

Remarks on Concentration Quenching in Chlorophyll *

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A quantitative analysis of fluorescence self-quenching of chlorophylls a and b in ether as well as chlorophyll a in lipid vesicles and liposomes has been carried out. It is demonstrated that concentration changes of the fluorescence quantum yield can be correctly described by a Förster-type excitation energy transfer process between chlorophyll molecules in the monomeric form if part of the transfers leads to energy degradation.

1. Introduction

Investigations of the concentrational quenching of photoluminescence (PL) in chlorophyll solutions *in vitro* are important and necessary because they can provide information on the mechanism of non-radiative excitation energy transfer (NEET) and also on the mechanism of PL quenching in natural light-absorbing systems [1–5]. Recently Beddard and Porter [6] and also Dalton [7] have attempted to describe quantitatively the classical results of Watson and Livingston [1] for chlorophyll PL concentration quenching (PLCQ) in ether. In [6] it was assumed that PLCQ in this system is caused by a Förster-type energy transfer process between like molecules terminating in a trap being a statistical pair of molecules closer than a critical separation R_t . The curve of relative quantum yield versus concentration was calculated using a Monte Carlo method and a good fit for $R_t = 10 \text{ Å}$ was obtained. Dalton [7], following earlier ideas of Vavilov [8] assumed that each step of NEET between chlorophyll molecules has a non-zero probability $1 - \alpha_0$ of energy degradation in a trap (α_0 corresponds to $\bar{\gamma}$ in [7]). With this assumption he obtained a relation, a modification of the Förster formula [9] for the quantum yield, which well fits the experimental results for $\alpha_0 = 0.96$ and a critical distance $R_0^{\text{exp}} = 46 \text{ Å}$. Now, the value of the critical distance R_0^{th} as determined from spectroscopic investigations [10] equals 51.7 Å . The difference between 46 Å and 51.7 Å is important for judging the correctness of the theoretical description.

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We present in this report a quantitative analysis of the PL self-quenching data for chlorophyll a and chlorophyll b in ether and also in other media under the assumption that beside the “monomer” quenching ($\alpha_0 < 1$) also quenching by non-luminescent dimers may occur.

2. Quantitative Analysis of the PL-self-quenching in Chlorophyll

The changes of the quantum yield η/η_0 with concentration, caused by the long-range excitation energy transfer from monomers D to dimers D'' may be described by [11]

$$\frac{\eta}{\eta_0} = \frac{1 - f(\gamma)}{1 - \alpha_0 \alpha f(\gamma)}, \quad (1)$$

where

$$f(\gamma) = \pi^{1/2} \gamma \exp(\gamma^2) [1 - \text{erf}(\gamma)], \quad (2)$$

$$\gamma = \gamma' + \gamma'' = (\pi^{1/2}/2) (c'/c_0' + c''/c_0''), \quad (3)$$

$$\alpha = \gamma'/\gamma = [(1 + 4 \gamma K_\gamma)^{1/2} - 1]/2 \gamma K_\gamma. \quad (4)$$

Here c' and c'' , c_0' and c_0'' are concentrations and critical concentrations of monomers D and dimers D'', respectively. The parameter K_γ is related to the dimerization constant $K = c''/c'^2$ by the formula $K_\gamma = 2 K c'^2/(\pi^{1/2} c_0'')$. Note that (1) takes into account the random distribution of the molecules D and D'' in solution and the excitation energy migration among monomers. To compare (1) with experimental values of η/η_0 it is necessary to know the concentrations c' , c'' , c_0' and c_0'' as well as the parameter α_0 . According to the data presented by Watson and Livingston the drop of the PL quantum yield for chlorophyll a as well as chlorophyll b in ether takes place at relatively high concentrations ($c > 2 \times 10^{-3} \text{ mol l}^{-1}$). We assume therefore that $c'' \ll c'$ and $\gamma \approx \gamma' \approx (\pi^{1/2}/2) c/c_0'$.

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Using the critical distance $R_0^{\text{th}} = 70 \text{ \AA}$ obtained from absorption and fluorescence data [2, 12, 13] for chlorophyll a in ether, the critical concentration $c_0' = 1.16 \times 10^{-3} \text{ mol l}^{-1}$ was obtained. For chlorophyll b in ether we assume the value $R_0^{\text{th}} = 55.5 \text{ \AA}$ (as for chlorophyll b in lecithin matrix [3]). According to Losev and Zen'kevich [14] the ratio of the critical distances for b and a chlorophylls in castor oil is 7/9. Assuming this ratio for the chlorophylls in ether too, the above value for chlorophyll b is obtained. For $R_0^{\text{th}} = 55.5 \text{ \AA}$, $c_0' = 2.32 \times 10^{-3} \text{ mol l}^{-1}$ is obtained. Figure 1 shows a comparison of

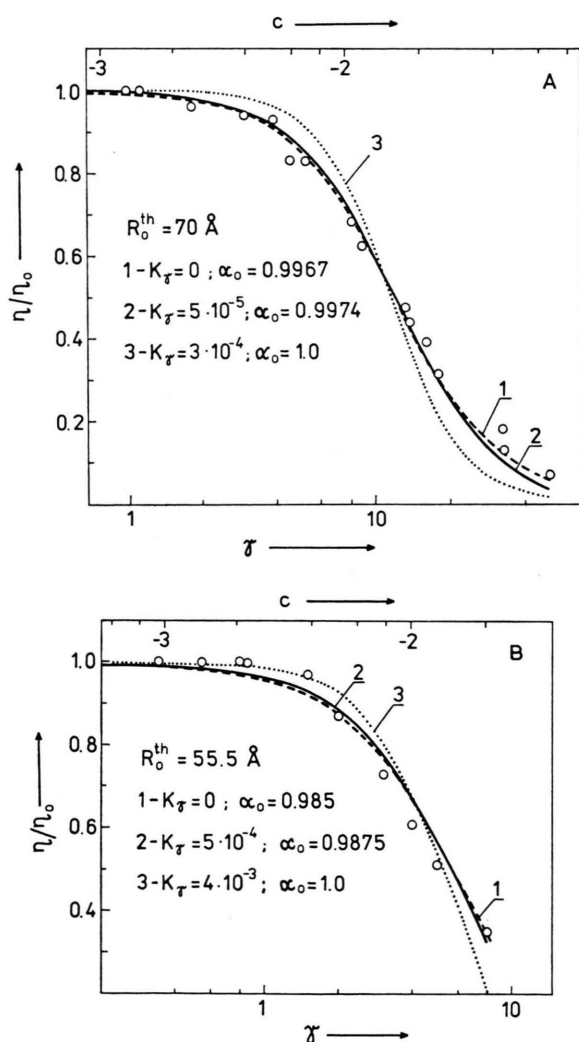


Fig. 1. PL quantum yield of chlorophyll a (Fig. A) and chlorophyll b (Fig. B) in ether vs reduced concentration γ ; \circ — experimental data from Ref. [1], curves 1, 2, 3 — calculated from (1); c in mol l^{-1} .

Watson's and Livingston's data with the relation (1) for the above values of the concentration c_0' . The best fit of (1) to the experimental results for chlorophyll a was obtained for $K_\gamma = 0$ and $\alpha_0 = 0.9967$, i. e. no quenching by dimers (curve 1 in Figure 1 A). Curve 2 corresponds to the case of combined "monomer" quenching and PL quenching due to energy transfer from monomers to dimers. Curve 3 was calculated under the assumption of the absence of "monomer" quenching ($\alpha_0 = 1$). Figure 1 B shows the comparison of the experimental data for chlorophyll b in ether with relation (1). The best fit was obtained for $K_\gamma = 0$ and $\alpha_0 = 0.985$. For chlorophyll a the best fit was obtained for $K_\gamma = 0$ and $\alpha_0 = 0.9953$ under the assumption of the more accurate value $R_0^{\text{th}} = 66 \text{ \AA}$ as given by Colbow [4]. Similarly good agreement with relation (1) was achieved for the results of Beddard, Carlin and Porter [5] for chlorophyll a self-quenching in lipid vesicles and liposomes taking $R_0^{\text{th}} = 65.1 \text{ \AA}$ from [4] (cf. data in the Table).

3. Discussion and Final Remarks

The effect of PLCQ in dye solutions is related mainly to the association of unexcited dye molecules and "monomer" quenching [11, 15, 16]. The phe-

Table. Values of parameters characterizing the PL self-quenching in chlorophylls.

system	R_0^{th} Å	R_0^{exp} Å	K_γ —	α_0 —	S —
Chlorophyll a in ether ^a	70	70	0.0	0.9967	0.0223
	70	70	5×10^{-5}	0.9974	0.0325
	70	70	3×10^{-4}	1.0	0.0758
	66	66	0.0	0.9953	0.0229
	51.7	46	0.0	0.9600	0.0187 ^c
	51.7	51.7	0.0	0.9600	0.1142
Chlorophyll b in ether ^b	55.5	55.5	0.0	0.9850	0.0359
	55.5	55.5	5×10^{-4}	0.9875	0.0376
	55.5	55.5	4×10^{-3}	1.0	0.0558
	—	69	0.0	0.995	0.0247 ^c
Chlorophyll a in lipid ^b					
-vesicles	65.1	65.1	0.0	0.9983	0.0449
	65.1	65.1	10^{-5}	0.9986	0.0553
-liposomes	65.1	65.1	0.0	0.9992	0.0408
	65.1	65.1	10^{-5}	0.9995	0.0414

^a Experimental data of Watson and Livingston [1].

^b Experimental data of Beddard, Carlin and Porter [5].

^c Calculated by Dalton [7]. For the same values of parameters we obtained for S the values 0.0246 and 0.0307 rather than 0.0187 and 0.0248 as quoted in the table.

S Standard estimates of error.

nomenon of PLCQ by ground state dimers has been well documented [15, 17–19]. This is not true in case of “monomer” quenching albeit this quenching mechanism has been assented by many authors [1, 3, 8, 20–22]. Recently Makshantsev et al. [23] proved theoretically that nonradiative electronic transitions in a solitary monomer molecule may result in PLCQ in a solution. Besides, it was proved experimentally that concentrational changes of the quantum yield, and also PL-decay times, may in some cases be described correctly only after taking into consideration both mechanisms [19, 24]. Therefore the optimum fit of relation (1) to data presented in [1, 5] was sought under the assumption that both mechanisms take part in the PLCQ process. It is evident from the data listed in the Table and from Fig. 1 that the best fit of relation (1) to experimental points has been obtained for $K_\gamma = 0$, i. e. by neglecting the participation of dimers in PLCQ process. We also took into consideration the case of quenching due entirely to NEET from monomers to dimers (curves 3). For chlorophyll a experimental points distinctly depart from curve 3. This is in favour of “monomer” quenching. However, the case of chlorophyll b is not as unambiguous. Curve 3, representing a poorer fit to the experimental data for the whole range of concentrations, is nonetheless distinctly “better” than the remaining curves for low concentrations. For this reason participation of dimers in the PLCQ process cannot be definitely excluded. On the other hand, for chlorophyll a in bilayer lipid vesicles and liposomes, similarly as for chlorophyll a in ether, the monomer quenching mechanism seems to prevail.

Dalton [7] compared * the data given by Watson and Livingston with relation (1) for $\alpha = 1$ ($K_\gamma = 0$). His method of fitting reduces to the choice of two independent parameters α_0 and R_0^{exp} . However, for the value $R_0^{\text{th}} = 51.7 \text{ \AA}$ quoted in the work mentioned the results differ significantly from the theoretical curve (cf. values of S in the Table). For chlorophyll b the best fit was achieved for $\alpha_0 = 0.995$ and $R_0^{\text{exp}} = 69 \text{ \AA}$.

It should be emphasized that the comparison of experimental data with relation (1) was made here

* The formula for the quantum yield as obtained by Dalton (Eq. (3) in Ref. [7]) is a particular case of expression (1) for $\alpha = 1$. Expression (1) had been derived many years earlier. It has, besides, a more secure theoretical basis [11].

for definite values of the critical distance R_0^{th} , obtained from spectroscopic data.

It can be seen from data for chlorophyll a in ether as presented in the Table that (for $K_\gamma = 0$) relatively small changes of α_0 correspond to large changes of R_0^{exp} , the fit of experimental data to relation (1) remaining nearly the same. Note, however, that the slope of the quenching curve is almost insensitive to variations of α_0 near 1, opposite to the “threshold” concentration which, together with the quenching curve, shifts substantially parallel to the abscissa (cf. Figure 2). This results in a considerable indeterminacy of the critical distance R_0^{exp} when fitting the theoretical curve to experimental points. Thus this method of comparison is not useful for the determination of R_0^{exp} .

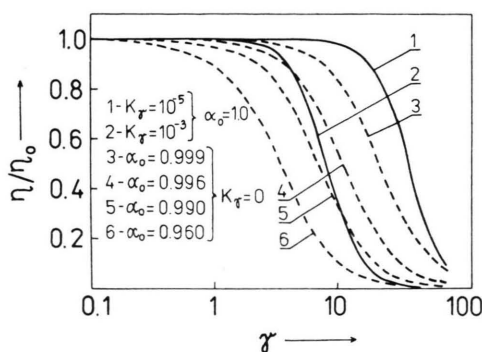


Fig. 2. PL-quantum yield versus γ for different K_γ and α_0 calculated from (1).

The analysis carried out above confirms in the case of chlorophyll a the opinion of Porter et al. [3, 5, 6] and also Watson and Livingston [1] as to the absence of dimers in systems specified in the Table. These authors presented some arguments in favour of the absence of chlorophyll dimers, such as the independence of absorption spectra of the concentration, the close similarity of the decay time and quantum yield curves, etc. Note that these arguments can also be used in case of systems characterized by weak dimerization. However, even a slight mole fraction of dimers, undetectable by spectroscopic methods, may be sufficiently large to explain the observed drop of the quantum yield (cf. Ref. [24]). The quenching curves should in such a case be distinctly steeper (see Figure 2).

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