Quenching of Perylene Fluorescence by Co$^{2+}$ Ions in Dipalmitoylphosphatidylcholine (DPPC) Vesicles

A. S. Holmes, D. J. S. Birch, and T. Salthammer

Received June 14, 1993; revised September 8, 1993

We report the fluorescence quenching of perylene by CoCl$_2$·6H$_2$O in small unilamellar DPPC vesicles via energy transfer. At the probe-to-lipid ratio of 1:200 and quencher to lipid ratios of ≥12.5:1, donor–donor energy transfer between clustered perylene molecules was observed as well as energy transfer from the perylene molecules to cobalt ions both above and below the main phase transition temperature of the lipid. The fluorescence quenching of perylene by CoCl$_2$·6H$_2$O in the lipid gel state is shown to be well described by Förster long-range energy transfer when both donor–donor and donor–acceptor energy transfer are considered. In the liquid crystalline phase diffusion of the molecules is described as well as energy transfer. The interaction radius $R_o$ for energy transfer from perylene to Co$^{2+}$ is found to be $\sim 13.4 \pm 1.1$ Å in the gel phase at 303 K, in good agreement with the theoretical value for $R_o$ of 13.9 Å. In the liquid crystalline phase at 323 K the lower value obtained for $R_o$, $\sim 11.3 \pm 1.6$ Å, is attributed to saturation of the Co$^{2+}$ ions at the interfacial region of the bilayer. A diffusion coefficient of $(1.06 \pm 0.15) \times 10^{-6}$ cm$^2$ s$^{-1}$ is obtained for perylene–cobalt diffusion in the liquid crystalline phase at 323K.

KEY WORDS: Perylene; cobalt(II) ions; lipid bilayers; energy transfer.

INTRODUCTION

Perylene has previously been used as a probe in studies of lipid bilayers and the basic fluorescence and anisotropy properties are well-known [1,2]. As a non-polar, nearly disk-shaped molecule, perylene can easily be integrated into vesicles. Below the phase transition temperature, $T_m$, diffusion in the membrane is slow, due to its highly ordered state, whereas above the $T_m$ the bilayer is more “fluid” and diffusion can occur.

The fluorescence quenching of perylene by transition metal ions via exciplex formation [3,4] or energy transfer [5,6] has been studied in detail in homogeneous solutions. We have recently reported the long-range energy transfer between perylene molecules and cobalt ions in DPPC liposomes in the gel phase [7]. In this preliminary study we also noted donor–donor energy transfer between the perylene molecules, which we attributed to clustering of the probe molecules in the gel phase of the bilayer. Such donor–donor energy transfer was also noted in fluorescence anisotropy studies of 2,5,8,11-tetra-t-butylerylene [8]. Most fluorescence quenching studies date in heterogeneous media have been in micellar solutions, where, for example, the quenching of singlet excited pyrene by metal ions [9–12] and the quenching of perylene fluorescence by diazonium salts [13] have been reported.

Theoretical expressions to describe dipole–dipole energy transfer in homogeneous media were first developed by Förster [14] and then by various authors in the regions where Förster’s theory breaks down [15–21], Förster’s model [14] being strictly valid only for negli-
gible diffusion at low donor and high acceptor concentrations. The models proposed by Huber [15,16] and Loring, Anderson, and Fayer (LAF) [19] are valid in the region where the donor–acceptor energy transfer is slow in comparison to the donor–donor energy transfer and diffusion is absent. Huber’s work is a restricted case of the LAF theory. These models have been verified experimentally in solution by Pandey et al. [22] and shown to be still useful in lipid bilayers [7]. When diffusion is occurring, then the models proposed by Gösele et al. [20,21] provide a simple and reasonable approximation. Two models are available, depending on the extent to which diffusion is influencing the energy transfer [20,21]. These models have been used experimentally by Tamai et al. [23] and Pandey and Pant [24] to describe diffusion-controlled energy transfer between rhodamine 6G and malachite green and between acriflavine and rhodamine 6G, respectively.

In the present paper, we assess the application of various isotropic energy transfer models to anisotropic media in a detailed study of the deactivation of singlet excited perylene by cobalt(II) ions in small unilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles. Previously we have shown that due to the large spectral overlap of perylene emission and cobalt absorption, the fluorescence of perylene is quenched by $[\text{Co(H}_2\text{O)}_6]^{2+}$ ions via Förster energy transfer in glycerol [6]. Membranes are of fundamental interest in this context because they offer a better-defined separation between donor and acceptor molecules. Understanding fluorescence probe kinetics might then lead to further methods of studying membrane and metal ion interaction. In this case the Co$^{2+}$ ions can be regarded as located at the polar headgroup of DPPC during the short fluorescence lifetime of perylene [25]. We are interested in the two states of the lipid bilayer: (i) the gel state (L$_g$ phase), where the effect of diffusion should be small and energy transfer is dominant, and (ii) the liquid crystalline state (L$_o$ phase), where we expect a significant contribution to the quenching from diffusion.

**KINETIC MODELS**

For the case of resonance energy transfer in the absence of diffusion, Förster gives the fluorescence response function as [14]

$$I(t) = I_o \exp \left( -\frac{t}{\tau_0} - 2\gamma \sqrt{\frac{t}{\tau_0}} \right)$$

where $\tau_0$ is the lifetime of the unquenched donor and $\gamma = [A]/C_{AO}$ with $[A]$ the acceptor concentration and $C_{AO}$ the critical acceptor concentration for energy transfer. The critical transfer distance $R_0$ can be expressed by the following equation:

$$R_0^2 = \frac{9\ln(10)\kappa^2 \eta_o}{128\pi^3 \chi_0 N \tau_0} \int_0^\infty F_d(\nu) \frac{e_d(\nu)}{\nu^2} \, d\nu$$

Here $\eta_o$ is the emission quantum yield of the donor in the absence of the acceptor, $\kappa$ is the orientation factor, and $\eta$ is the refractive index of the solvent. $F_d(\nu)$ is the emission intensity of the donor and $e_d(\nu)$ is the extinction coefficient of the acceptor at the wavenumber $\nu$. For donor and acceptor molecules which rotate freely and rapidly relative to the donor fluorescence lifetime, $\kappa^2 = 0.67$. This is the case above the phase transition in the lipid but not below, where the perylene motion is restricted [2]. However, when either the donor or the acceptor molecule is free to rotate, as is the case here with the Co$^{2+}$ ions, then the extreme values for $\kappa^2$ are 1.33 and 0.33, resulting in a maximum error in $R_0$ due to assuming $\kappa^2 = 0.67$ of approximately 12% [26,27]. The critical concentration $C_{AO}$ is related to $R_0$ by

$$C_{AO} = \frac{3}{2\pi^{3/2} \chi R_0^3}$$

When the rate of energy transfer from donor to donor is comparable to that from donor to acceptor, then the donor fluorescence decay is more appropriately described by a modified form of the Förster equation as proposed by Huber [15,16]:

$$I(t) = I_o \exp \left( -\frac{t}{\tau_0} - \sqrt{2 \gamma} \left( \frac{t}{\tau_0} \right)^{1/2} \right)$$

where the subscripts D and A refer to donor and acceptor, respectively. The reversible migration of energy among the donor molecules is accounted for by the reduced factor for the donor transfer [15].

When the effects of diffusion are also present in the system as well as energy transfer, then the expressions given by Gösele et al. [20,21] are more appropriate. There are two forms of the donor decay, depending on the extent to which diffusion is influencing the energy transfer kinetics. They are

$$I(t) = I_o \exp \left( -\frac{t}{\tau_0} - 4\pi D_R [A] N \tau_0 t \right) - 2\gamma \left( \frac{t}{\tau_0} \right)^{1/2}$$