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Spin-label CW microwave power saturation and rapid passage with triangular non-adiabatic rapid sweep (NARS) and adiabatic rapid passage (ARP) EPR spectroscopy

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ABSTRACT

Non-adiabatic rapid passage (NARS) electron paramagnetic resonance (EPR) spectroscopy was introduced by Kittell et al. (2011) as a general purpose technique to collect the pure absorption response. The technique has been used to improve sensitivity relative to sinusoidal magnetic field modulation, increase the range of inter-spin distances that can be measured under near physiological conditions (Kittell et al., 2012), and enhance spectral resolution in copper (II) spectra (Hyde et al., 2013). In the present work, the method is extended to CW microwave power saturation of spin-labeled T4 Lysozyme (T4L). As in the cited papers, rapid triangular sweep of the polarizing magnetic field was superimposed on slow sweep across the spectrum. Adiabatic rapid passage (ARP) effects were encountered in samples undergoing very slow rotational diffusion as the triangular magnetic field sweep rate was increased. The paper reports results of variation of experimental parameters at the interface of adiabatic and non-adiabatic rapid sweep conditions. Comparison of the forward (up) and reverse (down) triangular sweeps is shown to be a good indicator of the presence of rapid passage effects. Spectral turning points can be distinguished from spectral regions between turning points in two ways: differential microwave power saturation and differential passage effects. Oxygen accessibility data are shown under NARS conditions that appear similar to conventional field modulation data. However, the sensitivity is much higher, permitting, in principle, experiments at substantially lower protein concentrations. Spectral displays were obtained that appear sensitive to rotational diffusion in the range of rotational correlation times of 10^{-3} to 10^{-7} s in a manner that is analogous to saturation transfer spectroscopy.

1. Introduction

1.1. Background for non-adiabatic rapid sweep (NARS) electron paramagnetic resonance (EPR) spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed mutagenesis and spin-labeling can be used to study the structure and dynamics of biologically relevant proteins [1]. Although there are numerous EPR techniques available, it has become standard to investigate spin-labeled proteins by analyzing the continuous wave (CW) EPR spectrum.

Historically, CW EPR spectra have been collected by holding the microwave frequency constant and slowly sweeping a sinusoidally modulated magnetic field through the resonance condition. The signal is detected by collecting the first harmonic response with a phase-sensitive detector (PSD) set to the modulating frequency. Although this detection scheme improves baseline stability and overcomes $1/f$ noise, it carries a trade-off between signal height and spectral resolution as a function of the modulation amplitude.

The drawbacks associated with magnetic field modulation are well documented [2,3], and a number of alternative detection schemes have been developed to address some of those problems [4–6]. More recently, Hyde and co-workers developed Non-Adiabatic Rapid Sweep (NARS) EPR to overcome the drawbacks associated with field modulation [7]. In NARS, the polarizing magnetic field consists of the static magnetic field H_0, which is stepped slowly in time, plus a time-varying triangular magnetic field. Both fields must be homogeneous over the sample. The intent of the method is that the triangular sweep be sufficiently slow that the spins respond in the same manner as they would if only a slowly varying H_0 were present. The triangular sweep frequency is set at a value that is sufficiently high that $1/f$ noise is overcome. The full spectrum is obtained by collecting successive segments in a digital

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signal averager, followed by alignment and summation of overlapping spectral components. A pure absorption spectrum is obtained rather than the derivative-like spectrum that is conventionally acquired when using sinusoidal field modulation and phase-sensitive detection.

NARS provides several advantages over sinusoidal magnetic field modulation. Perhaps the greatest benefit, which results from time-averaging and collection of the pure absorption, is an improvement in sensitivity by a factor of four or more [7]. Collection of the pure absorption also avoids the line-shape–line-height compromise associated with field modulation. This enabled measurement of inter-spin distances at L-band [8] where linewidths are typically as narrow as one Gauss. Use of small modulation amplitudes in conventional EPR to ensure high spectral resolution resulted in insufficient signal amplitude to make this measurement. In addition, post-processing can be applied to the pure absorption spectrum to obtain the display of choice. In copper (II) spectra, the Moving Difference (MDIFF) algorithm was applied to the pure absorption spectrum to enhance the resolution of selected spectral features in a finite-difference display [9].

1.2. NARS spectroscopy under conditions of CW microwave power saturation

When using NARS with segmental acquisition, the CW microwave power saturation spectrum consists of the response of each point in the spectrum to increasing microwave power. There is no blurring of the saturation response as occurs from the use of magnetic field modulation in ordinary microwave power saturation experiments. For example, if one had a single homogeneously broadened line, the ordinary CW EPR spectrum when using field modulation under saturation conditions would be the off-resonance responses at the peaks of the derivative-like line, whereas in NARS it would be the response at the center of the EPR absorption line. The CW saturation response would be expected to be different in these two situations.

In the present work, we utilize NARS to investigate the saturation behavior of a spin-label attached to a slowly tumbling protein. Saturation studies have commonly been performed by measuring the peak-to-peak line height of the center line of the field modulated first harmonic as a function of the incident microwave power [10]. Structural information is obtained by observing the changes in this relationship in the presence of various paramagnetic relaxation enhancers.

We have discovered an interesting range of conditions in the very slow tumbling range of rotational correlation times where the turning points saturate differently from the regions between turning points. It is presumed that this effect arises from the spectral variation of the effective T_2 value. In this way, the turning points, which are characterized by slow anisotropic rotational diffusion in a defined solid angle, are revealed. This is a new result.

1.3. Experiments conducted at the transition between non-adiabatic rapid sweep and adiabatic rapid passage

The ability to study the role of the sweep direction also makes NARS suitable for investigating the onset of adiabatic rapid passage effects. There does not seem to be any prior experimental literature exploring the field of EPR spectroscopy at the interface of adiabatic and non-adiabatic sweeps of the polarizing magnetic field. The presence of passage effects in NARS spectroscopy is observed as a shift of spectral intensity in the direction of the sweep, whether up or down. This is the direction in which free induction decay (FID) signals are observed [5]. This phenomenon also occurs when detecting the dispersion using sinusoidal magnetic field modulation. Shifts of spectral intensity result in a change in phase of the signal [11,12]. A critical test of the presence of passage effects in NARS is reported here. If "up-field sweep" and "down-field sweep" pure absorption spectra are obtained (see Fig. 1) and displayed as a sweep in the same direction, then the spectra are substantially identical in the absence of passage effects. Another critical test is provided by the *g*-value moment theorem of Hyde and Pilbrow [13]. The theorem states that at the spectral point of the absorption rigid limit spectrum where the first moment is zero, the point corresponds to the trace of the g-tensor. Shifts of intensity due to passage lead to failure of the theorem.

Hyde and Dalton [14] observed that a range of conditions of very long rotational correlation time of a nitroxide spin label exists when the spectrum is substantially identical to that of a powder at low power but shows unusual passage effects at high power when using 100 kHz field modulation. They established that passage effects occur at the spectral turning points of the anisotropic Zeeman and hyperfine coupling but to a lesser degree at spectral points between turning points. This method is known as saturation transfer EPR spectroscopy (ST-EPR). It has been extensively used to study slow rotational diffusion. Reviews were provided by Beth [15] and by Marsh [16].

The use of sinusoidal modulation complicates the study of passage effects including ST-EPR. Weger described eleven different cases of passage and the various conditions where they apply [17]. Each case is determined by the incident microwave power, the relaxation properties of the spin system, and the spectral sweep rate. The superposition of a sinusoid on to the field sweep produces a non-uniform sweep rate, where the maximum rate is encountered at the zero crossover of the sinusoid and goes to zero at the extremities. This can create mixed states of passage within the modulation cycle, particularly at high incident powers. The combination of these passage states will be reflected in the line shape.

Passage effects in the NARS display offer an opportunity to advance this line of research, and this work provides an overview of the opportunity. The main advantages are: (i) that the sweep rate does not vary over the sweep cycle except for a change in sign,

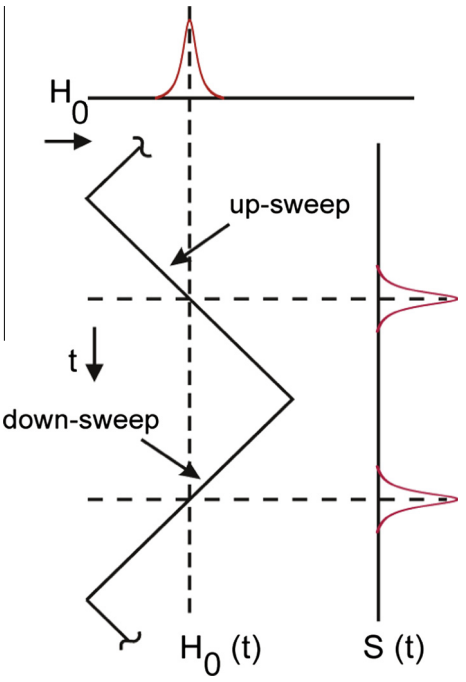

Fig. 1. Rapid passage effects can be investigated using the NARS technique by comparing the 'up-field sweep' of the triangle to the 'down-field sweep'.