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ORIGINAL PAPER

Discrepancy between experimental and theoretical excitation transfer rates in LH2 bacteriochlorophyll-protein complexes of purple bacteria

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Abstract Discrepancy is revealed between the values of excitation transfer times measured experimentally, and those calculated, for the atomic structures of B800 → B850 bacteriochlorophylls within the LH2 light-harvesting pigment–protein complex of the purple bacterium *Rhodopseudomonas acidophila*. The value 2.9–3.2 ps for the B800 → B850 excitation transfer, calculated on the basis of atomic structure of LH2, is about 4-times longer than that measured for this bacterium (0.7 ps). This discrepancy appears common in at least two purple bacteria. Possible sources responsible for this discrepancy are discussed. It may either signify some drawback/s/ in our notions about the precise in vivo structure of LH2 complexes, for example, possible changes of LH2 structure during crystallization, or it may reflect our ignorance of some mechanisms involved in excitation migration.

Keywords Purple bacteria · LH2 · Excitation migration

Introduction

In photosynthetic bacteria the bacteriochlorophylls (BChls) are organised by oligomeric, mostly helical, transmembrane proteins. Their photosynthetic units contain dominating light-harvesting pigments and reaction centres (RCs), which are responsible for the trapping of electronic excitations (EEs) from vast pigment "antennae" and subsequent EE conversion into charge carriers of opposite signs separated across the membrane (van Grondelle et al. 1994; Robert et al. 2003). In purple bacteria, α/β-heterodimers of transmembrane proteins apparently organize antenna BChls into ellipse-like complexes LH1 which contain the long-wave absorbing BChls, and ring-like LH2s (Cogdell et al. 2003). In bacteria with BChl*a*, LH1 usually have B875 in antenna pool and P870 special pairs in RCs; in accessory complexes LH2 antenna BChl fractions B850 and B800 are usually present (numerals 875, 870, 850 and 800 stand for the long-wave absorption peaks of corresponding BChl*a* fractions). One RC usually serves about 30 B875 in LH1 and up to 100–150 antenna BChls, in LH2s. Therefore, ascertaining the precise mechanisms of efficient EE delivery from these antenna molecules to RC special pairs becomes a task of high importance. EE migration proceeds in purple bacteria in the following way: B800* → B850* → B875* → P870* (van Grondelle et al. 1994) where superscript* stands for the first singlet excited state. Thus, a spectrum funnel is formed in vivo which accelerates and makes more efficient EE migration from antenna BChls to RC special pairs. Singlet EEs often form excitons delocalized in a few tightly bound BChls. In all organisms known "bottle necks" are present, i.e, the spans on EE way from antenna /B/Chls to RCs, where intermolecular distances exceed 15–20 Å. Correspondingly, the intermolecular EE migration in them has the slowest rate and is amenable to Förster's theory of slow inductive resonance (Förster 1960), see also in (Agranowich and Galanin 1982). Now this theory is often termed as FRET which should be interpreted as Förster [*not fluorescence(!) as some authors write*] Resonance Energy Transfer. LH2 antenna complexes were crystallized from *Rhodopseudomonas acidophila* (McDermot et al. 1995) and *Rhodospirillum molishianum* (Koepke et al. 1996) and their precise atomic structures were obtained with the aid

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of an X-ray method. It was shown (McDermot, et al. 1995, Koepke et al. 1996) that intramembrane helices of protein α/β-heterodimers arrange B800 and B850 BChl fractions in two circles. These circles lie rather flat within the membrane plane. In *Rps. acidophila* the B800 ring contains nine molecules (McDermot et al. 1995) liganded to α-apoproteins. The B850 fraction contains twice as many BChl molecules, with intermolecular distances of 8.9 Å in α/β-heterodimers and 9.2 Å between the closest B850s from neighbouring α/β-heterodimers (McDermot et al. 1995). The distances from any of nine B800 to the closest B850 molecules are about 17.6 Å (McDermot et al. 1995). The structural description of LH2 identified a single rhodopin-glucoside carotenoid molecule per α/β-heterodimer and some electron density which "…could have been a disordered second carotenoid" (McDermot et al. 1995). Excitation transfer in LH2 complexes was investigated in a great many of experimental and theoretical works, most of them are reviewed in the literature (van Grondelle et al 1994; Scholes and Fleming 2000; Robert et al 2003, which also contain a thorough theoretical analysis of this problem).

The main goal of the present work was, by using the above mentioned structural data and reliably established kinetic and energy constants, to calculate the average time for excitation delivery from B800 to B850 and to compare it with that obtained experimentally. Also, some means and details are suggested and discussed for obtaining, with reasonable precision, the value of the critical distance for heterogeneous intermolecular EE migration between B800 and B850 molecules in vivo.

Methods

The structural data for the LH2 complex from *R. acidophila* were obtained from the Brookhaven protein bank (http://www.rcsb.org/pdb/, identification number 1LGH) and from the PQS bank (identification number pqs.ebi.ac.uk,), respectively. Inter-chromophore distances and angles between their dipoles were determined with the aid of the RasMol program. Microsoft Excel program was used for the processing of the data used. The absorption spectrum of BChla in *n*-hexane was kindly given by Dr. Marina Krikunova. Fluorescence spectra were constructed according to the rule of mirror symmetry to the corresponding absorption spectra (see below).

Theoretical digression

The classical Förster theory (Förster 1960; Agranowich and Galanin 1982) yields for intermolecular EE migration:

$$(R_0)^6 \sim \frac{2/3\varphi_{fl}}{n^4}\int F_D(\nu)\varepsilon_A(\nu)\nu^{-4}\,d\nu \quad (1)$$

$$\kappa_{D,A} = \left(t_{D,A}\right)^{-1} \sim \frac{\xi(\psi,\theta_1,\theta_2)^2\varphi_{fl}}{n^4\left(L_{D,A}\right)^6\tau_D}\int F_D(\nu)\varepsilon_A(\nu)\nu^{-4}\,d\nu \quad (2)$$

where R_0 is the critical distance for EE migration between excited donor (D*) and non-excited acceptor (A) molecules; $\xi(\psi,\theta_1,\theta_2)^2 = [\cos\psi - 3\cos\theta_1\cos\theta_2]^2$ is the factor of mutual position of transition dipoles of excited D* and A* molecules; τ_D and φ_{fl} are D* lifetime and fluorescence yield, respectively; n is the refractive index of the host medium; $\varepsilon_A(\nu)$ and $F_D(\nu)$ are the molar absorption spectrum of A and normalized fluorescence spectrum of D* (integrals of $F_D(\nu)/\nu^3\tau_D$ and $\varepsilon_A(\nu)/\nu$ are proportional to the strengths of D* and A* dipoles for their corresponding $S_0 \rightarrow S^*_1$ singlet transitions); ν—optical frequency in cm^{-1}; $t_{D,A}$—the mean time for EE transfer from D* to A; $L_{D,A}$—the distance between the centres of D* and A* transition dipoles (central Mg atoms in BChl chromophores).

According to the definition, $K_{D,A} = (\tau_R)^{-1}$ provided $L_{D,A} = R_0$, where $\tau_R = \tau_D/\varphi_{fl}$ is the radiative lifetime of D* molecules. Besides $\xi_{D,A}(\psi,\theta_1,\theta_2)^2 = 2/3$ was used in (1) for this limiting case in accordance with Förster's definition for chaotic molecular ensembles. By replacing these symbols in formulae (2) and dividing correspondingly its left and right parts by those from the general formulae (2) one obtains one more Förster formulae for D* and A molecules with arbitrary values of $L_{D,A}$ and $\xi_{D,A}(\psi,\theta_1,\theta_2)^2$:

$$\left(\kappa_{D,A}\right)^{-1}/\tau_R = t_{D,A}/\tau_R = \frac{\xi_{D,A}(\psi,\theta_1,\theta_2)^2}{2/3}\left(L_{D,A}/R_0\right)^6 \quad (3)$$

Results

Calculation of the critical distance (hereafter designated as R_{het}) for the heterogeneous excitation transfer from photoexcited B800* to B850 molecules in LH2 complexes

The calculations were conducted with the aid of formula (3) because the distances between B800 molecules (21.2 Å) exclude formation of delocalized excitons and the spacing between them and the closest B850s (>17 Å; McDermot, et al. 1995) excludes noticeable excitonic and exchange interaction between their π-electronic circuits. Unfortunately 800 and 850 nm absorption bands overlap in the LH2 spectra, so that only the main part of B850 is real

while it is not easy to separate its shorter wavelength pedestal from the 800 nm band and its pedestal. Besides, one can hardly obtain the precise Stokes shifts from them and fluorescence spectra available from different publications. Förster's overlapping integrals depend on this shift. In order to decrease this source of uncertainty, the basic digital absorption spectrum used in this work was that for BChl*a* in *n*-octane kindly given by Dr. Krikunova. Its long wave peak is at 12,980 cm^{-1} and the band width is 735 cm^{-1}. Fluorescence spectra were constructed according to the mirror symmetry rule with Stokes shift equal to 35% of the band width as it was substantiated for BChl*a* in 14 different media in comprehensive work (Connolly et al. 1982). Both fluorescence and absorption spectra were shifted to the lesser frequencies, so that the peak of the latter one got to its in vivo position at 875 nm. Such substitution of the BChl*a* in vivo absorption spectrum appears to be reliable because: (a) being condensed along the frequency axis this spectrum corresponds to the main parts of B800 and B850 bands; (b) the integral absorption of $S_0 \rightarrow S^*_1$ band is conservative within 5% (Connolly et al. 1982). It is of note that the values of radiative lifetime ($\tau_R = 18 \times 10^{-9}\ s^{-1}$) calculated for B875 fraction in vivo (Zankel et al. 1967; Campillo et al. 1977), and in organic solutions of close-range dielectric permeability (Connolly et al. 1982) are the same.

The following standard parameters for in vivo conditions were associated with this BChl*a* with its peak shifted to 875 nm: $n \cong 1.36$ ($n^4 = 3.42$); $\xi(\psi,\theta_1,\theta_2)^2 = 2/3$; $\tau_R = 18 \times 10^{-9}$ s; $R_0[875] = 80$ Å (Zankel et al. 1967, Campillo et al. 1977). Hereafter $R_0[875]$ stands for Förster's critical distance for EE migration between homogeneous BChl*a* molecules having absorption peak at 875 nm and randomly distributed.

The calculation of R_{het} needed was then carried out in two steps:

1. First, the magnitude of intermediate $R_0[825]$ was determined for homogeneous EE migration between arbitrary BChl*a* molecules which have an absorption peak at 825 nm, i.e., about in the centre of the spectral region, in which B800* $\rightarrow$ B850 migration proceeds. One may see from formulae (1), that the only difference between expressions for the basic $R_0[875]$ and $R_0[825]$ appears due to the frequency factor ν^{-4} under the integral. Bearing in mind the rather narrow frequency range of integration, we may bring this factor out to the open from integral as the mean frequency for this range. Then, by dividing term by term expressions (1) for $R_0[825]$ and $R_0[875]$, one comes to the ratio:

$(R_0[825]/R_0[875])^6 \cong (825/875)^4$, and thus obtain,

$R_0[825] \cong 76.9$ Å.

2. Starting from this intermediate $R_0[825] \cong 76.9$ Å, we have to calculate the desired value of R_{het} for EE migration B800* $\rightarrow$ B850. Evidently, $F_D(800)$ for fluorescence and $\varepsilon_A(850)$ for absorption spectra must be used in formula (1) at this stage. By dividing term by term the corresponding expressions (1) for $R_0[825]$ and R_{het}, we obtain,

$$\frac{R_0[825]^6}{R_{het}^6} = \frac{76.6^6}{R_{het}^6} = \frac{\int F_D[825]\,\varepsilon_A[825]\nu^{-4}d\nu}{\int F_D[800]\,\varepsilon_A[850]\nu^{-4}d\nu} \quad (4)$$

The relative values of both integrals were calculated from BChla spectra in the *n*-octane band by compressing it in the frequency axis by 1.18-fold for B850 and 1.75-fold for B800 correspondingly. The band widths for B850 and B800 in *R. acidophila* obtained at 295 K are about 330 and 490 nm, respectively. It is interesting to note that these BChl*a* band widths in vivo are considerably narrower that in vitro in organic solutions. Compare with band widths of BChl*a* monomers in solutions (Connolly et al. 1982): ether 535 cm^{-1}; benzene 570 cm^{-1}; *n*-hexane 574 cm^{-1}; acetone 750 cm^{-1}; pyridine 600 cm^{-1} $CHCl_3$ 675 cm^{-1}; CH_2Cl_2 730 cm^{-1}; etOH(alcohol) 855 cm^{-1}.

Thus the value of critical distance R_{het} was determined:

$$B800^* \rightarrow B850: \quad R_{het} = 72.3\ Å$$

Mutual positions, $\xi_{D,A}(\varphi,\psi,\theta)^2$ of B800 and B850 molecules

Corresponding data were derived from the Brookhaven protein data bank. All the distances $L_{D,Ai}$, $(1 \le i \le 18)$ between either donor B800* and all acceptor B850 molecules and corresponding angles (ψ_i—between B800 and B850 dipoles and the line connecting their centres, ψ_i for dipole mutual orientations) were obtained for LH2 of

Table 1 The spacing ($L_{D,Ai}$) and interdipole angles between either B800 and seven closest $B850_i$ molecules in crystallized LH2 complexes from *Rhodopseudomonas acidophila*

$B850_i$	$\cos\theta_1$	$\cos\theta_2$	$\cos\psi$	$\xi(\psi,\theta_1,\theta_2)^2$	$L_{D,i}$ (Å)
1	0.708	0.581	0.325	0.826	39.1
2	0.656	0.502	0.560	0.182	32.4
3	0.512	0.741	0.856	0.080	25.5
4	0.198	0.327	0.976	0.611	20.1
5	0.175	0.040	0.877	0.733	19.0
6	0.427	0.624	0.835	0.001	22.9
7	0.402	0.717	0.369	0.246	29.0

R. acidophila. In Table 1 these parameters are presented for seven closest B850 molecules.

The individual rate constants were then calculated by using formulae (3) for all 18 pairs (B800/B850$_i$) in the LH2 circle, see Table 2, although the contribution from 11 of the more remote B850 molecules does not exceed 0.4%.

Thus,

$$t_{D,A} \cong \sum_{i}^{18} \left(\kappa_{D,Ai}\right)^{-1} \cong 3.6\,\text{ps}.$$

It is interesting to note that the value of $t_{D,A}$ thus obtained is nearly the same as that calculated by Förster' formulae in which all $\xi(\psi,\theta 1,\theta 2)$ are taken as statistic 2/3.

However, two corrections should be made:

1. The real time is known to be the reciprocal sum of rate constants for EE transfer in both up and down directions. Their estimation for $T = 300$ K yields: $K_{850/800}/K_{800/850} \cong 1/40$. With this correction one may obtain a slightly decreased result:

$t_{D,A} \cong 3.5$ ps.

2. According to Joo et al. (1996), the precise positions of BChl absorption peaks in *R. acidophila* spectrum were at 801 and 857.5 nm instead of the generally used 800 and 850 nm. This 5.5 nm increase in donor/accepter spectral separation caused a correction of R_{het} obtained via formula (4) and correspondingly about an 8% increase of the $t_{D,A}$. Thus the terminal value becomes:

$t_{D,A} \cong 3.77$ ps.

However, the experimental values of this parameter amounts from 0.6 ps, to 0.8 ps for *R. acidophila* and *Rhodobacter sphaeroides* (Shreve et al. 1991; Joo et al. 1996; Ma et al. 1998) and "this time constant is not very species dependent" (Fleming and van Grondelle 1997). Thus we come to a conclusion: The time interval for excitation transfer from B800* to B850 ensemble in LH2 complexes of purple bacterium *R. acidophila* is much shorter than the one calculated on the basis of its LH2 crystalline structure. We are now in a postition to analyze the reliability of such a discrepancy.

Table 2 Rate constants $K_{D,Ai}$ determined with formula (3) for the B800* → B850$_i$ excitation transfer and their sum (ΣK_i)

$K_{D,A1}$	$K_{D,A2}$	$K_{D,A3}$	$K_{D,A4}$	$K_{D,A5}$	$K_{D,A6}$	$K_{D,A7}$	ΣK_i	$t_{D,A}$
2.48	1.69	3.14	99.6	166.8	0.76	4.46	278.6	3.59 ps

Rate constants are in ns^{-1}

Adequacy of the theory used

For the correct application of Förster's theory, two criteria must be obligatorily met:

1. The interdipole distance should be considerably longer than the dipole length. The distances between B800 chromophore centres in this work ($L_{D,A} > 17$ Å) apparently meet this requirement. Besides, the precision of representation of the molecular interaction via that of their dipoles may be easily calculated as the function of L_{dip}/L_{DA} ratio for each particular mutual orientation of D and A dipoles. In our case the sum of errors for 18 BChl*a* pairs is <4%. Very encouraging results were obtained in recent work (Faure et al. 2004) in which excitation migration in 16 pairs of cofacial metalated bis-porphyrins were studied. The distance between their chromophore centres was gradually increased within 4–20 Å, by special spacer molecules which could support the linear construction of these polymolecular chains. The "switching" from Dexter's exponential mechanism to Förster's $(L_{D,A})^6$ mechanism was observed in these donor-acceptor pairs at inter-dipole distance about 6–7 Å only. Taking into account the red absorption limit of these porphyrins at about 600 nm, one may conclude that for BChla in vivo which have red border within 850–960 nm such limiting distance may be around 10 Å. In LH2 complex analyzed in this work the minimal inter-chromophore distance is 19 Å, i.e. this criterion is satisfied with a great excess.
2. The time required for quasi-equilibration of excited molecule must be shorter than the mean time for excitation migration. Nowadays this condition is treated more loosely. The analysis conducted in (Kenkre and Knox 1974; Knox and Gulen 1993) proved that the error within Förster's theory is reasonable even in cases when the equilibration and migration times are of the same order. The time limits for application of Förster's theory to Chls were established in (Kenkre and Knox 1974) for BChl*a*; it occurred to be shorter than 10^{-13} s which is considerably shorter than $(7–8) \times 10^{-13}$ s used in this work for EE migration from B800* to B850. Thus, this criterion is also satisfied in excess -and Ferster' theory fits well the problems of this paper.

Relevance and value of the refractive index used

One should remember that the principal parameter that was entered into all migration theories from Coulomb law is not the refraction index (above used n), but the index of dielectric permeability χ (see detailed analysis in the work

Knox and van Amerongen 2002). The microscopic presentation of parameter n has no physical meaning because the phenomenon of refraction may occur only on optical paths not shorter than the wavelengths of corresponding light. Förster has used the well known equality for non ferromagnetic media $\chi^2 = n^4$ which is rather reasonable for dye molecules in homogeneous aromatic solvents like benzene. However, n is determined for optical region, with light periods around 10^{-15} s, while in our case EE migration proceeds in time spans about 10^{-12} s. In such a time range, in addition to ever active electrons, mobile H-atoms having some non compensated charges are most appropriate candidates for field-induced shifts and thus, to dielectric polarization (Borisov 2004). It appears that their polarization (especially that in H_2O fraction which portion reaches 15–20% of the whole membrane weight, as well as in COOH and NH_2 protein fragments) may add much to the χ contribution induced solely by electrons in lipid–protein media of photosynthetic membranes. In them the value of "conditional" n may be as low as 1.29–1.32 (Borisov and Zuber 1993). Experiments with reversible vacuum desiccation of water from chromatophores of purple bacteria had estimated the portion of this water fraction as 0.4 (Clayton 1966; Aksenov. 2004). The account for polarization of their electrical dipoles (equal to 50% of the total water dipole, 1,84: 2 = 0.92 D) yields the increase in this particular χ value within 1.85–1.90. Note that in the optical region, water has $\chi = n^2 \cong 1.7$, but at the frequencies about 3×10^{11} s^{-1} it has $\chi \cong 4.3$–4.6 (Grant et al. 1978; Kuntz and Kautzmann 1974) which makes $\chi^2 \cong 20$. Thus, this estimation of the overall value of membrane χ^2 (with partial contribution from a portion of mobile H-atoms from water molecules) gives approximately,

$$\chi^2 \cong 3.5\,\text{ps}.$$

However, the χ microvalue may be higher in close vicinity of such H_2O molecules, or lower at longer distance from them.

Determination of the R_{het} value

R_{het} value is the most critical parameter in Förster theory, in particular, for EE migration between B800* $\rightarrow$ B850. In this article its magnitude was derived on the base of R_0 = 80 Å calculated earlier (Zankel et al. 1967; Campillo et al. 1977) for EE migration between homogeneous B875 BChla molecules. Note that in some works its value used was even greater, up to R_0 = 114 Å in (Kramer et al. 1984). Bearing in mind high value of orientation factor $\xi(\psi,\theta_1,\theta_2)^2$ in this work, one obtains the value R_0 = 93 Å for random ensembles. In the author's opinion even R_0 = 93 Å is an obvious overestimation, the more so that the frequency factor $(800/875)^{2/3}$ must be taken into account.

To eliminate the contradiction revealed in this work the value R_0 = 80 Å must be increased at least by 25–30%. The author has used an additional approach to verify the correctness of R_0 = 80 Å for BChl*a* in vivo. Widely investigated and repeatedly characterized dyes fluorescein, acridine orange, rhodamin have their R_0 within 40–50 Å. In particular, acridine orange embedded into nucleoproteid complex has the following parameters (Gursky et al. 1968): absorption band maximum at $\nu_{\max}$ = 503 nm, the band width = 2,000 cm^{-1}, Stokes shift = 1,070 cm^{-1}, molar extinction $\varepsilon(\nu)_{\max}$ = 56,000; fluorescence yield = 0.9, τ_{fl} = 5 ns and R_0 = 50 Å. These values were established in the work (Gursky et al. 1968) in experiments in which interchromophore distance and mutual orientation of molecular dipoles were well defined in ribonucleoproteid complex. The above mentioned figures for acridine orange were put into Eq. (1) and its left and right parts were divided term by term with those from the same Eq. (1) filled with BChla parameters. Nucleoproteid interior is known to be very hydrophobic, so it is reasonable to assume that its χ value is not much different from that in B875 containing membranes. The overlapping factor for BChl*a* was estimated to be about 1.78-times grater than that for acridine orange. Thus the value of R_0 for BChl*a* at 875 nm was estimated from the proportion thus created to be about 78,9 Å, i.e. very close to 80 Å, obtained earlier in Clayton's laboratory (Zankel et al. 1967).

So, both calculations of R_0 in this paragraph and those for R_{het} in the preceding one appear to ensure a reasonable safety margin for the above statement about the principal contradiction between experimental and theoretical magnitudes of $t_{D,A}$.

Discussion

It should be noted that the discrepancy noted above was reported for the first time for LH2 from purple bacteria, *R. sphaeroides* in Sundstrom's laboratory (Pullerits et al. 1997; Herek et al. 2000). Besides, it was estimated as about twofold in the theoretical work of Scholes et al. (1999) and Scholes and Fleming (2000). The present work confirms this earlier finding for LH2s of *R. acidophila*.

The authors of earlier work (Scholes and Fleming 2000; Pullerits et al. 1997; Herek et al. 2000; Scholes et al. 1997; Scholes et al. 1999, Robert et al. 2003) suggested and thoroughly analyzed several fine effects which may reduce this discrepancy. Among them the following are of importance.

Spectral heterogeneity of B800 and B850 molecules

This factor does play a noticeable role in EE migration. According to the data in (Rutkauskas et al 2004), separate

LH2 complexes exhibit about an 30-nm dispersion in positions of BChl fluorescence peaks. This site energy disorder apparently reflects some static diversity in LH2 structures. The influence of this diversity on the B800 → B850 EE migration was theoretically accounted for in the works (Pullerits et al. 1997; Herek et al. 2000; Scholes et al. 1997, 1999; Novoderezkin et al. 2003). It was demonstrated in them that site-energy disorder may increase the rate of B800 → B850 EE transfer. In our case, the value of the overlapping integrals in Eqs. (1,2) must increase for BChl fractions having slightly closer optical peaks than widely used at 800 and 850 nm. This effect was studied quantitatively. The above mentioned spectral distribution was modelled roughly by assuming three spectral fractions in B800 and B850 molecules: B790 (25%), B800 (50%), B810 (25%) for B800 and B840 (25%), B850 (50%) and B860 (25%) for B850. These fractions were chaotically distributed in the model of B800 and B850 circles used in this work and the kinetic data for four independent distributions were obtained. Two limiting models were analyzed.

1. Independent LH2 complexes. In such LH2 ensemble EE concentrations in B800 decayed with three different rates. Therefore the initial slope was about 22% higher in the range of 100–80% decay, and the averaged EE decay in net B800 ensemble becomes slightly non exponential. However in the decay range from 80 to 30% (more appropriate for real measurements) this increase did not exceed 2.5%.
2. All LH2 complexes efficiently coupled. In about 1 ps most of EEs got to the long wavelength fraction (B810) according to Boltzman energy distribution. If all these B810 are coupled to either of B840, B850, B860, the efficiency of EE transfer increases about 31–36%. According to the data in (Bahatyreva et al. 2004), LH2s and LH1s do not form regular arrays, but are distributed rather chaotically in the membrane. Therefore, we believe that the real effect of acceleration of EE B800–B850 transfer due to the spectral heterogeneity of B800 and B850 molecules should be lower, may be about 20–25%. Thus it may reduce the value of the crucial parameter $t_{D,A}$ from above mentioned 3.77 ps to $t_{D,A} \cong 2.9$–3.2 ps.

Carotenoids contribute to excitation migration?

According to (McDermot et al. 1995), in the LH2 of *R. acidophila* "…carotenoid molecule passes in van der Waals contact past the edge of the B800-bacteriochlorin ring. It then proceeds to cross over into the next α-apoprotein-band B850 bacteriochlorin ring (again in van-der-Waals contact)". Formally, in the Förster theory, the presence of carotenoids must entail a slightly negative influence on excitation migration because one may reasonably expect that π-electronic matter between B800 and B850 should increase the local dielectric permeability and thus hamper EE migration. However, after Scholes and Fleming (2000) the He et al. (2004) believe that π-electronic chains between BChl rings may also play a positive role in EE migration. One comes across as a rather similar problem in the theoretical treatment of intermolecular electron transfer. The analysis conducted by Walla et al. (2001) revealed that periodic polyene chains connecting molecules that exchange with electrons considerably stimulate this process. According to this study, their virtual influence causes noticeable (up to fractions of an electron-volt) lowering of the height of intermolecular potential barrier for electron tunnelling. Rather similar theory for super-exchange-mediated coupling via carotenoids was developed in the work of Scholes and Fleming (2000) and Scholes et al. (1997) for intermolecular transfer of electronic excitations. According to these works: "the space-electronic coupling through the π/π^* system of the bridging carotenoids may thoroughly work". Its possible efficiency in increasing of B800–B850 EE transfer was estimated in Scholes and Fleming (2000) as about 30%.

Within known theories, such an effect may be associated with participation of the "forbidden" carotenoid S1 (Ag)-state in EE transfer (Walla et al. 2000; Krikunova et al. 2002). In LH-II complexes of plants the EE transfer from S1 (Ag)-state of carotenoids to Chl*a* was proven to be very efficient (Walla et al. 2001). It is possible that some carotenoids of purple bacteria also have the S1 (Ag)-state not far from that in B800 BChls. For example, in *Chromatium minutissimum* two-photon absorbance due to the S1 (Ag)-state increases at about 710 → 745 nm (Krikunova et al 2002). By analogy with other organisms this forbidden band may expand in *Ch. minutissimum* right up to 800–820 nm.

If correct, the above suggestion predicts that, in vivo, carotenoids may execute one further fundamental function: to promote excitation migration at least between B800 and B850 in purple bacteria, but possibly in a more general way between some other antenna BChl and Chl molecules, thus increasing the overall efficiency of excitation delivery from plentiful antenna pigments to reaction centres in various photosynthetic organisms.

Excitons

The tendency has recently appeared to "cure" with delocalized excitons the problems related to EE transfer between BChl spectral fractions in vivo (see citations 15–32 in the work (van Grondelle and Novoderezkin 2001). In many recent quantum-mechanical models authors claim

that excitons are capable of increasing the range for efficient EE transfer as compared with that for the Förster theory of slow inductive resonance applied to monomer molecules. In particular, such an approach was suggested as a tool to resolve the discrepancy between experimental data and their theoretical treatment for the energy migration in LH1 complexes of purple bacteria (van Grondelle and Novoderezkin 2001; Novoderezhkin and Razjivin 1995; Chachisvilis et al 1997; Novoderezhkin et al. 1999). According to van Grondelle and Novoderezkin (2001), Novoderezhkin and Razjivin (1995), and Chachisvilis et al. (1997) the rate constant for B875 $\rightarrow$ P870 EE transfer may increase due to EE delocalization in B875 α/β-dimers. In B850 and B875 α/β-pairs the first singlet excited S^*_1-state is split into two levels. If an exciton covers 2–3 such α/β-pairs their S^*_1-states are split, respectively, into 4–6 sub-levels. Only two of them, slightly above the lowest one, are allowed, and the lower of the corresponding absorption bands dominate. Nevertheless, in LH2s the upper excitonic band of B850 complexes may also take part in the process of EE migration, provided it has a better overlap with the fluorescence band of excited donor, B800*. Unfortunately no direct experimental data were yet obtained about such minor bands. Possibly these bands are weak. The more so, that the area under S^*_1-bands of typical dyes are known to be very conservative. For example, in BChl*a* molecules dissolved in 14 solvents with differing dielectric constants the diversity in this area was within 5% (Connolly et al. 1982). Taking this into account, the area under the B800 band of LH2 should be equal to more than 50% of that for the B850 band, provided the latter one has donated to its minor counterpart near 800 nm a noticeable portion of its total strength. So, the question about the activity of the EE migration channel from B800* to B850 via its upper excitonic level is still open.

But generally, excitons in B850 BChl fraction can only strengthen the discrepancy, because the efficient dipole moment of excitonically coupled molecules is the vector sum of those of their individual chromophores. Thus the rate of reversed excitation migration, B850* $\rightarrow$ B800 will be increased.

Why this discrepancy was not reported in some other works?

The above discrepancy was not obtained for the same bacteria *R. acidophila* in the literature (Scholes and Fleming 2000; Scholes et al. 1999) and many others. It is believed that this is due to the following reasons:

Some physical parameters were varied. For example, the critical distance for EE migration was noticeably enlarged in the article (Kramer et al. 1984). Some authors have used severely limiting Lorentz formula which was reanimated in Juzeliunas and Andrews (1994a, b). This formula, $D = n^{-1}[(n^2 + 2)/3]^2$ where n is the index of optical refraction, was used for accounting for the dielectric permeability, instead of Förster's n^4 (compare for $n = 1.4$: $D = 1.24$ while in Förster's $n^4 = 3.84$). As it concerns many modern theoretical studies for EE migration in photosynthetic BChl ensembles, they use a definite strategy: some factors are varied in order to obtain accordance with the available optical and kinetic data. It is a reasonable mode of work, but it may have a disadvantage: in such a way it is hardly possible to reveal internal discrepancies, if they do exist between real and theoretical models.

The following mechanisms may be responsible for the above revealed discrepancy

However, it is evidently the model used, not a real LH2 system, which suffers from the above mentioned discrepancy. Possibly, one should follow the prescription of a well known British playwright and politician, Richard Sheridan who wrote "We often discover what will do, by finding out what will not do". So, by repelling from the negative result, we may suggest a joint action between the following mechanisms:

1. The 30% effect of carotenoids estimated theoretically in the work of Scholes and Fleming (2000) may contribute to the reduction of this discrepancy, but it needs experimental confirmation. In the course of an analysis of excitation migration between B800 and B850 molecules of LH2 complexes of purple bacteria, one has run across an exciting precedent whereby structural biochemistry and relevant kinetic data have provided a feedback to physics, and thus stimulate its further development, possibly of the new mechanism of polyene bridging in intermolecular transfer of electronic excitations between dye molecules like porphyrins to the upper split level which appears in a few excitonically coupled α/β-pairs of B850.
2. The media parameters are quite different in vivo and in media used for the preparation of artificial LH2 crystals. This circumstance, as well as possible diversity of LH2 complexes in bacterial membranes, may lead to formation of LH2 crystalline structures differing from those in vivo. Note that separate LH2 complexes exhibit about 30-nm dispersion in the position of their fluorescence peaks (Rutkauskas et al. 2004) (see also comment on heterogeneity and dynamics of LH1 complexes in Bahatyreva et al. 2004). This fact apparently reflects the diversity in LH2 structures in vivo, but only one of them (if any?) may be relevant for crystallization and its protein core may be changed in its course.

3. The most important point is: One may expect substantial conformational changes in α/β-heterodimers, affected by the transmembrane potential. Coulomb attraction of ± charges arising on the opposite sides of illuminated membrane may cause an effect of local membrane contractions. Possibly, it may result in considerable tilts of polypeptide chains thus decreasing the distance between a few of B800 and B850 molecules, or a few of α/β-heterodimers may step forth out of the membrane thus making a bridge for excitation B800 $\rightarrow$ B850 transfer. Note that the typical transmembrane potential of tens of mV corresponds to an electrical tension about 10^5 V/cm, which by two orders exceeds those in traditional electrostriction materials. The sigmoid kinetics of P875 photooxidation after switching actinic light is in line with this hypothesis. Prof. V. Samuilov suggests one must check this hypothesis by monitoring the B800* $\rightarrow$ B850 EE transfer in uncoupled chromatophores. E. Davydov's soliton mechanism [36] may also possibly be involved.

One more conclusion is suggested: Bacterial photosynthesis provides a unique possibility to check whether there are real similarities between the structures of polymolecular bioparticles in crystallized and the in vivo state. In fact, the set of energy, spectral and kinetic data reliably established for reaction centres of LH2 and LH1 particles are precisely coupled to their corresponding in vivo structures. In the crystalline state these dimensions are sometimes known with atomic resolution. Thus, by the use of all the above mentioned parameters applied to corresponding crystalline structures, one can calculate the values of the times of excitation transfer from B800 to B850, from B850 to B875, from B875 to the P870 special pair, and to compare these values with those obtained experimentally with natural particles, which possess natural structures. From the quantum mechanical approaches yet used, such a comparison may be reasonably executed with the aid of Förster's theory, whose parameters are strictly related to experimental ones. The coincidence of calculated and experimental lifetime values would prove similarity of crystalline and in vivo structures, and vice versa.

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