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Stochastic ESR Analysis of Rat Liver and Hepatoma Mitochondrial Lipids

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Abstract. A commonly used model for the interaction of the motional narrowing of ESR lines is shown to be qualitatively misleading. An analysis of lipid extracts of mitochondrial preparations labeled with 12-nitroxide stearic acid produced linear plots of the logarithm of the correlation time versus the reciprocal of the absolute temperature when analyzed with stochastic computer simulations. However, when the data were analyzed with isotropic Lorentzian line shape approximations, nonlinear plots were obtained.

Key words: ESR – Isotropic and anisotropic rotation – Lorentzian line shape – Stochastic method – Mitochondrial lipids.

In the past two decades there has been a growing interest in using the motional effects of spin labels to study biological membranes. The infinitely sharp resonance lines of unpaired free electrons are broadened by a "local field" from G anisotropies and nuclear dipole interactions. In the presence of molecular motion, the average value of the "local field" is less than the instantaneous field and the resonance lines will narrow.

The nitroxide molecule is often used for spin labeling applications because it has the unusual property of being a stable radical and has an ideal hyperfine scale. It can be affixed to biological molecules, such as stearic acid or cholesterol, and used as a biological analogue which will not perturb the nearby molecular environment.

When attempting to describe ESR line shapes two major problems are often encountered: 1) the calculations become very tedious except under special limits [11], and 2) one must understand the details of the averaging process induced by molecular motions. In some cases, data can be interpreted in terms of correlation times which roughly correspond to the time required for the nixtroxide radical to rotate through an arc of one radian. Assuming a motional narrowing model, Kivelson [11] and Freed and Fraenkel [3] showed that correlation times were proportional to spectral peak widths. Under the assumptions of isotropic motion and Lorent-

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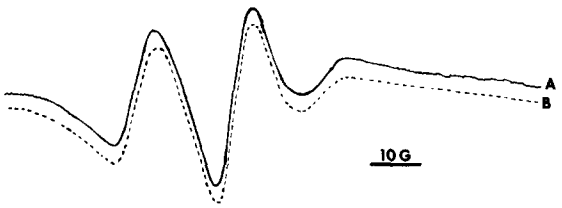

Fig. 1. (A) Experimental ESR spectrum of 12-nitroxide stearic acid labeled lipid extract of Morris hepatoma 21 mitochondrial preparation at 7.5° C **(B)** Stochastic computer simulated ESR spectrum for Brownian rotational diffusion with a correlation time of 7.5×10^{-9} s and an order parameter of 0.470. Approximately 50 different spectra with varying rotational diffusion constants and tilt angles would typically have to be simulated before a computer match could be obtained. Running times were about a minute per spectrum. Similar fits were obtained when lipid extracts obtained from rat liver mitochondrial preparations were studied. Mitochondrial preparations were obtained following homogenization of tissue with a Polytron®, and differential centrifugation [22]. Lipid extracts were prepared using methanol-chloroform extraction procedures [21]

zian line shapes one can obtain formulas for analyzing spectra based on peak heights and widths [18].

Recently mathematical models have been developed which are based on computer simulations and curve fitting. Some of these models assume rapid motion restricted by various angular limits [6–8, 12, 14] whereas other models consider spin labels undergoing diffusional rotations [2, 13, 20]. Of these models, the one of Freed et al. [2, 4] is undoubtly the most generally applicable and physically realistic. Freed's stochastic theory is valid in both the slow and fast motional regions and has been developed to include different models of rotational motion [5], as well as anisotropic rotation and the effect of a tilt angle [17]. Because of its mathematical complexity and the need for large regions of computer core, the stochastic method has not been applied to many systems of biological interest.

Cannon et al. [1] studied the fluidity and organization of mitochondrial membrane lipids of brown adipose tissue using nitroxide spin labels. They observed that analyses based on isotropic motional narrowing formalisms [3, 11] showed nonlinearities whereas stochastic computer simulations yielded linear plots of the log of the order parameter S vs $1/T$. The stochastic method also has been applied to a study of the influence of anesthetics and cholesterol on the degree of molecular organization and mobility of ox brain white matter [16].

We investigated lipid extracts of mitochondrial preparations obtained from Morris hepatoma 21 and rat liver which were labeled with 12-nitroxide stearic acid. The data were analyzed to obtain correlation times by using stochastic computer simulations with varying ordering parameters and rotational diffusion constants. The computer program used was obtained from Polnaszek [17]. When the data were analyzed by simple isotropic Lorentzian approximations [18], plots of the logarithm of the correlation time and reciprocal of the absolute temperature were nonlinear. However, stochastic analysis of the data showed that ordering parameters and correlation times varied linearly. Figure 1 is an example of an experimental spectrum and its computer generated match. Figure 2 illustrates the linear relationship between logarithm of the correlation time, obtained using stochastic computer simulations,

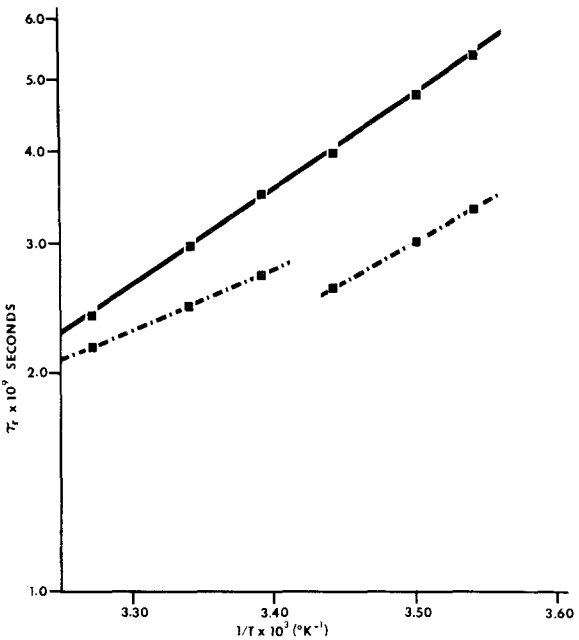

Fig. 2. Plot of logarithm of the correlation time τ_r, and reciprocal of the absolute temperature for 12-nitroxide stearic acid labeled lipid extract of rat liver mitochondrial preparation using stochastic computer simulations (▬▬); and motional narrowing line width and height measurements (■ ▬▬ ■). The computer simulated spectra required A tensor values of $A_x = 6.7$, $A_y = 5.6$, and $A_z = 32.0$ and G tensor values of $G_x = 2.0083$, $G_y = 2.0065$, and $G_z = 2.0030$. All spectra were simulated assuming a Brownian rotational diffusion model. In the presence of a weakly anisotropic diffusion, τ_r is a suitably chosen average over the rotational diffusion constants [17]

and reciprocal of the absolute temperature. Also shown is the plot obtained when the data were analyzed by simple isotropic Lorentzian approximations.

Numerous studies have appeared in recent years where apparent nonlinearities in correlation times have been associated with physical changes in membrane structure [9, 10, 15, 18, 19, 23]. The interpretation of the data of these studies is based primarily on isotropic motion and Lorentzian line shapes. It is entirely possible that some of these nonlinearities might be due to a breakdown in the equations used to analyze the data. A reanalysis of the spectra by more rigorous models, such as the stochastic, might well resolve this question.

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