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DYNAMIC IMAGING OF DIFFUSION IN ONE DIMENSION BY CW-ESR

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SUMMARY Dynamic Imaging of Diffusion by ESR has in the last several years emerged as a powerful new technique for accurate measurement of the translational diffusion coefficients of nitroxide radicals in isotropic and oriented (anisotropic) fluids. The methodology and some recent results on oriented model membranes are reviewed in this work.

INTRODUCTION

Detection and monitoring inhomogeneous concentration profiles of spin probes resulting from either diffusion and/or reduction is important in medicine and technology, for studying areas of altered physiology (which would be expected to exhibit different types of metabolic rates and processes), or reaction of tissues to different drugs (spin-labeled) on the one hand, and for studying oxidative degradation of polymers, through monitoring diffusion of the O_2 from the surface into bulk polymer, on the other. In addition, there is a need for development of ESR imaging methods for basic research efforts in the field of molecular dynamics. First, there is HESR (Heisenberg spin exchange) for studying translation diffusion over microscopic dimensions (1). Second, ESR is routinely used to obtain information about the rotational dynamics of spin probes (2). Therefore, in the study of molecular dynamics in condensed media by ESR, there is need foir a convenient technique for measuring macroscopic translational diffusion coefficients for spin probes, especially in anisotropic media, since this would enable simultaneous, yet independent, studies of rotational and translational motions on the same sample. Such studies have been carried out in our laboratory (3-5).

Dynamic imaging of diffusion involves dynamic samples with an inhomogeneous distribution of «impurities» (like spin probes, paramagnetic or optically active centers, radio tracers) in the host material. The term «dynamic sample» is used to describe a sample in which, with the passage of time, this inhomogeneous distribution will tend to a homogeneous distribution via translational diffusion. The use of ESR imaging for «dynamic samples» to investigate transport phenomena has been accomplished in just a few laboratories (6-10). The first experiments to measure diffusion coefficients of spin probes required either long experimental times (8, 11) (several days) or assumed an idealized distribution of the spin-probe concentration profile (6).Quite recently, a significant breakthrough in the development of the technique of dynamic imaging of diffusion by ESR (DID-ESR) was made by applying Fourier-space analysis of the data (9), and subsequently the methodology of the experiment and the numerical analysis of the data was improved (5, 10). Thus, it is possible to measure, within an hour, by DID cw-ESR, diffusion coefficients of the order of 10^{-7} cm^2s^{-1} with an accuracy better than a few percent, and of the order of 10^{-9} cm^2s^{-1} with an accuracy of 10-20%. This relatively short experimental time permits one to peform a multiple diffusion measurement on the same sample, e.g., to perform a series of measurements at different temperatures (3-5).

In this contribution we review the state of art of the technique of dynamic imaging of diffusion by cw-ESR in one dimension (DID cw-ESR), recently developed in our laboratory. We will also briefly discuss the application of DID cw-ESR to study translational diffusion in model membranes.

THEORETICAL BACKGROUND

The basic concept of DID cw-ESR is to monitor the time evolution of a non-uniform concentration of probes (spin probes) in one dimension using ESR spectroscopy. The time evolution of the concentration profile results from the translational diffusion of spin-probes, in a sample in which they are (initially) inhomogeneously distributed, as they tend to a (final) state of homogeneous distribution. A diffusion coefficient can then be determined from changes of the spin-probe distribution in time. The spin-probe can be used as a marker for the imag-

ing of diffusion only if the ESR signal is independent of the concentration. It is, therefore, very important that any time during the experiment, the concentration of spin-probes at any point in a sample be low enough that the line-broadening from Heisenberg spin-exchange (HSE) can be neglected. It is to the advantage of the analysis of the concentration profiles that the concentration of spin-probes is also low enough for the tranlsational diffusion to obey Fick's Second Law (12):

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}\,, \qquad [1]$$

where D is the diffusion constant.

The solution of Eq. [1] for the general case of an arbitrary initial distribution of spin-probes, in the absence of any boundary effects, can be written down as a convolution of the initial distribution C (x, t = O) with the Gaussian function, G (x; 2 Dt) (12):

$$\begin{aligned} C(x,t) &= \int_{-\infty}^{\infty} C(x',t=0)G(x-x';2Dt)dx' \\ &= \frac{1}{\sqrt{4\pi Dt}}\int_{-\infty}^{\infty} C(x',t=0)e^{-(x-x')^2/4Dt}dx'\,. \end{aligned} \qquad [2]$$

Eq. [2] may easily be adapted to any specific boundaries such as reflecting walls at x = 0 and L (13).

Therefore, one can take advantage of the fact that the concentration profile at any time can be considered as a convolution of the initial distribution with a Gaussian function (*cf.* Eq. [2]). Following the convolution theorem [14], the Fourier transform (*FT*) of both sides of Eq. [2]) yields:

$$C(k,t) = G(k;2Dt)\cdot C(k,t=0)\,, \qquad [3]$$

or,

$$\begin{aligned} \ln C(k,t) &= \ln G(k;2Dt) + \ln C(k,t=0) \qquad [4] \\ &= -4\pi^2 Dt\cdot k^2 + \ln C(k,t=0)\,, \end{aligned}$$

where k is the inverse wavelength and C (x, t) $\overset{FT}{\leftrightarrow}$ C (k, t) and G (x; 2 Dt) $\overset{FT}{\leftrightarrow}$ G (k; 2 Dt) are «Fourier transform» pairs.

The simplicity of the DID cw-ESR experiment lies in the fact that we are monitoring solely the component of diffusion in the direction of the one-dimensional magnetic field gradient, even though the sample is three-dimensional. Let us then consider a one-dimensional inhomogeneous concentration of spin-probes along some arbitrary direction x at time t, C (x, t). Let us also assume that a uniform magnetic field gradient parallel to x can be generated, $\nabla_x B$. Therefore, in the presence of the gradient, x becomes linearly mapped onto B and *vice versa* and one may then consider both variables equivalent: $x \nabla_x B = B - B_0$.

The absorption ESR spectrum in the presence of the magnetic field gradient, I_g (B, t), is a superposition of the signals of spin-probes at different positions; i.e. I_g (B, t) is the convolution of the absorption spectrum in the absence of magnetic field gradient, I_0 (B), with the concentration profile C (B, t) (8, 10):

$$I_g(B,t) = \int_{-\infty}^{\infty} C(B_a,t) I_o(B - B_a) dB_a / \int_{-\infty}^{\infty} C(B_a) dB_a \,. \quad [5]$$

In order to extract the concentration profile, we again take advantage of the convolution theorem (14). The Fourier transform of both sides of Eq. [5] gives:

$$\mathcal{I}_g(\kappa,t) = C(\kappa,t)\mathcal{I}_o(\kappa) \,, \quad [6]$$

where I_g (B, t) $\overset{FT}{\leftrightarrow}$ I_g (k, t), I_0 (B) $\overset{FT}{\leftrightarrow}$ I_0(k) and C (B, t) $\overset{FT}{\leftrightarrow}$ C (k, t) are «Fourier-transform pairs» and k is the «inverse wavelength» associated with B. [Note that since $B = x \nabla_x B$, then the inverse wavelength (associated with x) is $k = k\nabla_x B_x$].

Given two concentration profiles obtained at different times, t_i and t_j, Eq. [4] and Eq. [6] yield:

$$\ln \frac{|\mathcal{I}_g(k,t_i)|}{|\mathcal{I}_g(k,t_j)|} = -4\pi^2 D k^2 \Delta t_{ij} \,, \quad [7]$$

where $\Delta t_{ij} = t_i - t_j$, and $I_0(k)$ factors out. Consequently, the gradient-off spectrum is not necessary in obtaining D.

Although the principles of the DID cw-ESR experiment are simple, the experimental noise is a crucial constraint limiting the range of useful k-modes that provide accurate data on the diffusion coefficient (10). Suppose the change of gradient-on ESR spectrum during the time interval t_{ij} is small, then the lhs of Eq. [7] can be expanded in Taylor series:

$$\ln \frac{|I_g(k,t_j)|}{|I_g(k,t_i)|} \sim \frac{\Delta I(k,\Delta t_{ij})}{|I_g(k,t_i)|}, \qquad [8]$$

where ΔI (k, Δt_{ij}) is the difference of the amplitudes of the two spectra at times t_i and t_j. One notes from Eq. [8] that ΔI should be of the order of the experimental noise for a given k mode to be useful:

$$\frac{\Delta I(k,\Delta t_{ij})}{I_g(k,t_i)} \geq \epsilon, \qquad [9]$$

where ε is the inverse of the signal-to-noise ratio in k-space ($\varepsilon = 1/(S_n/N)$. Therefore, the lower limit of useful k modes, k_{min}, is determined at the point where the difference in the magnitude in the two paired spectra starts to overcome the experimental noise.

On the other hand, the amplitude of the Fourier transformed ESR spectrum does decrease with increase of k, so it will become lower than the noise level at sufficiently high k. The upper bound of useful k modes, k_{max}, may be set at the point where the amplitude is approximately equal to that of noise:

$$\frac{|I_g(k_{max},t)|}{|I_g(0,t)|} \sim \epsilon. \qquad [10]$$

A basic requirement for a successful measurement of diffusion is, of course, $k_{min} < k_{max}$.

More rigorous quantitative discussion and estimations of optimum experimental conditions show that both k_{min} and k_{max} depend not only on ε, but also on Δt_{ij}, the variance of the

concentration profile at the begining of experiment σ_i^2, and the ratio between the variance of the ESR line, Δ_B^2 (i.e. the rms width of Gaussian ESR line in the absence of a field gradient) and the magnetic field gradient $\nabla_x B$. For example, the applied magnetic field gradient is an external source of inhomogeneous broadening of the ESR spectrum. The stronger the magnetic field gradient for a given concentration profile, the lower will be k_{max}, and vice versa.

To optimize the experiment, one has to make the difference between k_{max} and k_{min} a maximum. We have shown elsewhere that this is achieved if [10]:

$$\frac{(\nabla_z B)^2}{\Delta_B^2} = \left[\sqrt{\frac{\alpha}{\beta \ln \epsilon_\kappa}} + 1\right]\left[\alpha - \sqrt{\frac{\alpha\beta}{\ln \epsilon_\kappa}}\right]^{-1} \qquad [11]$$

$$\simeq [D\Delta t_{ij}\sqrt{2\epsilon \ln \epsilon}]^{-1} \quad \text{for } \epsilon \gg 1 \text{ and small } \sigma_i^2 ,$$

where $\alpha = \varepsilon_k D\Delta t_{ij} - \sigma_i^2$ and $\beta = 2D\Delta t_{ij} + \sigma_i^2$ (Note that Eq. [11] has a physical solution only if $\alpha > 0$, i.e. $D > \sigma_i^2(t)/(\varepsilon_k \Delta t_{ij})$).

The error for eack k mode can be monitored by analyzing the data in two steps (5). First, the diffusion coefficient for each pair is calculated by plotting $\ln \frac{I_g(k,ti)}{I_g(k,tj)}$ (cf. Eq. [7] with respect to $k^2\ \Delta t_{ij}$ for arbitrarily fixed k_{max}, (i.e. the k modes less than k_{max} are taken into account). Second, the first step is repeated for a range of values for k_{max}, and $D(k_{max})$ is obtained by averaging the values from all time-pairs for each k_{max}. Figure 1 shows how $D(k_{max})$ goes through a plateau over a limited range of k modes (for which $k_{min} \leq k \leq k_{max}$). It also shows that the plateau region corresponds to the minimum rms deviation in the calculated diffusion coefficients at a given k_{max}. Therefore, one can objectively choose the D at the plateau region as a reliable diffusion coefficient.

APPLICATION TO DIFFUSION OF CHOLESTEROL IN MODEL MEMBRANES

An excellent example of the applicability of DID cw-ESR to detailed studies fo molecular dynamics is offered by our investigations of the effects of cholesterol on the dynamics and the structural properties of the sterol type spin probe CSL in phospholipid/cholesterol type (POPC/cholesterol and/or DMPC/

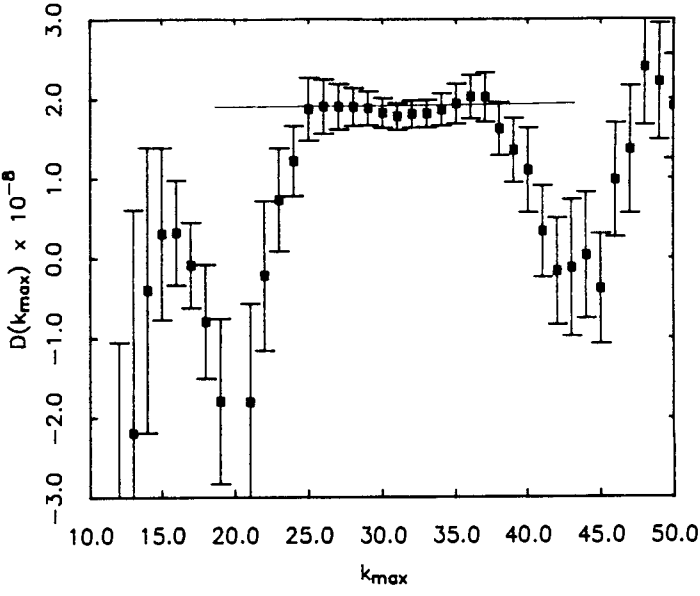

FIG. 1. Average behavior of the $D(k_{max})$ as a function of k_{max} for CSL diffusing in 1:1 POPC:DMPC (5). 9 pairs of lg(k, t) were used in the calculations. Error bars correspond to the standard deviation of the average of 9 points. Note that the plateau is also distinguished by a significant decrease in the random errors over the range.

POPC/cholesterol; POPC stands for 1-palmitoyl-2-oleoyl-*sn*-glycero-phopshatidylcholine, and DMPC for 1,2-dimyristoyl-*sn*-glycero-phosphatidylcholine) oriented multilayer model membranes (3-5). Sample preparation and instrumental details are given elsewhere (5, 10) Due to the relatively small translational diffusion constants of this spin label in the model membranes (~ 10^{-8} cm^2 s^{-1}), we were able to perform multiple measurements of the translational diffusion coefficient, D, on a single sample. Furthermore, by analyzing the gradient-*off* spectra collected in the course of the DID cw-ESR experiment, we also simultaneously obtained for the same sample, the order parameter, S and the rotational diffusion constant, $R_\perp$, utilizing ESR spectral simulation methods (2). Since the spin probe was chosen purposely to mimic the cholesterol behavior, this enabled in-depth considerations of the effects of the membrane composition and temperature on the dynamic molecular structure of membranes. Even though the diffusion coefficient is measured from the time evolution of the concentration profile, it is a tracer diffusion coefficient (self-diffusion of tracer molecule) rather than a mutual diffusion coefficient, since the concentration of spin probes is kept so low that the spatial chemical potential gradient is negligible; we shall assume that the

spin-labeled sterol (CSL) properly displays the diffusive properties of cholesterol (4):

For the different model membranes we studied, we found a remarkable correlation between the lateral diffusion coefficient and the order parameter, i.e., $D_\perp = D^o_\perp \exp[-E_\alpha(x, T)/RT]$, where the activation energy is $E_\alpha(x, T) = \alpha(T)S^2(x, T) + \beta$ and $\alpha(T) = a + b/T$ (4, 5).

A similar correlation was also found between $R_\perp$ and S as a function of both the mole fraction of cholesterol x and the temperature T in the liquid crystalline phase (4, 5). This provides a clear demonstration that the model membranes are *simple* nonideal solutions (3-5). Our results demonstrate the preferential association of cholesterol molecules (including CSL) with each other in POPC solvent. As result, the environment of CSL changes significantly as a function of x, from that of flexible POPC molecules, to the more rigid cholesterol molecules, which is manifested by a decrease of both D and $R_\perp$ with increasing x. On the other hand, the tendency of cholesterol to aggregate means that the POPC-rich regions are less influenced by cholesterol molecules than would otherwise be expected. Experiments with CSL spin label in DMPC/POPC/cholesterol ternary mixtures show a weaker effect of cholesterol on D, indicating that addition of the saturated lipid DMPC to the unsaturated lipid POPC enhances the mixing of cholesterol in PC model membranes (5).

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