TIME DEPENDENCE OF TRIPLET-SINGLET EXCITATION TRANSFER FROM COMPACT POLY rA TO BOUND DYE AT 77 K

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ABSTRACT The nonexponential phosphorescence decay of a highly folded form of polyriboadenylic acid (poly rA) with noncovalently bound dye is explained by a novel application of a well-known theory of electronic excitation transfer based on the Förster mechanism. This theory, originally used to describe singlet-singlet energy transfer from donor molecules to an acceptor in a solution, is here applied to the transfer of triplet excitation from the adenine (in poly rA) to the singlet manifold of either of the bound dyes, ethidium bromide or proflavine. New experimental data are presented that allow straightforward theoretical interpretation. These data fit the form predicted by the theory, $U(t) \exp(-Bt^{1/2})$, where $U(t)$ is the decay of the poly rA phosphorescence in the absence of dye, for a range of relative concentrations of either dye. The self-consistency of these theoretical fits is demonstrated by the proportionality of B to the square root of the Förster triplet-singlet overlap integrals for transfer from poly rA to each of the dyes, as demanded by the theory. From these self-consistent values of B, the theory enables one to deduce the mean packing density of nucleotides in this folded poly rA, which we estimate to be $\sim 1\ \mathrm{nm}^{-3}$. We conclude that some variation of the method described here may be useful for deducing packing densities of nucleotides in other compact nucleic acid structures.

INTRODUCTION

Polyriboadenylic acid (poly rA) collapses into a folded conformation when frozen in an aqueous medium containing sodium acetate and glucose (1). In this conformation both singlet and triplet electronic excitation energy of the adenine bases in the polymer can efficiently transfer to singlet states of certain bound dyes, with $r \equiv$ [dye]/[base] $> 0.2\%$, by a direct process assumed to be the Förster mechanism (1, 2). Triplet-triplet transfer from base to dye is not expected to be important in these systems because of the quite limited stacking in the folded polymer (1).

In previous work (1, 2) we showed that singlet and triplet excitation transfer to dye excited-singlet states occurs with either ethidium bromide or proflavine bound to poly rA in frozen glucose-acetate medium. We calculated Förster overlap integrals and estimated Förster critical distances for both the singlet-singlet and singlet-triplet transfers in these systems (1). We thus became interested in developing a new method for deriving system structural parameters from energy transfer kinetics in compact nucleic acid systems. This method does not require covalent bonding of the dye to the nucleic acid (3). It takes advantage of the natural energy-transfer orientational-averaging properties of a highly folded nucleic acid (see Theory). A possible disadvantage of the method, that it requires measure-

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ments to be made at cryogenic temperatures, can perhaps be overcome by appropriate choice of a second bound dye to serve as excitation energy donor. The specific nucleic acid and solvent studied here were originally chosen for different reasons (2), but are also significant for this problem because of the highly efficient energy transfers observed with bound dye.

Because of an anomalous loss of transfer efficiency at relatively large values (≥ 0.01) of molar dye-to-adenine-base ratio r, especially for triplet transfer to bound proflavine (reference 1; also see Discussion), it is difficult to fit theoretical models of energy transfer to the steady-state data and thereby to derive structural information. Time-dependent measurements are better for this purpose because potentially much more data can be obtained for small values of r, and information can be derived for fixed r. Although some time-dependent data were taken in earlier work (1), an experimentally simple method was used that makes detailed theoretical analysis very difficult, so we adopt a different procedure here (see Methods).

In this paper we present the results of measurements of the time course of the phosphorescence decay of compact poly rA with various concentrations of bound ethidium bromide or proflavine at 77 K. The decay of phosphorescence of the donor (poly rA) is measured in preference to the sensitized delayed fluorescence of the acceptor (bound dye) for ease of theoretical analysis, which is carried out with a novel application of a well-known theoretical energy transfer model (see Theory). Using this model, we fit the time dependence of the decay due to energy transfer, verify that the transfer is by a one-step Förster process, and deduce the values of associated parameters. This includes an estimate of the volume per nucleotide in the compact structure.

THEORY

The theoretical model we use was first proposed by Förster (4) and later generalized by Eisenthal and Siegel (5). This model was originally used to describe singlet-singlet excitation transfer from a donor molecule to acceptor molecules in solution. Its two principal assumptions are that there is irreversible Förster transfer from each donor to acceptors, and that donors and acceptors are distributed randomly in position and orientation. The first assumption is well satisfied by our system, and the second one approximately so[1] if the individual poly rA strands are sufficiently compact. We apply this theoretical model to energy transfer from a donor triplet state to acceptor singlets. This involves no new theoretical result, because both singlet-singlet and triplet-singlet transfer can proceed via the Förster mechanism (6).

According to this theory, the time decay of the donor excited state in response to instantaneous excitation is (5,7)

$$S(t) = S_0 \exp[-(t/\tau) - \sqrt{\pi}\kappa' c(t/\tau)^{1/2}], \tag{1}$$

[1] This remains true in spite of the small deviation from the assumption that results, for triplet-singlet transfer, from the prior shorter-range singlet-singlet transfer (see Discussion).

where τ is the unquenched donor state lifetime and c is the reduced concentration,

$$c = (4\pi/3)\bar{R}_0^3 c_A. \tag{2}$$

In Eq. (2), c_A is the acceptor concentration (molecules/unit volume) and the Förster critical distance is given by

$$\bar{R}_0^6 = (2/3)\phi I_{ov}/n^4, \tag{3}$$

where ϕ is the emission quantum yield of unquenched donor state per excited donor state (phosphorescence yield per triplet formed for triplet-singlet transfer), n is the refractive index of the medium, and I_{ov} is the normalized spectral overlap integral of donor emission with acceptor absorption,

$$I_{ov} = 8.785 \times 10^{17} \int F_D(\nu)\epsilon_A(\nu)\, d\nu/\nu^4 \text{ (in nm}^6\text{)}. \tag{4}$$

Here, $\epsilon_A(\nu)$ is the acceptor molar extinction coefficient on a wave number scale and $F_D(\nu)$ is the normalized donor emission (8).

The $\sqrt{t}$-dependence and the constant κ' that appear in Eq. 1 arise from the theoretical assumption of fixed, random positions and orientations of the donor and acceptor dipoles. The constant has the value $\kappa' = 0.8458$ (reference 7). (It is incorrectly calculated to be unity in reference 5.) The quantity κ' should not be confused with the orientation factor κ^2 that appears in the expression for Förster transfer from one donor to one acceptor. As is well-known, κ^2 has the mean value 2/3 for rapidly rotating dipoles. It is merely convention (5,7) in this theory to define $\bar{R}_0$ with the explicit factor 2/3. The $\sqrt{t}$-dependence only arises in three dimensions, i.e., only if an acceptor receives energy from donors randomly arrayed about it in all three spatial dimensions. If donors and acceptors are confined to a plane, it is easily seen from the formalism of reference 4 that $t^{1/2}$ becomes $t^{1/3}$; if they are confined to a straight line it is seen to be $t^{1/6}$. (In any number of dimensions, if donors and acceptors are arrayed in some regular fashion, rather than a $\sqrt{t}$-dependent term in the exponent, there is usually just an additional term linear in time.) The fact that our data are consistently fit by a $\sqrt{t}$-dependent expression (see Results) is further corroboration of the highly folded conformation of poly rA in our samples.

METHODS

Samples of poly rA and bound dye, either ethidium bromide or proflavine, dissolved in an aqueous solution of 10 mM sodium acetate and 0.25% glucose, were prepared as described previously (1,2). Concentrations of poly rA and dye were determined spectrophotometrically at room temperature by using the molar extinction coefficients $\epsilon_{257} = 9.6 \times 10^3$ for poly rA, $\epsilon_{480} = 5.6 \times 10^3$ for ethidium bromide, and $\epsilon_{444} = 4.1 \times 10^4$ for proflavine (1,2). The concentration of poly rA as nucleoside was typically 0.1 mM. Samples were frozen in quartz tubes of 4 mm inside diameter, either slowly in a laboratory freezer, or rapidly by immersion in liquid nitrogen. Slowly frozen samples gave more reproducible results.

Samples were excited at 77 K with a frequency-quadrupled Nd:YAG laser ($\lambda_{ex} = 266$ nm) having pulses of width ~150 ns (Chromatix, Mountain View, Calif.). Poly rA phosphorescence was observed through an interference filter having a full-width at half maximum of 10 nm (Oriel Corp. of

America, Stamford, Conn.; 420 or 440 nm) and detected by photon counting with a photomultiplier (RCA Solid State, Somerville, N.J.; 8852) whose photocathode was cooled to ~210 K. Photomultiplier anode pulses were discriminated and the resulting logic-level pulses were counted by a 1,024-channel analyzer (Nuclear Data, Inc., Schaumburg, Ill.; model 2400) used in its multiscaling mode. Each octant of the analyzer memory was used to store the results of a separate run (unquenched poly rA, poly rA + dye, or background) in a typical eight-run experiment.

Because Eq. 1 holds only when the donors are initially excited randomly with respect to the acceptors (5), we did not follow the procedure used in our earlier work (1). In that study, the sample was pulsed repeatedly until the phosphorescence achieved a steady state, a condition in which donors far from acceptors are preferentially populated. This did not matter in the earlier work, since we were not then interested in quantitative details of the decay. We wished to take advantage of the fact that under a steady-state initial condition, the sample emits much more light than it does when excited by a single, brief pulse of light. For purposes of this study, however, such a steady-state initial condition would lead to a substantial complication in the comparison of theory and experiment, because Eq. 1 would then have to be replaced by a much less tractable expression (5). To perform the experiments reported here, we therefore excited the sample with a single laser flash, accumulated data for about 10 s, allowed the phosphorescence to decay for another 20 s ($\tau \sim 2.5$ s for unquenched poly rA in glucose-acetate solution—see reference 9 and Discussion), and then re-excited the sample with a second flash. This procedure was repeated 10 or more times on a given sample and the data summed electronically to obtain adequate signal/noise. The multiscalar sweep was triggered photoelectrically by each laser flash with light from a beam splitter in the excitation path. The stored data were subsequently analyzed by computer.

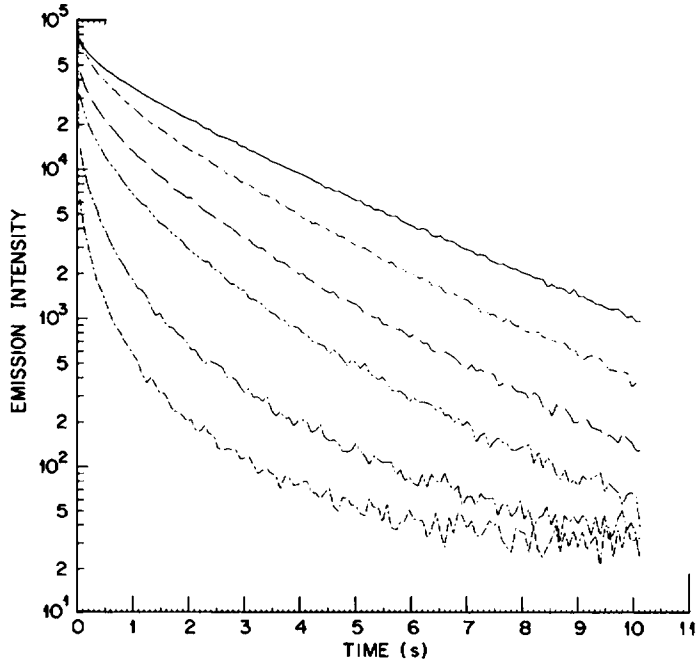

FIGURE 1 Phosphorescence decay of poly rA with various fractional concentrations, r, of bound ethidium bromide. Top curve, $r = 0$; $r = 0.0055$, 0.0117, 0.0164, and 0.0410 in next four lower curves, respectively. The bottom curve shows background glow from a glucose-sodium acetate solution without poly rA or dye. All measurements at 77 K.

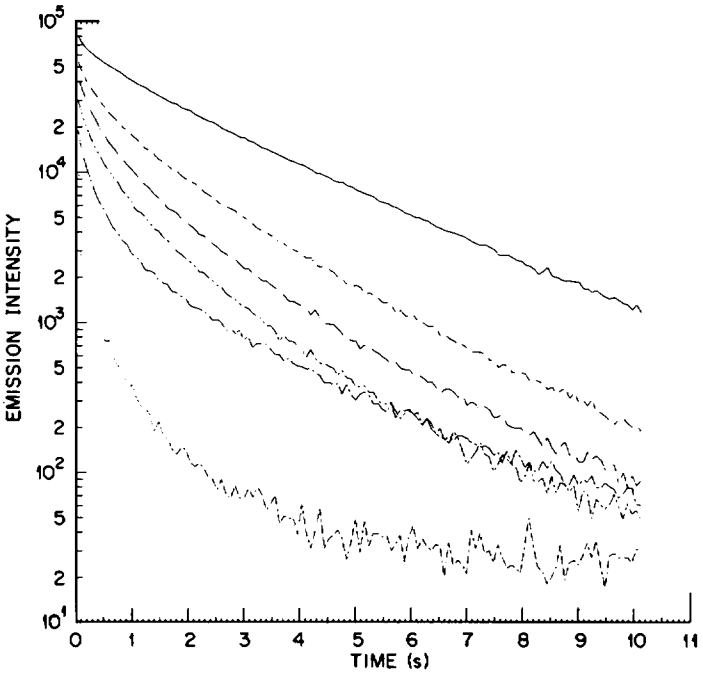

FIGURE 2 Same as in Fig. 1 for bound proflavine. Fractional concentrations of proflavine for the four intermediate curves are, from top to bottom (at $t = 0$), $r = 0.0027, 0.0041, 0.0102$, and 0.0205.

RESULTS

Data from a typical set of experimental runs are shown in Figs. 1 and 2. The top curve in each figure shows the phosphorescence decay of unquenched poly rA, and the bottom curve in each case shows the "background glow" from a frozen sample of glucose-acetate solution without poly rA or dye. The intermediate curves in Fig. 1 show the phosphorescence decay of poly rA with the indicated relative concentration, r, of bound ethidium bromide; those in Fig. 2 show the same for poly rA with bound proflavine. The initial ordinate values are the number of counts collected in the first channel, i.e. the first 80 ms after a laser flash, uncorrected for variations in laser-flash intensity. The initial values generally decrease with increasing r because of the decreasing initial poly rA triplet population that results from prior quenching of the poly rA singlet by the bound dye, i.e., singlet-singlet transfer (1,2). With ethidium bromide (Fig. 1), samples decay more rapidly as r increases, except for the largest r-value sample. With proflavine (Fig. 2), the decay rate noticeably declines at large r over the same time range (see Discussion).

Analysis of the data in terms of the theoretical model is shown in Figs. 3 and 4. As described below, the dashed curves in both figures are plots of the data shown in Figs. 1 and 2 (plus curves for two additional r-values for each dye) corrected for background glow and processed to remove the effect of the overall unquenched poly rA decay; the solid lines are theoretical fits to the processed data.

As can be seen from Figs. 1 and 2, the background glow decays faster than the poly rA phosphorescence. Rather than attempting to model the time-dependence of the background

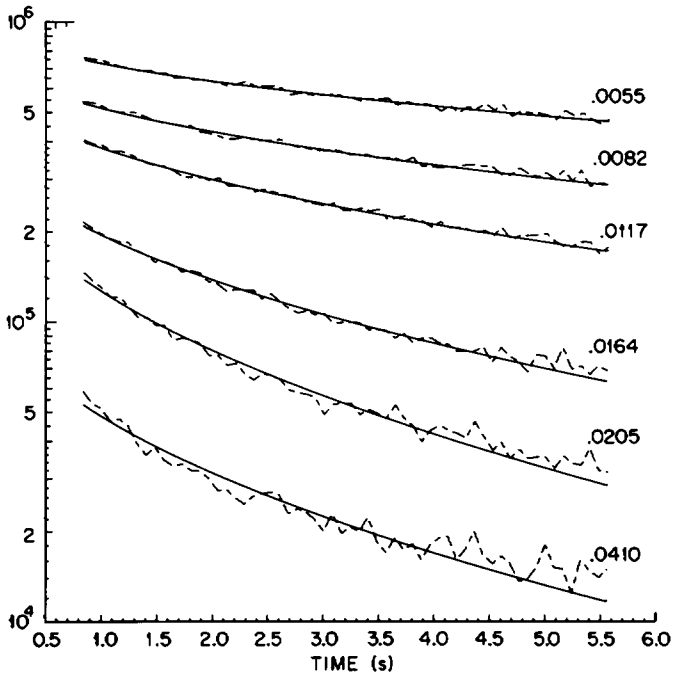

FIGURE 3 Analysis of poly rA phosphorescence decay in terms of the Förster "$\sqrt{t}$-theory" for quenching by bound ethidium bromide. Dashed curves are data corrected for background glow and processed to remove the effect of the overall unquenched poly rA decay (see text). Solid curves are least-squares fits to the processed data. Each pair of curves is labeled by the relative dye concentration, r.

decay, we have simply ignored the first 10 channels (0.8 s), during which time the background decays by nearly an order of magnitude. The average level of the background during the interval, 8 s $\leq t \leq$ 10 s, is taken as the base line and subtracted from all of the quenched poly rA data. Although this method of correcting for background is not ideal, it is probably about the best that can be done with the available information.

The nonexponentiality of the unquenched phosphorescence decay of poly rA in glucose-acetate solution at 77 K has been noted previously (1,9). As written, Eq. 1 applies to a state which, when unquenched, decays exponentially. However, if it is assumed that the observed unquenched decay is the superposition of several exponential decays, each having the same radiative lifetime τ_0 (e.g., due to a multiplicity of local environments), and that the dye molecules bind randomly with respect to each of the unquenched emitting classes, Eq. 1 is readily generalized. With the aid of Eqs. 2 and 3 and the relation,

$$\phi = \tau/\tau_0, \tag{5}$$

Eq. 1 becomes

$$S(t) = S_0 \exp[-(t/\tau) - Bt^{\frac{1}{2}}], \tag{6}$$

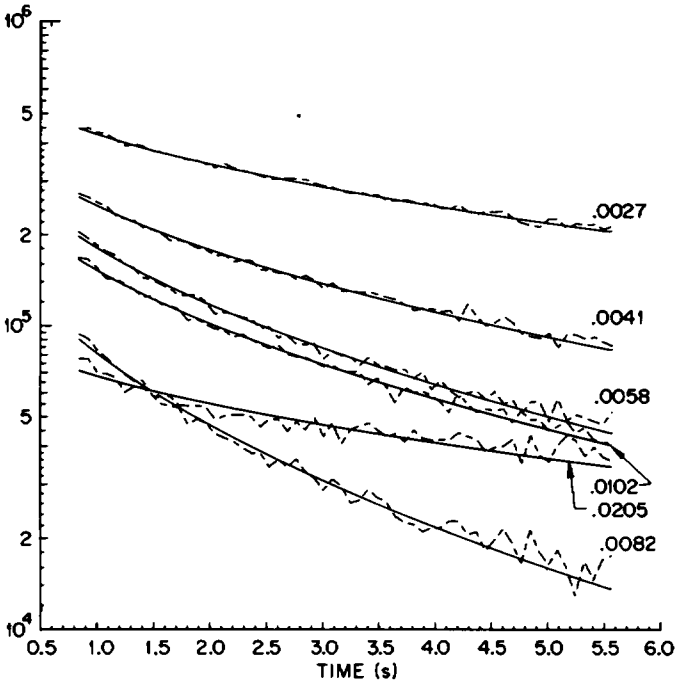

FIGURE 4 Same as in Fig. 3, for bound proflavine.

for single-exponential unquenched decay, where

$$B = (4\pi\kappa' c_A/3n^2)(2\pi I_{ov}/3\tau_0)^{\frac{1}{2}}. \tag{7}$$

From Eqs. 6 and 7 it is seen that the energy transfer term in the decay expression is really independent of the unquenched decay lifetime, i.e. in Eq. 1 the presence of $\tau^{-\frac{1}{2}}$ is artificial because c is proportional to $\bar{R}_0^3$, hence to $\tau^{\frac{1}{2}}$. Thus, if the unquenched decay is $P(t)$, Eq. 6 becomes

$$S(t) = P(t)\exp(-Bt^{\frac{1}{2}}). \tag{8}$$

According to Eq. 8, the quenched decay is still a product of two factors, one of which is simply the unquenched decay. To reduce our data, we therefore proceeded as follows: the quenched decays were first corrected for background glow as described above. An unquenched decay taken from a sample prepared at the same time was also corrected for background. We then divided each corrected quenched decay, channel-by-channel, by the corrected unquenched decay. Each of these time-dependent quotients was multiplied by the same arbitrary constant (10^6) and plotted as a dashed line in Figs. 3 and 4.

If Eq. 8 applies, each dashed line should be approximately fit by a function proportional to $\exp(-Bt^{\frac{1}{2}})$. The solid lines in Figs. 3 and 4 are such fits. Each fit was determined by the method of linear least-squares for log (ordinate) versus (abscissa)$^{\frac{1}{2}}$, weighted simply by the

TABLE I

PARAMETERS FOR POLY rA PHOSPHORESENCE QUENCHING BY BOUND ETHIDIUM BROMIDE

Fractional dye concentration, r	B	B/r	c_{eff}(2.5 s)	Q_{eff}(2.5 s)
	$s^{-\frac{1}{2}}$	$s^{-\frac{1}{2}}$		
0.0055	0.320	58.2	0.338	107
0.0082	0.434	52.9	0.458	96.7
0.0117	0.574	49.1	0.605	89.4
0.0164	0.832	50.7	0.877	92.6
0.0205	1.094	53.4	1.154	97.4
0.0410	1.045	25.5	1.102	46.5

value of the ordinate. Tables I and II show the negative slopes, B, of these least-squares fits as functions of r for each of the two dyes studied. Since

$$r = c_A/c_D, \tag{9}$$

where c_D is donor concentration, it follows from Eq. 7 that the ratio B/r is theoretically independent of r. For this reason B/r is also tabulated.

DISCUSSION

The results summarized in the first three columns of each Table (I and II) can be analyzed further. For a sample whose unquenched decay is a single exponential, the reduced concentration is

$$c = (\tau/\pi)^{\frac{1}{2}}(B/\kappa'), \tag{10}$$

from Eqs. 2, 3, and 7. Although, strictly speaking, a reduced concentration cannot be defined in this way with a multiexponential unquenched decay, one can nonetheless obtain an idea of the magnitude of c for a given unquenched decay component. According to both our own data and that reported earlier (9), the unquenched poly rA decay is reasonably well fit by the two-component function, $0.25 \exp(-t/0.5 \text{ s}) + 0.75 \exp(-t/2.5 \text{ s})$. The 2.5-s component thus appears to be the predominant one. [The 2.5-s value is also quite close to

TABLE II

PARAMETERS FOR POLY rA PHOSPHORESCENCE QUENCHING BY BOUND PROFLAVINE

Fractional dye concentration, r	B	B/r	c_{eff}(2.5 s)	Q_{eff}(2.5 s)
	$s^{-\frac{1}{2}}$	$s^{-\frac{1}{2}}$		
0.0027	0.539	200	0.568	360
0.0041	0.803	196	0.847	358
0.0059	1.037	176	1.094	324
0.0082	1.312	160	1.384	292
0.0102	0.980	96.1	1.034	175
0.0205	0.505	24.6	0.533	45

that reported (2.6 s [9]) for the single-exponential decay of poly rA in a glassy solvent at 77 K]. It is therefore convenient to define an "effective reduced concentration" as if the unquenched decay were a single exponential of time-constant 2.5 s. Accordingly, the quantity,

$$c_{\text{eff}}(\tau) \equiv (\tau/\pi)^{1/2}(B/\kappa'), \tag{11}$$

with $\tau = 2.5$ s, is also tabulated in Tables I and II. We emphasize that this definition is made solely for comparative purposes.

In a system whose donors have a single unquenched decay component, one can theoretically define a quantity Q from c that has an interesting physical interpretation. This is readily seen by considering a hypothetical system that achieves a steady-state population of donor emitting states. For such a system, the relative emission intensity of the donors is (5),

$$I(c) = I_0\{1 - \pi^{\frac{1}{2}}\, x \exp(x^2)[1 - \text{erf}(x)]\}, \tag{12}$$

where $x = 0.7496\, c$ and "erf" is the error function. At the critical concentration, i.e. at $c = 1$, $I/I_0 = 0.326$, which means that 67.4% of the donor excitation has been transferred to acceptors (donors quenched). As pointed out above, B/r, and hence c/r, is theoretically independent of r. One can thus think of c/r as the equivalent number of donors quenched by one acceptor, such that 67.4% of the excitation initially on the donors is ultimately received by the acceptor. (The other 32.6% is channeled into the processes that make up the unquenched lifetime, τ.) It is more usual to think in terms of a 50%-quenching "range." Since $I/I_0 = 0.5$ when $c = 0.577$, the number of donors quenched of 50% of their initial excitation by one acceptor is

$$Q \equiv (c/0.577)/r = 1.73\, c/r. \tag{13}$$

Again, even though these concepts do not extend simply to multiexponential unquenched decay, one may define

$$Q_{\text{eff}}(\tau) \equiv 1.73\, c_{\text{eff}}(\tau)/r. \tag{14}$$

The quantity Q_{eff} (2.5 s) is tabulated in the final column of each of Tables I and II.

For ethidium bromide, except for the largest r-value studied, Q_{eff} is independent of r, as expected (Table I). For proflavine, deviations from this behavior are observed at smaller values of r (Table II). For both dyes, the deviations appear to be greater if one reduces the data in a somewhat different way than described in the Results. If instead of dividing the data for a quenched sample channel-by-channel by the unquenched decay, one fits the latter by a two or even three-exponential function and divides the quenched sample data by that function, the resulting values of Q_{eff} for each dye are much less constant. This suggests that there are more than two or three components in the unquenched phosphorescence decay of our poly rA samples.

It remains to explain the inconstancy of Q_{eff} at large r. As the Tables show, not only the Q-values but even the values of c_{eff} diminish at large r. The latter effect, which is a reflection of the declining decay rate at large r (noted in the Results), is much more pro-

nounced for proflavine than for ethidium bromide. While we cannot be certain of the explanation with the data available, we have a plausible hypothesis, viz. partial depletion of the dye ground state indirectly resulting from singlet-singlet energy transfer from poly rA to dye. As already pointed out in the Results, there is an efficient and very rapid singlet-singlet transfer of excitation from poly rA to dye for $r \gtrsim 0.01$ (2), which lowers the initial population of poly rA triplet available for subsequent triplet-singlet transfer to dye. That initial population of course arises from excited-singlet-to-triplet intersystem crossing in poly rA. Excited-singlet-to-triplet intersystem crossing also occurs in the acceptor, at least for proflavine whose phosphorescence, when bound to poly rA, is easily observed (1). If the dye triplet lifetime is not much shorter than that of poly rA ($\sim$2.5 s), the former state can become heavily populated at the expense of dye ground state on the same time scale, as follows: singlet-excited dye is formed quickly, either by singlet-singlet transfer from poly rA or, to a slight extent, by direct absorption; excited-singlet-to-triplet intersystem crossing then occurs in the dye, also rapidly; the resulting dye triplet then lives long enough to depopulate dye ground state on the time scale of triplet-singlet transfer from poly rA to dye. Because such transfer requires the acceptor initially to be in its ground state, the loss in population of the latter lowers the overall transfer rate. The effect would almost certainly be accentuated by the repeated pumping of the dye singlet that occurs during a single 150-ns long laser flash.

From Eqs. 7, 9, 11, and 14,

$$Q_{\text{eff}} = 1.73\,(4\pi c_D/3n^2)(2\phi I_{\text{ov}}/3)^{\frac{1}{4}}. \tag{15}$$

From this it follows that the ratio of Q_{eff}'s for two different acceptors in an otherwise identical donor system equals the square root of the ratio of I_{ov}'s. The calculated values of I_{ov} for transfer from the poly rA triplet to dye singlets are $I_{\text{ov}} = 6{,}600$ nm^6 for ethidium bromide and $I_{\text{ov}} = 63{,}000$ nm^6 for proflavine (1). Thus, $[I_{\text{ov}}(\text{proflavine})/I_{\text{ov}}(\text{ethidium bromide})]^{\frac{1}{2}} = 3.1$. The average value of Q_{eff} for triplet to ethidium bromide transfer is (excluding the last value in Table I) $\langle Q_{\text{eff}} \rangle = 96.6$. Similarly, excluding the last value in Table II, $\langle Q_{\text{eff}} \rangle = 302$ for proflavine, and $\langle Q_{\text{eff}}(\text{proflavine}) \rangle / \langle Q_{\text{eff}}(\text{ethidium bromide}) \rangle = 3.1$, in agreement with theory. This close an agreement is probably fortuitous, since, as noted above, proflavine quenching does not exhibit theoretical behavior to as high a value of dye concentration as does ethidium bromide. Nonetheless, the 10% or so higher ratio obtained if one excludes, say, the last two values in Table II from $\langle Q_{\text{eff}} \rangle$ still agrees with the calculated ratio within the probable errors of both.

Eq. 15 also provides a basis for estimating the volume per nucleotide, c_D^{-1}, in the compact structure. By using the values of I_{ov} and $\langle Q_{\text{eff}} \rangle$ given above for ethidium bromide, and assuming $n = 1.33$, one obtains

$$c_D^{-1} = 2{,}800\sqrt{\phi}\,\text{Å}^3. \tag{16}$$

Unfortunately, the quantity ϕ for poly rA in glucose solution at 77 K has not been measured. However, since the value of c_D^{-1} from Eq. 16 is not very sensitive to that of ϕ, one can estimate the value of c_D^{-1} to within a factor of two or so by assuming $\phi \sim 0.1$, which gives $c_D^{-1} \sim 1{,}000$ Å^3. This is about five times the volume per nucleotide in completely stacked poly rA, the conformation in which it is found at neutral pH near 0°C (10). Our estimate implies that the poly rA in our frozen solvent, while partially folded, is not as compact as

is theoretically possible, i.e., in close-packed segments each of which is almost completely stacked.

We conclude that Förster's "$\sqrt{t}$-theory," originally used to describe electronic excitation energy transfer between donor and acceptor molecules in solution, can be used to analyze quantitatively such processes in compact nucleic acid structures, and thereby to deduce nucleotide packing densities in more complex systems.

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REFERENCES

1. VAN NOSTRAND, F., and R. M. PEARLSTEIN. 1978. Phosphorescence quenching of poly rA in glucose-sodium acetate solution at 77 K. *Photochem. Photobiol.* **28**:407–411.
2. VAN NOSTRAND, F., and R. M. PEARLSTEIN. 1976. Singlet excitation transfer from poly rA to bound dye at 77 K. *Chem. Phys. Lett.* **39**:269–272.
3. BEARDSLEY, K., and C. R. CANTOR. 1970. Studies of transfer RNA tertiary structure by singlet-singlet energy transfer. *Proc. Natl. Acad. Sci. U.S.A.* **65**:39–46.
4. FÖRSTER, TH. 1949. Experimentelle und theoretische untersuchung des zwischenmolekularen übergangs von elektronenanregungsenergie. *Z. Naturforsch. A.* **4**:321–327.
5. EISENTHAL, K. B., and S. SIEGEL. 1964. Influence of resonance transfer on luminescence decay. *J. Chem. Phys.* **42**:652–655.
6. BENNETT, R. G., R. P. SCHWENKER, and R. E. KELLOGG. 1964. Radiationless intermolecular energy transfer. II. Triplet-singlet transfer. *J. Chem. Phys.* **41**:3040–3041.
7. HEMENGER, R. P., and R. M. PEARLSTEIN. 1973. Time-dependent concentration depolarization of fluorescence. *J. Chem. Phys.* **59**:4064–4072.
8. FÖRSTER, TH. 1965. Delocalized excitation and excitation transfer. *In* Modern Quantum Chemistry. O. Sinanoğlu, editor. Part 3. Academic Press, Inc., New York. 93–137.
9. KLEINWACHTER, V., J. DROBNIK, and L. AUGENSTEIN. 1968. Emission spectra from synthetic polynucleotides and deoxyribonucleic acid in aqueous solutions. *Photochem. Photobiol.* **7**:485–497.
10. STANNARD, B. S., and G. FELSENFELD. 1975. The conformation of polyriboadenylic acid at low temperature and neutral pH. A single-stranded rodlike structure. *Biopolymers.* **14**:299–307.