaeroides* strain 2.4.1. The carotenoid present in this sample is spheroidene, which has ten conjugated double bonds. Following excitation with a 3 ns laser pulse the absorbance changes due to the formation of ^{3}Bchl*a** were monitored at 850 nm and those due to the formation of ^{3}Car* at 550 nm. At 850 nm there is an initial fast bleaching due to the formation of ^{1}Bchl*a** that then, within the time resolution of the measurements, relaxes to form ^{3}Bchl*a**. It decays back to the ground state in the nanosecond time-scale. The rise of the ^{3}Car* at 550 nm shows two phases: an initial fast phase, due to interfering changes that arise from Bchl*a* excited states, and a slower nanosecond rise, due to the formation of ^{3}Car*. At 25 K the decay of the ^{3}Bchl*a** is biphasic. These two phases have half-lives of 143 ns and 414 ns and each phase has approximately equal amplitude. The rise of ^{3}Car* signal

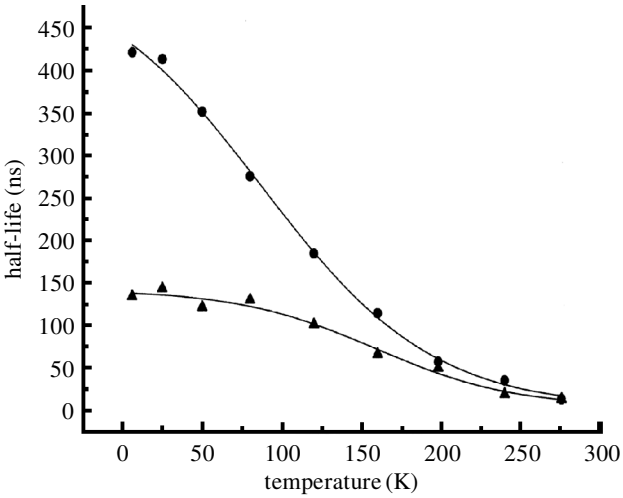

Figure 6. The temperature dependence of the decay of the laser-induced ^{3}Bchl*a** in the LH II complex from *Rhodobacter sphaeroides* strain 2.4.1. Filled circle, the slow phase of the ^{3}Bchl*a** decay; filled triangle, the fast phase of the ^{3}Bchl*a** decay. This figure presents the results of analysing the kinetics at 850 nm as shown in figure 5 as the temperature of the sample was varied between 4 and 393 K. Full details of the curve-fitting methods are presented in Bittl *et al.* (2000).

is also biphasic. In this case the two half-lives are very similar to those seen for the decay of ^{3}Bchl*a**, namely 124 ns and 414 ns. Clearly there is a close kinetic correspondence between the decay of ^{3}Bchl*a** and the rise of ^{3}Car*, exactly as required by the mechanism outlined in § 1. This triplet–triplet exchange reaction is temperature dependent (figure 6) and this kinetic correspondence between the ^{3}Bchl*a** decay and the rise of ^{3}Car* is maintained at all temperatures studied. As the temperature is lowered from 293 to 4 K the decay of ^{3}Bchl*a** slows down from 14 (monophasic) to 296 and 400 ns (biphasic). The exact temperature dependence of the Bchl*a* to carotenoid triplet–triplet exchange reaction varies depending upon the type of LH II complex studied. There are two extremes: the rate of formation of ^{3}Car* in the LH II complex of *Chromatium purpuratum* is too fast to measure at all temperatures; on the other hand, the rate of ^{3}Car* formation in the LH II complex from *R. sphaeroides* strain GIC becomes so slow at cryogenic temperatures that the ^{3}Bchl*a** lasts for more than 1 μs (this complex is identical to that from strain 2.4.1 except that spheroidene is replaced with neurosporene, which has nine conjugated double bonds; Cogdell *et al.* 1981).

The origin of this temperature dependence is not immediately obvious. The formulation of the electron exchange mechanism does not have an explicit temperature-dependent term (Dexter 1953). The spectral overlap, however, could show some temperature dependence. The absorption bands of LH II, especially at 850 nm, are affected by temperature (Wu *et al.* 1997). At cryogenic temperatures the 850 nm band sharpens and shifts to the red. These changes, though, are complete by 150 K whereas the temperature dependence of the triplet–triplet exchange reaction continues down to 4 K. Recent calculations of the spectroscopic properties of the LH II complexes from *R. acidophila* have suggested that in order to get a satisfactory 'fit' to the actual experimental data, the 'site-energy' of the β-bound B_{850} Bchl*a* molecule must be lower that that of the α-bound B_{850} Bchl*a* molecule (Koolhaas *et al.* 1998). In the structure of LHC II the single carotenoid present per αβ-apoprotein pair only comes into Van der Waals' contact with the α-bound B_{850} Bchl*a* molecule. If the triplet Bchl*a* was located on the lower energy B_{850} Bchl*a* (i.e. the β-bound one) then the first step in the triplet–triplet energy transfer from ^{3}Bchl*a** to Car would involve an 'uphill' transfer from the β-bound B_{850} Bchl*a* to the α-bound one. This would then result in a temperature dependence. A similar explanation has been proposed to explain the temperature dependence of the Bchl*a* to carotenoid triplet–triplet energy transfer that occurs in purple bacterial reaction centres (Frank *et al.* 1993). These kinetic experiments have demonstrated directly, for the first time, to our knowledge, in the purple bacterial antenna system, that the kinetic requirements for this triplet–triplet exchange reaction are fulfilled.

This work was supported by the Biotechnology and Biological Sciences Research Council, the Humboldt Foundation, the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

REFERENCES

Bittl, R., Schlodder, E., Geisenheimer, I., Lubitz, W. & Cogdell, R. J. 2000 Transient EPR and absorption studies of carotenoid triplet formation in purple bacterial antenna complexes. *J. Phys. Chem.* B. (In the press.)

Borland, C. F., McGarvey, D. J., Truscott, T. G., Cogdell, R. J. & Land, E. J. 1987 Photophysical studies of bacteriochlorophyll *a* and bacteriopheophytin *a*—singlet oxygen generation. *J. Photochem. Photobiol.* B **1**, 93–101.

Borland, C. F., Cogdell, R. J. Land, E. J. & Truscott, T. G. 1989 Bacteriochlorophyll *a* triplet state and its interactions with bacterial carotenoids and oxygen. *J. Photochem. Photobiol.* B **3**, 227–245.

Cogdell, R. J. & Frank, H. A. 1987 The function of carotenoids in photosynthesis. *Biochim. Biophys. Acta* **815**, 63–79.

Cogdell, R. J., Hipkins, M. F., Macdonald, W. & Truscott, T. G. 1981 Energy transfer between the carotenoid and bacteriochlorophyll within the B800–850 light-harvesting pigment–protein complex of *Rps. sphaeroides*. *Biochim. Biophys. Acta* **634**, 191–202.

Cogdell, R. J., Isaacs, N. W., Howard, T. D., McLuskey, K., Fraser, N. J. & Prince, S. M. 1999 How photosynthetic bacteria harvest solar energy. *J. Bacteriol.* **181**, 3869–3879.

Deisenhofer, J., Epp, O., Miki, H., Huber, R. & Michel, H. 1985 Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature* **318**, 618–622.

Dexter, D. L. 1953 A theory of sensitised luminescence in solids. *J. Chem. Phys.* **21**, 836–860.

Ermler, U., Frittsch, G., Buchanan, S. K. & Michel, H. 1994 Structure of the photosynthetic reaction centre from *Rhodobacter sphaeroides* at 2.65 Å resolution: cofactors and protein–cofactor interactions. *Structure* **2**, 925–936.

Feher, G., Allen, J. P., Okamura, M. Y. & Rees, D. C. 1989 Structure and function of bacterial photosynthetic reaction centres. *Nature* **339**, 111–116.

Foote, C. S. 1976 Photosensitised oxidation and singlet oxygen: consequences in biological systems. In *Free radicals and biological systems* (ed. W. A. Pryor), pp. 85–133. New York: Academic Press.

Foote, C. S. & Denny, R. W. 1968 Chemistry of singlet oxygen. VII. Quenching by β-carotene. *J. Am. Chem. Soc.* **90**, 6233–6235.

Frank, H. A., Chynwat, V., Hartwich, G., Meyer, M., Katheder, I. & Scheer, H. 1993 Carotenoid triplet state formation in *Rhodobacter sphaeroides* R-26 reaction centres exchanged with modified bacteriochlorophyll pigments and reconstituted with spheroidene. *Photosyn. Res.* **37**, 193–203.

Frank, H. A, Young, A. J., Britton, G. & Cogdell, R. 1999 *The photochemistry of carotenoids.* Dordrecht, The Netherlands: Kluwer.

Griffiths, M., Sistrom, W. R., Cohen-Bazire, G. & Stanier, R. Y. 1955 Function of carotenoids in photosynthesis. *Nature* **176**, 1211–1214.

Koepke, J., Hu, X., Muenke, C., Schulten, K. & Michel, H. 1996 The crystal structure of the light-harvesting complex II (B800–850) from *Rhodospirillum molischianum*. *Structure* **4**, 581–597.

Koolhaas, M.-H. C., Frese, R. N., Fowler, G. J. S., Bibby, T. S., Georgekopoulou, S., Van der Zwan, G., Hunter, C. N. & Van Grondelle, R. 1998 Identification of the upper exciton component of the B850 bacteriochlorophylls of the LH2 antenna complex, using a B800-free mutant of *Rhodobacter sphaeroides*. *Biochemistry* **37**, 4693–4698.

Krinsky, N. I. 1978 Non-photosynthetic functions of carotenoids. *Phil. Trans. R. Soc. Lond.* B **284**, 581–590.

McAuley, K. E., Fyfe, P. K., Ridge, J. P., Cogdell, R. J., Isaacs, N. W. & Jones, M. R. 2000 Ubiquinone binding, ubiquinone exclusion, and detailed cofactor conformation in a mutant bacterial reaction centre. *Biochemistry*. (In the press.)

McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J. & Isaacs, N. W. 1995 Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature* **374**, 517–521.

Monger, T. G., Cogdell, R. J. & Parson, W. W. 1976 Triplet states of bacteriochlorophyll and carotenoids in chromatophores of photosynthetic bacteria. *Biochim. Biophys. Acta* **449**, 136–153.

Wu, H.-M., Ratsep, M., Jankowiazk, R., Cogdell, R. J. & Small, G. L. 1997 Comparison of the LH2 antenna complexes of *Rhodopseudomonas acidophila* (strain 10050) and *Rhodobacter sphaeroides* by high pressure-absorption—hole-burning and temperature-dependent absorption spectroscopies. *J. Phys. Chem.* **101**, 7641–7653.

Discussion

A. Laisk (*Department of Plant Physiology, University of Tartu, Estonia*). Why was your curve that showed the triplet lifetimes not exponential? I expected that temperature dependence caused by a difference in energy levels must be exponential.

R. J. Cogdell. For simplicity I only showed the decay kinetics plotted as an overall half-time. In fact there are two decay phases and, when they are plotted separately, the dependencies are indeed more exponential.

J. Barber (*Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK*). Has the temperature dependence of chlorophyll–carotenoid triplet transfer been measured in LHC II? Clearly such measurements would help clarify whether chlorophyll *a* or chlorophyll *b* are located close to the bridging luteins.

R. J. Cogdell. Similar measurements have not been done on LHC II. I too believe that they could be very informative.

C. Buc (*Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK*). Should the different energy level of Bchl band to the α-subunit and the β-subunit, respectively, not show a low temperature absorption spectroscopy? How come the bright triplet localizes on the α-subunit band Bchl?

R. J. Cogdell. The absorption properties are due to exciton interactions of the whole ring of Bchls, and even though the different ones may have different site energies, this will not cause a splitting of the absorption band. We expect, though, that the localized triplet state will be preferentially located on the lowest energy Bchl especially at low temperature.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES