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High-field ESR on aligned membranes: A simple method to record spectra from different membrane orientations in the magnetic field

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Abstract

A combination of isopotential spin-dry ultracentrifugation (ISDU) and microtome techniques was used to facilitate the collection of high field/high frequency (170 GHz) ESR spectra corresponding to different orientations of the membrane normal relative to the magnetic field. This technique is particularly valuable for aligned biological samples in vitro. At 170 GHz, conventional sample preparation techniques based solely on ISDU constrained the sample to be oriented so that the membrane normal was parallel to the applied magnetic field due to the geometry and the millimeter wave field distribution of the Fabry–Pérot resonator used in these experiments. This orientational constraint limited the information that could be obtained from aligned membranes at high field. The combined ISDU/microtome technique overcame this limitation. Spectra from spin-labeled Gramicidin A and the spin label cholestane in aligned DPPC membranes provide a demonstration of the technique. We also discuss some virtues of high field/high frequency ESR on aligned membranes compared to X-band.

Keywords: High field/high frequency ESR; Aligned membranes; Gramicidin A

1. Introduction

Multifrequency spin-label ESR is a valuable and well-proven approach to study the structure and molecular dynamics of biological membranes and membrane proteins [1]. Recording and analyzing ESR spectra of the same system over a range of ESR frequencies can effectively separate different modes of molecular motion, such as the overall tumbling of the entire molecule vs. the relative motion of its parts or the specific mobility of nitroxide tethers, and can facilitate unambiguous assignments of ordering and dynamical parameters via spectral analysis.

Utilization of well-aligned membrane samples not only dramatically improves ESR spectral resolution, but also provides particularly valuable information on the orientation of the nitroxide moiety relative to the membrane normal. Such information, particularly in the slow-tumbling regime, is difficult to extract from spectra obtained from vesicles. Because all orientations of the membrane normal relative to the magnetic field are averaged in vesicles, the orientation of the nitroxide moiety manifests itself only as a result of anisotropic molecular motion around the principal axes of the molecular frame. As one approaches the rigid limit, the vesicle spectrum converges to a ''powder'' spectrum, which is not sensitive to any properties of molecular structure, except for the magnetic tensors of the nitroxide.

The vesicle spectrum is a superposition of spectra corresponding to different orientations of the membrane normal relative to the external magnetic field (MOMD model [2,3]). Ambiguity in model parameters derived from spec-

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tral fitting is not unusual in vesicles. On the other hand, simultaneous fitting of ESR spectra of an aligned membrane sample in different orientations is in general less susceptible to such ambiguity [3,4].

There is a variety of methods to obtain well-aligned membrane samples. For the purpose of ESR spectroscopy, the best alignment on model membranes [4] can be achieved by ISDU or the isopotential spin dry ultracentrifugation method, introduced by Clark et al. [5]. The method is based on a simultaneous application of ultracentrifugation and drying and gives significantly improved membrane alignment compared to that achievable by either method alone. Another important advantage for the method is its ability to keep good membrane alignment in relatively thick membrane layers. Samples of DPPC membrane prepared by ISDU containing various spin labels with subsequent hydration, showed virtually identical ESR spectra in the whole range of membrane thicknesses 100–2000 μm. Thus, unlike other alignment methods, which are appropriate for samples only a few molecular layers thick, ISDU can be used to generate aligned samples consisting of many molecular layers. This feature is of special value for ESR, because one can work with an absolute number of spins large enough to facilitate spectral acquisition without increasing the spin concentration to levels that would cause spectral broadening. In the present work, cutting such thick discs of ISDU aligned membranes at a desired orientation yields flat thin slices, large enough to handle and study by ESR spectroscopy.

At X-band (~9.5 GHz) one can get ESR spectra corresponding to different orientations of the membrane normal relative to the magnetic field direction merely by rotating the sample in the resonator. Higher frequencies, however, require very thin (<100 μm) flat samples with B_0 directed perpendicular to the plane of the sample in order to minimize dielectric losses in the resonant structure.

Previously, Barnes and Freed [6,7] designed Fabry–Pèrot resonators for a 250 GHz ESR spectrometer to accommodate a thin sample that must rest with its flat surface perpendicular to the optical axis of the incident FIR beam. This geometry makes it possible to obtain high field/high frequency (HFHF) ESR spectra of macroscopically ordered samples at director tilts of 0° and 90°. However, this elegant solution is difficult to implement for the most common types of HF ESR spectrometers based on conventional resonators [8].

To obtain high field spectra in an orientation different from 0° (where the membrane normal corresponds to the sample normal), we apply a novel microtome technique to ISDU aligned samples. This simple technique allows samples to be prepared at any director tilt value, does not need special instrumentation, and can be used on ESR spectrometers at any frequency. The microtome, a mechanical device for cutting thin slices to be examined under microscope, has a long history in biomedical applications [9]. We have found that cryosection on fully hydrated membranes, where water-rich tissues are hardened by freezing and cut frozen, is the most suitable and convenient for our purposes.

2. Materials and methods

2.1. Materials

DPPC was obtained from Avanti polar Lipids (Birmingham, AL), CSL (3β-DOXYL-5α-cholestane) was bought from Aldrich, GAsl (spin labeled gramicidin A) was synthesized as described in [10].

2.2. Preparation of aligned membranes

ISDU—aligned lipid membranes were prepared as described in [4]. The technique utilizes sedimentation of the membrane fragments (in the gel phase) with simultaneous evaporation of the water phase in a vacuum ultracentrifuge. Measured amounts of DPPC and spin labeled compounds in a molar ratio 200:1 were dissolved in chloroform/methanol 3:1 v/v. The solvent was evaporated by nitrogen gas flow. To ensure complete removal of the solvent the sample was evacuated for 10–12 h. One milliliter of deionized water per 5 mg of lipid was added for the preparation of thin aligned samples which were studied at the 0° orientation. A higher concentration of 30 mg lipid in 1 ml water was used to produce thicker aligned samples which were later cut via the microtome technique. The mixture was sonicated 12 min above the chain melting temperature at a frequency of 20 kHz, and an incident power of 20 W/cm^2. For the ISDU procedure, a Beckman L8M-70 centrifuge with standard SW27 rotors at 15,000 rpm was used. The procedure took 24 h for DPPC at 20 °C. After ultracentrifugation, the alignment of the dry membranes was checked by a polarizing microscope (Nikon, Instrument Division, Garden City, NJ). Only those samples that exhibited the distinctive interference pattern indicative of an aligned membrane were used in the ESR experiments. The samples were initially kept at 100% relative humidity for 24 h and then transferred into water for 12 more hours. Typically, 25 mg of the dry lipid mixture per centrifuge bucket (volume 0.9 ml) yield a final ~300 μm thick hydrated aligned sample with area ~1.1 cm^2.

2.3. Microtome procedure

Thin cuts (~80 μm) of aligned membranes were made on a cryostat microtome (International Equipment, Model CTD), with an American Optical steel microtome knife. Initially, a lump of ice was frozen on the microtome cold plate (−15 °C) and shaped in the form of a rectangle (perpendicular cut) or a wedge, with one side resting on the cold plate and another side forming a smooth plane. The wedge angle corresponds to the desired director tilt angle. A flat piece of hydrated aligned membrane was put on the vertical or inclined plane and embedded in ice in this fixed position by dripping water onto the sample mounted