Proc. Nat. Acad. Sci. USA
Vol. 73, No. 2, pp. 271–273, February 1976
Chemistry

Intramolecular energy transfer and molecular conformation

(orientation factor/fluorescence/depolarization/decay/polymers/macromolecules)

R. E. DALE* AND J. EISINGER†

* Mergenthaler Laboratory for Biology and McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218; and † Bell Laboratories, Murray Hill, New Jersey 07974

Communicated by John J. Hopfield, November 5, 1975

ABSTRACT **A discussion of the range of applicability of Förster long-range energy transfer in the determination of macromolecular dimensions and conformational dynamics is given. Emphasis is laid on the effect of restrictions in the orientational freedom of donor and acceptor and on the importance of the orientational averaging regime. The usefulness and limitations of *polarized* emission measurements in this regard are discussed.**

A paper which appeared recently in this journal (1) reports the experimental determination of the efficiency of energy transfer between donor (D) and acceptor (A) luminophores attached to the ends of synthetic oligopeptides of various lengths. Directly observed nanosecond decay kinetics of D emission were analyzed to yield mean values for the end-to-end distances (R) and their distributions about the means. The experimental technique and method of analysis were recommended by the authors as offering a way of studying the conformation of oligopeptides in solution. While energy transfer experiments are indeed among the few methods by which intramolecular distances in macromolecules in solution may be studied (2, 3), the experimental and analytical methods offered by Haas *et al.* (1) cannot provide valid estimates of either R or the distribution in R. The present note attempts to dispel several misconceptions about the applicability of long-range (Förster) intramolecular energy transfer (4) as a tool for distance determination or "spectroscopic ruler" (5), some of which appear not only in the paper under discussion but in a variety of earlier reports, e.g., lately, refs. 6–10. It will also outline briefly how appropriately chosen *polarized* energy transfer experiments *can* yield useful information about macromolecular conformation.

Consider first a single, *stationary* D–A pair separated by a well-defined distance R and attached to a macromolecular framework. The rate of energy transfer from D to A may conveniently be written as

$$k_T = k_D(R_0/R)^6 \qquad [1]$$

where k_D is the decay rate of D in the absence of A and R_0 is the so-called Förster distance which corresponds to $k_T = k_D$, i.e., to a transfer efficiency of 50%. The dependence of R_0 on the relative orientations of the transition dipoles of D and A (represented by the unit vectors **D** and **A**) and their unit separation vector, **R**, may be expressed by

$$R_0^6 = C\kappa^2 \qquad [2]$$

where κ^2 is the orientation factor for dipole–dipole interaction defined by

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

and where C is a combination of universal and solvent constants with spectroscopic parameters characteristic of D and A but independent of their orientation and separation. Eq. [1] may then be rewritten as

$$k_T = Ck_D\kappa^2R^{-6} \qquad [4]$$

and the efficiency of energy transfer (T) is given by

$$T = \frac{k_T}{k_D + k_T}. \qquad [5]$$

In the experimental determination of an intramolecular separation R one deals with an *ensemble* of such macromolecules (i), each of which is endowed in the most general case with a D_i,A_i pair separated by some R_i and characterized by some κ_i. Consider first, by way of illustration, the case in which the separation is the same and constant for all D–A pairs, the case which has most usually been of interest, e.g., refs. 6–10. Here, the motion of the substrate is slow compared with k_D and k_T, but the motion of **D** and **A** relative to the substrate (or to **R**) may be either rapid or slow corresponding to *dynamic* or *static* orientational averaging regimes.

In the *dynamic* limit it is easy to see that each D_i,A_i pair samples all allowed orientations *before* transfer occurs so that the transfer efficiency T_i for each molecule is the same. In other words, the *dynamically averaged transfer efficiency* for the ensemble of molecules is, according to Eqs. [4] and [5],

$$\langle T_i\rangle_d = \langle T\rangle_d = \frac{\langle\kappa_i^2\rangle}{C^{-1}R^6 + \langle\kappa_i^2\rangle} \qquad [6]$$

where $\langle\kappa_i^2\rangle$ is the average of κ_i^2 over all *allowed* orientations $\mathbf{D}_i$ and $\mathbf{A}_i$. If the allowed orientations extend over all space, corresponding to isotropic distributions of D_i and A_i, $\langle\kappa_i^2\rangle$ is $\frac{2}{3}$. This is the case illustrated for the ensemble shown in Fig. 1a. If the orientational freedom of D_i and/or A_i is limited for steric or other reasons, appropriate upper and lower limits for the average can be calculated if the extent of orientational freedom of D_i and A_i relative to the (static) matrix is known or assumed (11–13). Once limits have been set on $\langle\kappa_i^2\rangle$ and $\langle T_i\rangle$ is measured, upper and lower bounds for R may be determined from Eq. [6] or, if the decay of D is measured in the presence and absence of A, they may be determined from Eqs. [1] and [2] together with the identity

$$k_{D(A)} = k_D + k_T \qquad [7]$$

where $k_{D(A)}$ is the (still first order) rate parameter for the decay of D in the presence of A.

The situation is quite different for an ensemble of *statically* averaged D_i,A_i pairs, as depicted for the isotropic case in Fig. 1b. Since D_i and A_i do not reorient during the transfer time, the rate and efficiency of energy transfer will differ from molecule to molecule. For the ensemble of mole-

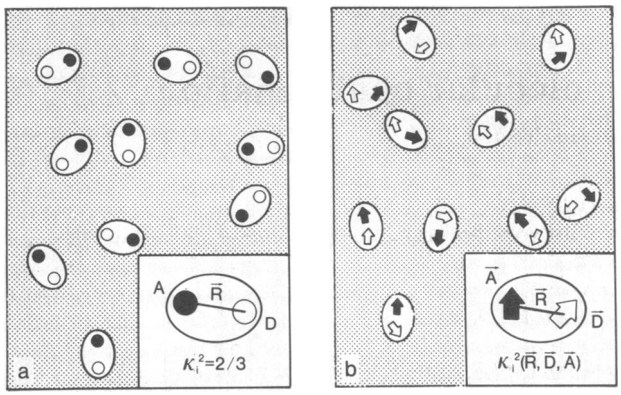

FIG. 1. Schematic representations of ensembles of macromolecules, each of which is endowed with a specifically bound donor D_i and acceptor A_i separated by a distance R along the unit vector **R**. **R** is considered fixed with respect to the molecular matrix. (a) The transition dipole directions **D**,**A** can take up all orientations relative to **R** during the donor lifetime, corresponding to a dynamic averaging regime. (b) **D** and **A** also have isotropic distributions but their orientations relative to **R** do not change during the donor lifetime (static averaging regime). While the transfer rate k_T for each D_i,A_i pair is the same in (a), its value in (b) depends on the relative orientations **D**,**A** and **R**, as well as on the separation R, so that no R-independent average value for the orientation factor κ^2 exists for the statically averaged ensemble shown there. As discussed in the *text*, this precludes the use of energy transfer rates for the determination of R.

cules, therefore, the *statically averaged transfer efficiency* is

$$\langle T_i \rangle_s = \left\langle \frac{\kappa_i^2}{C^{-1}R^6 + \kappa_i^2} \right\rangle. \qquad [8]$$

Since the average expressed here depends on the range and ratio of values of κ_i^2 and $C^{-1}R^6$, it is not possible in general, as it was for $\langle T_i \rangle_d$, to obtain $\langle T_i \rangle_s$ directly in terms of $\langle \kappa_i^2 \rangle$ and R. *The static limit, therefore, does not readily lend itself to intramolecular distance determinations* except when $k_T \ll k_D$, in which case $\langle T_i \rangle_s$ approaches $\langle T_i \rangle_d$ and, to a good approximation, the analysis given for the dynamic limit is valid.

The above considerations apply equally well to the case of variable R. Moreover, it should be noted that both κ_i and $\langle \kappa_i^2 \rangle$ can be expected to correlate to some extent with R_i, either reinforcing or opposing the change in transfer rate and efficiency elicited by changes in R.

Haas *et al.* (1) use what they consider to be an appropriate static average value of 0.476 for $\langle \kappa^2 \rangle$ purported to relate to isotropic distributions of **D** and **A**. The averaging regime was justified by the observation of relatively high polarizations of the emission of D and A in the glycerolic solvent used originally in order to prevent *translational* motion of D and A during the transfer time. The randomness of relative orientations was tacitly justified by the observation that A emission due to transferred D excitation energy was "completely" depolarized. Citing the work of the present authors (12), in which in any case only transfer depolarization results for the dynamic limit are given, this was incorrectly inferred to mean that correlation of the orientations of D and A is unlikely, a conclusion which is not borne out either on examination of ref. 12 or of the results presented elsewhere for the static limit (13). Even leaving this aside, however, and granting isotropic (or near-isotropic) orientational freedom, the value of 0.476 quoted is inappropriate for the models of R-distribution considered. It was derived for the case of transfer from donors to an ensemble of acceptors randomly distributed in both distance and orientation (14–16). It arises, not as the average of κ^2 but as $(\langle \kappa \rangle)^2$, the square of the average of κ, which appears in the solution of an integral of the form:

$$\alpha \int R^2 \exp[-\beta \kappa^2 R^{-6}] dR = 1 - \gamma\kappa \qquad [9]$$

where α, β, and γ are constants. Only insofar as this integral, or another giving rise to a function containing *only the first power of* κ, appears in the expression for the decay of D emission, is it appropriate (for isotropic orientational distributions) to assign the value of 0.476 ($=0.690^2$) to $(\langle \kappa \rangle)^2$. Such is patently not the case for the expressions derived using the end-to-end distribution functions quoted by Haas *et al.* (1) in their Table 1.

While a nonexponential donor emission decay was observed by Haas *et al.* (1) and found to be consistent with first order decay kinetics when certain distributions in R were introduced, the points raised above should make it clear that, at the very least, no quantitative significance can be ascribed to the parameters characterizing these distributions. Indeed, the improvement in fit to the experimentally observed decay curve might equally well have been obtained with an *invariant* separation and limited angular distributions of D and/or A, which must in any case exist for purely steric reasons. In fact, even an *isotropic* orientational model with invariant R would give rise to a nonexponential donor decay in the static limit.

How, then, in general, can the information obtainable from energy transfer experiments be analyzed to permit arriving at valid conclusions about intramolecular separations? While this problem has been discussed in great detail elsewhere (11–13, 17), it would seem appropriate to list the most important requirements here.

(*i*) The averaging regime (static or dynamic) obtaining for D and A reorientation must be established. Intermediate cases are virtually impossible to analyze and the static limit does not lend itself to distance determinations from transfer efficiency measurements, except in the limit of low transfer efficiency.

(*ii*) Experimental energy transfer data in the dynamic limit can be analyzed if a specific model for the relative orientations and orientational freedom of D and A is assumed. Graphical solutions for a wide variety of models are available in the literature (12, 13). Currently, however, these only lead to extreme upper and lower bounds for $\langle \kappa^2 \rangle$ and thus merely limit to some extent the uncertainty in R obtained in this way. The model chosen should, of course, be justified where possible by determinations of the orientational freedom of D and A from measurements of the depolarization of their emission (12, 13).

(*iii*) The above uncertainty in R may be further reduced by measuring also the depolarization of transferred excitation energy in the dynamic limit which provides a way of limiting the number of models since they must be consistent with both this and the observed depolarizations of D and A. Since κ^2 can be described as a function of three angles of which only two are independent (see Eq. [3]) while the transfer depolarization depends only on the other one, a unique model for the relative orientation of D and A and a unique value of R cannot be obtained even by means of polarized energy transfer experiments. It must be emphasized, however, that this uncertainty is *intrinsic* to the energy

transfer method and reflects a real lack of knowledge of the relative dispositions of *D* and *A*.

Returning, in conclusion, to the experiments of Haas *et al.* (1), it would seem that, in view of the arguments presented above and the further possibility which they did not exclude, that there might predominantly exist a *small* number of discrete conformational types rather than a continuous distribution, no definitive interpretation can be given to their observations in the oligopeptide systems studied. It appears to be beyond the scope of existing theory or experiment to analyze the observed donor decay kinetics unambiguously. It is unfortunate that, in this case, interpretation of observations in the dynamic limit would undoubtedly be obscured by averaging of the relative translational as well as reorientational motion of the donor and acceptor, although this also has been attempted recently, albeit with a more cautious interpretation of the results (18).

1. Haas, E., Wilchek, M., Katchalski-Katzir, E. & Steinberg, I. Z. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1807–1811.
2. Beardsley, K. & Cantor, C. R. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 39–46.
3. Wu, C-W. & Stryer, L. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1104–1108.
4. Förster, Th. (1951) *Fluoreszenz Organischer Verbindungen* (Vandenhoeck & Rupprecht, Göttingen).
5. Stryer, L. & Haugland, R. P. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 719–726.
6. Bunting, J. R. & Cathou, R. E. (1974) *J. Mol. Biol.* **87**, 329–338.
7. Alfimova, E. Ya., Syrtsova, L. A., Pisarskaya, T. N. & Likhtenshtein, G. I. (1974) *Mol. Biol.* **8**, 537–544.
8. Moe, O. A., Jr., Lerner, D. A. & Hammes, G. G. (1974) *Biochemistry* **13**, 2552–2557.
9. Matsumoto, S. & Hammes, G. G. (1975) *Biochemistry* **14**, 214–224.
10. Cantley, L. C. & Hammes, G. G. (1975) *Biochemistry* **14**, 2976–2981.
11. Eisinger, J. & Dale, R. E. (1974) *J. Mol. Biol.* **84**, 643–647.
12. Dale, R. E. & Eisinger, J. (1974) *Biopolymers* **13**, 1573–1605.
13. Dale, R. E. & Eisinger, J. (1975) "Polarized Excitation Energy Transfer" in *Biochemical Fluorescence: Concepts*, eds Chen, R. F. & Edelhoch, H. (Marcel Dekker, New York), Vol. I, chap. 4, pp. 115–284.
14. Galanin, M. D. (1955) *Sov. Phys.-JETP (Engl. Transl.)* **1**, 317–325.
15. Maksimov, M. Z. & Rozman, I. M. (1962) *Opt. Spectrosc.* **12**, 337–338.
16. Steinberg, I. Z. (1968) *J. Chem. Phys.* **48**, 2411–2413.
17. Blumberg, W. E., Dale, R. E., Eisinger, J. & Zuckerman, D. M. (1974) *Biopolymers* **13**, 1607–1620.
18. Guillard, R., Leclerc, M., Loffet, A., Leonis, J., Wilmet, B. & Englert, A. (1975) *Macromolecules* **8**, 134–140.