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Theory of Fluorescence Polarization of Oriented Pigment Molecules in Spherical Arrays

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Summary. Theoretical results are presented which are appropriate for the analysis of the static polarized fluorescence experiment with oriented pigment molecules in spherical arrays (vesicles). Though the global orientation mediated over the whole sphere is isotropic, the fluorescent molecules may have preferred local orientation with respect to the local plane. As in a former paper, concerning fluorescence polarization in planar arrays, three basic (local) orientation distributions of the electronic transition moments are investigated, which may be expected to describe a wide class of real cases with sufficient accuracy. Analytic expressions for the degree of polarization are derived. One important result is that the degree of polarization may be extremely dependent on the local orientation of transition moments. Hence the usual method of determination of microviscosities from experiments with vesicles with the use of the theory of fluorescence polarization for macromolecules in solutions should be regarded with great caution.

Key words: Fluorescence polarization – Vesicles – Local orientation.

Introduction

In a recent paper [1] we have developed a quantitative theory of the static polarized fluorescence experiment with fluorescent molecules in planar arrays. With this theory it has been possible to study orientation and mobility of molecules incorporated into artificial plane membranes. – Often it may be more convenient to perform fluorescence experiments with molecules bound to spherical membranes (e.g. vesicles)[1]. Hence in this paper a theory of fluorescence polarization of oriented molecules in spherical arrays is presented.

In this connexion "orientation" means local orientation with respect to the local membrane plane, whereas the global orientation distributions, mediated over the whole sphere, are assumed to be isotropic.

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[1] Results and further references concerning such experiments are reported in the review articles of G. K. Radda and J. Vanderkooi [2] and A. Azzi [3]

As in the well known theory of fluorescence polarization with macromolecules in solutions [4—5] only one independent degree of polarization is available from the experiment. Therefore the information from the polarized fluorescence experiment is much smaller than from experiments with oriented membranes (e.g.) [6].

Similarly as in [1] the method applied in this paper is as follows: From the infinite number of possible (local) orientation distributions we have choosen three model distributions, which on the one hand are as simple as possible, but on the other hand may be expected to be sufficient for the quantitative analysis of a wide class of real cases. We restrict to the case where the transition moments of absorption and emission within the molecules are parallel. A reorientation of transition moments during the excited state is assumed to be done by rotational motion of the molecules.

Theoretical Foundations

The basic assumptions for the theoretical description of the experiments are essentially the same as in I. But now the fluorescent sample consists of a number of *spherical* vesicles within an *isotropic medium.* Hence the stationary distribution of transition moments of absorption is isotropic (spherical symmetry). Throughout this paper we assume that the vesicles themselves do not move (rotate) during the lifetime of fluorescence τ. Furthermore the influence of translational motions of the pigment

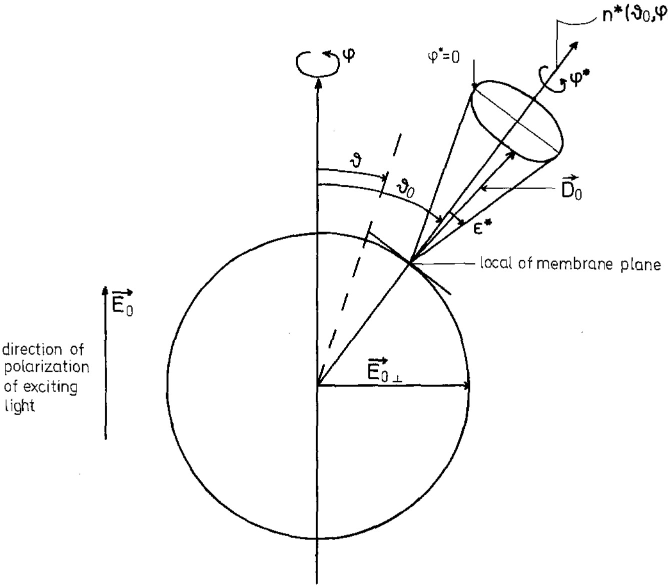

Fig. 1. Introduction of global polar coordinates (ϑ, φ), adapted to the polarization of exciting light, and of local coordinates (ε^*, φ^*) adapted to the local membrane plane. $\vec{E}_0$, $\vec{E}_{0\perp}\vec{D}_0$ are normalized vectors with $\vec{E}_0$ parallel to the direction of polarization of exciting light and $\vec{E}_{0\perp}$, perpendicular, $\vec{D}_0$ denotes the direction of a transition moment in local position ε^*, φ^*

molecules within the (spherical) membrane of the vesicles on possible reorientations is neglected. Both assumptions are justified because the typical lifetimes of fluorescence (between 1 and 100 ns) are in general small compared with the characteristic times governing translational motions of pigments and motions of the vesicles themselves.

In Figure 1 spherical coordinates ϑ, φ are introduced with the axis $\vartheta = 0$ parallel to the direction of polarization of the linearly polarized exciting light. The fluorescence process taking place in the experiment is as follows: The linearly polarized monochromatic light beam excites a stationary isotropic orientation distribution F_A of dipole moments of absorption. The intensity of absorption of a single dipole moment is proportional to $\cos^2 \psi$, where ψ is the angle between the electric vector of exciting light and the dipole. The *intensity of absorption* defined by

$$I_A = P_A \cdot F_A \cos^2 \psi \tag{1}$$

is obviously independent of φ (rotational symmetry with $\vartheta = 0$ as axis of symmetry). The factors of proportionality P_A, P_M in (1) and below in (2) are not specified. They depend on different quantities such as apparatus constants, quantum yield of fluorescence etc.

During the lifetime τ of the excited state a complex rearrangement of transition moments can take place, resulting in an intensity of emission I_E. I_E determines the fluorescence intensity I_M passing through a polarizing analyzer by

$$I_M = P_M \cdot \int_{\vartheta = 0}^{\pi} \int_{\varphi = 0}^{2\pi} I_E \cos^2 K \sin \vartheta \, d\vartheta \, d\varphi \tag{2}$$

The plane of the analyzer is oriented perpendicularly to the direction from the fluorescent sample to the analyzer. K is the angle between a transition moment of emission (direction ϑ, φ) and the direction of polarization of the analyzer. The densities F_A, I_A and I_E are defined as densities on the unit sphere.

Because the fluorescent sample is assumed to be completely isotropic, the emission I_E resulting from the rearrangement of transition moments must be independent of φ as well as the absorption I_A. This symmetry condition on the one hand yields an extraordinary simplification of the theory. On the other hand the information which can be derived from the experiment is smaller compared with the information which is contained in the polarized fluorescence experiment with molecules in oriented arrays (see [1]).

Hence all information is obviously contained in the polarized intensities $I_{\parallel}$, $I_{\perp}$ with direction of polarization parallel, perpendicular to the polarization of exciting light respectively [5]. In analogy to the polarized fluorescence experiment with macromolecules in solutions we define as the relevant quantity the degree of polarization P:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \tag{3}$$

P is independent of the factor of normalization P_M in (2).

Explicite Model Calculations

Additionally to (ϑ, φ) in Figure 1 are introduced local spherical coordinates (ε^*, φ^*) adapted to the local membrane plane. We will perform model calculations for three different local orientation distributions with respect to the local membrane plane, which are shown in Figure 2. All orientation distributions have local rotational symmetry with the normal n^* (ϑ_0, φ_0) as axis of rotation. In the *first* case the dipoles are assumed to be homogeneously distributed in ε_0^* around a mean position ε_0^*. Then the local districution F_A^1 (ε^*) is:

$$F_A^1(\varepsilon^*) = \begin{cases} \dfrac{1}{4\,\pi\,\Delta\varepsilon}\cdot\dfrac{1}{\sin\varepsilon^*} & \text{for} \quad (\varepsilon_0^* - \Delta\varepsilon) \le \varepsilon^* \le (\varepsilon_0^* + \Delta\varepsilon) \\ 0 & \text{else} \end{cases} . \tag{4}$$

Second a circular rectangular distribution is considered, i.e. homogeneous distribution on the unit sphere with a solid angle 2π (1—cos $\Delta\gamma$) around a mean position (ε_0^*, φ_0^*). Introducing polar angles $\bar{\varepsilon}$, $\bar{\varphi}$ with the local mean direction (ε_0^*, φ_0^*) as polar axis the stationary distribution F_A^2 belonging to the mean position (ε_0^*, φ_0^*) is:

$$F_A^2(\varepsilon_0^*, \varphi_0^*, \bar{\varepsilon}) = \begin{cases} \dfrac{1}{2\,\pi(1 - \cos\Delta\gamma)} & \text{for} \quad \bar{\varepsilon} \le \Delta\gamma \le \pi \\ 0 & \text{else} \end{cases} . \tag{5}$$

Third we regard a delta-shaped distribution $F_A^3(\varepsilon^*)$ of transition moments

$$F_A^{3*} = \frac{1}{\sin\varepsilon^*}\,\delta(\varepsilon^* - \varepsilon_0^*), \tag{6}$$

where the molecules are assumed to make local rotational diffusion in φ^*, i.e. around the normal n^*.

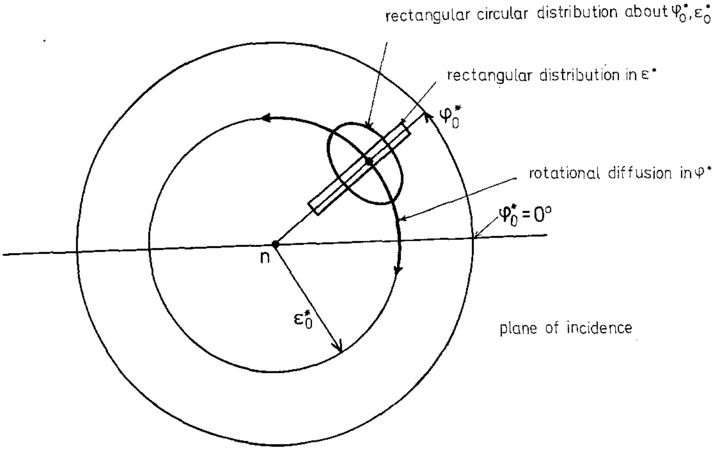

Fig. 2. Different local model distributions of transiton moments around a local mean position ε_0^*, φ_0^*, presented in a perpendicular projection from the unit sphere to the local membrane plane

The first two model distributions correspond to those discussed in I. The third case we have recently treated for plane membranes [6].

Introducing the coefficient of Brownian rotational diffusion D_φ and the lifetime of fluorescence τ the result for the local intensity of emission I_E^{3*} is according to [7] in local coordinates

$$\begin{aligned} I_E^{3*}(\vartheta_0, \varepsilon^*, \varphi^*) = \Bigg\{ & \frac{\sin^2 \varepsilon^* - \sin^2 \vartheta_0}{2} + \cos^2 \varepsilon^* \cdot \cos^2 \vartheta_0 \\ & + \left(\frac{1}{1 + D_\varphi \cdot \tau}\right)(2 \sin \varepsilon^* \cdot \cos \varepsilon^* \cdot \cos \varphi^* \cdot \sin \vartheta_0 \cdot \cos \vartheta_0) \\ & + \left(\frac{1}{1 + 4\, D_\varphi \cdot \tau}\right) \sin^2 \varepsilon^* \cdot \sin^2 \vartheta_0 (\cos^2 \varphi^* - \tfrac{1}{2}) \Bigg\} . \end{aligned} \tag{7}$$

We emphasize that the three considered distributions are oriented only with respect to the *local* membrane plane. Globally, i.e. mediated over the whole sphere they all yield isotropic distributions. Hence for all models, in case no (observable) rearrangements take place during the fluorescent state, the degree of polarization P is 0.5. The first two models are treated for lifetimes of fluorescence being great compared with the characteristic relaxation times of transition moments. For the angle ψ between a transition moment in local orientation (ε^*, φ^*) and $\vec{E}_0$ holds:

$$\cos \psi = + \sin \varepsilon^* \cdot \cos \varphi^* \cdot \sin \vartheta_0 + \cos \varepsilon^* \cdot \cos \vartheta_0 . \tag{8}$$

The calculations are facilitated, if we take into account that obviously the degree of polarization in the first two models depends only on $\Delta\varepsilon$, $\Delta\gamma$ respectively and not on the mean local orientation ε_0^*, because globally all orientations are equally represented. Hence for simplification of calculations we choose the mean local orientation to be $\varepsilon_0^* = 0$. In contrast to this in the third model the degree of polarization depends on the local orientation. For $\varepsilon^* = 0$ the local intensities of emission are for the first and second model:

$$I_E^{1*}(\vartheta_0, \varepsilon^*, \varphi^*) = \begin{cases} \begin{aligned} & \frac{\cos^2 \varphi^* \cdot \sin^2 \vartheta_0 + \cos^2 \vartheta_0}{2} \\ & - \frac{\cos^2 \varphi^* \cdot \sin^2 \vartheta_0 - \cos^2 \vartheta_0}{2} \cdot \left(\frac{\sin 2\,\Delta\varepsilon}{2\,\Delta\varepsilon}\right) \end{aligned} & \text{for} \quad -\Delta\varepsilon \le \varepsilon^* \le \Delta\varepsilon \\ 0 \qquad \text{else} & \end{cases} \tag{9}$$

and

$$I_E^{2*}(\vartheta_0, \varepsilon^*) = \begin{cases} \frac{1}{3} + \cos \Delta\gamma (1 + \cos \Delta\gamma) \left(\frac{1}{3} - \frac{\sin^2 \vartheta_0}{2}\right) & \text{for} \quad \varepsilon^* \le \Delta\gamma . \\ 0 \quad \text{else} & \end{cases} \tag{10}$$

For calculation of P the polarized intensities $I_\parallel$ and $I_\perp$ must be known. Additionally to $\vec{E}_0$, being parallel to the direction of polarization of incident light a normalized vector $\vec{E}_{0\perp}$ being perpendicular, is introduced (see Fig. 1). Then the intensities $I_\parallel$ and $I_\perp$ are given by integration of the local intensities of emission I_E^* over all orientations in the following way:

$$I_{\parallel}=\frac{1}{4\pi}\int_{\varphi_0=0}^{2\pi}d\varphi_0\int_{\vartheta_0=0}^{\pi}\sin\vartheta_0\,d\vartheta_0$$
$$\left(\frac{1}{4\pi}\int_{\varepsilon^*=0}^{\pi}\int_{\varphi^*=0}^{2\pi}I_E^*(\vartheta_0,\varepsilon^*,\varphi^*)\cdot(\vec{E}_0\cdot\vec{D}_0)^2\cdot\sin\varepsilon^*\,d\varepsilon^*\,d\varphi^*\right) \tag{11a}$$

and

$$I_{\perp}=\frac{1}{4\pi}\int_{\varphi_0=0}^{2\pi}d\varphi_0\int_{\vartheta_0=0}^{\pi}\sin\vartheta_0\,d\vartheta_0$$
$$\left(\frac{1}{4\pi}\int_{\varepsilon^*=0}^{2\pi}\int_{\varphi^*=0}^{\pi}I_E^*(\vartheta_0,\varepsilon^*,\varphi^*)\cdot(\vec{E}_{0\perp}\cdot\vec{D}_0)^2\cdot\sin\varepsilon^*\,d\varepsilon^*\,d\varphi^*\right). \tag{11b}$$

The scalar product between an orientation $\vec{D}_{0\perp}$and $\vec{E}_0$, $\vec{E}_{0\perp}$, respectively may be expressed in local coordinates as follows:

$$(\vec{E}_0\cdot\vec{D}_0)(\vartheta_0,\varepsilon^*,\varphi^*)=\cos\varphi^*\cdot\sin\varepsilon^*\cdot\sin\vartheta_0+\cos\varepsilon^*\cdot\cos\vartheta_0 \tag{12a}$$

$$(\vec{E}_{0\perp}\cdot\vec{D}_0)(\vartheta_0,\varphi_0,\varepsilon^*,\varphi^*)=-\cos\varphi^*\cdot\sin\varepsilon^*\cdot\cos\varphi_0\cdot\cos\vartheta_0$$
$$+\sin\varphi^*\cdot\sin\varepsilon^*\cdot\sin\varphi_0+\cos\varepsilon^*\cdot\cos\varphi_0\cdot\sin\vartheta_0. \tag{12b}$$

With (12) the evaluation of the integrals in (11) is elementary but rather lengthy for all three model distributions. The final results for the degrees of polarization are:

First model (local rectangular distribution in ε^*):

$$P=\frac{1+3A^2}{7+A^2},\quad A=\frac{\sin 2\Delta\varepsilon}{2\Delta\varepsilon}. \tag{13}$$

Second model (local circular rectangular distribution):

$$P=\frac{3B^2}{20+B^2},\quad B=\cos\Delta\gamma(1+\cos\Delta\gamma),\qquad \Delta\gamma\leqslant\pi. \tag{14}$$

Third model (local delta-shaped distribution in ε^*, rotational diffusion around the local normal to the membrane):

$$P=\frac{\left\{\begin{aligned}&4\cos^4\varepsilon^*+\sin^4\varepsilon^*-4\sin^2\varepsilon^*\cos^2\varepsilon^*\\&+\left(\frac{1}{1+D_\varphi\cdot\tau}\right)12\cos^2\varepsilon^*\cdot\sin^2\varepsilon^*+\left(\frac{1}{1+4D_\varphi\cdot\tau}\right)3\sin^4\varepsilon^*\end{aligned}\right\}}{\left\{\begin{aligned}&8\cos^4\varepsilon^*+7\sin^4\varepsilon^*+12\sin^2\varepsilon^*\cos^2\varepsilon^*\\&+\left(\frac{1}{1+D_\varphi\cdot\tau}\right)4\cos^2\varepsilon^*\cdot\sin^2\varepsilon^*+\left(\frac{1}{1+4D_\varphi\cdot\tau}\right)\sin^4\varepsilon^*\end{aligned}\right\}}. \tag{15}$$

The results are plotted in Figures 3, 4, 5. In all cases holds $P=1/2$, when no rearrangement can take place ($\Delta\varepsilon=0$, $\Delta\gamma=0$, $D_\varphi\cdot\tau=0$ or $\varepsilon^*=0$). A complete depolarization in the third model is possible only for $\mathrm{tg}^2\,\varepsilon^*=2$ ($\varepsilon^*\approx 54{,}3^\circ$) and $D_\varphi\cdot\tau\to\infty$.

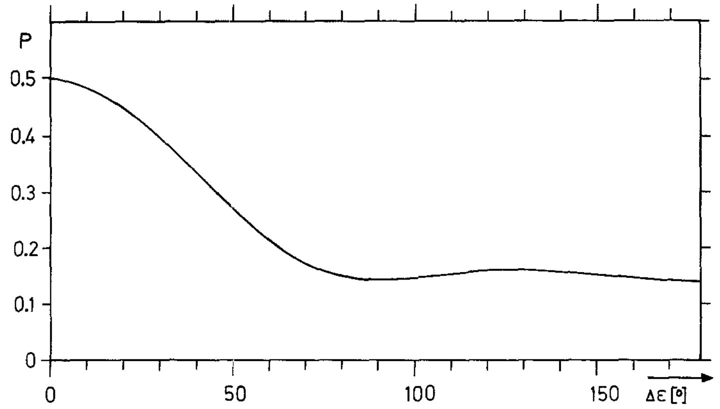

Fig. 3. Degree of polarization P as a function of $\Delta\varepsilon$ for model distribution 1

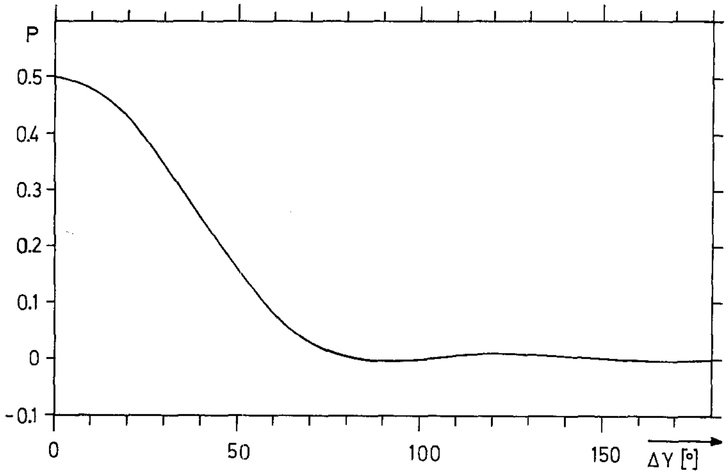

Fig. 4. Degree of polarization P as a function of $\Delta\gamma$ for model distribution 2

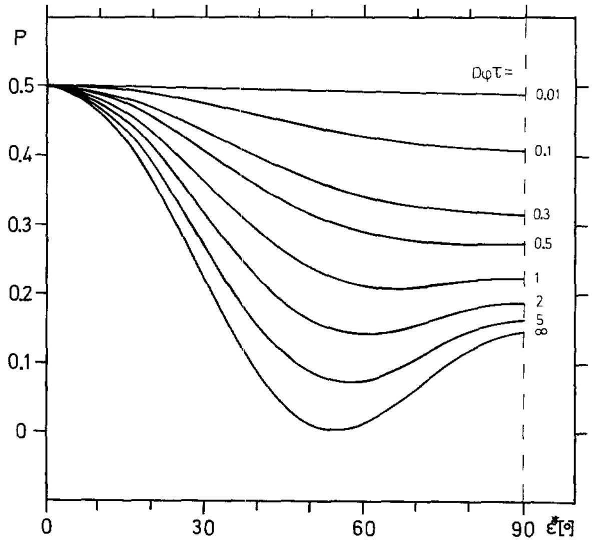

Fig. 5. Degree of polarization P as a function of local orientation ε^* for different values of $D_\varphi \cdot \tau$

Discussion

Because the static polarized fluorescence experiment with molecules in spherical arrays yields only two independent polarized intensities, and hence only one degree of polarization, it is in general not possible to derive unique information about orientation and mobility without having available additional information from other experiments. Generally, the measured degree of polarization can be fitted by all three calculated model distributions, e.g. for a degree of polarization $P \approx 0.3$: First model $\Delta\varepsilon \approx 45°$, second model $\Delta\gamma \approx 35°$, third model: orientations ε^* from about 25° up to 90° with varying mobility $D_\varphi \cdot \tau$ from ∞ to ≈ 0.4. Furthermore the results for the third model distribution in Figure 5 show, that from the degree of polarization P alone determinations of microviscosities (see e.g. [8, 9] are rather difficult and should be done with great caution, if no further informations about preferred local orientation and rotational degrees of freedom are available. A further complication arises when molecules are bound to the vesicle membranes in two different populations (orientations). In this case a change in the degree of polarization may be coupled to a change in the preference of one of these populations and not to a change in microviscosity.

In any way fluorescence polarization experiments with oriented systems, e.g. plane membranes [1, 10] or cylindrical (nerve) membranes [11, 12] contain much more independent informations about orientation and mobility of the fluorescent probes. Nevertheless the polarized fluorescence experiment with vesicles can provide us with helpful information, if from other experiments (e.g. with plane membranes) or from chemical and geometric properties of the fluorescent probes informations are available about the nature of the incorporation into membranes. On the basis of these additional informations one can take into account one of the calculated model distributions for the theoretical interpretation of the measured degree of polarization. E.g. for the first and second model the spread $\Delta\varepsilon$ or $\Delta\gamma$ of the non delta-shaped distributions may yield information about the degree of binding to the membrane. For the delta-shaped distribution one gets information about the rotational mobility around the normal to the membrane if the orientation of transition moments with respect to this normal is known.

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