DIE NATURWISSENSCHAFTEN

63. Jahrgang 1976 Heft 5 Mai

Some Reflections on the Work of Theodor Förster

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Theodor Förster's great contributions to the science of electronically excited molecules are summarized. As an example of the applications of his work, the problem of energy transfer in the chloroplast is discussed.

Over the last quarter of a century few branches of science have enjoyed more progress than the physics and chemistry of excited molecules. Photochemistry, which previously was concerned mainly with the final products or the dark reactions of intermediates such as free radicals, has become a new science of the excited state. No single person contributed more to this progress than Theodor Förster.

When I was asked by the European Photochemistry Association to deliver this Theodor Förster Memorial Lecture I was very pleased to accept because I had known and admired Theodor and his work for many years. I first met this intellectually powerful but modest man at a meeting of the Deutsche Bunsengesellschaft in Lindau in 1952. At that time he was studying the changes in acidity or basicity of a molecule when it becomes electronically excited and his name is firmly associated with the work through the method he used for calculating the change in equilibrium constant in the excited state by means of the thermodynamic cycle now known as the Förster cycle. He was also able to show how the new equilibrium is established in a time comparable with the lifetime of the excited state, that is to say in just a few nanoseconds. He did this work several years before Manfred Eigen's direct studies of proton-transfer reactions and it was particularly exciting to me because I was interested in the development of flash methods for the direct observation of events of this kind. Unfortunately our methods were more than a thousand times too slow at that time but I remember how optimistic Professor Förster was that even the short times of proton-transfer reactions in excited states would eventually be resolvable by these methods. Today his optimism has been more than vindicated and several members of his school, particularly Albert Weller, have used them in just the way he predicted.

The last time we met was twenty years later, at the Sixth International Congress on Photochemistry in Bochum in 1972, when I had the pleasure, as President, of presenting him with the Finsen Medal. Dr. E.J. Bowen gave the oration before the presentation and concluded with these sentences.

"The last words of the poet-philosopher Goethe were 'Macht doch den Fensterladen auch auf, damit mehr Licht hereinkomme'. Professor Förster has certainly answered that call by the light he has thrown on photochemical processes. He has opened several windows. For long he has been a Donor to science. Now, to mark his merits, we ask him to be an Acceptor."

This was, of course, an eloquent reference to what is the most important of all Förster's contributions to photochemistry, his development of the theory of electronic energy transfer between two molecules which we call the donor and the acceptor. The remarkable thing about such transfers is that they can occur over what, on a molecular scale, are very great distances, sometimes as much as 100 Å. Developing earlier ideas of J. Perrin and F. Perrin, Förster showed that a comparatively weak interaction between two molecules could be sufficient for transfer to occur provided some kind of *resonance* condition is fulfilled. After absorption of light, vibrational relaxation occurs very rapidly in solution and then resonance between the transitions in the two molecules will bring about a transfer of energy, providing some coupling between them is present. The coupling is strongest if the corresponding electronic transitions in the two molecules are allowed for electric dipole radiation. Then the dipole-dipole interaction will depend on the third power of the separation of the two molecules. The probability of transfer is proportional to the square of this interaction energy and so the energy-transfer probability depends on the sixth power of the distance.

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* The First Theodor Förster Memorial Lecture, given in Copenhagen, Munich, etc. September/October 1975

The other important factor, the extent to which the resonance conditions are fulfilled can, he showed, be estimated remarkably simply by the overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor. Quantitative treatment of these ideas by Förster led to his important equations for the calculation of transfer rates and distances, and for the quenching of fluorescence of one molecule by another. For quenching of a molecule D by a molecule A at separation R_i, the quenching rate constant $k_Q(\sec^{-1})$ is given by

$$k_Q = \frac{1}{\tau_0}\left(\frac{R_0}{R_i}\right)^6$$

where

$$R_0^6 = \frac{(9{,}000 \ln 10)K^2}{128\pi^5 n^4 N}\int_0^\infty F_D(\nu)\varepsilon_A(\nu)\frac{d\nu}{\nu^4}.$$

Here n is the refractive index, K^2 is an orientation factor, N is the Avogadro number and the integral gives the overlap of the emission spectrum of the donor D with the absorption spectrum of the acceptor A.

Förster extended the theory to the case of quenching by a solution of molecules Q in random distribution and derived an equation for the relative fluorescence yield:

$$\frac{\phi}{\phi_0} = 1 - \sqrt{\pi}\,\gamma e^{\gamma^2}(1 - \mathrm{erf}(\gamma))$$

where

$$\gamma = \frac{(A)}{(A_0)} = \frac{2\pi^{\frac{3}{2}} N R_0^3 (A)}{3{,}000}.$$

These important contributions were made whilst Förster was at the Max Planck Institute for Physical Chemistry in Göttingen. The Director of the Institute was Karl-Friedrich Bonhoeffer, to whom Förster had been an assistant at Frankfurt University when he was 23 and when Bonhoeffer was the Professor and himself only 34. They went together to the University of Leipzig when Bonhoeffer was made Director of the Institute of Physical Chemistry there in 1934, and remained together until Förster became a full Professor at the University of Posen in 1942. But immediately after the war he joined Bonhoeffer again, now in Göttingen, and his years there were some of the most fruitful and productive of his life. His classical monograph "Fluoreszenz organischer Verbindungen" was published near the end of this period and had an enormous influence on the subject.

Förster accepted a chair in the University of Stuttgart in 1951 and, three years later, made his third important contribution to the study of molecular behaviour in the excited state. He discovered that pyrene shows different fluorescence spectra in dilute and in concentrated solutions and showed that this is due to the formation of dimers between excited and ground-state molecules, although no such dimers are formed between two ground-state molecules. This was the concept of "excimers" which he had already suggested two years earlier on theoretical grounds. During the last five years he worked on several other aspects of the excited state, particularly on diabatic chemical reactions (reactions which involve a change in electronic excitation) but probably the three contributions for which he will most be assured of a place in the history of of chemistry, and which have so greatly increased our understanding of molecular behaviour in the excited state, are proton transfer, energy transfer and excimer formation in electronically excited molecules.

The details of these processes are so well known to all photochemists that it would not be interesting for you if I pursued them further now. And were I to attempt an adequate review of all the developments in chemistry, physics and biology which have arisen from his work it would occupy many lectures. Instead I should like to take just one problem which always interested Theodor Förster and which particularly interests me at the present time in which almost every aspect of Förster's work is illustrated and is indeed vital to any understanding of it. It is the important problem of the primary processes of photosynthesis. It immediately involves us in a second problem, general to all molecules but potentially very important in the photosynthetic process, the problem of concentration quenching, which is again a theme running through many aspects of Förster's work.

Förster wrote a paper as early as 1947 in the Zeitschrift für Naturforschung called "Ein Beitrag zur Theorie der Photosynthese". This appeared one year before his first full account of the resonance-energy transfer theory although this theory is used, in the paper, in a discussion of the energy-transfer processes involving chlorophyll in the primary processes of photosynthesis. It seems clear that his interest in this most important of all photochemical processes provided much of the inspiration for his later work. It continues to provide a stimulus today because the nature of the primary processes of photosynthesis are not unravelled yet and there are anomalies which must be a challenge to any scientist interested in excited-state behaviour.

The problem which I wish to consider is that of how the photon first absorbed by the leaf or other photosynthetic system finds its way to the chemical reaction centre. It is well established that there are far more (approximately 250) absorbing chlorophyll and other pigment molecules than there are chemical reaction centres and the efficiency of photosynthesis is far higher than would be the case if only one quantum in 250 were effective. Following Emerson and Arnold, therefore, it is generally accepted that there is a photosynthetic unit consisting of about 250 molecules of chlorophyll and other pigments which harvest the light quanta and transmit them efficiently by some energy-migration process to the reaction centre.

This energy-migration process must be very fast indeed. Figure 1 shows the decay curves of fluorescence

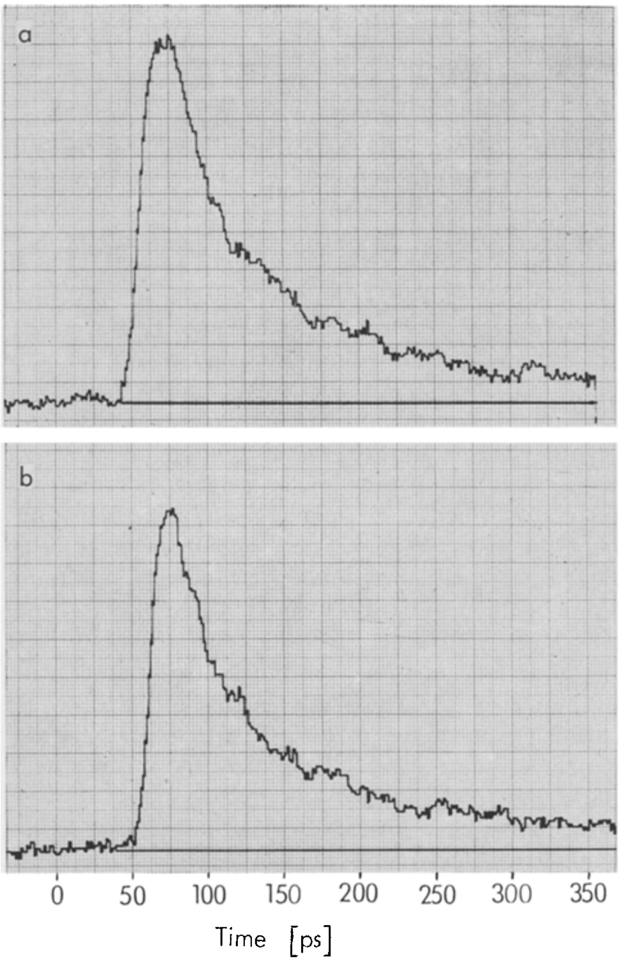

Fig. 1. Fluorescence decay of intact algae, (a) *Porphyridium cruentum*, (b) *Chlorella pyrenoidosa*

of the algae *Chlorella* and *Porphyridium* measured by Beddard, Porter, Tredwell and Barber [1] using picosecond pulses from a Nd mode-locked laser. The lifetimes are 108 and 92 picoseconds, respectively. (There are two separate systems involving two electron-transport processes in series, probably with somewhat different lifetimes but this need not concern us here.) This means that, for energy to migrate over 250 molecules, the time for a single transfer must be less than 1 ps (and probably much less since some sites will be visited more than once). However, this in itself presents no serious problem. The average concentration of chlorophyll in the chloroplast is about $0.1\,M/\mathrm{dm}^3$ which, for a random distribution, corresponds to an average near-neighbour separation of 14 Å and a time for inductive resonance transfer between these near neighbours of 1 ps for chlorophyll *a* ($R_0 = 70$ Å, $T_0 = 19$ ns). Since the local concentration of chlorophyll in the photosynthetic unit may be higher than the average and, perhaps more importantly, other transfer mechanisms such as multipole resonance and exchange transfer will become operative at these small separations, the light-harvesting mechanism is not incompatible with the observed lifetimes.

There is another problem, however, which arises out of studies of chlorophyll in solution in vitro and which confounds all attempts to reproduce the light-harvesting mechanism outside the chloroplast and this is concentration quenching. In vitro, the fluorescence of chlorophyll solutions in polar solvents including lipids, rigid polymers and two-dimensional solutions, is half-quenched at concentrations in the region of $10^{-2}M$ and the quantum yield is less than 1 % at $10^{-1}M$. Such behaviour seems incompatible with high yields of photosynthesis. Furthermore, yields of fluorescence in the chloroplast, when chemical traps have been closed, are well in excess of this and may be as high as 10 %. If we are to understand this anomaly, we must understand first the mechanism of concentration quenching in general and of chlorophyll in vitro in particular.

Mechanisms of Concentration Quenching

The various mechanisms by which molecular fluorescence may be self-quenched at high concentrations, some of them well established, are summarized in Table 1. Type-1 quenching occurs when the fluorescent molecules form dimers or oligomers which are non-fluorescent or which fluoresce in a different region of the spectrum. Dimers of fluorescein and similar molecules and their equilibria were studied in detail by Förster. If the equilibrium of dimer formation is K the expression for the relative yield of monomer fluorescence as a function of the total concentration C of the solute expressed as monomer is given by

$$\frac{\phi}{\phi_0}=\frac{1}{4KC}\left[(8KC+1)^{\frac{1}{2}}-1\right].$$

The first detailed studies of the relative fluorescence yield of chlorophyll in solvents such as alcohols and ether were carried out by Livingston and Watson [2] and we shall use their data to try to distinguish between the mechanisms of quenching. They showed that the expression above did not account for their data which were, however, fitted to the empirical expression

$$\frac{\phi}{\phi_0}=\frac{1}{1+4{,}300\,C^2}.$$

Other evidence against Type-1 quenching in solutions of chlorophyll in polar solvents is the absence of departures from Beer's law or of spectroscopic evidence of dimeric species at higher concentrations, although it is well known that dimers and oligomers are formed in non-polar solvents, especially when carefully dried.

Table 1. Mechanism of concentration quenching

Type 1	Ground-state complex	$M+M \rightleftharpoons M_2$
Type 2	*Molecular* diffusion to form an excited complex	$M^*+M \rightleftharpoons MM^* \rightarrow 2M$
Type 3	*Excitation* diffusion to trap	$(M^*+M \rightarrow M+M^*)_n$
a) Trap is extraneous quencher		$M^*+Q \rightarrow M+Q$
b) Trap is dimer or oligomer		$M^*+M_2 \rightarrow M+M_2^{\ddagger} \rightarrow M+M_2$
c) Trap is statistical pair at R_p		$M^*+M \overset{R_p}{\cdots} M \rightarrow M+MM^* \rightarrow 3M$

Type-2 quenching is typified by the excimer formation studied by Förster in pyrene and already referred to. By this mechanism, concentration quenching can occur even when there is no interaction between ground-state molecules. The excimer may be non-fluorescent or may fluoresce in another spectral region. No excimer fluorescence of chlorophyll is known, although we have looked for it, but it would be expected in the infra-red region and the fluorescence efficiency may be very low. Type-2 quenching does not, however, account for concentration quenching in chlorophyll since it involves *molecular* diffusion and there is ample evidence that the quenching in chlorophyll is not diffusion-controlled. It is only necessary to note that the concentration range of quenching is similar in solid or very viscous solutions such as poly-(methylmethacrylate) and lecithin [3] and in fluid solvents such as ether [2].

Only mechanisms of Type 3 remain to account for concentration quenching of chlorophyll in a wide range of polar solvents. In this mechanism there is no bulk diffusion of molecules but the excitation is able to diffuse from molecule to molecule by a random walk or exciton wave. There is no reason why energy transfer between identical molecules, by a Förster or any other mechanism, should lead to a change in the fluorescence lifetime or yield. However, if quenching molecules (traps) are present, even in small concentration, a migration of the excitation from molecule to molecule provides a diffusional mechanism of quenching at these traps.

Three sub-cases of Type-3 quenching may be considered depending on whether the trap is an impurity, a dimer/oligomer or a statistical-pair trap. The term statistical-pair trap refers to the fact that, in a random array of molecules in solution, even when there are no interactions between the molecules in their ground state, there is a statistical probability that some of the molecules will have nearest neighbours so close that, when one of the pair is electronically excited, interaction may occur leading to quenching. The most likely mechanism for this would be collapse of the pair into an excimer configuration (such as is known to occur with pyrene in rigid solvents [4]) but any interaction such as charge transfer may increase the probability of radiationless conversion whether a true excimer is formed or not.

Impurities, as traps for Type-3 quenching, can probably be excluded as a general cause of concentration quenching in chlorophyll because the concentration range of quenching varies so little over a wide range of solvents and is similar in chlorophyll *b* and in pheophytins [3]. Dimers will certainly form traps when they are present in high enough concentration but calculations of the type to which I shall refer in a moment show that, at the higher concentrations studied, the fraction of molecules which are traps must exceed 10 % and yet there is no spectral change in polar solvents such as is found when dimers of chlorophyll are formed in solvents such as carbon tetrachloride. In most of the cases which interest us, those studied by Livingston in alcohols for example, it is therefore probably necessary to invoke quenching of mechanism Type 3(c) where the traps are statistical pairs.

We must now enquire whether this mechanism is able to account quantitatively for the concentration-quenching data of Livingston and others. We assume transfers according to the Förster inductive-resonance mechanism (which should certainly be valid except perhaps at the very highest concentrations) and quenching by all pairs of molecules within a critical separation R_t. Although random-walk theory is highly developed it has been concerned almost exclusively with regular arrays of particles. Approximate methods of obtaining a solution to the random distribution, random-walk problems in closed form introduce uncertainties which it is difficult to assess and calculations have therefore been carried out by Dr. Godfrey Beddard in our laboratory [5], which use a Monte-Carlo method of computation.

The object is to calculate the rate of a random walk of excitation in a random three-dimensional array of molecules in which the intermolecular transfer rate is proportional to R^{-6}. By selection of pseudo-random numbers from a uniform distribution, 1620 molecules are positioned among 27×10^6 coordinates in a regular three-dimensional lattice. This is equivalent to placing points in a cube consisting of 300 planes with separation D Å. Excitation is introduced by randomly selecting one point. Rates are then calculated for all possible processes available to the initially excited molecule *via* fluorescence, intersystem crossing and resonance-energy transfer to all molecules in a sphere of radius 35 D Å around the excited molecule. The energy is then transferred to a new molecule and this process is repeated until it is terminated by fluorescence, intersystem crossing or trapping by a pair of molecules closer than a distance R_t. The whole random walk is now repeated using further selection of arrays and the results averaged. The complete process is repeated for a range of concentrations, the concentration being changed by varying D. The lattice is large enough so that the probability of excitation reaching the edge of the lattice is negligible.

Complete fluorescence-quenching curves for chlorophyll *a*, calculated in this way by Beddard [5], are shown in Figure 2. A separation of the statistical-pair trap is chosen for the calculation of each curve and the process is stopped whenever the excitation reaches such a pair. In Figure 2 we have compared our calculations with the results of Watson and Livingston [2] for chlorophyll self-quenching in ether. It will be seen that the shape of the curve of relative quantum yield against concentration is well reproduced and a quantitative fit is obtained for a statistical-pair trap separation of 10 Å. This may be compared with the diameter of the porphyrin ring in chlorophyll which is 15 Å.

We now return to the question of the mechanism of light harvesting by chlorophyll molecules in the photosynthetic unit. At a chlorophyll concentration in the

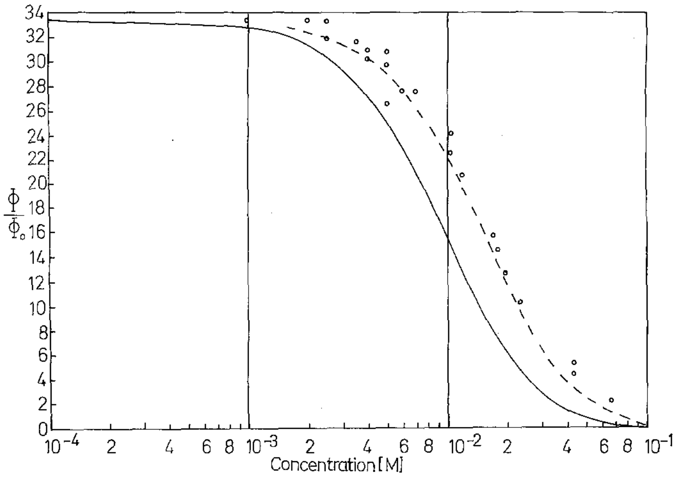

Fig. 2. Relative fluorescence yield (%) of chlorophyll *a* as a function of concentration. o experimental data of Watson and Livingston, —— calculated curve with $R_0 = 70$ Å and trap = 14 Å, ------ calculated with $R_0 = 70$ Å and trap = 10 Å

chloroplast of 10^{-1} molar, the fraction of molecules in statistical-pair traps with separation of 10 Å would be 0.22 (if they were in random distribution) and efficient photosynthesis would be impossible. Transfer to a chemical trap must occur rapidly enough to compete efficiently with trapping which is not consistent with the rather high fluorescence yield of the chloroplast and the lifetimes of 100 picoseconds or more.

Two molecules, which are within the statistical-pair quenching distance, may be prevented from quenching if they are separated by another molecule. Infra-red, X-ray diffraction and other structural studies have shown [6] that chlorophyll molecules, whatever their environment, tend to coordinate the Mg atom, either to another chlorophyll or to a solvent molecule, so that chlorophyll with only four bonds coordinated to Mg probably rarely exists. We therefore suggest that chlorophyll molecules in the chloroplast antennae, although at close distance, are separated from each other by strongly coordinated molecules which prevent collapse to an excimer or other quenching configuration. Since it is not yet decided whether antennae chlorophylls are associated with protein, with lipid or with lipid/protein interface it is not profitable to speculate further about structure at this stage.

I think this one example which I have chosen shows very well how intimately the threads of Förster's ideas are woven into any discussion of the fabric of molecular behaviour in the excited state. Although his untimely death makes us sad, all who knew him remember him as a contented man, blessed with the happiest of marriages to Martha for more than 30 years and with two sons both of whom have already distinguished themselves in science.

There is little in our world which is immortal except natural knowledge and the arts; this is one of the beliefs which attracts us to science. It is a source of happiness to all his scientific colleagues and friends that they can continue to turn to Theodor Förster, through his written works, for advice, for example and for inspiration.

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Received December 12, 1975