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Concentration Quenching in Chlorophyll-*a* and Relation to Functional Charge Transfer *In Vivo*

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Abstract

Chlorophyll-*a* in ordinary solvents exhibits concentration quenching. Dimeric chlorophyll is reasonably well confirmed as the quenching species, by a critical reanalysis of available data on concentration dependence and on spectral features, in ordinary solvents, and in several analogous quenching environments. This quenching in the dimer in vitro is somewhat less firmly analyzed as due to a new fast internal conversion. Much peripheral evidence supports transient charge transfer as the cause of internal conversion. The same evidence points to a strong similarity to functional charge transfer in vivo. I suggest that inability to extract P680 may be due to its conversion to a form resembling P700 by addition of water.

A number of straightforward experiments are suggested to test these proposals. In particular, it is desirable to test for the existence of a vibronic perturbation (from a higher $n\pi^*$ state) in the dimer, as an alternative to charge transfer for explaining the "observed" internal conversion. Such a vibronic cause would raise interesting problems for phototrap function in vivo.

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Introduction

Fluorescent species in solution often aggregate at high concentrations, effecting a self-quenching [1] of their fluorescent yield (ϕ_{fl}).* The aggregates compared to monomers have lower radiative rates or higher nonradiative rates of decay [1, 2]. Förster transfer of excitation from monomers to quenching aggregates enhances the fractional quenching well above the fractional concentration of aggregates. The kinetics of transfer can be complicated by several effects. Nevertheless, the qualitative increase of quenching with increase of formal concentration can indicate the *nature* of quenching species —dimer, trimer, *n*-mer. Also aiding identification is spectral evidence (excitonic splits in weak quencher fluorescence and perhaps absorption). The *mode* of quenching—decreased radiative strength or increased nonradiative decay—is identifiable from triplet yields and net lifetimes. It then remains to discover the *internal* mechanism—cancellation of radiative dipoles, lower energy gap S_1–T_1, shift in vibronic structure of S_1, etc. This requires vibronic detail in spectra, chemical insight, and more.

For chlorophyll-*a* (Chl-*a*) in ordinary, basic solvents (having Lewis basic groups such as O or N) [3], I have more critically identified the normal, "π-stacked" dimer as quenching species, by reanalyzing existing experimental data. Gross concentration dependence of fluorescent yield is supportive semiquantitatively. Dimers of partly analogous natures in nonbasic (hydrocarbon, halocarbon) solvents [4–6] and at photosystem traps in vivo [7] are likewise quenchers. (In contrast, Chl-*a*'s of the antenna in vivo are very likely prevented from close approaches and show no quenching among themselves.) Spectral data are consistent with dimer quenching, if not uniquely so.

The mode of quenching is established a bit less firmly as increased decay by internal conversion, for both dimeric forms in vitro. The fluorescent radiative rate itself is unmeasured, but the weak fluorescent spectrum (for dimers in nonbasic solvents, at least) is normal. There are no obvious strong excitonic interactions from antiparallel radiative dipoles that would reduce the radiative rate. Reliable triplet yields in concentration-quenched, basic solutions or of dimers in nonbasic solvents have yet to be measured, though

*Abbreviations used: ϕ_{fl}: yield of fluorescence; S_n, T_n: *n*th excited singlet, triplet states; Chl-*a*: chlorophyll-*a*; A_0: rate of fluorescence; k_{ix}, k_{ic}: rates of intersystem crossing, internal conversion; M, Q, D: monomer, quencher, dimer; ϕ_{rel}: relative yield of fluorescence; $[i]$: concentration of species i; f: formal concentration; K_d: dimerization constant; μ: transition-dipole vector; CQ: concentration quenching; BChl: bacteriochlorophyll; P680, P700, P870: trap species in photosystem II, photosystem I, or in bacteria; PS: photosystem; C-T, charge transfer; Phe-*a*: pheophytin-*a*.

qualitative results [8, 9] for the latter rule out increased decay via triplets. Firmer identification of mode will require several straightforward experiments which I hereby suggest.

The internal mechanism I propose to be transient, dissipative, charge transfer between partners in the dimer. Electrochemical potentials [9, 10] and distance for electron tunneling in dimers are appropriate. The special dimer at traps in bacterial photosystems shows fast charge transfer [11–13], including a slower dissipative return path. (Ratios of radiationless to phosphorescent decay from triplets of dimers—in nonbasic solvents [14]—versus monomers—in basic solvents [6]—show an anomaly.) However, another complete explanation is still feasible. Lim and co-workers [15–17] studied perturbation of excited singlet vibronic structure by a nearby, higher $n\pi^*$ state, leading to increased internal conversion. I suggest a few more direct experiments to decide upon mechanism.

The significance of facile charge transfer within the dimer in vitro for understanding functional, stable charge separation in vivo is apparent. Some details are noted in the section on probable charge transfer for internal conversion.

Dimers as Quenchers

Excitations on isolated molecules are subject to decay by fluorescence at rate A_0, by intersystem crossing to triplet at rate k_{ix}, and by internal conversion to ground state at rate k_{ic}. From this simultaneous first-order competition, the fluorescent yield is simply

$$\phi_{fl} = \frac{A_0}{A_0 + k_{ix} + k_{ic}} = A_0 \tau$$

where τ is the net lifetime of excited state. For dimers to be effective quenchers compared to monomers, they must (1) accept excitation from monomers readily and irreversibly (or if reversibly, they must hold it for times comparable to their net lifetime τ_Q); (2) fluoresce poorly, due to lower A_0 than monomer, higher $k_{ix} + k_{ic}$, or both, or fluoresce in a spectral region far from the monomer's. Here I shall discuss part (1), leaving part (2) for the following section.

Excitations on monomers M migrate among all the monomers with occasional transfers to the smaller concentration of quenchers Q. Migrations and transfers are both predominantly by Förster transfer [18]. In contrast to transfer by direct collisions it sufficiently outpaces normal monomer

decay to achieve quenching. The essential independence of concentration quenching (CQ) from solvent viscosity [19] bears out the dominance of the Förster mechanism.

Potentially quenching transfers are terminal if the quencher is a deep trap of several kT depth or else fast decaying. Otherwise, detrapping and further migration are possible. In the first, irreversible case, transfer rate k_t relative to monomer decay rate $k^0{}_m$ determines the degree of quenching for excitations initially on monomers:

$$\phi_{\mathrm{rel}}=\frac{\phi_{\mathrm{fl}}}{\phi^0_{\mathrm{fl}}}=\frac{k^0_m}{k^0_m+k_t} \qquad \text{for zero-fluorescent Q.}$$

When transfer M*→Q is describable by mass action kinetics as k_t [M*][Q], we obtain the Stern–Volmer form

$$\phi_{\mathrm{rel}}=\frac{k^0_m[\mathrm{M}^*]}{k^0_m[\mathrm{M}^*]+k_t[\mathrm{M}^*][\mathrm{Q}]}=\frac{1}{1+k_t[\mathrm{Q}]\tau_{\mathrm{M}}}$$

When the quenchers form a significant fraction of initial absorbers, one corrects ϕ_{rel} by multiplying the preceding equation by a ratio of absorbing cross sections $\Sigma_{\mathrm{M}}/(\Sigma_{\mathrm{M}}+\Sigma_{\mathrm{Q}})$, where $\Sigma_i=[i]\sigma_i$ in terms of molecular cross sections σ_i..

When detrapping can occur the kinetics are more complex but still analytic. They may predict a CQ trace ϕ_{rel} versus formal concentration (monomer plus twice dimer) which is close to simple Stern–Volmer form or quite different. Furthermore, when one accounts for the wide distribution of transfer times for the process M*→Q (due to the distribution in distances), the simple Stern–Volmer form is seen to be quite inappropriate. The latter assumes a single average transfer time. Förster [20, see Eq. 14] derived a form for the time-dependent concentration of excited donor species (monomer) which is not readily expressed in terms of the single average concentration of quenchers. His result includes an unusual $\exp(\alpha\sqrt{t})$ time dependence due to the convolution over the distribution of first-order relaxation times.

Quenching in Chl-a

Empirically, Watson and Livingston [3] found that for Chl-*a* in ether,

$$\phi_{\mathrm{rel}}\simeq\frac{1}{1+\gamma f^2}$$

where f is formal concentration, equal to [M]+2[dimer D]. Ballschmiter et al. [21] and Broyde and co-workers [22] have studied aggregation in the ground state by chemical means (infrared spectra, osmometry). They have

established that monomer and a little dimer predominate out to fairly high values of $f \simeq 0.02F$ (formal). At low f, we have by the dimerization equilibrium

$$[\mathrm{D}] = K_d[\mathrm{M}]^2 \simeq K_d f^2$$

This fits the Stern–Volmer form if the dimer is quencher, and one may identify $\gamma = k_t \tau_M K_d$. Quantitative interpretation of γ is risky because of the error introduced by the average transfer time approximation in Stern–Volmer kinetics, as just discussed. The more accurate kinetics of Förster [20] can be interpreted with more confidence when his results are modified as by Seely [23] and myself herein for donor-to-donor migration of excitation that facilitates quenching. This interpretation is now developed quantitatively.

Förster assumed that a small concentration of excited donors [d] transfers its excitation irreversibly to a larger concentration of acceptors [a]. Furthermore, for a given d–a pair at distance R the rate of transfer is $(1/\tau_0)$-$(R_0/R)^6$, the form familiar from all discussions of Förster migration. He obtained for the relative yield of fluorescence

$$\phi_{\mathrm{rel}} = 1 - qe^{q^2}\sqrt{\pi}\,\mathrm{erfc}(q)$$

where erfc is the complementary error function. Here, q is the ratio of acceptor concentration to a critical value, $[a]/c_0$, and $c_0 = \text{constant}/R_0^3$. When excitations can transfer among donors, thus achieving a greater mobility toward acceptors, net quenching is increased. Seely [23] argued primarily from his own empirical data that a simple rescaling of c_0, hence of q, is merited, $c_0 \to c_0([\mathrm{a}]/[\mathrm{d}])^{1/2}$ and $q \to ([\mathrm{a}][\mathrm{d}])^{1/2}/c_0$. This form is in error when $[\mathrm{a}] \gg [\mathrm{d}]$, however. I argue as follows for a new form. Net transfer from a given donor is increased in the ratio $([\mathrm{d}]+[\mathrm{a}])^2/[\mathrm{a}]^2$, in the presence of a net concentration $[\mathrm{d}]+[\mathrm{a}]$ *all* acting as acceptors as far as the initial donor is concerned (if donor–donor transfer is irreversible). The concentration ratio appears as the square because elementary transfer rates scale as R^{-6}, and net transfer rates summed over R values scale as the square of bulk concentration. Only a fraction $[\mathrm{a}]/([\mathrm{a}]+[\mathrm{d}])$ of such transfers achieve quenching. Hence the elementary rate of transfer from initial donor scales as $([\mathrm{d}]+[\mathrm{a}])/[\mathrm{a}]$, and q straightforwardly as the square root of this:

$$q = \left(\frac{[\mathrm{d}]+[\mathrm{a}]}{[\mathrm{a}]}\right)^{1/2} \frac{[\mathrm{a}]}{c_0} = \frac{\{([\mathrm{d}]+[\mathrm{a}])[\mathrm{a}]\}^{1/2}}{c_0}$$

This form reproduces both Förster's and Seely's limits. It is valid under the same (quite good) assumptions as used by Förster, plus the assumption that excitation migrates irreversibly among the donors toward ultimate acceptors, i.e., that returns of excitation to earlier donors are a negligible fraction of all migrations. This is true when quenching is strongly draining the neighboring

acceptors. When quenching is just beginning to appear as a function of increasing concentrations (low ratio [a]/[d]), it will be overestimated.

The modified Förster kinetic scheme can be used to fit ϕ_{rel} as a function of formal concentration f, if the concentrations [d] and [a] are known as functions of f. I identify quenching acceptors as dimers and donors as monomers, such that

$$[d]=[M]=\frac{-1+(1+8K_d f)^{1/2}}{4K_d}$$

$$[a]=[D]=\tfrac{1}{2}(f-[M])$$

$$q=\frac{\{([M]+[D])[D]\}^{1/2}}{c_0}$$

The yield of fluorescence is the modified Förster form for yield from monomers, $\phi_{rel}(q)$, multiplied by the fraction of all absorption done by monomers, $[M]/f$ (the fraction directly absorbed by dimers is all quenched). Therefore, only two parameters c_0 and K_d can be used to fit $\phi_{rel}(f)$. Table I gives three fits to the empirical data of Ref. 3. Both K_d and c_0 are not well determined simultaneously, because ϕ_{rel} is primarily a function only of the ratio K_d/c_0^2, at intermediate concentrations f. K_d itself is expected to lie in the range 5–15 M^{-1} in the acetone solvent used in Ref. 3, based on values of K_d

Table I. Concentration quenching of relative fluorescent yield as a function of formal concentration f[a]

	ϕ_{rel} (and dimeric fraction)			
f	Ref. 3	$K_d=4.5\ M^{-1}$ $c_0=0.01$ M	$K_d=9\ M^{-1}$ $c_0=0.014$ M	$K_d=0.44\ M^{-1}$ $c_0=0.003$ M
0.0051	0.93	0.88 (0.042)	0.85 (0.078)	0.90 (0.004)
0.0107	0.68	0.69 (0.081)	0.66 (0.142)	0.71 (0.010)
0.0117	0.62	0.66 (0.088)	0.63 (0.151)	0.68 (0.010)
0.0174	0.47	0.48 (0.121)	0.47 (0.200)	0.48 (0.015)
0.0197	0.39	0.43 (0.133)	0.42 (0.217)	0.41 (0.017)
0.0235	0.31	0.34 (0.152)	0.34 (0.243)	0.32 (0.020)
0.0427	0.18	0.13 (0.229)	0.14 (0.337)	0.10 (0.035)
0.0660	0.07	0.05 (0.295)	0.06 (0.411)	0.03 (0.052)

[a]First column: experimental data of Ref. 3, which includes the correction for self-absorption. Remaining columns: author's theoretical simulation by modified Förster kinetics (see text). Parentheses contain calculated values of fraction dimerized.

determined in a range of basic solvents [21, 22]. The last fit sets K_d to the statistical value, 0.44 M^{-1}, used by Beddard and Porter [24] implicitly in an alternate model discussed shortly. This low value applies to hypothetically noninteracting chlorophylls, with pairs separated by less than 10 Å arbitrarily considered to form the dimer. In contrast to "normal" values of K_d, this low value gives results that are good at low and medium f, but not high f. In truth, one expects the model to be poor only at low f, where revisitation of sites during migration of the excitation can occur but is neglected. Accordingly, the "normal" K_d values may be regarded as giving the better fit, which is least accurate only at low f. The value 9 M^{-1} is slightly preferred. Either value, however, implies a high value for c_0, which should agree well with values of c_0 for concentration depolarization of fluorescence. The latter lie in the range of 0.001 M. My values imply a Förster transfer radius near 34 Å, rather than the 60 Å and larger as commonly accepted [18]. The discrepancy remains to be explained.

Beddard and Porter [24] performed a fully numerical simulation of all the Förster transfer processes to avoid shortcomings of mass action models. They assumed that quenching is terminal, that there is no detrapping. (At the half-quenching concentration I estimate that detrapping is only 5% of the dimer decay rate.) They also assumed that Chl-*a* molecules were distributed at random instead of partly clustered by chemical dimerization. They fit Watson and Livingston's data by taking statistical pairs closer than 10 Å to be quenchers. Unfortunately, the effective statistical dimerization constant thus implied is only 0.44 M^{-1} versus $K_d \simeq 10\ M^{-1}$ for chemical forces. Chemical dimers therefore radially perturb the distribution of pairs at separations they consider important. Their agreement with experiment is somewhat fortuitous, but their technique could be corrected for chemical dimerization to provide the most stringent test of the hypothesis that dimers are the quenching species.

Partial Analogies in Other Dimerizing Systems

In very pure nonbasic solvents, Chl-*a* is dimerized down to very low concentration [21, 25–27] and oligomerized at modest concentrations in nonpolarizable nonbasic solvents such as aliphatic hydrocarbons. It is also poorly fluorescent [4, 5], $\phi_{fl} \simeq 0.01$ compared to 0.33 for monomer in basic solvents. This dimer is broadly of the same structure as dimer in basic solvents. The macrocyclic tetrapyrrole structures of the two units in basic dimer are surely overlapped. Macrocycles in nonbasic dimers are also partially overlapped, according to infrared and NMR environmental probes [7, 25, 27]. Angles of skewness between transition dipoles are moderately near perpen-

dicularity (far from parallelism certainly) in both cases [22]. This angle is about 78° in basic solvents [28].

Of course, bonding in the two classes of dimers is quite different. Nonbasic dimers have specific coordination of a magnesium on one monomer with a carbonyl on the other [7, 25]. Basic dimers have only the weak interaction of π-electron-cloud polarization, because both magnesiums are near coordinative saturation with the solvent ligands attached [7]. Free energies of formation determining K_d values bear this out, being much more negative for nonbasic dimers (but enthalpies in both cases are near zero; binding is largely an entropic effect). The two Chl-*a*'s in nonbasic dimers may be slightly nearer each other than in basic dimers: the nonbasic dimers have 45% as large an excitonic split in the red band as that shown by basic dimers (360 versus 800 cm^{-1}), whereas the projection of transition dipoles $\mu_1 \cdot \mu_2$ is only one-third as large. Of course, $\mu_1 \cdot \mu_2$ is not the complete interaction if the dipoles are (1) not perpendicular to the intermolecular axis or (2) of finite extent [29]. Without knowning more geometry of the dimers little more can be said.

Nonfluorescence of the nonbasic dimers may or may not be due to the same internal causes as in basic dimers. I will argue in the following section that the latter at least have increased internal conversion, although I lack direct experimental data verifying decreased fluorescent lifetime. The same lack occurs for nonbasic dimers, unfortunately.

Concentration quenching also seems to occur among Chl-*a* (and related) molecules localized on polymer chains in solution [30, 31]. Quantitative analysis appears to be too difficult, certainly, to do convincingly and simply enough regarding the hypothesis of dimers as quenchers. For example, on random coil polymers, there is necessarily a Gaussian profile of gross Chl-*a* concentration as a function of distance from the center of the coil. Furthermore, the value for R_0 which Seely derived from various types of studies is 42 Å, in apparent disagreement with the majority of determinations [18] by other means (lecithin matrix, monolayers on various liquids). This casts some doubt on the analyzability of these experiments in general.

A second useful analogy to CQ by dimers in basic solvents is the behavior of Chl-*a* or bacteriochlorophyll (BChl) in vivo, which also goes beyond analogy to new insights on species and kinetics. On the whole, chlorophylls in photosynthetic membranes are present at 5–6% by weight, or about 0.05 F bulk concentration. One might expect strong CQ. Yet only *functional* quenching by photosystem traps seem evident, as quantum yields for such are near unity. Fluorescent strength of the bulk "antenna" chlorophylls is apparently normal. For photosystem II of green plants, ϕ_{Fl} is near 6%, of which perhaps one-third is discounted [32] as "dead" fluorescence by molecules uncoupled

to trapping.† The remanent 4% yield and 600-psec lifetime [33, 34] imply a natural radiative lifetime of 15 nsec, which is normal. PS I is similar, with lower ϕ_{fl} and lifetime. Prevention of dimerization which would form adventitious traps in the antenna is quite surely a primary role for the antenna Chl–protein complexes. I will comment on this later.

At the trap of photosystem I in green plants, the P700 protein serves to enforce a special dimerization, perhaps involving an extra nucleophile such as water [7, 35, 36]. This dimer performs the functional quenching by separating charges—perhaps first internally as I will propose by analogy to CQ in solution, then quickly to primary electron accepting species. Evidence for the special dimer includes excitonic couplings in absorption and circular dichroism [35] and spin delocalization over two molecules in ESR [37] and ENDOR, all correlated strongly with oxidation of P700 [35] (or P870 for BChl). Similar evidence obtains for bacterial photosystems. The trap P700 or P870 traps excitation virtually irreversibly (back-reaction yield [38] about 10^{-4}) by stabilized charge separation which (1) is achieved in several picoseconds, and (2) leaves negligible transition dipole for fluorescence and is evidently a new electronic state.

Mode of Quenching—A New Internal Conversion?

Förster [39] and McRae and Kasha [2] have explained how excitonic interactions upon aggregation can cause quenching. Dimers at separation R with antiparallel (or nearly so) transition dipoles μ_i for the transition $S_1 \rightarrow S_0$ have two excitonic states to first order,

$$\Psi_{\pm} = \frac{1}{\sqrt{2}} (\psi_1^* \psi_2 \pm \psi_1 \psi_2^*)$$

at energies $\pm(\mu_1 \cdot \mu_2 - 3\mu_{z1}\mu_{z2})/(4\pi\varepsilon_0 R^3)$ relative to isolated $S_0 + S_1$ molecules. Ψ_- is higher in energy and carries about double the oscillator strength of one monomer in absorption, proportional to $|\mu_1 - \mu_2|^2/2$. However, excitations quickly relax radiationlessly to Ψ_+ which has negligible oscillator strength proportional to $|\mu_1 + \mu_2|^2/2$. Fluorescence is readily quenched if

†Under intense laser excitation of *Chlorella pyrenoidosa* [33, 34], the excitations in the antenna form a dense population and thus annihilate each other, quenching the antenna fluorescence. About one-third of net fluorescence remains even at highest intensities, and its strength is quite independent of sample optical properties. Therefore it is not due to "lasing" of the excited antenna. It must (?) be from Chl molecules that do not communicate with each other or with the traps.

k_{ix}, k_{ic} remain close to monomeric values. Additionally, k_{ix} may even be enhanced [2], as the gap in energy is decreased [40] between the excited state (now Ψ_+) and nearest triplet (little affected by weak excitonic interactions among triplets).

However, chlorophyll-*a* in basic dimers and in analogous dimers shows no clear evidence of either excitonic effect on rates of decay:

1. While excitonic splits in absorption wavelengths are evident in basic dimers [22] and all analogs [5, 28, 35], the oscillator strengths z of both $\Psi_\pm$ are always broadly comparable. In the absence of rearrangement in the excited state (see comment 3), fluorescent rates would be normal within a factor of about 2. Nearly normal z for the lower, relaxed state Ψ_+ is necessary to get good acceptance rates from excitations of monomers also. Infrared studies on bonding [25] entities in nonbasic dimer verify a skew structure with noncancellation of dipoles.

2. The excitonic split in energy of the state Ψ_+ compared to S_0+S_1 is modest, only several hundred reciprocal centimeters (in absorption). This could accelerate k_{ix} notably *only* if it brought about a near degeneracy of Ψ with a higher triplet of Chl-*a* (the lowest T_1 is about 5000 cm^{-1} below Ψ) possessing an anomalously strong coupling to Ψ. Ordinarily, once a singlet and triplet are close in energy, a closer approach will not strongly affect rates [41].

Direct evidence regarding intersystem crossing in the dimer is lacking, but indirect evidence indicates no enhancement. Pugh [8] and Livingston and Fujimori [9] showed for the dimer in *nonbasic* solvents that triplet yield is actually reduced, in a qualitative experiment. Studies by Bowers and Porter [42] and by Usacheva et al. [43] which were intended to be quantitative were vitiated by traces of bases (water?) in their Chl-*a* solutions, as evidenced by the low solubilities they found for Chl-*a* in their alkane solvents. There are no reliable studies whatever on dimers in basic solvents. In vivo, antenna triplets have not been observed in any significant amount [44], most likely because (a) disposition of excitations to the trap is too fast, and (b) any triplets formed are quenched by carotenoids in times far shorter than observation.

3. After excitation, the dimer could conceivably rearrange ("excimerically") to a configuration in which dipoles are quite fully canceled. The dimer in basic solvents would stand to decrease its energy favorably and would not have to break coordinative bonds to rearrange. The dimer in nonbasic solvents would have to break three such bonds [25, 45] but show no rearrangement; its weak fluorescence occurs at only slightly shifted wavelengths (673 versus 665 nm, at the peak). Full verification that no *fraction* of the dimers rearranges to a new configuration (with fluorescence displaced disconnect-

edly far in wavelength) would require comparing ϕ_{fl} ($\simeq 0.01$) for *observed* fluorescence to lifetime τ (unknown). For the basic dimer, neither ϕ_{fl}, τ_{fl}, nor λ_{fl} is known. Because the nonbasic dimer with its strong bonding may closely resemble the functional dimer in vivo, measurement of its lifetime is desirable. Fluorescence of the basic dimer will be quite difficult to measure: fluorescence from remanent monomer should easily mask it, and self-absorption will be quite strong at the necessarily high concentrations.

Fluorescence of P700, an analog to dimers in vitro, is unmeasured, being undoubtedly weak.

Much reduced fluorescent yield ϕ_{fl} and moderate yield of triplet ϕ_T implies a quenching by a new or enhanced route of internal conversion—either $S_1 \rightarrow S_0$ directly or through a intermediate state other than a triplet. In the basic monomer, k_{ic} is nearly negligible: $\phi_{ic} = 1 - \phi_{fl} - \phi_T = 1 - 0.32 - 0.64 \simeq 0.04$ [42, 43, 46]. Yet one may justly be skeptical of much strengthened internal conversion in any dimer if no additional evidence is given, because photophysical environments in monomer and dimer seem quite similar. Witness the smallness of excitonic energy splits in the dimer; substantial preservation of Franck–Condon factors in the red band; and preservation [5] of the Herzberg–Teller satellite [46] (red band II) to the $S_1 \rightarrow S_0$ transition.

Probable Charge Transfer for Internal Conversion

I have argued that rapid internal conversion is a most likely mode for quenching of dimer fluorescence in solvents and in vivo. I am now obliged to propose and support a mechanism.

Transient electron transfer can occur dissipatively; radiationless relaxation from the excited singlet $S_{\pi\pi *}$ to the charge transfer (C-T) state is followed by another radiationless relaxation to the ground state. Such a mechanism has been implicated for cases in which electron donor and acceptor are not the same species [47–49]. The particular case of bacteriopheophytin/*p*-benzoquinone [50, 51] relates broadly to Chl-*a* pairs, and has been well characterized kinetically.

This quenching mechanism requires that a C-T state be accessible below the usually lowest $\pi \rightarrow \pi^*$ singlet, but only in dimeric Chl-*a*. In monomers, the C-T state (now for widely separated ions) must either lie above $S_{\pi\pi *}$ in energy or be kinetically inaccessible due to the rapid drop in electron transfer rate over large distances. Either case is possible. That a low lying C-T state can exist at all in the dimer can be inferred, though it has not yet been sought spectroscopically. Chl-*a* can function as both electron donor and acceptor, at least in separate reactions [10]. As such it is a prime example of amphipathy

in redox behavior [7, 52]. In nonbasic (aprotic) solvents, Saji and Bard [53, and references cited therein] found that the *reduction* potential for ground state Chl-*a* going to Chl-a^- anion radical is -0.90 V (converted to voltage against normal hydrogen electrode). The *oxidation* potential for excited Chl-a^* going to Chl-a^+ is positive and larger, 1.05 V, if one uses the potential -0.83 V for ground state oxidation augmented by the 1.88 V of electronic excitation (procedure of Seely [10]). In basic solvents, the data are a bit older and less clear, but for reduction of Chl-*a* in ethanol, Seely estimates -1.01 V. This may be corrected to -0.83 V for irreversibility, as done by Kiselev et al. [54, 55]. For oxidation in methanol, Seely estimates $+1.21$ V. Solvents of the same general type should affect potentials only slightly, so that the difference just discussed is ignored. In any event, one expects, by adding potentials, that the reaction Chl-a^* + Chl-$a \rightarrow$ Chl-a^+ + Chl-a^- is spontaneous in both basic and nonbasic solvents. In dimers, moreover, the close approach of the ion pair would further stabilize the pair electrostatically and favor the reaction. Close approach is also necessary for the kinetic rate to compete with intersystem crossing. Hopfield [56] with Potasek [57] has shown that the range of electron transfer is quite short for the best substantiated mechanism of vibronically assisted electron tunneling. For the example of transfer between cytochrome *c* and $Fe(CN)_6^{4-}$ there is a sharp decrease beyond 10 Å. In either dimer of Chl-*a*, basic or nonbasic, the tunneling distance is likely less than 7–8 Å.

Charge transfer in vivo at dimeric traps P700 or P870 is essentially unidirectional instead of cyclic, dissipative. The stabilization of the initial transfer can be enforced by the nearby chain of redox enzymes, draining away charges faster than they can recombine. In the better studied bacterial case [11–13, 58], C-T from $BChl_2$ to BPhe is clearly established. The intermediacy of C-T inside $BChl_2$ is uncertain; there is some admixture [59, 60] of $BChl^-$ to the spectrum of $BPhe^-$ to be seen in the "first" state P^F formed within 10 psec.

Direct spectral evidence for charge transfer in vitro does not yet exist, partly for lack of real motivation. Even were it to be sought, the absorption from ground state to C-T state would undoubtedly be very weak (compare the cytochrome case [57]), perhaps 10^{-4} the strength of the $\pi \rightarrow \pi^*$ transition and buried under the wings of same. Characteristic ionic absorption spectra from C-T to higher states might be sought as they are in vivo with rapid sequential pulses to excite and then measure.

A probable scheme of electronic levels in dimers is given in Fig. 1. The radiationless sequence $S_{\pi\pi*} \rightarrow S_{C-T}$ (singlet charge transfer state) $\rightarrow S_0$ is rapid. The first stage is very rapid, on the order of nanoseconds, in order to compete well with intersystem crossing. The second relaxation can be slower,

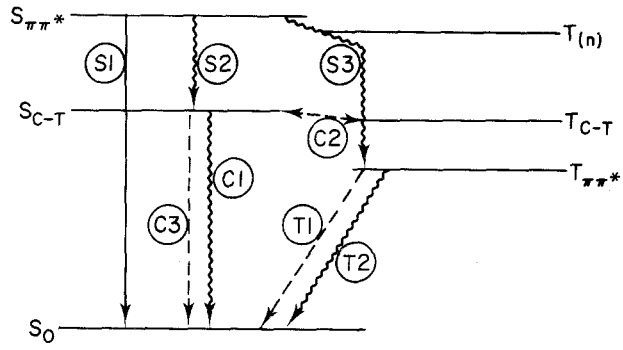

Figure 1. Proposed electronic level scheme for dimeric chlorophyll-*a*. Process *S*1 is ordinary fluorescence; *S*2 is the proposed fast conversion to singlet charge transfer state; and *S*3 is ordinary intersystem crossing by way of various higher triplet states. Process *C*1 is a fast internal conversion, faster than the competing spin scrambling *C*2 and the weak radiative path *C*3 (responsible, on reversal, for a weak absorption band). Phosphorescence *T*1 competes poorly with radiationless decay *T*2. Approximate partial lifetimes are, in the order just given 10^{-8}, 10^{-9}, 10^{-9}; $\ll 10^{-6}$, $< 10^{-6}$, 10^{-4}; 10^{+1}, 10^{-3} sec.

providing that it is more rapid than spin scrambling between the nearly degenerate singlet and triplet C-T states. Otherwise, S_{C-T} and T_{C-T} would "equilibrate" to yield more than three-fourths triplets. A reasonable lifetime for spin scrambling, hence the process $S_{C-T} \rightarrow S_0$ also, is less than 1 μsec; compare the BPhe-benzoquinone case [51], or bacterial reaction centers where it may be only picoseconds for the marginally dissimilar pair $BChl_2^+$-$BPhe^-$ [61]. Because T_{C-T} in this scheme decays to S_0 directly and via S_{C-T} as a real intermediate, its decay rate is at least as high as that of spin scrambling, giving it a lifetime of less than 1 μsec. Therefore, it cannot be the lowest triplet, which lives [46] about 1.5 msec in the nonbasic solvent dimer. An ordinary $\pi \rightarrow \pi^*$ triplet lies lowest.

The singlet $n \rightarrow \pi^*$ state drawn above $S_{\pi\pi^*}$ is invoked in an alternative hypothesis for quenching, discussed in the section on alternative vibronic quenching.

Phosphorescence from $T_{\pi\pi^*}$ should have a relatively low yield, radiationless relaxation to S_0 being favored instead. In monomers, where C-T states are irrelevant, this is true; the yield is only 5×10^{-5} [62]. In dimers in basic solvents, ϕ_{ph} is unmeasured, but should be similarly low, because the state $T_{\pi\pi^*}$ can now borrow extra radiationless and radiative strength from S_{C-T} in the same proportion as S_{C-T} shows itself, i.e., favoring radiationless decay. These extra borrowings are added, crudely, to radiative strength already borrowed from $S_{\pi\pi^*}$ and to radiationless strength from spin-vibronic coupling

directly to S_0. Dimers in nonbasic solvents should also have low values of ϕ_{ph}, because they are similar to basic monomers in triplet lifetime (monomers [62] live 1.7 msec) and in energy (wavelength is near 950 nm for both [62, 63]). However, nonbasic dimers have qualitatively higher yields, which is hard to explain with any level scheme.

Diagnostic Experiments to Be Done

I have previously noted the lack of data on accurate triplet yields for either type of dimer, and on fluorescence from nonbasic dimers. In order to test the charge transfer hypothesis explicitly, one might profitably look for C-T absorption in excited dimers resembling absorptions by anion plus cation, as happens [11–13, 58] in bacterial P870. To verify that the short range of C-T favors quenching in dimers but not in the presumably more widely separated Chl-*a* of antennae in vivo, one might determine the Chl-*a* separations in a/b antenna–protein complex, at least between magnesium atoms. In *Chlorobium* [64] at least, the BChl-bearing protein enforces a minimal separation of 12 Å. Computational verification that C-T proceeds by Hopfield's tunneling mechanism will require measurement of the weak $S_0 \rightarrow S_{C-T}$ absorption. Finally, pheophytin-*a* might be examined for similarity of quenching kinetics and for C-T involvement, since it shares the relevant photophysical properties with Chl-*a*: redox potentials that favor C-T, weak π–π dimerization, and occurrence of concentration quenching in all solvents.

Significance for Function in Vivo

If dimers in vitro facilely transfer charge among themselves and retain the state for microseconds, one need not search for large environmental effects in vivo favoring stable separation of charge. One might more profitably examine why the separation functions so well under a large change in environment and operating potential (0.4 V) between PS I and PS II. The inability to date to extract traps for PS II could be rationalized as destruction of a subtle environmental difference between P700 and P680. Perhaps P680 lacks a nucleophilic bridge (H_2O or an amino acid residue) between dimers (as postulated [7, 35–37, 65] but unproven for P700), which difference enforces a change in operating potential but is labile under extraction with aqueous solvents, P680 becoming derivatized to resemble P700.

Notes on an Alternative Vibronic Quenching

Lim and co-workers [15–17] have shown that $n\rightarrow\pi^*$ excited states lying above $\pi\rightarrow\pi^*$ states may interact configurationally to perturb the $\pi\rightarrow\pi^*$ vibronic structure strongly. Franck–Condon factors and Born–Oppenheimer couplings of $S_1(\pi\pi^*)$ to S_0 become sufficiently favorable to give fast internal conversion. Chl-*a*, by virtue of its four nitrogens, possesses $n\rightarrow\pi^*$ states which may function by internal conversion. (In the past, they were argued [6, 66, 67] to be so low in energy as to be the lowest singlets in nonbasic environments, hence quenching fluorescence by being poor radiators, not internal converters.) It is now questioned that they lie low enough in energy to interact vibronically. Molecular orbital calculations [45, 68, 69], very crude compared to state of the art for small molecules, place them too high relative to $\pi\pi^*$, but these calculations have additional defects† in treating $n\pi^*$ states. Spectral evidence will be hard to obtain, such as by two-photon absorption.

There are several diagnostics for Lim's mechanism. First is a vibrational band in fluorescence from nonbasic solutions corresponding to out-of-plane bendings that mix $n\pi^*$ and $\pi\pi^*$ states (by rehybridizing orbitals on nitrogen). This can be sought in the quasi-line spectra of Chl-*a* by the Sh'polskii technique. Unfortunately, these spectra have proven difficult to obtain; only one solvent has been used [70], of uncertain dryness. A second diagnostic is a position-dependent isotope effect (especially with deuterium) on fluorescent yield. The poorer Franck–Condon factors for deuterium–carbon bending modes would retard radiationless decay, and enhance fluorescence. This likewise has not been done for dry Chl-*a*. A third diagnostic is less specific to Lim's mechanism, namely, strong increase of radiationless decay with rising temperature [71]. "Hot bands" of the lower $\pi\pi^*$ state mix better than the ground vibrational state with the upper $n\pi^*$ state. The rate of decrease $d\phi_{fl}/dT$ for nonbasic dimer should be very much larger than for basic monomer lacking the Lim coupling. It is unmeasured to date.

Were this vibronic coupling to be the cause of quenching, the significance of CQ and nonbasic dimer quenching for function of Chl-*a* in vivo would be quite different than proposed earlier. Functional C-T would not be presaged or previewed in vitro, but avoidance of vibronic quenching in the special pair would demand explanation.

†Molecular orbital calculations are unreliable in locating $n\rightarrow\pi^*$ states specifically, and in general for ordering the higher excited states, even when done fully a priori in "large" basis sets (say, double zeta) that far exceed computability at present for Chl-*a*. In the complete Hartree–Fock limit, they omit correlations which are important for nearly degenerate states. In semiempirical calculations, on the other extreme of simplicity, the *n* orbitals are simply hard to parametrize.

Conclusions

I have examined the existing experimental data on quenching of fluorescence in vitro, concluding critically that dimerization (close association) is responsible by causing rapid internal conversion which is essentially absent in monomers. Internal conversion is strongly indicated, by much circumstantial and some direct evidence, to proceed by an intermediate charge transfer state. Quite similar conversion in vivo at the traps P700 and P680 may be verifiable. The lability of P680 to extraction may be explicable as an insertion of water in the special dimer, giving a P700 form. An alternative means of internal-conversion quenching in vitro by a vibronic perturbation of an $n\pi^*$ state is less likely, but signifies that a quite different "fine adjustment" is necessary in vivo to avoid wasteful quenching. Verification of internal conversion as model will require experimental determination of fluorescent lifetime, wavelength, and yield for quenching species. Verification of the charge transfer intermediate state will likely require an absorption spectrum of the excited dimer and/or spectroscopic observation of a very weak charge transfer band below 15,000 cm^{-1} in energy.

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