Dynamics of Fluorescence Polarization in Macromolecules

( rotational diffusion/relaxation/theory/formula)

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Communicated by H. S. Gutowsky, April 8, 1972

ABSTRACT Reexamination of the theory of fluorescence time dependence owing to rotational diffusion of rigid macromolecules reveals deficiencies or hidden restrictions in each of the previous treatments. The correct master equation has five exponential decay terms, with preexponential factors that depend upon the diffusion constants and, in a completely symmetrical fashion, upon the orientations of absorbing and emitting dipoles.

If a dye molecule is bound in a definite way to a rigid macromolecule, and an isotropic solution of such molecules is excited by a short pulse of light polarized along a laboratory axis, the fluorescence will be polarized; the polarization anisotropy [r(t)] will then evolve in time in a way determined by the characteristic reorientation times of the macromolecule (1). Although the problem appears to be straightforward, previous treatments have not agreed upon the form of r(t). In a recent paper (2), Tao gives a general expression [Eq. (46) of ref. 2] for r(t) for the completely asymmetric body as a function of its three principal rotational diffusion constants (D1, D2, D3), the direction of the emitting dipole (\(y\)) in the body, and the angle \(\lambda\) between absorbing and emitting dipoles. Previously, Lombardi and Dafforn (3) had derived a quite different expression [their Eq. (12) for \(p = 1\), with \(r(t) = 0.3 QA\)] for the same quantity. Tao's expression has five different exponential decay terms; Lombardi and Dafforn's has only two. Tao's expression contains the direction of the absorption dipole only in a simple overall constant factor \(P_x(\cos \alpha)\), while Lombardi and Dafforn's has each decay term modified in a different way by the polarization properties of the absorber. Finally, Lombardi and Dafforn's equations, previous discussions of special cases (1, 4), and the results of a discontinuous jump model for reorientation (5) all allow for situations where the polarization ratio after the initial pulse is zero, but grows to a maximum and then decays back to zero in time; according to Tao (2), if r(0) = 0, then r(t) = 0 for all time. To resolve these discrepancies, we have carried through an independent solution to the problem and have obtained for r(t) a new expression, which is completely general and which contains as different special subcases corrected versions of the expressions given previously by Lombardi and Dafforn (3) and Tao (2). The formalism and results apply to a wide variety of experiments, not restricted to macromolecule fluorescence polarization decay; full details and discussion will be published later. The purpose of the present communication is to clarify the record by giving our new expression and briefly comparing it with previous treatments.

Our result for the problem posed by Tao (2) is as follows:

\[
5/6 \ r(t) = \sum_{i=1}^{3} c_i \exp(-t/\tau_i) + [(F + G)/4] \\
\exp(-[6D - 2\Delta t]t) + [(F - G)/4] \exp(-[6D + 2\Delta t]t), \quad [1]
\]

where

\(r(t)\) is the polarization anisotropy (2) at time \(t\).

\(D \equiv (D_1 + D_2 + D_3)/3\), the mean rotational diffusion constant.

\(\Delta \equiv (D_1^2 + D_2^2 + D_3^2 - D_1D_2 - D_1D_3 - D_2D_3)/3\).

\(c_i\), \(c_2\), \(c_3\) are the direction cosines of the absorbing dipole with respect to the principal rotation axes.

\(\alpha_1\), \(\alpha_2\), \(\alpha_3\) are the corresponding direction cosines of the emitting dipole.

\(\tau_i = 1/(3D + 3D_i)\).

\(F = \sum_{i=1}^{3} \alpha_i^2 \tau_i = \frac{1}{\lambda}\).

\(\Delta = \sum_{i=1}^{3} D_i(\alpha_i^2 \epsilon_i^2 + \alpha_j^2 \epsilon_j^2 + \alpha_k^2 \epsilon_k^2) - D; \ \alpha_i \neq j \neq k \neq i\).

This expression can be derived by the procedure indicated by Lombardi and Dafforn (3). Our result, however, differs from theirs because we assume one absorption and one emission dipole vector fixed in general directions in the body, whereas special symmetry of either the absorption or emission probability is implicit in their treatment, and indeed was appropriate for the molecules that they considered. This special symmetry is that in which either the absorption or the emission probability (for a given orientation of polarizer or analyzer) is invariant to two-fold rotation of the molecule about each of its principal axes of diffusion. Such symmetry would be provided by averaging 8 of our expressions (each \(\alpha_i = \pm \alpha_i\) or each \(\epsilon_i = \pm \epsilon_i\)), whereupon the first three decay terms would vanish; the last two terms are identical with the expression of Lombardi and Dafforn (except for a trivial clerical error in their equation).

Tao's derivation (2) is correct only if absorption and emission directions in the body coincide \(\alpha_i = \epsilon_i\) for all \(i\); i.e., Tao's \(\lambda = 0\), in which case both expressions are readily seen to be the same. In any other case, Tao errs in assuming a simple one-to-one correspondence between the distribution
of body orientations and the distribution of orientations of the emitting dipole.

In summary, we verify that each of the five decay times (no more than three of them independent) appearing in Tao’s paper (2) contributes an exponential term to the time dependence of the fluorescence polarization, but find a more complex form for the preexponential factors, which are unaffected by interchange of absorber and emitter directions. That Lombardi and Dafforn (3), and Weber (5) found only two decay times does not reveal any error; rather, it reflects special symmetry restrictions inherent in initial conditions in the one case (3) and the 6-position jump model in the other (5). [The incorrect assertion by one of us (5) that the differential diffusion model yields only two decays was caused by missing the restrictions implied in Lombardi’s treatment.] We further note that in most circumstances, only two decay rates will be distinguishable within realistic experimental error and, furthermore, that any one of these experimental rates will usually be proportional to some weighted average of the 5 intrinsic decay constants. Moreover, α and ε are not usually known, in which case the weighting factors are not well known. In fact, the two decay times in Weber’s model (5) are functions of α and ε, and may be regarded as representing the two phenomenological decay rates obtained from a given experiment.

We thank Prof. John Lombardi of the University of Illinois and Prof. Richard Bersohn of Columbia University for exchanges of views, and Dr. Terence Tao of the MRC Laboratory of Molecular Biology (Cambridge, England) for checking our derivation and verifying our results; all parties are now in agreement on our Eq. 1. We acknowledge support from the National Science Foundation.