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THE INTRINSIC LIFETIMES OF BACTERIOVIRIDIN-660 AND CHLOROPHYLL *a* IN DIFFERENT SOLVENTS

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SUMMARY

Intrinsic lifetimes, τ_0, for bacterioviridin-660 (chlorobium chlorophyll-660) in diethyl ether, acetone, methanol, and chlorophyll *a* in diethyl ether, acetone, methanol, pyridine were calculated. The spectral data of different authors were used. The possible sources of errors in τ_0 determinations were examined. According to our calculations the averaged value of τ_0 of bacterioviridin-660 in ether is 18 ± 2 ns. Taking into account new data for the Stokes shift and specific absorption coefficient the intrinsic lifetime of chlorophyll *a* in ether was found to be 15.6 ± 0.5 ns.

INTRODUCTION

Einstein[1] in 1917 derived the fundamental equations relating the transition probabilities for induced absorption and emission with that for spontaneous emission, if the refractive index of the surrounding medium is unity. Perrin[2,3] and Lewis and Kasha[4] modified the equation to allow for the refractive index of the medium containing the absorbing atoms or molecules. For transitions between states of the same multiplicity, *e.g.* singlet–singlet transitions as in fluorescence, the relation obtained in terms of experimental parameters[2,4–7] is:

$$\frac{1}{\tau_{10}} = 2.88 \times 10^{-9}\, \bar{\nu}_{10}^{2}\, n_{10}^{2} \int \varepsilon(\bar{\nu}) \mathrm{d}\bar{\nu} \qquad (1)$$

where τ_{10} is the intrinsic lifetime of the $1 \rightarrow 0$ transition, $\bar{\nu}_{10}$ is the wavenumber of spontaneous emission of radiation, n_{10} is the refractive index at $\bar{\nu}_{10}$, $\varepsilon(\bar{\nu})$ is the molar extinction coefficient at $\bar{\nu}$, and the integral is taken over the $0 \rightarrow 1$ absorption band.

The derivation of eqn. (1) assumes that the absorption band is sharp, and that the fluorescence occurs at the same wavenumber as the absorption. This means

that eqn. (1) is valid only for sharp atomic transitions and to resonance fluorescence. Förster[8] obtained a modified equation applicable to molecular fluorescence, on the assumption that there is mirror symmetry between the fluorescence and absorption bands such as that discovered experimentally by Levshin[9]. The modified equation is:

$$\frac{1}{\tau_0} = 2.88 \times 10^{-9} n^2 \int \frac{(2\bar{\nu}_0 - \bar{\nu})^3}{\bar{\nu}} \varepsilon(\bar{\nu}) d\bar{\nu} \qquad (2)$$

where τ_0 is the intrinsic lifetime, n is the mean refractive index of the medium over the fluorescence and absorption bands, $\bar{\nu}_0$ is the wavenumber of the "mirror symmetry" point between the fluorescence and absorption bands, and the integral is taken over the $0 \rightarrow 1$ absorption band. Other authors[10,11] have derived alternative equations which do not directly involve the assumption of mirror symmetry.

It is important to measure τ_0 for the photosynthetic pigments, as the fluorescence quantum yield, Φ_{fl}, may be calculated from the equation: $\Phi_{fl} = \tau/\tau_0$ where τ is the fluorescence lifetime. The direct measurement of the Φ_{fl} value (particularly *in vivo*) is associated with many difficulties which reduce the precision of Φ_{fl} determinations in comparison with the above-mentioned indirect method. The Φ_{fl} value of the chlorophyll fraction which is directly related to the reaction centre correlates with the photosynthesis efficiency as was shown for the photosynthetic bacteria[12] where the excitation energy migrates *via* singlet levels of bacteriochlorophyll[13].

In the present paper intrinsic lifetimes, τ_0, for bacterioviridin-660 (chlorobium chlorophyll-660) and chlorophyll *a* in different solvents from the spectral data obtained by different authors were calculated from eqn. (2) and the possible sources of errors in their determination were examined.

EXPERIMENTAL

The absorption spectra of bacterioviridin-660 (BVr-660) were measured using a Cary-15 spectrophotometer. The characteristics of the absorption spectra obtained are presented in Table 3. To determine the Stokes shift value, the fluorescence spectra of BVr-660 were measured in a spectrofluorimeter designed in this laboratory[14].

RESULTS AND DISCUSSION

The following factors influence the absolute τ_0 value calculated from eqn. (2):

(1) how accurately the integrating absorption spectrum corresponds to the $0 \rightarrow 1$ band;

(2) the Stokes shift (the shift between the main absorption and fluorescence maxima);

(3) the accuracy of the absorption coefficient measurements;

(4) the temperature at which the absorption spectra were measured;

(5) the dependence of the τ_0 value on the environment.

The spectral difference between the pigment preparations obtained by different authors appreciably affects the calculated τ_0 value.

Let us consider one by one the effects of these factors on the τ_0 value.

Different electronic transitions leading to various absorption bands characterizing the absorption spectrum can be detected experimentally with the help of polarization optics and fluorescence spectra. Additional information can be obtained from an analysis of the influence of interaction with solvent molecules on the shape and position of the absorption bands. π–π transitions, which are parallel to the plane of the molecule, can be distinguished from others, which may be perpendicular, by measuring the dichroism of the pigment dissolved in an artificially oriented system ("liquid crystal", a system in a strong electric field etc.). The mutual orientation of the electronic transitions can be determined by measuring the degree of fluorescence polarization p as a function of the wavelength of the exciting light. When the absorbing and emitting oscillators are the same, $p = +0.5$, provided the Brownian rotation is negligible during the lifetime of the excited state, and the concentration is so low that no energy transfer occurs. If the absorbing oscillator is perpendicular to the emitting one, $p = -0.33$[2]. Within the absorption band system of a single transition, the polarization value should be constant.

The interaction with solvent molecules, which is mainly determined by the dielectric properties of the solvent, is especially strong in polar solvents. There is experimental evidence that different types of transitions are affected in a different way.

Interaction with other pigment molecules affects the shape and the position of the absorption bands, usually broadening and shifting them to the red. It seems likely that such shifts are mainly determined by dipole–dipole interactions of the π-electron systems of the pigments. However, if aggregates are formed, the absorption spectrum may be changed more drastically[15].

The existence of transitions to states different from the fluorescing one can be sometimes detected by using arguments involving mirror symmetry between the fluorescence and absorption bands.

The absorption and fluorescence spectra of chlorophyll *a* (Chl-*a*), dissolved in different solvents, have been obtained by many investigators.

Measurements of Chl-*a* dichroism in liquid crystals, such as ammonium oleate and lecithin, indicate that absorption occurs in the plane of the molecule for all bands in the visible part of the spectrum[8]. Thus all these bands are most probably caused by π–π transitions, at least for the major fraction of absorption. However, in the fluorescence spectrum of Chl-*a* there is no distinct mirror image

corresponding to the 578 nm absorption band[16]; hence this band, all or in part, belongs to a transition different from the fluorescing one. This conclusion is supported by the fluorescence polarization spectrum data[17–19]. The degree of fluorescence polarization drops around 580 nm to a slightly negative value. From the mirror symmetrical shape of the fluorescence spectrum of Chl-*a* it can be estimated that about 35% of the absorption at 578 nm is due to the second electronic transition[16]. This value is in a good agreement with the one calculated from the degree of polarization, assuming the occurrence of two perpendicular transitions[20].

The fluorescence polarization spectrum of Chl-*a* shows a minimum[18] or a shoulder[19] around 620 nm. This could indicate that one more transition is also present near 620 nm overlapping the $0 \rightarrow 1$ one. This suggestion is supported by the fact that in some cases the 620 nm absorption band is doubled[21].

The shape of the absorption spectrum of the main chlorophyll pigment of green sulphur bacteria, chlorobium chlorophyll-660, is generally similar to that of Chl-*a*. The main difference is the absence of a small band around 580 nm in BVr-660. The spectrum of fluorescence polarization indicates that the corresponding band is displaced to the red and is masked by the vibrational level of the first electronic transition[16].

Taking into account the foregoing, the Chl-*a* extinction coefficient for the 578 nm band was decreased by 35% while calculating the integral:

$$\int \frac{(2\bar{\nu}_0 - \bar{\nu})^3}{\bar{\nu}} \varepsilon(\bar{\nu}) d\bar{\nu}.$$

This increased the τ_0 value by 1 to 3% (see Tables 2 and 3). Other corrections for the shape of the integrating Chl-*a* absorption spectra were not introduced. The BVr-660 absorption spectra were not changed while calculating the analogous integral, as a similar correction for BVr-660 is significantly smaller than that for Chl-*a*. When calculating the τ_0 value for BVr-660 (τ_0^{BVr}) the molecular weight of BVr-660 was assumed to be 793.4, which fits the empirical formula of BVr-660 ($C_{49}H_{60}O_4N_4Mg$).[22]

The Stokes shift for Chl-*a* in diethyl ether at room temperature obtained by different authors varies from 4.5 to 7.0 nm[16,23,24,26], and BVr-660 in diethyl ether from 3.0 to 7.0 nm[16,25,27]. Fortunately, this two-fold change in the Stokes shift value does not alter the τ_0 value by more than 2% (see Tables 2 and 3).

The measured values of specific absorption coefficient, α for the main red absorption maximum of the pigments in diethyl ether and acetone obtained by different authors are presented in Table 1. Thus the α_{red}^{max} value of Chl-*a* in ether varies within the limits of 6%; for Chl-*a* in acetone, 10%, for BVr-660, 11%. This introduces appropriate errors into the τ_0 calculation.

The spectral differences between the Chl-*a* samples results in the 5% fluctuations in the $\tau_0^{Chl\text{-}a}$ value. These fluctuations are more appreciable for BVr-660,

TABLE 1

SPECIFIC ABSORPTION COEFFICIENTS, α_{red}^{max}, FOR THE MAIN RED ABSORPTION MAXIMA OF CHLOROPHYLL *A* AND BACTERIOVIRIDIN-660

Chlorophyll *a*				Bacterioviridin-660	
Diethyl ether, + 25°C		Acetone, + 25°C		Diethyl ether, + 25°C	
α_{red}^{max}	ref.	α_{red}^{max}	ref.	α_{red}^{max}	ref.
102.1	30	94.0	32	112.5	25
100.9	24	92.5	31	98.6	34
96.6	29	90.7	28		
95.3	21	85.7	21		
		84.2	33		

as the available literature data give two types of BVr absorption spectra in ether: (i) showing a marked absorption above 700 nm with a maximum near 750 nm[25,35], and (ii) not showing this[16,27,34,36]. Besides, the investigated absorption spectra of BVr-660 in ether markedly differ by the absorption in the peaks at 660 and 622 nm (Table 3). These spectral differences result in 15% deviation in the τ_0^{BVr} in ether.

The effect of temperature on the τ_0 value was examined only for two specimens: Chl-*a* and BVr-660 in ether at temperatures of −196°C and +20°C. For the solutions of same pigment at temperatures of +20°C and −196°C:

$$\frac{\tau_0^{rt}}{\tau_0^{nt}} = \frac{n^2_{nt}}{n^2_{rt}} \times \frac{\int_{nt} \frac{(2\bar{\nu}_0 - \bar{\nu})^3}{\bar{\nu}} \varepsilon(\bar{\nu}) d\bar{\nu}}{\int_{rt} \frac{(2\bar{\nu}_0 - \bar{\nu})^3}{\bar{\nu}} \varepsilon(\bar{\nu}) d\bar{\nu}} \tag{3}$$

where index "rt" means room temperature (+20°C) and index "nt" means nitrogen temperature (−196°C).

Using the Litvin's and Guljaev's data[26,27] we calculated for Chl-*a* in ether the ratio of

$$\gamma \equiv \int_{rt} \frac{(2\bar{\nu}_0 - \bar{\nu})^3}{\bar{\nu}} \varepsilon(\bar{\nu}) d\bar{\nu} \Big/ \int_{nt} \frac{(2\bar{\nu}_0 - \bar{\nu})^3}{\bar{\nu}} \varepsilon(\bar{\nu}) d\bar{\nu} = 1.08$$

for BVr-660 in ether, $\gamma = 1.10$. Our assessments showed that the n^2 value does not increase by more than 10% as one passes from room temperature to the nitrogen temperature (in our case this change is no more than 5%). Thus the effects of the changes in the n^2 and γ values are opposed. It can be shown from eqn. (3) that the τ_0 values for the Chl-*a* and BVr-660 samples examined in ether increase slightly as one passes from +20°C to −196°C (the maximum possible increase is 10%).

TABLE 2

NATURAL LIFETIME, τ_0, FOR CHLOROPHYLL *a* IN DIFFERENT SOLVENTS

Solvent	Refractive index, n	λ_{abs}^{max} (nm)	α_{red}^{max} (lg^{-1} cm^{-1})	Stokes shift (nm)	$\frac{\alpha_{red}^{max}}{\alpha_{505}}$	$\frac{\text{blue max}}{\text{red max}}$	Limits of integrating for $\int \frac{(2\bar{\nu}_0-\bar{\nu})^3}{\bar{\nu}} \times \varepsilon(\bar{\nu})d\bar{\nu}$ (nm)	$\tau_0 \times 10^9$ (s)	Ref.
Diethyl ether, + 25°C	1.354	660	102.1[30]	4.5[23]	52.3	1.32	450–700	15.4	30
				7.0[16]				15.6	
Diethyl ether,				4.5				14.9	38
24–26°C	1.354	660	102.1	7.0	52.7	1.33	460–700	15.1	
Acetone, 24–26°C	1.359	661.5	84.2[33]	8.5[23]	43.9	1.26	460–700	15.2	
			85.8[21]					14.9	
Methanol, 24–26°C	1.329	664	74.5[21]	10.0[23]	45.0	1.00	460–700	16.5	
Diethyl ether, + 20°C	1.354	660	102.1	7.0	40.0	1.31	440–700	15.6	26
Diethyl ether, + 20°C	1.354	661	102.1	7.0	47.3	1.30		15.4	28
					57.5	1.31	460–715	15.8	
					57.2	1.31		15.4	
Acetone, + 20°C	1.359	662.5	90.7[28]	8.5	42.2	1.25	460–715	14.8	
			84.2					15.9	
Pyridine, + 20°C	1.509	671	93.7[28]	10.0	34.4	1.33	480–715	12.1	
			86.1[21]					13.1	

TABLE 3

NATURAL LIFETIME, τ_0, FOR BACTERIOVIRIDIN-660 IN DIFFERENT SOLVENTS

Solvent	λ_{abs}^{max} (nm)	a_{red}^{max} (l g^{-1} cm^{-1})	Stokes shift (nm)	$\frac{a_{660}}{a_{622}}$	blue-max / red max	Limits of integrating for $\int \frac{(2\bar{\nu}_0-\bar{\nu})^3}{\bar{\nu}} \varepsilon(\bar{\nu})d\bar{\nu}$ (nm)	$\tau_0 \times 10^9$ (s)	Ref.
Diethyl ether, + 20°C	660	112.5[25]	3.0[25]	5.6	1.53	425–700	16.3	36
			7.0[16]				16.5	
	660	112.5	3.0	4.7	1.55	400–700	12.2	25
			7.0				12.5	
	660	98.6	7.0	6.3	1.54	525–700	21.5	34
	661	112.5	6.0[27]	6.2	1.40	440–700	18.5	27
		98.6[34]					21.1	
		112.5	7.0	5.3	1.57	460–700	15.4	Our data, 1973
		98.6					17.5	
Methanol, + 20°C	667.5	86.0[25]	10.0	4.5	1.13	470–700	15.8	
Acetone, + 20°C	662	92.6[25]	6.0	4.7	1.48	470–700	16.8	

The dependence of τ_0 value on the solvent was followed for Chl-*a* in ether, acetone, pyridine, methanol and for BVr-660 in ether, acetone, methanol. It is difficult to examine the influence of the solvent nature on τ_0 from the literature data available, as α values presented in the literature are most often given without indication of the measurement accuracy. This prevents comparison of τ_0 values which differ from another by less than 15–20%. For Chl-*a* in ether only one α value with an indication of the measurement accuracy is presented in the literature[30]: $\alpha_{red}^{max} = 102.1 \pm 0.8\,1\,g^{-1}\,cm^{-1}$. This provides the accuracy in intrinsic lifetime $\Delta\tau_0 = \pm 0.1$ ns. For Chl-*a* in acetone $\alpha_{red}^{max} = 84 \pm 6$[33]. This error gives a corresponding $\Delta\tau_0 = \pm 1.1$ ns. For BVr-660 analogous data concerning the measurement accuracy of presented α values are lacking.

The calculated τ_0 values for Chl-*a* and BVr-660 are listed in Tables 2 and 3, respectively. The characteristics of the absorption spectrum (for which the given τ_0 value is obtained) and the parameters which are used for calculating τ_0 from eqn. (2) are indicated for every τ_0 value.

In our opinion, a τ_0 value of 15.6 ns calculated for Chl-*a* in ether should be used. This value is slightly different from that obtained by Brody[37] as the improved value of Stokes shift for Chl-*a* in ether was used and the correction for the 578 nm absorption band was introduced. The maximum error in calculating τ_0 for Chl-*a* in ether from eqn. (2) is equal to 6%. The main error is associated with the α_{red}^{max} value dispersion obtained by different authors.

The averaging of τ_0^{BVr} values for the investigated absorption spectra of BVr-660 in ether gives:

$$\tau_0 = 16.6 \pm 1.7 \text{ ns for } \alpha_{red}^{max} = 112.5$$

and

$$\tau_0 = 18.9 \pm 1.9 \text{ ns for } \alpha_{red}^{max} = 98.6$$

The great error in the τ_0^{BVr} measurement is due to spectral differences of the BVr-660 samples investigated.

Thus, at the present day, the following intrinsic lifetime of bacterioviridin-660 in diethyl ether may be used:

$$\tau_0^{BVr} = 18 \pm 2 \text{ ns}$$

Further refinements of the α value will provide a means for the determination of τ_0^{BVr} with greater accuracy.

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