Self-Aggregation of Spin Labeled Alamethicin in ePC Vesicles Studied by PELDOR

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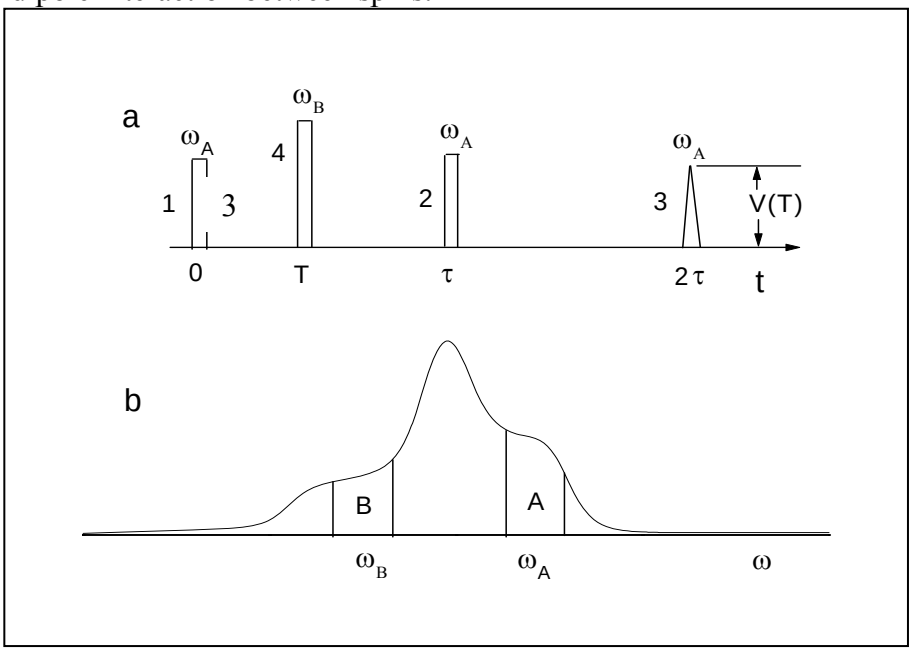
Supporting information

Sample preparation

The synthesis and properties of the spin-labeled alamethicin A16 studied in this work are described in [1]. We used Egg L-α phosphatidylcholin ("Sigma") and prepare the 20 mM Tris-HCl buffer containing 140 mM NaCl and 1 mM EDTA (pH 7.0). The samples of MLV were prepared as described in [2].The necessary amount of A16 was added to lipid solution in chloroform. Chloroform was removed by preliminary flush-drying with an argon gas followed by high vacuum pumping at room temperature for half an hour. After adding the Tris-buffer to the lipid film the dispersion equilibrate under argon at +4C during 12 h for complete lipid hydration. The milky suspension of LMV's are formed under the argon after three vortexing procedures with addition freeze-thaw cycle between vortexings. The samples containing about 80 μl of suspension were quickly freeze and then used in ESR and PELDOR measurements.

PELDOR experiments.

The PELDOR studies were carried out using the PELDOR spectrometer described in [3-5]. We use the simplest 3-pulse version of the PELDOR technique i.e. a usual two-pulse technique of electron spin echo at frequency ω_A with the addition of a pumping third pulse at frequency ω_B. The first and second pulses form spin echo originated from spins A at the frequency ω_A. The pumping pulse at the frequency ω_B rotates spins belonging to another region of ESR spectrum. The pumping pulse changes the dipole-dipole interaction of some spins and as a result, the spin echo amplitude starts to depend on both the magnitude of the dipole-dipole interaction between spins and the (*T*) position and the pumping pulse intensity. The PELDOR signal is the spin echo signal *V(T)* in the presence of pumping pulse and contains information on the dipole-dipole interaction between spins.



The durations of the first and second pulses forming the spin echo signal were 40 and 70 ns, respectively. The duration of the pumping pulse was 30 ns. The position of the pumping pulse corresponded to the maximum amplitude in the ESR spectrum. The frequency difference was 65 MHz.

The important parameter for PELDOR data analysis is a degree of ESR spectrum excitation by B pulse. As for homogeneous frozen solutions the PELDOR signal decay is exponential function of the type $V=\exp(-\alpha p_b CT)$, where α is the known numerical coefficient, $\alpha=1.65\times10^{-12}$ cm^3/ s and C is known concentration of A16 p_b was estimated to be 0.054±0.002 [4]. We use this p_b data for analysis as the shape of the ESR spectra of the solutions of spin labeled alamethicin A16 frozen to 77 K in ePC vesicles is typical of ESR spectra in the case of homogeneously disordered nitroxyl radicals in the solid phase.

References

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