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SOLVENT EFFECTS ON THE LOWEST SINGLET-SINGLET TRANSITION IN CHLOROPHYLS a AND b*

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Absorption and fluorescence spectra involving the lowest energy singlet state at chlorophyll a (ch a) and chlorophyll b (ch b) in twenty-seven solvent of different dielectric constants and refractive index have been recorded. The solvent-dependent position of the absorption and emission maxima are discussed in terms of dispersive solute-solvent interactions.

1. *Introduction*

One of the major behavioural differences of chlorophyll *in vivo* and *in vitro* concerns the efficiency with which excitonic energy is transferred from the initial site of photon absorption to the site of photochemical reaction. While this process is extremely efficient *in vivo*, providing the well-known "funnel effect", attempts to simulate the phenomenon *in vitro* always result in considerable energy quenching. The widely held qualitative explanation of this is that in the natural system the chlorophyll molecules (or aggregated systems) are oriented by the lipid b-membrane in such a way that inter-molecular energy migration is favoured, but self-quenching processes are not. It has been postulated that the self-quenching in synthetic systems takes place by exciton-exciton annihilation at excimer forming sites, where chlorophyll molecules are in particularly close proximity. Consequently it seems to these authors that the first step in design of a synthetic system with some of the energy migration characteristics of naturally occuring chlorophyll must

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[*] Dedicated to Professor Aleksander Jabłoński on the occasion of his 80th birthday.

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be the creation of a sequence of chlorophyll molecules in a specific geometry permitting resonance energy transfer, but not satisfying the more stringent requirements for excimer formation. One possible method of achieving this is to create a chain of chlorophyll molecules by complexing in some specific way to a preformed polymer chain. It is a research project investigating the synthesis and photophysical properties of such polymeric chlorophyll complexes of which this work forms the first part.

In order to measure the electronic and energy transfer characteristics of chlorophyll molecules in a complexed sequence it is necessary to make precise measurements of the absorption and fluorescence emission spectra. Before this can be done it is essential to characterise in detail the spectral effects of solvation, complexation and aggregation. In the evaluation of the effects on the electronic transition of both the static and electronic polarizability of the surrounding medium it is possible to derive information relating to the transition involved, and in particular to the ionisation potential of the chlorophyll molecule. Such information then permits the reverse derivation of environmental electronic interactions from the observations made on systems of unknown solvation or complexation effects.

In this work precise measurements of the absorption and emission spectra of chlorophylls a and b in 27 different solvents are analysed in such a way as to evaluate the dependence of the lowest energy singlet-singlet transition on the electronic polarizability of a solvent environment which behaves as a continuum, to illustrate a simple means of detecting specific solvation effects in which a direct intermolecular interaction perturbes the electronic transition, and to evaluate the important transition parameters such as the ionisation potential of the ground state chlorophyll molecule.

2. *Experimental*

Chlorophylls a and b were extracted from spinach using acetone, and then purified by chromatography on sucrose and polyethylene columns [1]. The products were free of detectable pheophytins and xanthophylls, and were stored in a desiccator over silica gel.

Solvents of A R or spectroscopic grade (Table I) were dried over Linde 4A molecular sieves and were redistilled before use. Spectroscopic observations were made on freshly prepared 10^{-6} molar solutions which had not been de-aerated. Such low concentrations were necessary to minimize aggregation in the less polar solvents, and to avoid disturbance of the emission spectra due to the strong overlap of the absorption and emission bands.

Absorption measurements of the lowest energy singlet-singlet transition (QY-red band) were made on a Beckman Acta IV spectrophotometer equipped with a derivative mode module. Uncorrected fluorescence spectra were obtained on a Perkin Elmer MPF44 fluorescence spectrophotometer calibrated using the 450.1 and 467 nm xenon lines. The chlorophyll solutions were excited at right angled excitation/emission at the maximum of the second (Soret-QX) band using 2 nm bandpass on both the excitation and emission monochromators. The fluorescence spectrum was not sensitive to the precise excitation wavelength within the Soret band. Emission spectra were recorded on ratio-mode, so automatically correcting for fluctuations in the xenon lamps intensity.

TABLE I

Positions of absorption ($\tilde{\nu}_A$) and fluorescence ($\tilde{\nu}_F$) bands of the lowest energy singlet-singlet electronic transitions in chlorophyll a and b in various solvents

No.	Solvent	$^{n}D_{20°C}$*	Chlorophyll a		Chlorophyll b	
			$10^{-3}\times\tilde{\nu}_A$ [cm^{-1}]	$10^{-3}\times\hat{\nu}_F$ [cm^{-1}]	$10^{-3}\times\tilde{\nu}_A$ [cm^{-1}]	$10^{-3}\times\tilde{\nu}_F$ [cm^{-1}]
1	n-pentane	1.385	15.197	15.100	15.617	15.515
2	n-hexane	1.3754	15.151	15.079	15.600	15.509
3	n-heptane	1.3867	15.144	15.057	15.593	15.524
4	cyclohexane	1.4266	15.158	15.034	15.594	15.503
5	iso-octane	1.39	15.160	15.079	15.617	15.491
6	carbon tetrachloride	1.4607	15.048	14.908	15.515	15.396
7	chloroform	1.4464	15.042	14.853	15.451	15.308
8	dichloromethane	1,4242	15.069	14.903	15.475	15.337
9	1,1-dichloroethane	1.4164	15.105	14.936	—	—
10	benzene	1.5011	15.042	14.908	15.479	15.360
11	chlorobenzene	1.5248	15.037	14.880	15.455	15.325
12	bromobenzene	1.5577	15.010	14.847	—	—
13	1,2-dichlorobenzene	1.549	15.006	14.858	15.422	15.302
14	aniline	1.5863	14.841	14.630	15.151	14.936
15	methanol	1.3288	15.048	14.814	15.384	15.140
16	ethanol	1.3610	15.051	14.853	15.384	15.152
17	n-propanol	1.3854	15.037	14.847	15.384	15.186
18	ethyl ether	1.3497	15.174	15.048	15.605	15.494
19	ethyl acetate	1.3728	15.128	14.992	15.515	15.372
20	acetone	1.3591	15.128	14.938	15.518	15.337
21	pyridine	1.5092	14.925	14.766	15.255	15.114
22	4-ethylpyridine	1.5009	—	—	15.262	151441
23	1-ethyl-2-pyrrolidone	1.4652	15.055	14.892	—	—
24	acetonitrile	1.345	15.128	14.953	15.494	15.313
25	dimethylsulfoxide	1.4783	15.033	14.853	15.384	15.186
26	dimethylformamide	1.4304	15.048	14.876	15.420	15.232
27	nitromethane	1.3935	15.101	14.936	15.479	15.267

* Handbook of Chemistry and Physics, 56th Ed., CRC Press.

In order to calibrate for the wavelength dependence of photomultiplier sensitivity Rhodamine G and Mythylene Blue were used as standards [2] and a correction function computed as follows:

$$E(\lambda)_{\text{corr}} = E(\lambda)_{\text{uncorr}} \times q(\lambda), \tag{1}$$

where $E(\lambda)_{\text{corr}}$, $E(\lambda)_{\text{uncorr}}$ are the corrected, uncorrected normalized spectra respectively, and $q(\lambda)$ is the correction function. Over the range of chlorophyll a and b emission $q(\lambda)$ can be approximated by the linear function

$$q(\lambda) = \alpha\lambda + \beta. \tag{2}$$

Denoting the long-wavelength half of the emission spectrum as a Gaussian function

$$E(\lambda)_{\text{uncorr}} = \exp\left(-\frac{\lambda-\lambda_{\text{max}}}{2^{1/2}\sigma}\right)^2, \tag{3}$$

where λ_{max} is the wavelength of maximum emission in the uncorrected spectrum, σ is a standard deviation expressed in terms of the half width at half height, $W_{1/2}$

$$\sigma = W_{1/2}/(2\ln 2)^{1/2}. \tag{4}$$

Solving $dE(\lambda)_{\text{corr}}/d\lambda = 0$ yields

$$(\lambda_{\text{max}})_{\text{corr}} = -\beta/2\alpha+\lambda_{\text{max}}/2+\tfrac{1}{2}\left\{\left(\frac{\beta}{\alpha}-\lambda_{\text{max}}\right)^2+4\left[\left(\frac{\beta}{\alpha}\right)\lambda_{\text{max}}+\sigma^2\right]\right\}^{1/2}. \tag{5}$$

In these experiments conversions of spectra to an energy scale [3] and application of equation (5) resulted in a correction, $(\lambda_{\text{max}})_{\text{corr}}-\lambda_{\text{max}}$, of less than 0.2 nm. Since the difference in wavelength maximum in absorption and emission could be measured only to 0.5 nm this correction is negligible.

3. *Results*

A. Spectral observations

Spectral parameters of chlorophyll a and b in a wide range of solvents are listed in Table I. For each system the absorption maximum of the Q_Y band (red band) and the emission maximum of the main fluorescence band are reported. The absorption maxima of chlorophyll a are consistent, within the experimental error, with those reported previously by Seely et al. [4]. The emission spectra of chlorophyll a in CCl_4 are in excellent agreement with those reported by Broyde and Brody [5], although there are differences in C_2H_5OH and C_6H_6.

The emission spectra of both chlorophylls are broadened as the polarity of the solvent increases. This has been explained in terms of the solvent-dependence of the transition moment [6, 7]. However in this work we emphasize the effect of solvent on the position of band maxima rather than on band shape.

In hydrocarbon solvents certain poorly resolved bands were observed on the long wavelength tail of the principal absorption band. These are presumably due to chlorophyll a oligomers [4], which exist in such solvents even at very high dilution. A further source of spectral error at such low chlorophyll concentrations is the formation of an adduct with traces of water. It is now well documented [8, 9] that such species absorb at longer wavelength than non-complexed chlorophyll, so that either hydration or dimerization [10] could perturb the long wavelength spectra. However, although no long-wavelength absorption shoulder could be observed in benzene or carbon tetrachloride, analyses were restricted to the peak maxima.

In the same way a long wavelength shoulder observed in emission could be due to aggregation or hydration [5, 9] or to phosphorescence [5]. Again this was ignored and attention focussed on the clearly resolved maximum of the emission peak.

B. Correlations with solvent polarizability

The correlations to be expected between absorption and fluorescence wavelengths on the one hand, and the solvent polarizability on the other, depend on the nature of the solute-solvent interaction. Where this involves dispersion forces, or the transition is not accompanied by solvent relaxation, the correlation is with the electronic (dynamic) polarizability rather than with the static dielectric constant. For a chromophore located in a quasi-spherical cavity this can be ascertained by plotting [11] the transition wavelength against either $(\varepsilon-1)/(2\varepsilon+1)$ or $(n^2-1)/(2n^2+1)$ where ε, n are the static dielectric constant and optical refractive index of the solvent. In this case good straight lines are obtained in the latter case, but the correlation is much inferior in the former. Consequently in the following argument all correlations are with the dynamic polarizability.

Although a good linear plot with high regression coefficient is obtained when the frequencies of absorption maxima of chlorophyll a are plotted against $(n^2-1)/(2n^2+1)$, the correlation with frequencies of emission maxima is much inferior, as is true for chlorophyll b in both absorption and emission. Two reasons for a poor correlation are immediately apparent. There may be some solvents for which the solute/solvent interaction is so specific that the normal dispersion relationships [11] are inapplicable, or the assumption that the chlorophyll molecule undergoes a transition in a quasi-spherical cavity may be incorrect, particularly for transitions originating in the excited state. Consequently we have investigated the consequences of considering the chlorophyll solute as existing in an ellipsoidal cavity in the solvent continuum.

This is achieved by modifying [11] the polarization term, $(n^2-1)/(2n^2+1)$ by an appropriate function, $F(n^2, A)$ containing the refractive index and the ellipsoid asymmetry, A.

$$A = \frac{abc}{2}\int_0^\infty \frac{ds}{(s+a)\,[(s+a^2)\,(s+b^2)\,(s+c^2)]^{1/2}}\,, \tag{6}$$

where a, b, c are the semi-axes of the ellipsoid. This integral was discussed by Liptay [11] with the transition vector axis $a \neq b = c$. For chlorophyll, with the transition vector in the plane of the major ring system, we have used a closed solution for the case $a = b \neq c$ and $a, b > c$

$$A = \frac{a^2 c}{(a^2-c^2)^{3/2}}\left\{\frac{\left(\dfrac{1}{a}\right)(a^2-c^2)^{1/2}}{2}\left[1-\left(\frac{1}{a}\right)(a^2-c^2)^{1/2}\right]^{1/2} + \tfrac{1}{2}\sin^{-1}\left(\frac{1}{a}\right)(a^2-c^2)^{1/2}\right\}. \tag{7}$$

Although the geometrical term is dimensionless, insight into appropriate axial ratios can be obtained from consideration of molecular dimensions. Consequently examples have been selected from the known dimensions [12] of the chlorophyll chromophore ($1.5 \times 1.5 \times 0.5$ nm) making due allowance for possible separations of the solvent continuum. The regression analyses for these examples are listed in Table II.

TABLE II

Correlation between absorption and emission frequencies and solvent polarizability for different ellipsoidal cavities

Chlorophyll	Ellipsoid axes [Å]		A	Absorption				Emission		
	$2a=2b$	$2c$		D [cm^{-1}]	$\tilde{\nu}_0$ [cm^{-1}]	regression coefficient	E_I [eV]	D [cm^{-1}]	$\tilde{\nu}_0$ [cm^{-1}]	regression coefficient
a	15	5	0.1999	−3962.7	15631.9	−0.905	3.4	−4725.1	15591.4	−0.619
	21.2	5	0.1503	−5251.8	15647.7	−0.905	2.7	—	—	—
	15	7	0.2643	−3031.5	15613.3	−0.905	3.6	—	—	—
	21.2	7	0.1984	−3991.3	15632.4	−0.905	2.7	—	—	—
b	15	5	0.1999	−3829.5	16027.5	−0.556	—	−3997.8	15872.3	−0.333

The linear relationship between the frequency shift in absorption and emission ($\Delta\nu_{A,F}$) can be expressed as

$$\Delta\nu_{AF} = Df. \tag{8}$$

The polarizability function, f, is given by

$$f = \frac{3a^3(n^2-1)A(1-A)}{abc[(1-A)n^2+A]} \times \frac{n^2-1}{2n^2+1} = F(n^2, A)\frac{n^2-1}{2n^2+1}, \tag{9}$$

and the interaction energy function, $-D$, by

$$-D = \frac{1}{abchc}\,\frac{E_a}{(E_I-E_a)E_I}\left[\frac{3\hbar^2e^2}{2m} + (2E_I-E_a)\,|\mu_{a,g}|^2\right], \tag{10}$$

where E_a, $\mu_{a,g}$ are the energy and transition moment of the absorption and E_I is the dissociation energy of the ground state molecule, h, $\hbar$, m, e, c have their usual meaning. Thus measurement of D, E_a, and knowledge of $\mu_{a,g}$ [12], allows calculation of E_I. As can be seen from Table II, the value obtained from plausible ellipsoid dimensions is ~ 3 eV, being rather insensitive to the dimensions c, and so solvation of the plane, but sensitive to a, the dimension in the direction of the transition vector (which is actually 11.5° out of the plane of the major ring system).

4. Discussion

It can be seen from figures 1, 2 that the linear relationship, equations (8)–(10), holds for the correlation between the frequency of the important singlet-singlet transition and the refractive index of the solvent in the majority of solvents. The hydrocarbons (with evidence of solute aggregation) and certain polar ligands are specific exceptions which are immediately apparent from the figures. Thus the linear relationship can be used with any new solvent system as a test for specific intermolecular interactions.

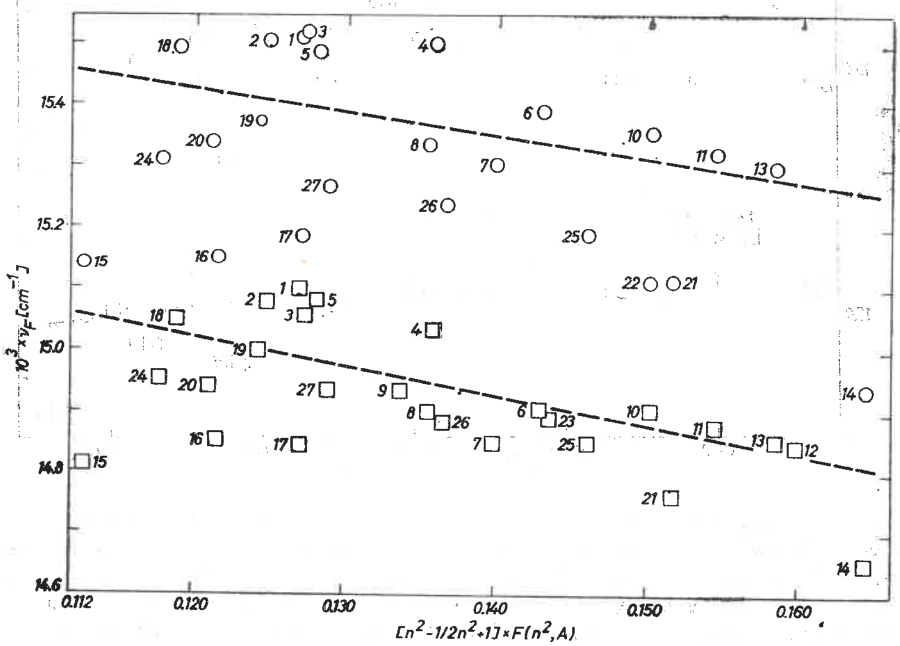

Fig. 1. Correlation of red band (Q_Y) absorption maximum of chlorophyll a–□ and chlorophyll b–○ with solvent function $[(n^2-1)/(2n^2+1)] \times F(n^2, A)$, 1-n-pentane, 2-n-hexane, 3-n-heptane, 4-cyclohexane, 5-iso-octane, 6-carbon tetrachloride, 7-chloroform, 8-dichloromethane, 9-1,1-dichloroethane, 10-benzene, 11-chlorobenzene, 12-bromobenzene, 13-1,2-dichlorobenzene, 14-aniline, 15-methanol, 16-ethanol, 17-n-propanol, 18-ethyl ether, 19-ethyl acetate, 20-acetone, 21-pyridine, 22-4-ethyl pyridine, 23-1-ethyl-2-pyrrolidone, 24-acetonitrile, 25-dimethylsulfoxide, 26-dimethylformamide, 27-nitromethane. Dotted line obtained by least square fitting

The use of the relationship for the evaluation of fundamental molecular properties involves a number of approximations amongst which must be included the modelling of the solvent as a polarizable continuum, and the representation of the transition vector as lying in an ellipsoid of somewhat arbitrarily selected dimensions. In addition input parameters, such as E_a and $\mu_{a,g}$ have been selected for a non-polar solvent (diethyl ether) rather than vacuum which should really apply to a meaningful ionization potential. Nevertheless, the particular ionization potential, E_I, here evaluated, does combine with

E_a and $\mu_{a,g}$ to increase our knowledge of the electronic transition behaviour of chlorophyll in such a way as to allow further prediction and explanation of photochemical behaviour "in vitro".

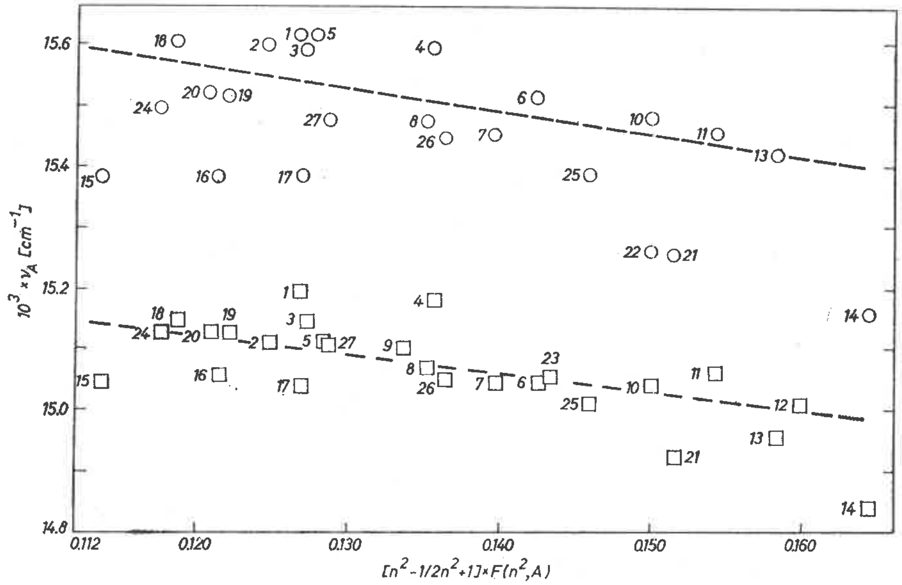

Fig. 2. Correlation of fluorescence maximum of chlorophyll a–□ and chlorophyll b–○ with solvent function $[(n^2-1)/(2n^2+1)]\times F(n^2, A)$, 1-n-pentane, 2-n-hexane, 3-n-heptane, 4-cyclohexane, 5-iso-octane, 6-carbon tetrachloride. 7-chloroform, 8-dichloromethane, 9-1,1-dichloroethane, 10-benzene, 11-chlorobenzene, 12-bromobenzene, 13-1,2-dichlorobenzene, 14-aniline, 15-methanol, 16-ethanol, 17-n-propanol, 18-ethyl ether, 19-ethyl acetate, 20-acetone, 21-pyridine, 22-4-ethyl pyridine, 23-1-ethyl-2-pyrrolidone, 24-acetonitrile, 25-dimethylsulfoxide, 26-dimethylformamide, 27-nitromethane. Dotted line obtained by least square fitting

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