

Intramolecular energy transfer in molecules with a large number of conformations

(orientation factor/fluorescence decay/oligopeptides/molecular dimensions)

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ABSTRACT The equations representing the decay kinetics of fluorescence of a donor luminophore in the presence of an acceptor in the same flexible molecule are discussed. The assumption that distances between luminophores attached to chain ends are not correlated with the relative orientations of luminophores is tested by theoretical calculations for the unperturbed chains Tyr-(Ala)_n-Tyr with *n* equal to 4 and 9. It appears that for these chains, in which the conformation is dominated by entropic factors, the above assumption is satisfactory even for chains with a small number of repeating units (*n* = 4).

It has been suggested that it should be possible to determine experimentally the distribution of the end-to-end distances in flexible molecules from the efficiency of energy transfer between a donor (D) and an acceptor (A) luminophore (1) or from the decay kinetics of the donor in the presence of an acceptor (2), when the luminophores are attached to the molecular ends.

Subsequently, Haas *et al.* (3) reported mean values of the distances and their distributions about the means $\langle f(R) \rangle$ obtained from the observed nanosecond decay kinetics of D emission in the presence of A in synthetic oligopeptides containing from four to six repeating units. However, Dale and Eisinger (4) critically examined how far Förster's long-range energy transfer (5) can be applied in the determination of macromolecular dimensions and questioned the validity of the results obtained. Eisinger and Dale (4) stressed the effect of restrictions in the orientational freedom of the luminophores, which obscures the interpretation of the decay kinetics in terms of molecular dimensions. The present note attempts, by studying theoretically appropriate model compounds (6), to discuss the assumptions underlying the calculations of molecular dimensions from decay kinetics.

Let us consider a single D-A pair separated by a distance *R*. If the emission band of the donor and the absorption band of the acceptor are both characterized by a single transition dipole moment vector of constant direction across the bands, the probability of energy transfer depends on the relative orientation of D and A through the orientation factor κ defined by (7)

$$\kappa = \mathbf{D} \cdot \mathbf{A} - 3(\mathbf{D} \cdot \mathbf{R})(\mathbf{A} \cdot \mathbf{R}) \quad [1]$$

in which **D**, **A**, and **R** are unit vectors along the transition dipole directions of D and A and along the separation vector **R**, respectively.

In a viscous solution the spatial distribution of the distances and orientations of all D-A pairs is fixed and independent of time on the time scale of nonradiative energy transfer experi-

ments (1-100 nsec). The decay kinetics determined under such experimental conditions correspond therefore to an averaging regime that is static (7) with respect both to the translational and to the rotational motion of the luminophores, $\langle I(t) \rangle_s$,

$$\langle I(t) \rangle_s = k \exp\left(-\frac{t}{\tau}\right) \int_0^\infty \int_0^4 f(R, \kappa^2) \times \exp\left\{-\kappa^2 \frac{t}{\tau} \left(\frac{S_0}{R}\right)^6\right\} d\kappa^2 dR \quad [2]$$

in which *k* is a constant, τ is the lifetime of the excited state of D in the absence of transfer, $f(R, \kappa^2)$ is the probability of a conformation defined by *R* and κ^2 , and S_0 is related to Förster's critical distance R_0 (5) by

$$R_0^6 = \kappa^2 S_0^6 \quad [3]$$

Inspection of Eq. 2 shows that the analysis of decay kinetics in flexible molecules in terms of molecular dimensions is possible only when the distances *R* and the orientations κ^2 are not correlated. It is reasonable to assume that this will hold in the case of chain molecules in which the luminophores are separated by a large number of valence bonds, rotating more or less freely. The number of conformations accessible to such molecules is large. When *R* and κ^2 vary independently, the decay data can be analyzed in terms of the distribution of distances $f(R)$ with the aid of Eq. 4.

$$\langle I(t) \rangle_{s, \text{ran}} = k \exp\left(-\frac{t}{\tau}\right) \int_0^\infty \int_0^4 \times f(R) f_{\text{ran}}(\kappa^2) \exp\left\{-\kappa^2 \frac{t}{\tau} \left(\frac{S_0}{R}\right)^6\right\} d\kappa^2 dR \quad [4]$$

in which $f_{\text{ran}}(\kappa^2)$ is a random distribution of orientations. Such a distribution can be computed from an analytical expression, as shown by Guillard and Englert (8). The equation used by Haas *et al.* (3) for the interpretation of experimental decay curves is

$$\langle I(t) \rangle_{s', \text{ran}} = k \exp\left(-\frac{t}{\tau}\right) \int_0^\infty f(R) \times \exp\left\{-\langle \kappa^2 \rangle_{\text{ran}} \frac{t}{\tau} \left(\frac{S_0}{R}\right)^6\right\} dR \quad [5]$$

in which $\langle \kappa^2 \rangle_{\text{ran}}$ is the average value of κ^2 for the isotropic situation when all orientations of transition dipoles of A and D are equally probable ($\langle \kappa^2 \rangle_{\text{ran}} = 2/3$).

Two distinct physical situations can be described by Eq. 5. For D-A luminophores with single dipole moments, Eq. 5 applies when rapid and isotropic rotation of the luminophores cancels the effect of orientation on the transfer (dynamic rotational averaging). In the idealized extreme case when the absorption and emission are described by three orthogonal transition dipole moments of the same magnitude, an intrinsic

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averaging of κ^2 of each A-D pair makes the probability of transfer independent of orientation, even in the absence of fast rotation (9).

In order to examine the effect of correlation between distances and orientations on the decay kinetics in a static regime for chains with a limited number of repeating units, we have considered a donor and an acceptor characterized by single transition dipole moments. We have therefore used the methods described previously (6) to compute $\langle I(t) \rangle_s$, $\langle I(t) \rangle_{s,\text{ran}}$, and $\langle I(t) \rangle'_{s,\text{ran}}$ given by Eqs. 2, 4, and 5 for an unperturbed (10) oligopeptide chain Tyr-(Ala) $_n$ -Tyr, with n equal to 4 and 9. The luminophore Tyr has in fact only one transition dipole moment (6); its conformational properties are well established, while the back transfer cannot affect the results of the present study. The decay kinetics are obtained from a representative set of chains by Monte Carlo methods and are shown in Fig. 1 for the chain Tyr-(Ala) $_9$ -Tyr for three different values of $\langle R \rangle/R_0$; R_0 was computed with Eq. 3 assuming that $\kappa^2 = 2/3$. The values are computed from a large number of chains in the sample (5000–20,000) and are independent of this number. The value of $\langle \kappa^2 \rangle$ is 0.74 and that of $\langle R \rangle$ is 26.7 Å.

Inspection of Fig. 1 shows that Eq. 4 is a good approximation to the exact decay kinetics given by Eq. 2 for chains with $n = 9$. For these chains, $\langle I(t) \rangle_{s,\text{ran}}$ differs from $\langle I(t) \rangle_s$ by at most 0.02 (for $\langle R \rangle/R_0$ between 0.75 and 1.25) so that the difference between the two values is 4% when $\langle I(t) \rangle_s$ equals half its initial value. The differences between the decay curves $\langle I(t) \rangle_s$ and $\langle I(t) \rangle'_{s,\text{ran}}$ are larger, especially when $\langle R \rangle/R_0 < 1$. In shorter chains, when $n = 4$, the decay curves can still be approximated by Eq. 4, and the differences from the exact values (0.03 at most) are not much larger than for chains with $n = 9$. The observed randomization of the orientations in the chains studied is mainly due to the fact that the number of variable angles of rotation around the valence bonds of the backbone is sufficiently large, as has been verified by maintaining the side chains of the luminophores in a fixed (low energy) conformation.

It should be pointed out that in most chain molecules the number of conformations is smaller than in the corresponding unperturbed chain, since long-range interactions are neglected in the unperturbed chain model. We have therefore also computed the transfer properties for Tyr-(Ala) $_n$ -Tyr with n equal to 4 and 9 by Monte Carlo methods including all interactions (11); the results will be presented elsewhere (M. Leclerc, S. Prémilat, and A. Englert, unpublished data). For these chains the differences between the decay kinetics computed with Eq. 2 and with Eq. 4 are hardly greater than those computed for the unperturbed chains if the only important long-range interactions are the stacking interactions between the luminophores.

We conclude, therefore, that Eq. 4 can be used for the interpretation of the decay kinetics in terms of the distribution of distances $f(R)$ for oligopeptides with a limited number of repeating units in which the conformation is dominated by entropic factors if the transitions of the luminophores are characterized by single dipole moments. Of course, even for such chains the transfer data cannot unambiguously yield the entire distribution $f(R)$, and this point should be discussed in detail when transfer data are being analyzed.

All other situations of short chains in a random coil state, in

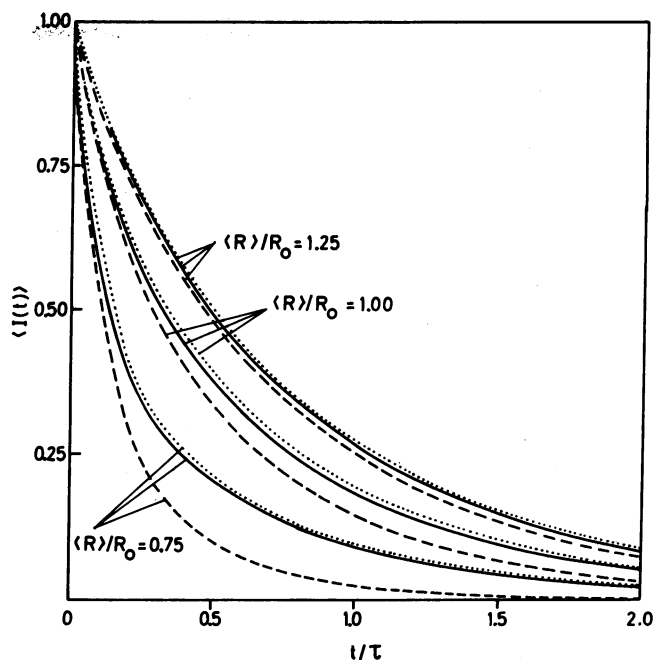


FIG. 1. Fluorescence decay curves calculated for an unperturbed chain Tyr-(Ala) $_9$ -Tyr as represented by $\langle I(t) \rangle_s$ (—), $\langle I(t) \rangle_{s,\text{ran}}$ (....), and $\langle I(t) \rangle'_{s,\text{ran}}$ (- - -) for various values of the ratio $\langle R \rangle/R_0$. R_0 was defined by Eq. 3 with $\kappa^2 = 2/3$.

which the transitions of either of the luminophores have to be described by more than one dipole moment, can be examined with the assumption that distances and orientations are not correlated. It seems very plausible that the decay kinetics determined by Haas *et al.* (3) are well represented by Eq. 5, since these luminophores, naphthyl and dansyl, are three-dimensional and two-dimensional oscillators, respectively (12).

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