

DEPENDENCE OF THE KINETICS OF SINGLET-SINGLET ENERGY TRANSFER ON SPECTRAL OVERLAP*

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Abstract.—Electronic excitation energy can be transferred between chromophores separated by distances of the order of 30 Å. Förster proposed that the transfer occurs by a dipole-dipole resonance interaction which depends on certain spectroscopic and geometric properties of the donor-acceptor pair. His prediction that the rate of transfer depends on the inverse sixth power of the distance between the chromophores was verified previously. In this work, we tested a second prediction of Förster's theory, namely, that the transfer rate is proportional to J , the magnitude of the overlap between the emission spectrum of the energy donor and the absorption spectrum of the energy acceptor.

The energy donor was an N-methylindole moiety, and the acceptor was a ketone. These chromophores were fused to a rigid steroid that separated them by 10.2 Å. Rate constants for singlet-singlet energy transfer in this system were obtained by nanosecond flash spectroscopy. J was varied over a 40-fold range simply by altering the solvent. We found that the transfer rate is proportional to J , as predicted by Förster's theory. The results bear on the potential use of this energy transfer process to measure distances in biological macromolecules. It is evident that the length of such a spectroscopic ruler can readily be controlled by varying the magnitude of the spectral overlap integral of the energy donor-acceptor pair.

Singlet-singlet energy transfer can occur over distances of the order of 30 Å.¹⁻³ Förster has proposed that the transfer occurs by a dipole-dipole resonance interaction between the energy donor and acceptor chromophores.⁴ In his theory, the rate constant for transfer k_T is related to geometric and spectroscopic factors by⁵

$$k_T = r^{-6} JK^2 n^{-4} k_F \times 8.71 \times 10^{23} \text{ sec}^{-1} \quad (1)$$

where r is the distance (in Å) between the centers of the donor and acceptor transition moments, K^2 is the dipole-dipole orientation factor, n is the refractive index of the medium, and k_F is the rate constant (in sec⁻¹) for fluorescence emission by the donor. The spectral overlap integral J , which measures the extent to which the donor and acceptor transitions are in resonance, is given by

$$J = \frac{\int F(\lambda) \epsilon(\lambda) \lambda^4 d\lambda}{\int F(\lambda) d\lambda} \quad (2)$$

where $F(\lambda)$ is the fluorescence intensity of the energy donor at wavelength λ (in cm), and $\epsilon(\lambda)$ is the molar decadic extinction coefficient (in cm⁻¹ M⁻¹) of the energy acceptor.

We have previously shown that singlet-singlet energy transfer depends on the inverse sixth power of the distance between the donor and acceptor chromophores.³ In this paper, we report an experimental study of the dependence of the kinetics of singlet-singlet energy transfer on the magnitude of the spectral overlap integral. The energy donor in this study was an N-methylindole moiety, and the energy acceptor was a ketone group. The donor and acceptor chromophores were fused to a rigid steroid that separated them by 10.2 Å (I, Fig. 1). The emission spectrum of the energy donor depended markedly on the nature of the solvent, which made it possible to vary the magnitude of J as much as 40 times simply by altering the solvent. The rate constants for transfer were determined by measuring the kinetics of fluorescence of I and II. We found that the transfer rate is proportional to the magnitude of the spectral overlap integral, as predicted by Förster's theory.

Materials and Methods.—*Synthesis of the indole steroids:* 1'-Methylindolo (3',2':2,3)-2(5 α)-androst-17-one (I) was prepared by the Fischer indole synthesis.⁶ The site of fusion of the indole to the steroid was determined previously.^{7,8} Equimolar quantities of 5 α -androst-3,17-dione (1 gm) and 1-methyl-1-phenylhydrazine were dissolved in 5 ml acetic acid. The solid that formed on standing at room temperature overnight was filtered, washed with methanol, and dried in air, yielding 861 mg (67%) product. Recrystallization from methanol gave 789 mg of colorless needle clusters. The analytical sample melted at 258–260°. The absorption maximum in ethanol was at 286 nm, $\epsilon = 6103 \text{ cm}^{-1} M^{-1}$. Calculated analysis for $C_{26}H_{33}NO$: C, 83.16; H, 8.86%. Found: C, 83.06; H, 8.99%.

1'-Methylindolo (3',2':2,3)-2(5 α)-androst-17 β -ol (II) was prepared in a similar way from 5 α -androst-17 β -ol-3-one. Melting point 263–266°. The absorption maximum in ethanol was at 286 nm, $\epsilon = 6250 \text{ cm}^{-1} M^{-1}$. Calculated analysis for $C_{26}H_{35}NO$: C, 82.71; H, 9.34%. Found: C, 82.76; H, 9.40%.

Androsterone (III) was purchased from Mann Research Laboratories.

Spectroscopic measurements: Spectra and lifetimes of air-equilibrated solutions were

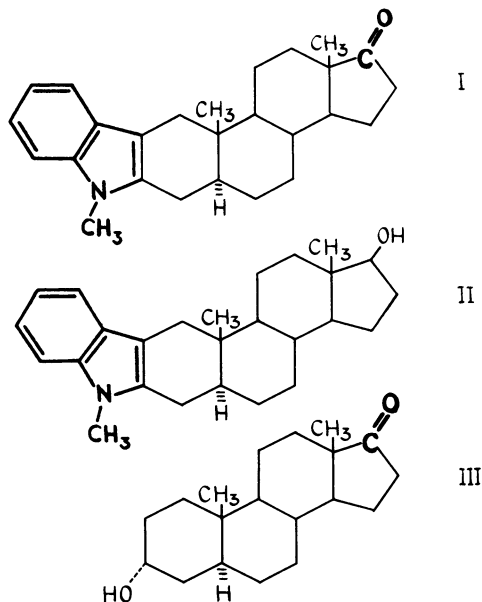


FIG. 1.—Structure of the energy donor-acceptor pair (I), the steroid containing the energy donor alone (II), and the steroid containing the energy acceptor alone (III).

taken at $24^\circ \pm 1^\circ\text{C}$. Absorption spectra were obtained on a Cary model 15 recording spectrophotometer. An expanded scale was used for the measurement of absorbances less than 0.1. Solvents were spectrograde.

Fluorescence emission spectra were obtained on a recording spectrofluorimeter described previously.⁹ Spectra were corrected for the variation with wavelength in the sensitivity of the detection system. Absolute quantum yields were determined using p-terphenyl in cyclohexane as a standard¹⁰ of quantum yield 0.92.

The kinetics of fluorescence emission were measured with a nanosecond fluorimeter which employs a modified image converter tube for light detection.¹¹ The detection system has a time resolution of 0.1 nsec. An oxygen spark-gap lamp was used as the light source.¹² The light pulse had a measured full width at half-height of 1 nsec and a decay time of 0.8 nsec. The nanosecond data were analyzed in terms of a convolution integral that took into account the shape of the exciting light pulse as seen by the detection system.¹² The exciting light was filtered through a Corning 7-54 filter and 1 cm of $4 \times 10^{-4} M$ N-methyl-anthraniloyl aziridine in acetonitrile. This excitation filter combination had a maximum transmittance of 57% at 300 nm and a minimum transmittance of 1.5% at 370 nm. The emission filter was a Corning 7-39. In the nanosecond kinetic studies, $10^{-4} M$ solution of the indole steroids were used, while for steady-state measurements $10^{-5} M$ solutions were used.

Results.—The absorption spectrum of the ketone overlaps the fluorescence emission spectrum of the N-methylindole moiety (Fig. 2), thus fulfilling one of the requirements for energy transfer by the Förster mechanism. The emission spectrum of the donor is shifted to the red in going from nonpolar to polar solvents^{13, 14} (Fig. 3), while the absorption spectrum of the ketone shows a small shift to the blue. The result is a decrease in the extent of the spectral overlap (Fig. 2). The presence of the ketone in I has no effect on the shape of the emis-

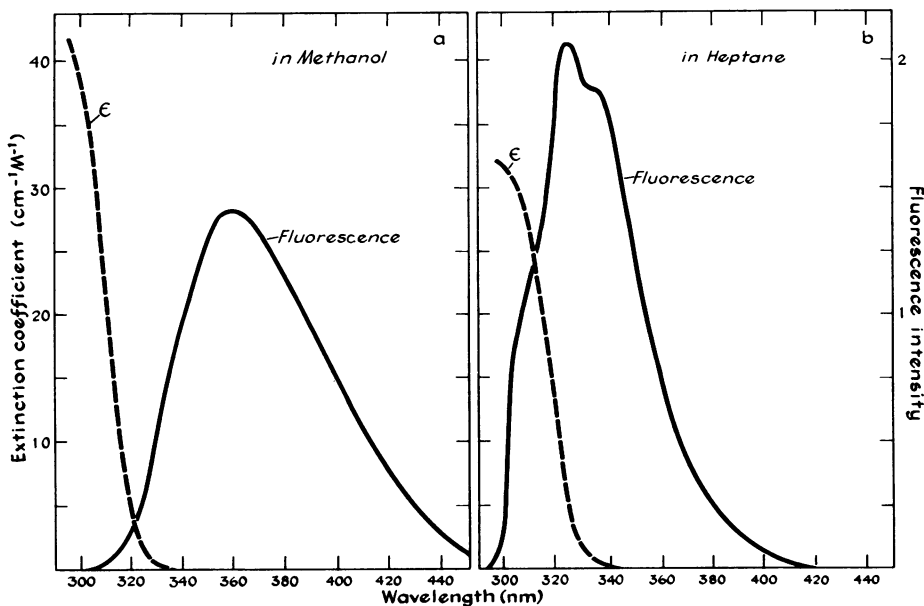


FIG. 2.—Overlap of the emission spectrum of the N-methylindole energy donor (II) and the absorption spectrum of the ketone energy acceptor (III) in (a) methanol and (b) heptane. III is nonfluorescent.

sion spectrum of the donor, nor on the absorption spectrum at wavelengths shorter than 310 nm. The ketone is nonfluorescent.

The energy transfer that occurs in I was expressed in two ways. The fluorescence quantum yield (Q) of I is less than that of II, where there is no energy acceptor. Furthermore, the excited state lifetime (τ) of the fluorescence of I is shorter than that of II because of the presence of a new pathway that depopulates the singlet excited state of the N-methylindole moiety. These two effects are illustrated in the emission spectra of I and II in ethyl ether (Fig. 4) and in the nanosecond kinetic data for these compounds (Fig. 5). The quantum yields and excited state lifetimes of I and II in a series of solvents are given in Table 1.

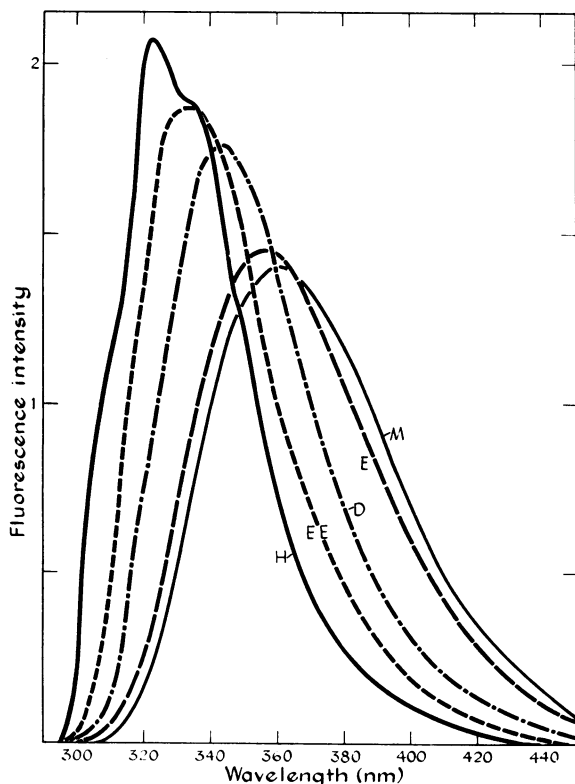


FIG. 3.—Fluorescence emission spectra of II in n-heptane (*H*), ethyl ether (*EE*), p-dioxane (*D*), ethanol (*E*), and methanol (*M*). The spectrum of II in ethyl acetate is virtually identical to that obtained in dioxane. The intensities have been normalized so that the areas under the curves are equal. The excitation wavelength was 290 nm.

The efficiency of energy transfer based on the steady-state quantum yield measurements is given by $E_{SS} = 1 - (Q_I/Q_{II})$, while the efficiency based on the lifetimes of I and II is given by $E_K = 1 - (\tau_I/\tau_{II})$. As shown in Table 1, the transfer efficiency varies from 0.055 in methanol to 0.608 in heptane. There is very good agreement between E_{SS} and E_K . The observed energy transfer was clearly intramolecular at the concentrations used in these studies, since there was no detectable transfer in solutions containing $10^{-4} M$ II and $10^{-4} M$ III.

The rate constant for transfer is obtained directly from the excited state lifetimes of I and II. τ_I and τ_{II} are related to the rate constant for transfer k_T , the rate constant for fluorescence emission k_F , and the sum of the rate con-

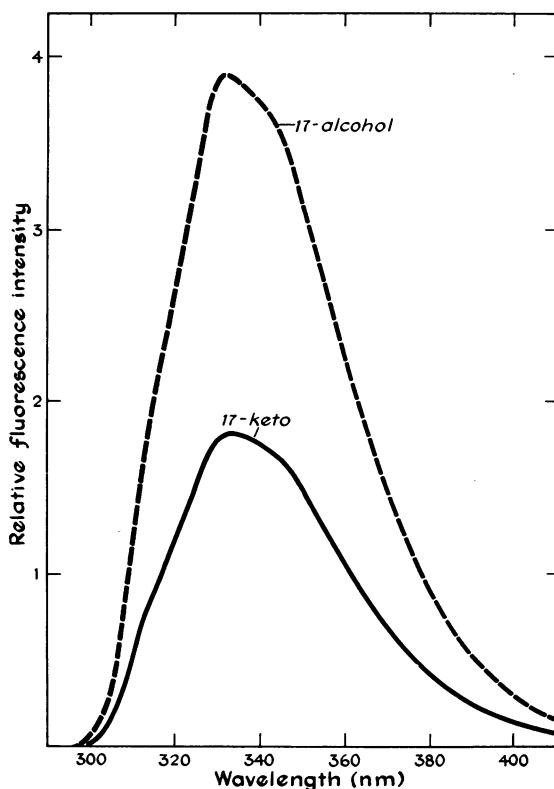


FIG. 4.—Fluorescence emission spectra of I (—) and II (---) in ethyl ether. The intensity of the emission of I is less than that of II because of energy transfer to the ketone in I. The excitation wavelength was 290 nm.

stants for all nonradiative processes other than energy transfer k_T , by the expressions

$$\tau_I = (k_F + k_I + k_T)^{-1} \quad (3)$$

$$\tau_{II} = (k_F + k_I)^{-1} \quad (4)$$

and therefore,

$$k_T = \frac{1}{\tau_I} - \frac{1}{\tau_{II}} \quad (5)$$

The transfer rate constants determined in this way range from $1 \times 10^7 \text{ sec}^{-1}$ in methanol to $55.2 \times 10^7 \text{ sec}^{-1}$ in heptane (Table 2).

Spectral overlap integrals were calculated from the fluorescence spectra of II and the absorption spectra of III. J ranges from $1.5 \times 10^{-19} \text{ cm}^6 \text{ mmole}^{-1}$ in methanol to $60.3 \times 10^{-19} \text{ cm}^6 \text{ mmole}^{-1}$ in heptane (Table 2). Values of k_F were calculated from $k_F = Q_{II}\tau_{II}^{-1}$.

Discussion.—The significant finding is that the rate constant for transfer is proportional to the magnitude of the spectral overlap integral J (Fig. 6 and Table

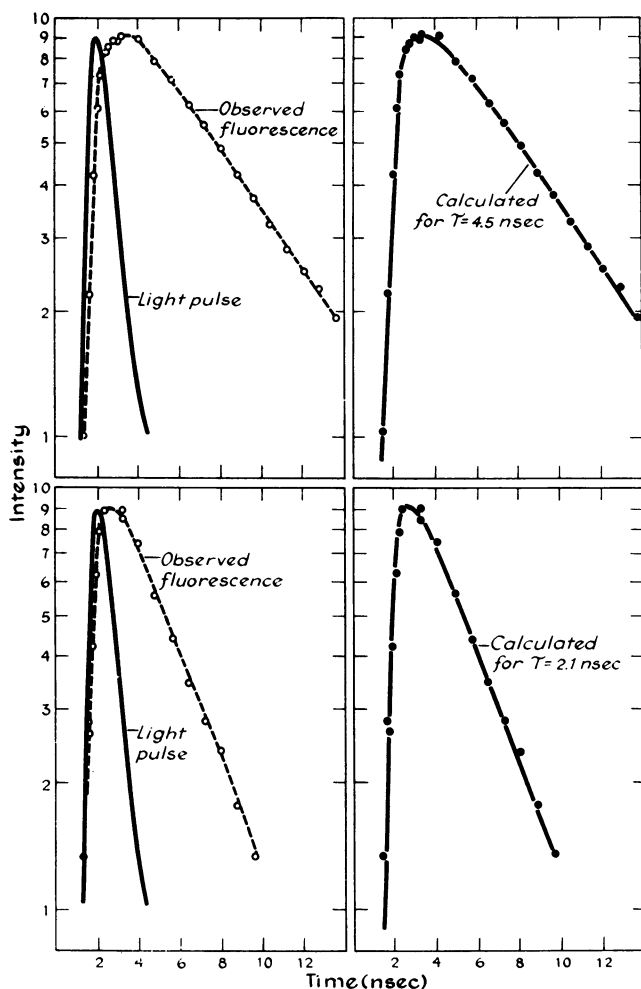


FIG. 5.—Nanosecond emission kinetics of I and II in ethyl ether. *Upper left:* light pulse (—) and fluorescence of II (O—O—O). *Lower left:* light pulse (—) and fluorescence of I (O—O—O). *Upper right:* the agreement between the observed emission of II (●) and a decay curve calculated for $\tau = 4.5$ nsec (—). *Lower right:* the agreement between the observed emission of I (●) and a decay curve calculated for $\tau = 2.1$ nsec (—). τ for I is shorter than for II because of energy transfer.

2). This relationship holds over at least a 40-fold change in J . The slope in the plot of $\log k_T$ versus $\log J$ is 1.10 (Fig. 6). A slope of 1.0 is expected if equation (1) holds and if the geometric and spectroscopic factors other than J in equation (1) are constant in the series of solvents studied. Actually, k_F increases as the polarity of the solvent decreases (Table 2), which may account for the finding that the slope in Figure 6 differs slightly from 1.00. In fact, the agreement is closer when $\log k_T$ is plotted against $\log (Jk_F)$, yielding a slope of 0.96.

The transfer rate for I in dioxane calculated from equation (1) is $2.2 \times 10^7 K^2 \text{ sec}^{-1}$. The value of K^2 in I is not known, and so the quantitative validity of

TABLE I. Fluorescence properties of I and II in a series of solvents.

Solvent	τ_I (nsec)	τ_{II} (nsec)	Fluorescence Quantum Yield		Transfer Efficiency	
			Q_I	Q_{II}	E_K	E_{SS}
Methanol	5.3	5.6	0.397	0.420	0.053	0.055
Ethanol	5.6	6.5	0.440	0.510	0.14	0.138
Dioxane	3.6	5.4	0.360	0.535	0.33	0.328
Ethyl acetate	3.3	4.7	0.286	0.418	0.30	0.315
Ethyl ether	2.1	4.5	0.247	0.515	0.53	0.52
Heptane	1.1	2.8	0.136	0.346	0.606	0.608

TABLE 2. Transfer kinetics and spectral overlap integral of I in a series of solvents.

Solvent	Transfer rate, $k_T(\text{sec}^{-1})$ $\times 10^{-7}$	Spectral overlap integral, $J(\text{cm}^6 \text{mmole}^{-1})$ $\times 10^{19}$	$k_F(\text{sec}^{-1})$ $\times 10^{-7}$	n_D	k_T/J $\times 10^{-26}$	$k_T/(Jk_F)$ $\times 10^{-13}$
Ethanol	2.5	3.0	7.85	1.362	0.83	1.06
Dioxane	9.6	13.0	9.9	1.423	0.74	0.75
Ethyl acetate	11.3	12.8	8.9	1.372	0.88	0.99
Ethyl ether	25.5	30.0	11.5	1.349	0.83	0.72
Heptane	55.2	60.3	12.4	1.387	0.92	0.74

equation (1) cannot be ascertained at this time.¹⁵ However, it might be possible to determine experimentally the magnitude of K^2 by polarized absorption and emission spectroscopy of crystals of II and III.

These results bear on the potential use of energy transfer processes as spectroscopic rulers in biological macromolecules.^{1, 3, 16} It has been suggested that singlet-singlet and triplet-singlet transfer¹⁷ can be used to estimate distances between 10 and 60 Å. A number of donor-acceptor pairs with different R_0 values (distance at which the transfer is 50% efficient) are needed to span this range, because of the steep dependence of energy transfer on distance. For estimating distances as short as 10 Å, it is evident from this study that the carbonyl group with an ϵ of 30 $\text{cm}^{-1} M^{-1}$ is a suitable energy acceptor if the donor emits near 300 nm. Distances as large as 60 Å should be within the scope of donor-acceptor pairs in which the acceptor has an ϵ of the order of

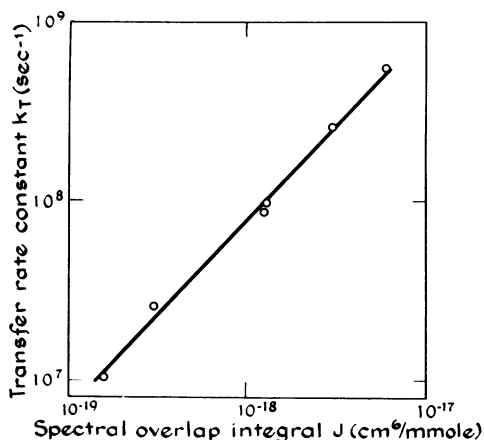


FIG. 6.—Dependence of the rate of energy transfer in I on the magnitude of the spectral overlap integral.

50,000 $\text{cm}^{-1} M^{-1}$. Triplet-triplet energy transfer, which occurs by an exchange interaction, is appropriate for distances shorter than about 10 Å.^{18, 19}

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¹⁵ The observed transfer has been attributed to a dipole-dipole coulombic interaction rather than to an exchange interaction, because the latter mechanism would probably be too slow to account for the measured transfer rates. An order of magnitude estimate of the transfer rate for an exchange interaction in I, based on the rate expression of Inokuti, M., and F. Hirayama (*J. Chem. Phys.*, **43**, 1978 (1965)), yields a value between 1.1×10^4 and $7.7 \times 10^6 \text{ sec}^{-1}$, depending on whether the distance between the chromophores is taken as 10 Å (center-to-center) or 7 Å (closest approach). Since the observed rate in dioxane is $9.6 \times 10^7 \text{ sec}^{-1}$, it seems unlikely that the transfer occurs by an exchange interaction.

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