Energy Transfer in Fluorescent Derivatives of Uracil and Thymine

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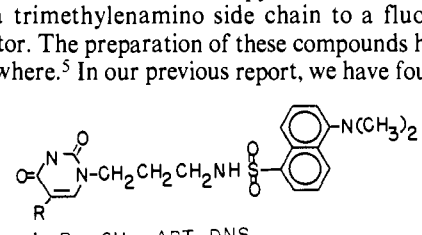
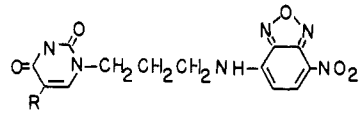
Abstract: In the bichromophoric compounds of uracil and thymine, I-IV, fluorescence of the dye moieties was sensitized efficiently by light absorbed in the pyrimidine moieties, the efficiencies ranging from ~30% in the dansyl derivatives (I and II) to 59 ~ 95% in the NBD derivatives (III and IV). In the uracil derivatives, II and IV, quenching of the uracil photohydration was also observed; the efficienty of quenching was about the same as the efficiency of fluorescence sensitization. If the mechanism of energy transfer is that of Forster dipolar interaction, the lifetimes of the donor states are indicated to be in the order of 10^{-10}, considerably longer than the reported lifetimes of the thymine and uracil fluorescent excited states. These experiments show that the excited state precursor for both fluorescence sensitization and photohydration is a singlet excited state of the uracil; they reduce the probability that a "hot" ground state of uracil is the precursor for photohydration.

The nature of the excited state of uracil, thymine, or cytosine, which is the precursor to photohydrate formation, and probably to formation of other photonucleophile addition products,[1] has been under study for some time.[1,2] This study has been hampered by the very low fluorescence yield of these bases and their nucleosides in aqueous solution at room temperature.[3] Attempts to study the excited states of these bases via excitation energy transfer to a fluorescent or phosphorescent acceptor has been unsuccessful. Although orotic acid derivatives and some nucleotides can be used to sensitize europium ion emission,[4a] this transfer has been found to be transfer within a complex[4b] and bases or nucleosides unable to complex with europium ion do not sensitize its emission. None of these bases or nucleosides were able to sensitize emission from biacetyl in aqueous solution.[4b] We have therefore resorted to intramolecular energy transfer in the effort to characterize the excited state precursors of uracil photohydration, in particular, and to this purpose we have synthesized the compounds I-IV, in which the pyrimidine is covalently bonded via a trimethylenamino side chain to a fluorescent energy acceptor. The preparation of these compounds has been reported elsewhere.[5] In our previous report, we have found that light energy absorbed in the uracil or thymine part of these molecules is efficiently transferred to the fluorescent acceptor part of the molecule.[6] We now report here an experimental study on excitation energy transfer of pyrimidines and its relevance to photohydration reaction.



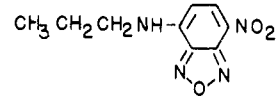
I R = CH_3 APT-DNS

II R = H APU-DNS

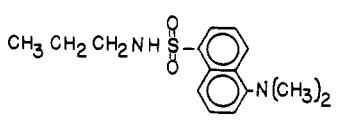


III R = CH_3 APT—NBD

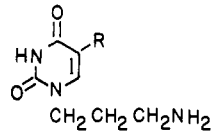
IV R = H APU—NBD



V PA-NBD



VI PA-DNS



VII R: $-CH_3$ APT

VIII R: -H APU

Experimental Section

Materials. Water was double distilled from acid dichromate. Absolute ethanol from U.S. Industrial Chemical Co., used as received, showed neither trace UV absorption above 230 nm nor any fluorescence. Acetonitrile from Matheson Coleman and Bell was refluxed with phosphorus pentoxide and distilled to remove traces of fluorescent impurities. Fisher reagent ethylene glycol was passed through alumina and distilled under reduced pressure before using. Rhodamine B, pontachrome blue black (PBBR), and *m*-nitro-*N*,*N*-dimethylaniline were purchased from Aldrich and used without further purification. Compounds I-VIII were prepared in our laboratory; detailed synthetic procedures and analytical data (including the absorption spectra) have been described elsewhere.[5]

Spectra. All absorption spectra were recorded on a Hitachi Perkin-Elmer (Coleman 124) or a Cary 118 spectrophotometer, with slits of either 0.5- or 2-nm bandwidth and slow scanning control. Fluorescence emission and excitation spectral studies have been done with Hitachi Perkin-Elmer MPF-3 spectrofluorophotometer equipped with ratio mode recording. For the recording of fluorescence excitation spectra, the excitation slit was set at 2 nm to give a bandwidth similar to that used in absorption recording.

Excitation Correction. Correction for variation in source-excitation monochromator combination in the fluorometer was made with either PBBR-aluminum chelate or rhodamine B as a reference material. The correction with PBBR was a dilute solution method where the absorption and excitation spectra of PBBR-Al chelate were compared.[7] Rhodamine B was also used as a correction standard; the procedure is more handy. A concentrated solution of rhodamine B in ethylene glycol (8 mg/mL) was used in a short path length cell (5 mm); emission at 640 nm was recorded with 2-nm excitation slit (24-nm slit in emission side) and a glass filter (Hitachi) cutting below 350 nm was placed between sample and emission monochromator to eliminate second-order scattered light showing up in emission. Correction curves obtained with the PBBR and rhodamine B systems generally agreed well at 240-400 nm. The correction factor thus obtained gave a good agreement between the anthracene excitation spectrum and absorption spectrum. The corrected excitation spectrum of VI, however, deviated significantly from its absorption spectrum, using the same correction curve. It is possible that the high optical density at 250 nm (Tables I and II) compared to the much lower OD at 330 nm combined with extremely low source energy in the 245-nm region could cause greater experimental error. On the other hand, the magnitude of the deviation (~30% at 245 nm) suggests that the fluorescence quantum yield of compound VI may not be constant over the entire absorption range. Therefore, calibration of the excitation spectra of the dansyl derivatives was done using VI itself as a reference; the absorption spectrum of VI was compared to the excitation spectrum of very dilute solution

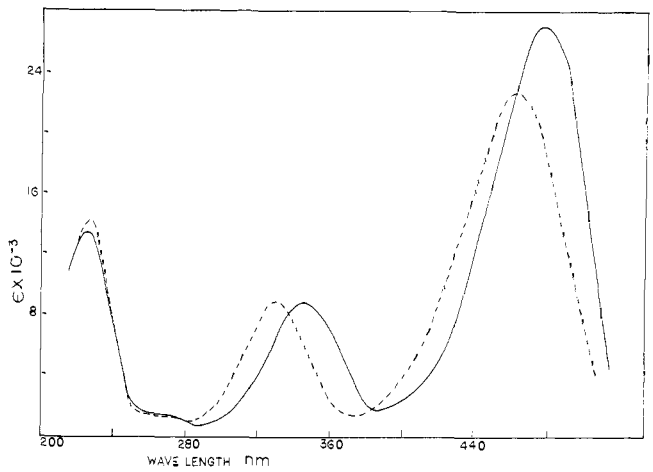


Figure 1. Absorption of acceptor fluorescent dye PA-NBD (V): 100% ethanol, - - -; 30% (vol) ethanol, —.

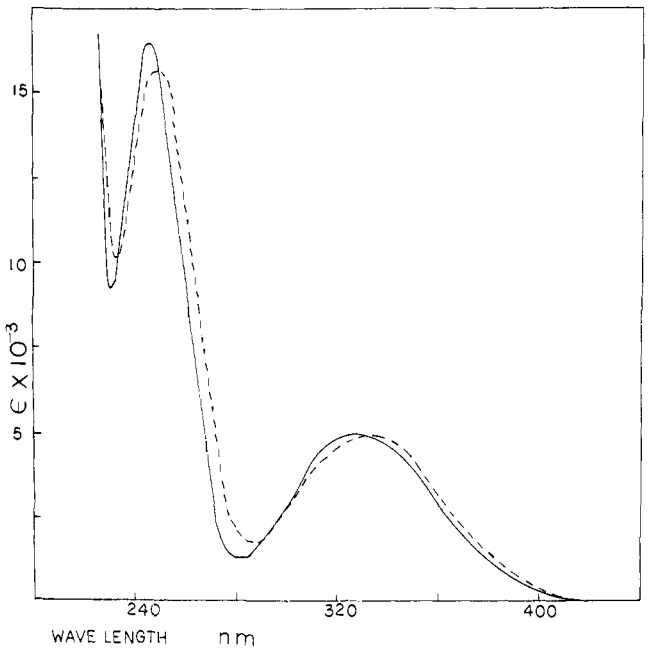


Figure 2. Absorption of acceptor fluorescent dye PA-DNS (VI): 100% ethanol, - - -; 30% (vol) ethanol, —.

(OD ~0.02 at 330 nm) in the same solvent and the calibration factors were calculated at 5-nm intervals.

Detector Correction. Correction of the detector sensitivity of the fluorescence spectrometer was made using the reported fluorescence spectra of quinine bisulfate, *m*-nitro-*N*,*N*-dimethylaniline, and PBBR-Al chelate.[7] The fluorescence spectra of the three reference compounds cover the wavelength range 400–600 nm; the fluorescence spectra of reference materials were recorded under the same conditions where the "standard" spectra were taken. The quantum yield of fluorescence was determined from the corrected fluorescence spectra of sample solutions using a quantum yield value of 0.52 for quinine bisulfate fluorescence in 0.1 N sulfuric acid (10^{-4} M).[8]

Determination of Molar Extinction Coefficients. Evaluation of the possibility of stacking interactions between the pyrimidine and dye parts of compounds I–IV required careful evaluation of the hypo- or hyperchromism of these four compounds. We have calculated the degree of perturbation on absorptivity by comparisons of the molar extinction coefficients at particular wavelengths. In the case of the dansyl compounds, I and II, simple use of the molar extinction coefficients is less reliable owing to the intense absorbance of the dansyl compounds at both of the λ_{max} values. In these cases the hypochromism was determined both from the extinction coefficient and also by integration of the absorption curves. Samples for the precision measurements of the extinction coefficients were weighed on a microbalance, dissolved in the appropriate solvents, and the absorbances measured on a Cary 118 spectrophotometer with a precision of 0.005 absorbance units.

Table I. Absorption, λ_{max}

Compd	nm: 30% (vol) ethanol, aqueous	nm: Ethanol	nm: Aceto-nitrile
PA-NBD (V)	344	332	332
	478	464	462
APT-NBD (III)	340	330	330
	475	460	458
APU-NBD (IV)	340	330	330
	474	460	460
PA-DNS (VI)	246	250	251
	328	335	336
APT-DNS (I)	250	255	257
	330	335	337
APU-DNS (II)	250	255	256
	330	335	336
APC-DNS (IX)	245	249	248
	330	335	335

Table II. Molar Extinction Coefficients, Hypochromism

Compd	245 nm	250 nm	270 nm
APT (VII)	2 938	4 290	9 019
PA-NBD (V)			2 076
APT-NBD (III)			10 834
V + VII			11 095
% H[a]			2.4
PA-DNS (VI)	15 450	14 450	4 003
APT-DNS (I)	17 154	17 772	12 781
VI + VII	18 388	18 740	13 022
% H[b]		3.4	

[a] % H = $[1 - \epsilon_{DA}/(\epsilon_D + \epsilon_A)] \times 100$. [b] % H calculated from integrated absorption, 230~300 nm, instead of extinction coefficient in the equation.

Uracil Photohydration. The determination of relative rates of photohydration for different uracil derivatives were carried in an irradiation system composed of a high-pressure mercury lamp (GE type B6), Bausch & Lomb monochromator (180~400 nm), and an appropriate optical system. The 3-mL uracil solutions were magnetically stirred during irradiation. The absorbances of all samples at irradiating wavelength (280 nm) were equal and conversions of the starting materials were <10% (25–35 min). Deaeration with nitrogen had no effect on the photoreaction. The pH of sample solutions was adjusted to 6.5 using either 0.1 N HCl or potassium carbonate solution. For compounds II and IV, correction for the inner filter effect of acceptor absorption at irradiating wavelength was made, using the optical densities of separate chromophores.

Results

Absorption and Emission Spectra. The absorption spectra of V and VI together with donor–acceptor paired compounds have been examined in different solvents, and the data are shown in Table I. Absorption spectra of both VII and VIII are little affected by solvent change and are not included in the table. When the water content in aqueous ethanol solvent is increased from 0 to 70% (vol), the absorption maxima of V in the visible and UV are red shifted by 12~14 nm (Figure 1); absorption maxima of VI are blue shifted (4–7 nm) (Figure 2 and Table I). For π,π^* transitions, a red shift is expected for increase of solvent polarity whereas, n,π^* bands are expected to blue shift. Chen and other workers reported that fluorescence of dansylsulfonamides is a π,π^* transition.[9] Both UV bands of VI are intense (ϵ_{245} 15 000, ϵ_{328} 4350) and the fluorescence band is red shifted in polar solvent in accordance with a π,π^* transition (Table III). We can not explain the small blue

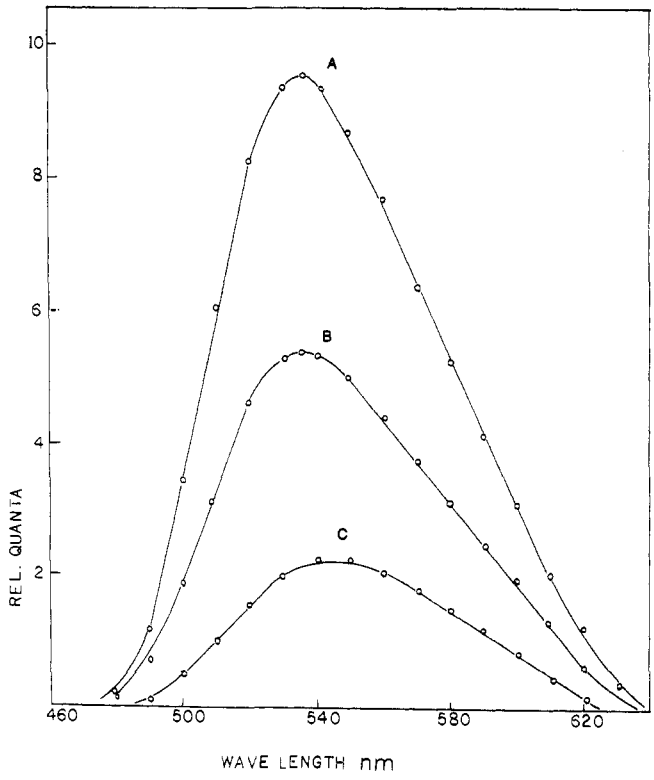


Figure 3. Fluorescent spectra in different solvents: curve A in acetonitrile, curve B in ethanol, curve C in 30% (vol) ethanol in water. APT-NBD (III), 6-nm band width on both excitation (340 nm) and emission slit.

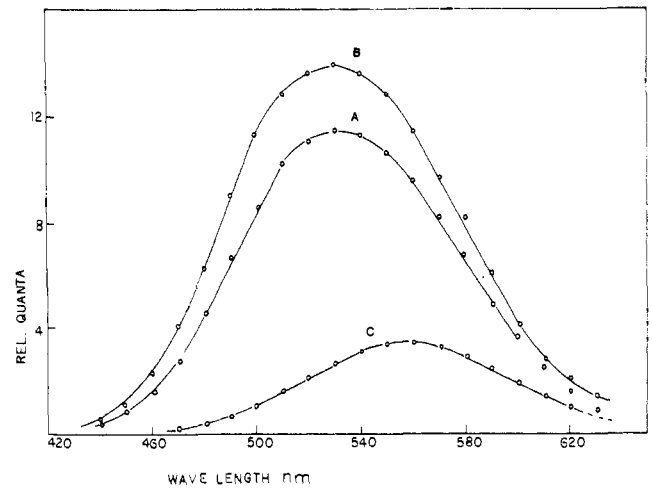


Figure 4. Fluorescent spectra in different solvents: curve A in acetonitrile, curve B in ethanol, curve C in 30% (vol) ethanol in water. APT-DNS (I), 6-nm band width on both excitation (330 nm) and emission slit.

Table III. Fluorescence Quantum Yield Φ_f and λ_{max} [a]

	APT-NBD [b]		APT-DNS [c]	
Solvent	λ_{max}	Φ_f	λ_{max}	Φ_f
Ethanol	535	0.32	530	0.38
Acetonitrile	535	0.57	533	0.29
30% ethanol	540	0.12	558	0.05

[a] Concentrations $\simeq 5 \times 10^{-6}$ M (OD excitation ≤ 0.05). [b] Excitation at 340 nm with slit bandwidth 6 nm on both excitation and emission side. [c] Excitation at 330 nm with slit bandwidth 6 nm on both excitation and emission side.

Table IV. Quantum Yield of Intramolecular Energy Transfer, [a] Φ_{et}

Compd	30% (vol) ethanol	Aceto-nitrile	Ethanol
APT-NBD (II)	0.94	0.92	0.94
APU-NBD (III)	0.60	0.59	
APT-DNS (IV)	0.38	0.32	0.37
APU-DNS (VI)	0.31	0.19	0.19
APC-DNS (VII)	0.31	0.19	0.21

[a] Concentration = 5×10^{-6} M (OD excitation ≤ 0.05).

shift in the DNS absorption bands. The combination of absorption blue shift with fluorescence red shift means that the already large Stoke's shift of DNS ($\approx$200 nm) is even larger in polar solvents. Discussion of this matter in detail is beyond the scope of our report, but this may well be an indication that equilibrium state of both ground and excited state is more stabilized in polar solvent than the corresponding Franck-Condon states.

Kenner and Aboderin[10] found both fluorescence and absorption of benzylamino-NBD derivatives were red shifted in polar solvents and the similar trend is true in our amino-NBD derivatives, as shown in Figures 1 and 3 and Table 1. The spectra in Figures 3 and 4 and the quantum yield of fluorescence (Table III) show that the fluorescence intensities of both NBD and DNS derivatives are very sensitive to solvent polarity and are less in more polar solvents.

Efficiency of Energy Transfer. In all NBD- and DNS-pyrimidine bichromophoric compounds (I-IV), there is no detectable fluorescence from the pyrimidine moiety[11] where fluorescence emission at 340 nm of $\sim 5 \times 10^{-4}$ quantum yield might be detected. Therefore, the energy transfer was monitored solely by observation of the sensitized fluorescence of NBD and DNS part. As the excitation spectrum in Figure 5 indicates, the fluorescence intensity of APT-NBD from excitation at 270 nm (thymine moiety) is obviously larger than can be accounted for by excitation of the NBD moiety alone. Since the acceptor chromophore (NBD moiety) also absorbs light at the λ_{max} of pyrimidine, the efficiency of excitation transfer (Φ_{et}) of APT-NBD (III), as an example, must be expressed as follows (A = absorbance):

$$\Phi_{et} = \frac{(\text{fl. excitation})_{\text{APT-NBD}} - A_{\text{PA-NBD}}}{A_{\text{APT-NBD}} - A_{\text{PA-NBD}}} \qquad (1)$$

When the fluorescence excitation curves of PA-NBD and APT-NBD are normalized at the 340-nm peak, the numerical value of Φ_{et} for APT-NBD can be calculated from areas under excitation and absorption curves illustrated in Figure 5.

$$\Phi_{et} = \frac{\text{area, curve 2} - \text{area, curve 3}}{\text{area, curve 1} - \text{area, curve 3}} \qquad (2)$$

Values of Φ_{et} for compounds I-IV were similarly calculated from the appropriate spectra. The integration limits for the area measurement were 250-300 and 230~290 nm for NBD and DNS derivatives, respectively.[12] For determination of energy-transfer efficiency, absorbance of the samples was kept below 0.05 (concentration 5×10^{-6} M) to eliminate the possibility of intermolecular quenching. Values of the energy transfer efficiencies thus determined are shown in Table IV. The intramolecular energy transfer is remarkably efficient in all cases, ranging from 0.1 to 0.94. In the case of the NBD derivatives (III, IV), the absorption of the NBD moiety is flat with low intensity at 270 nm where the pyrimidine moiety absorbs. Thus the sensitization is easily visible, and the quantitative estimation of Φ_{et} is reasonably accurate. In the case of the dansyl derivatives (I, II), the sensitization is qualitatively apparent but the quantitative estimation was more difficult owing to intense absorption of DNS (Figure 6); for this reason the figures for the quantum yields of energy transfer for I and

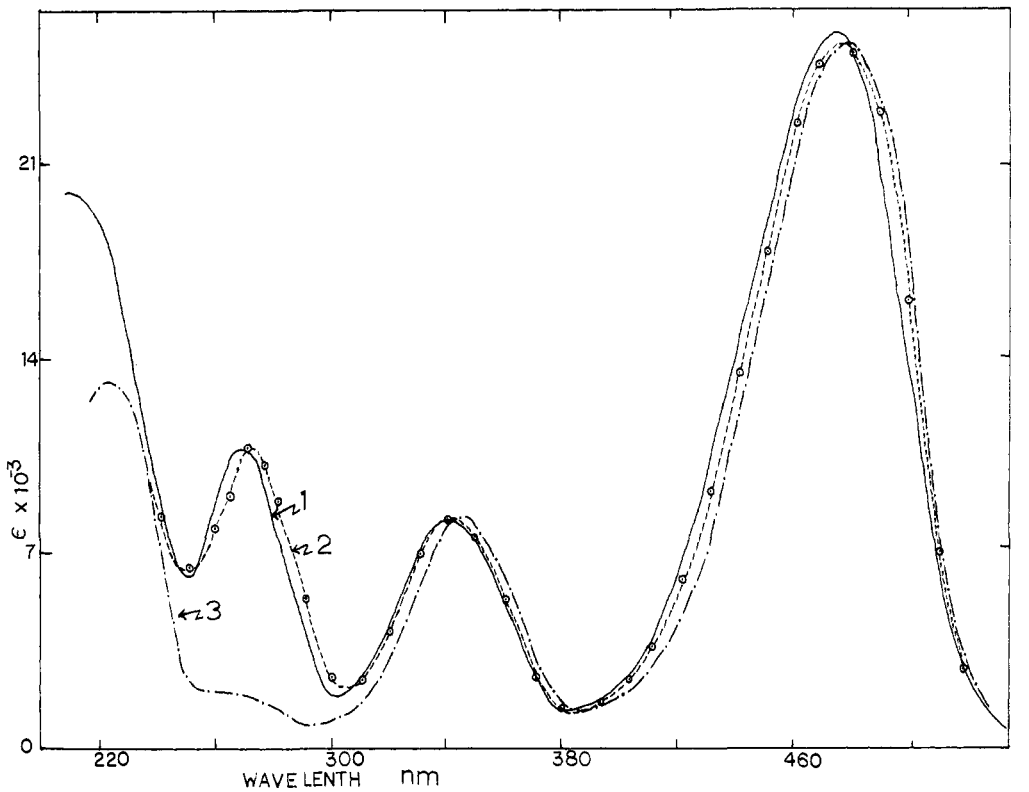


Figure 5. Absorption and fluorescent excitation spectra of biochromophoric thymine derivative APT-NBD (III): curve 1 (—) absorption; curve 2 (⊙- -⊙) fluorescence (540 nm) excitation; curve 3 (- · -). Fluorescence excitation of acceptor (PA-NBD, V). Absorption and fluorescence excitation slits 2 nm.

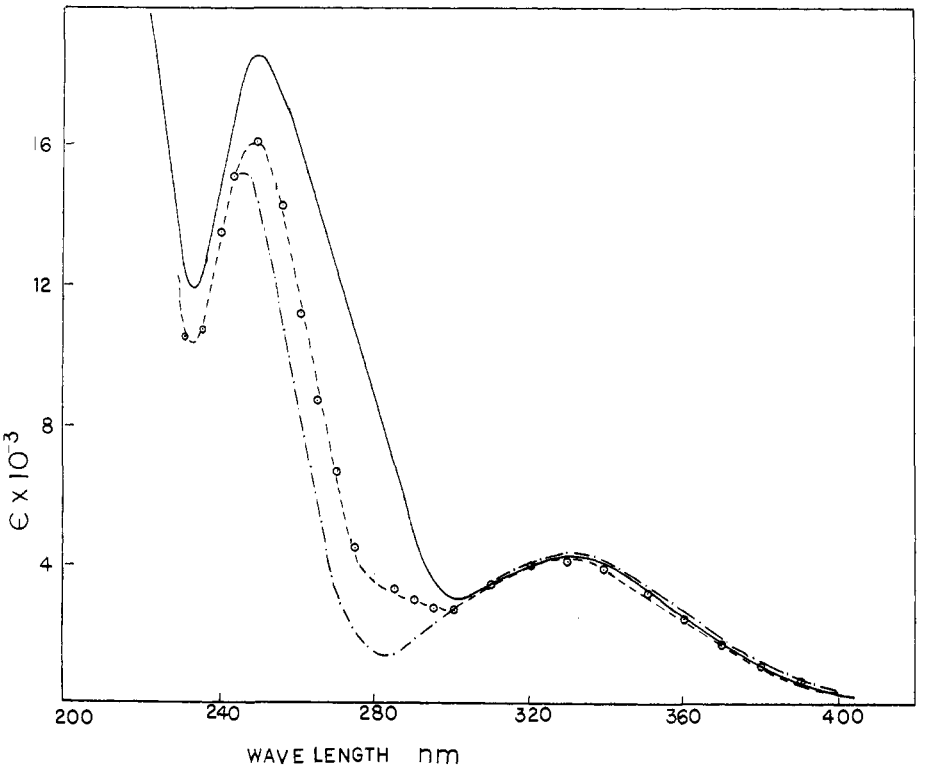


Figure 6. Absorption and fluorescent excitation spectra of biochromophoric thymine derivative APT-DNA (I), (—) absprotion, (⊙- -⊙) fluorescence (540 nm) excitation, (- · -) fluorescence excitation of acceptor (PA-DNS, VI). Absorption and fluorescence excitation slits 2 nm.

II in Table III are less accurate than those for III and IV. Energy transfer in III and IV appears to be more efficient than that in I and II. Thymine derivatives appear to exhibit a higher efficiency for energy transfer than uracil derivatives. The quantum yield of fluorescence for NBD derivatives is invariant over the excitation wavelength range, 250–500 nm, but that of the dansyl derivatives appears to be a little lower for 250-nm excitation than for 330-nm excitation (cf. Experimental Section).

The intensity of fluorescence of these compounds could not

Table V. Rates for the Energy Transfer

Compd	Φ_{et}	Φ_h^0 ($\times 10^4$)[a]	k_f, s^{-1} [a]	R, Å[b]	k_{et}, M^{-1} s^{-1} [c]
APU-NBD	0.6	1.2	1.2×10^8	6.7	1.62×10^{12}
APT-NBD	0.94	1.7	1×10^8	4.8	9.9×10^{12}

[a] Reference 18. [b] Calculated from eq A in ref 17. [c] Forster transfer rate from eq 4.

be enhanced by acetone sensitization, though acetone is a known triplet donor and sensitizer of triplet-state formation in uracil and thymine.[2]

There was no evidence of intermolecular excitation energy transfer between VII and V in mixtures of these two up to 10^{-3} M in concentration. With samples of such high optical density, emission from the front surface area of the cuvette was monitored instead of emission from the core of the sample volume. Since 10% or more energy transfer could be detected by our procedure, the absence of any observable fluorescence sensitization in these mixtures sets an upper limit of $\sim 10^{-8}$ s for the lifetime of any donor state of VII capable of transferring energy to V assuming that such energy transfer would be diffusion limited.[13]

Donor–Acceptor Interactions. The absorption spectra of the individual donor (VII) and acceptor (V) chromophores remained unchanged in the compound combining these chromophores, APT-NBD (III), and the fluorescence spectrum of III coincides with that of the fluorophore alone (V). The observed hypochromism in absorption for the chromophores—e.g., VII + V vs. II—was very low (~2% as shown in Table II). In some other systems, such as the bispyrimidines reported by Leonard and Cundall,[14] small stacked populations caused as much as 10% hypochromism; so the very small hypochromism observed in III means there is probably no extensive interaction between the two component chromophores in APT-NBD (III). The very small interactions between the chromophores in III and IV might be detectable by ^{1}H NMR measurements[15] except that the solubility of these two compounds in water is so low that conventional ^{1}H NMR measurements were not possible; FT techniques with very large samples can be considered for each measurements, but are not available to us at present. The ^{1}H NMR spectrum of APT-NBD (III) in trifluoroacetic acid (δ_{CH_3} 2.00, $\delta_{6\,H}$ 7.57) was found to be very similar to that of APT (δ_{CH_3} 2.00, $\delta_{6\,H}$ 7.55). Even though ^{1}H NMR data in such a solvent may not be meaningful for observations in aqueous ethanol, it should be noted that the efficiency of energy transfer in both solvents was about the same.

The fluorescence efficiency of APT-NBD was less in 1/1 (v/v) mixtures of ethanol/ethylene glycol compared with that in pure ethanol, but the efficiency of energy transfer was virtually the same in both solvent systems. This means that the efficiency of energy transfer is viscosity independent. The transfer efficiency was also the same in 30% (v/v) ethanol/water, water, ethanol, acetonitrile, and trifluoroacetic acid. In 30% (v/v) ethanol/water, the energy transfer efficiency was nearly constant over a pH variation of 1–9.5; at pH values higher than 9.5, the NBD moiety of APT-NBD became unstable since a reversible change in absorption spectrum took place at pH values >9.5.

Close molecular interactions—particularly those of the stacking type most likely in our systems (hydrophobic interactions)—should be influenced by the change of solvent properties, such as polarity, protic character, hydrogen bonding character, and viscosity, which we have employed. The lack of such effect suggests strongly that such close molecular interactions are not extensive—if present at all—in the compounds III and IV.

Resonance Energy Transfer. In the absence of indications for the existence of a preferred stacked conformation which would allow assignment of the energy-transfer mechanism to an exchange mechanism and since transfer by the trivial mechanism is ruled out by the very low fluorescence efficiencies of the donor moiety, then the most probable mechanism for the energy transfer is the Forster dipole–dipole interaction which operates efficiently over the distances involved (~10 Å). As will be discussed below, if the mechanism of the energy transfer is a Forster mechanism, then the calculated rates of energy transfer provide information about the possible lifetimes of the donor moieties in III and IV.

The compound APT-NBD (III) meets the several spectroscopic requirements for Forster energy transfer. The NBD moiety has intense absorption at 330 nm where the fluorescence maxima of uracil and thymine lie; the pyrimidine moiety can be selectively excited; the pyrimidine moiety is nearly nonfluorescent, whereas the NBD moiety has strong fluorescence at a wavelength well separated from the donor emission wavelength (Table III); and the most probable distance between donor and acceptor moieties is not short for Forster dipole–dipole interaction.

The Forster equation[16] provides a value for R (donor–acceptor distance) and for the rate constant for energy transfer (k_{et}), according to eq 3 and 4[17]

$$R = \left[(8.8 \times 10^{-28}) \frac{K^2}{n^4} \Phi_f \Omega \left(\frac{1 - \Phi_{et}}{\Phi_{et}} \right) \right]^{1/6} \quad (3)$$

$$k_{et} = (8.8 \times 10^{-28}) \frac{K^2}{n^4} \Omega \frac{K_f}{R^6} \quad (4)$$

where Φ_f is the donor fluorescence quantum yield in the absence of energy transfer, Ω is the overlap integral for donor fluorescence and acceptor absorption, K is the orientation factor for the donor and the acceptor, n is the refractive index of the medium, and Φ_{et} is the quantum efficiency of the energy transfer. For APT-NBD, $\Phi_f = 1.75 \times 10^{-4}$;[18] $\Omega = 6.9 \times 10^{-12}$ cm^6 (determined by the methods described by Conrad and Brand);[19] $K^2/n^4 = 0.21$ for aqueous solution, assuming random orientation ($K = 2/3$); and $\Phi_{et} = 0.94$ (Table IV). The spectral overlap of thymine fluorescence[20] and NBD absorption is shown in Figure 7. Using these values for the parameters, the calculated value for R is 4.8 Å.

Using the value of 4.8 Å for R, and eq 4, the calculated value for k_{et} is 9.9×10^{12} s^{-1} (Table V) (k_f is the rate constant for donor fluorescence).[18]

The uncertainties about use of these equations revolve mostly about the value for the orientation factor, K. The independence of the energy transfer efficiency from variation in solvent parameters, discussed above, militates against a preferred orientation of the donor–acceptor moieties and supports choice of a random orientation; molecular model studies show that there is no steric hindrance for rotation along the carbon chain. Diffusional effects have been shown to result in modification of the Forster equations,[21] but in our cases the lack of a viscosity effect on energy-transfer efficiencies shows that diffusional effects are not important.

The results of these calculations lead to an unrealistically high value for the rate constant for energy transfer and an unrealistically low value for R, and in both case the source of these unreal values can be traced to the use of the reported values for lifetime of the thymine/uracil excited singlet state and the reported quantum yields of fluorescence.[18] Both the

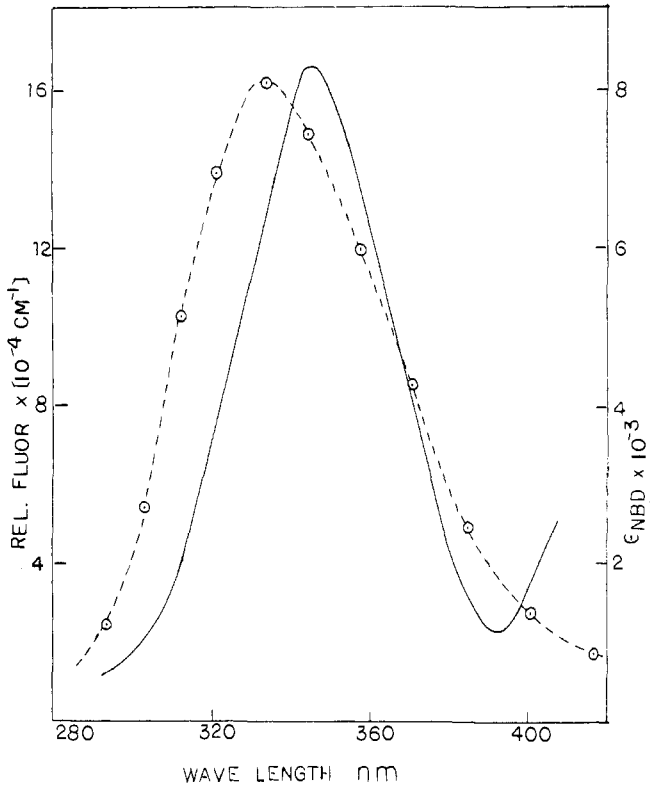


Figure 7. Overlap of thymine fluorescence and absorption of PA-NBD. Thymine fluorescence[20] (- - -); absorption of PA-NBD (—).

values for the energy-transfer rate constant and the value for R become more realistic if a longer lifetime is allowed for the donor excited state. Use of models predicts a distance for 9.8 Å for the fully extended four atom chain in APT-NBD (III). Full extension of the chain is supported by the lack of solvent effects on the energy-transfer efficiency and by analogy with the dansyl-tryptophan system.[18] For a fully extended chain where the donor-acceptor distance is 9.8 Å, a donor lifetime of 10^{-10} s is required for an energy-transfer efficiency of 94%.[22] A candidate for such an excited state of long lifetime is an excited state of an enolic isomer,[23] but this postulate could not explain the high efficiency of energy transfer since the concentration of such an isomer must be very low; such an excited state is not likely a precursor for photohydration since 2-keto-4-ethoxy-1,2-dihydropyrimidine and 2,4-diethoxypyrimidine do not undergo photohydration.[24]

Other possibilities for a long-lived excited state would be an n,π^* state, or a state in which n,π^*, and π,π^* character are mixed.

The failure of thymine and uracil to fluoresce efficiently[3] has always presented the enigma of how such fast internal conversion can take place from an excited state which is energetically well separated from the ground state (85 kcal/mol). Polarization studies on the fluorescence of a single crystal pyrimidines has led to the suggestion that both $^1(\pi,\pi)^*$ and $^1(n,\pi)^*$ states are involved in the emission.[25] The extremely short lifetime and weak fluorescence is not a typical characteristic of a $^1(\pi,\pi)^*$ state, but is a common phenomena among compounds which contain $^1(n,\pi)^*$ levels in the vicinity of lowest excited levels of $^1(\pi,\pi)^*$ states.[26] It has been suggested that mixing π,π^* with n,π^* states is a cause of radiationless decay resulting in broad band and short excited lifetime.[27] Although direct spectroscopic evidence is not available, the extremely short lifetime may be an evidence that $^1(\pi,\pi)^*$ is not the lowest excited singlet state or a well-isolated state in thymine and uracil.

Quenching of Uracil Photohydration. Quantum yields for photohydration of 1,3-dimethyluracil (DMU) (a secondary actinometer)[28] in water and in 20% (v/v) ethanol-water are shown in Table VI. APU (VIII) undergoes similar photohydration with a slightly higher yield, but the uracil residue in APU-NBD is photohydrated with a greatly reduced yield—the photochemical reaction of the uracil moiety is apparently quenched by the NBD moiety of the molecule. Photohydration of the uracil in the APU-DNS (II) molecule is also quenched, although to a less extent.

Table VI. Efficiency of Uracil Photohydration (10^{-4} M Solutions)

Compd, solvent	Rel rate	Quantum yield
1,3-DMU, water (10^{-4} M)	1.00	4×10^{-3} [a]
1,3-DMU, 20% EtOH (vol)	0.81	3.23×10^{-4}
1-APU, 20% EtOH (vol)	1.31	5.23×10^{-3}
APU-NBD, 20% EtOH (vol)	0.31	1.22×10^{-3}

[a] Literature value for 10^{-4} M DMU = 4×10^{-3}.[28]

Scheme I

$$D \xrightarrow{\nu} D^* \quad \text{(a)}$$

$$D^* \rightarrow D^0 + \Delta \quad (k_{rd}') \quad \text{(b)}$$

$$D^* \rightarrow D^0 + h\nu'' \quad (k_f') \quad \text{(c)}$$

$$D^* + H_2O \rightarrow DH_2O \quad (k_h') \quad \text{(d)}$$

$$D^* + A \rightarrow D^0 + A^* \quad (k_{et}' = k_{et}[A]) \quad \text{(e)}$$

$$A^* \rightarrow A^0 + \Delta \quad (k_{rd}) \quad \text{(f)}$$

$$A^* \rightarrow A^0 + h\nu \quad (k_f) \quad \text{(g)}$$

A quantitative measure of this quenching Φ_{hq} can be obtained by assuming that the quenching of the photohydration in these compounds is the result of the quenching of an excited-state precursor of the photochemical reaction; i.e., energy is transferred from the pyrimidine excited state to the dye moiety of the molecule according to the simplified kinetic scheme shown in Scheme I where D, A, D*, and A* denote ground and excited state of donor and acceptor moieties and k_{rd}', k_f', k_h', and k_{et}' are rate constants of radiationless decay, fluorescence, photohydration, and energy transfer of donor excited state and k_{rd} and k_f are radiationless decay and fluorescence rate constant of acceptor. The efficiency of the energy-transfer process can then be expressed by eq 5. The quenching of photohydration (Φ_{hq}) will also represent the energy transfer as shown in eq 6 where Φ_h and Φ_h^0 are quantum yield of photohydration in the presence and absence of the quencher, respectively.

$$\Phi_{et} = k_{et}'/(k_f' + k_{rd}' + k_h' + k_{et}') \quad (5)$$

$$\Phi_{hq} = 1 - (\Phi_h/\Phi_h^0) = k_{et}'/(k_f' + k_{rd}' + k_h' + k_{et}') = \Phi_{et} \quad (6)$$

$$\Phi_h^0 = k_h'/(k_f' + k_{rd}' + k_h')$$

$$\Phi_h = k_h'/(k_f' + k_{rd}' + k_h' + k_{et}')$$

Comparison of quantum yield of the photohydration quenching and of the energy transfer is shown in Table VII; the values are remarkably similar.

Discussion

The following discussion will be mainly confined to the results obtained in the APU-NBD (IV) and APT-NBD (III) systems, since in those cases the existence and quantitative yields of fluorescence sensitization are unambiguous; the

Table VII. Quenching of Photohydration[a]

Compd	Quenching of photohydration,[b] Φ_{hq}	Quantum yield of energy transfer for fluorescence sensitization (from Table IV), Φ_{et}
APU-NBD	0.76 (±0.05)	0.60 (±0.05)
APU-DNS	0.34 (±0.05)	0.31 (±0.03)

[a] 30% ethanol solution. [b] Quenching = $1 - (\Phi_h/\Phi_h^0)$. Φ_h^0 and Φ_h are photohydration quantum yields in absence and presence of energy transfer (eq 6), respectively.

quantitative estimation of the extent of fluorescence sensitization is more difficult for the dansyl derivatives.

In APU-NBD the quantum yield of uracil photohydration is markedly reduced compared with the yield of the process in APU (VII) and the extent of photohydration quenching is the same as the excitation energy transfer measured by fluorescence sensitization in the same molecule (Table IV). This concomitant quenching of photohydration with the intramolecular energy transfer supports the proposed reaction scheme a-g, particularly the processes d and e which postulate that the energy-transfer and the photohydration process compete for the same excited-state precursor. The lack of acetone sensitization of the energy-transfer process and the high observed efficiency of the energy transfer process rule out the donor triplet state as the precursor, since the reported quantum yield of intersystem crossing in thymine/uracil is very low (10^{-3}).[18]

The important conclusion of the work presented here is that both photohydration and fluorescence sensitization in APU-NBD (IV) have a common *excited singlet* state as precursor. This conclusion does not entirely eliminate an intermediate "hot" ground state[29] as immediate precursor for the photohydration process (derived from the excited singlet-state precursor), but the probable very short lifetime for such an intermediate upper vibration state and the observed very similar extent of photohydration quenching and fluorescence sensitization in APU-NBD make such an intermediate state unlikely.

Another outstanding fact in the case of the NBD derivatives is the high efficiency of energy transfer. This high efficiency is unexpected in view of the reported extraordinarily short lifetimes of the excited donor pyrimidines, $\sim 10^{-12}$ s. The most efficient energy transfer seen was in APT-NBD with a quantum yield of transfer of 94%—an unprecedented efficiency for quenching of a uracil (or thymine) excited singlet state.

The evidence presented here suggests that the mechanism of fluorescence sensitization is that of a Forster transfer, that the donor excited singlet state has a longer lifetime than that reported for the fluorescent singlet states of thymine and uracil, and that there is little evidence for the existence of a stable preferred conformation of donor-acceptor moieties which would favor exchange interaction for energy transfer.

However, we do not feel that the assumption of a Forster transfer mechanism is unassailable. Conformations which bring the donor and acceptor parts close enough together for energy transfer by the exchange mechanism may occur frequently enough in a random fashion to result in some fractions of the energy transfer to occur by the exchange process yet infrequently enough to be undetectable by detection methods which we have employed. Energy transfer rates of 10^{12} s^{-1} estimated in some aromatic crystals (benzene) have been ascribed to moderate exchange interaction 8-12 cm^{-1},[30] which may not be detected in low resolution spectra. It should be remembered, however, that in our compound, APT-NBD, the energy gap between donor and acceptor is >10 400 cm^{-1} (30 kcal/mol) compared with the near 0 energy gap in these aromatic hydrocarbon crystals. Furthermore, our systems are much more loose; the donor-acceptor parts are not held in place by crystal field forces. The best way to explore this possibility would be to replace the flexible chain in our compounds with a more rigid spacing link, such as a steroid skeleton; such an endeavor is in progress in our laboratory.

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(13) A general expression of a bimolecular quenching of the excited state, $(D^0/D) = 1 + k_q\tau_D[Q]$ is reduced to $0.11 = k_q\tau_D[Q]$ for 10% quenching. D^0 *and* D = donor fluorescence in the absence and presence of quencher, respectively, k_q = rate of constant of quenching, [Q] = quencher concentration, and τ_D = donor lifetime in the absence of quenching. If $k_q = 10^{10}$ (diffusion rate) and $[Q] = 10^{-3}$ mol/L, then $\tau_D = 1.1 \times 10^{-8}$ s.
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$$R_0 = [8.8 \times 10^{-28}(k^2/n^4)\Phi_f\Omega]^{1/6} \quad \text{(A)}$$

$$\Phi_{et} = \frac{(1/\tau)(R_0/R)^6}{(1/\tau) + (1/\tau)(R_0/R)^6} = \frac{(R_0/R)^6}{1 + (R_0/R)^6} \quad \text{(B)}$$

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