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Review

# Electron spin resonance of spin-labeled lipid assemblies and proteins

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ABSTRACT

Spin-label electron spin resonance (ESR) spectroscopy is a valuable means to study molecular mobility and interactions in biological systems. This paper deals with conventional, continuous wave ESR of nitroxide spin-labels at 9-GHz providing an introduction to the basic principles of the technique and applications to self-assembled lipid aggregates and proteins. Emphasis is given to segmental lipid chain order and rotational dynamics of lipid structures, environmental polarity of membranes and proteins, structure and conformational dynamics of proteins.

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## 1. Introduction

Electron spin resonance (ESR) is a spectroscopic technique that detects the resonant absorption of microwave radiation by a substance with at least one unpaired electron spin placed in a static magnetic field. It is widely used in different fields from basic research to industrial applications [1]. For systems that are ESR active, the technique is suitable to study the structure of solid and liquid samples and is useful to investigate dynamic processes. In biophysics, ESR represents a valid tool to characterize transition metal complexes, active site of metalloproteins, and radicals formed under a variety of conditions [1–4]. Its field of application has been extended to diamagnetic biosystems, such as lipid membranes, proteins, nucleic acids, by using the spin-labeling technique [5–7]. A spin-label is a stable free radical that can be introduced at a specific site in biological macromolecules and allows ESR to be applied. Spin-labels and spin-labeled molecules can be synthesized and are also commercially available. Versatile species are those based on the nitroxide –NO radical [5,6,8]. Advances in molecular biology concerning protein mutagenesis has determined a strong impulse in ESR spectroscopy through site-directed spin labeling (SDSL) methodology. In SDSL any protein residue can be selectively substituted with cysteines for spin-label attachment [9–11].

Spin-label ESR is commonly used at 9 GHz (X-band), but spectrometers working at lower frequency (1 GHz, L-band; 3 GHz, S-band) or higher frequency (24 GHz, K-band; 35 GHz, Q-band; 94 GHz, W-band; 130 GHz, D-band; 250 GHz, J-band) are also available. They are designed to operate in the continuous wave (cw), in the linear and non-linear regime under progressive saturation conditions, or saturation transfer. Some spectrometers operate in the pulsed Fourier Transform (FT) mode and in the time-resolved domain.

Spin-label ESR spectroscopy is largely driven by the sensitivity of the label to its surroundings. The motional sensitivity of the technique extends on a timescale range from $10^{-12}$ to $10^{-6}$ s (i.e., $T_2$ spin–spin relaxation timescale) to $10^{-6}$–$10^{-3}$ s (i.e., $T_1$ spin–lattice relaxation timescale). This timescale matches the one characteristic of molecular motions of lipid and protein components in membranes. The length-scale range goes from ca. 5–80 Å, important for structural investigations of biomacromolecules. Intra- and intermolecular distance determinations are possible; the distances sampled in singly labeled cw- and FT-ESR are increased by using double labeling and pulsed double ESR techniques [12–15].

X-band ESR of nitroxide spin-labels holds a prominent place among the spectroscopic techniques for the investigation of the structure and function of enzymes and proteins, slow translational motions of lipids and proteins (lateral lipid and protein diffusion in membranes, anisotropic protein rotations) and fast anisotropic lipid rotations (long axis rotations, angular motions, chain segmental motions), lipid/protein interactions, and local polarity of membrane and protein regions [5–7,14,16–19]. For the improved orientational resolution and motional sensitivity on a faster timescale, high field/high frequency spin-label ESR gives insights into nonaxial rotations and both lateral and transverse ordering of the

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phospholipid chains in membranes, rapid internal motions of proteins, and environmental polarity [20–26]. The librational dynamics at cryogenic temperatures and the direct detection of solvent accessibility to specific biosystem sites [27–36] are addressed by electron spin echo-based methods of pulsed FT-ESR [37]. An overview of different ESR methods applied to several biological systems can be found in [38,39].

In this work the basic principles underlying the conventional, cw-ESR spectroscopy of nitroxide spin labels at 9 GHz are presented along with applications to self-assembled lipid aggregates and proteins.

## 2. Nitroxide spin-labels

Most biological systems do not possess an intrinsic paramagnetism which is necessary for ESR spectroscopy. The technique is therefore applied by using a stable free-radical or "spin-label", i.e., an external paramagnetic probe which contain one unpaired electron spin residing in a molecular orbital. The spin-label can be inserted in the sample and acts as a reporter group. The corresponding ESR signal originates exclusively from the label site, so that site-specific, ensemble-averaged molecular and dynamical information can be obtained [5,6,8].

The most common used spin-label moieties are based on the nitroxide radical, –NO, where the unpaired electron spin is localized on the 2pπ orbital of the nitrogen atom, $^{14}N$. The –NO group is flanked by quaternary carbon atoms, which protect the radical and account for the stability of the label. The –NO group is enclosed either in a six-membered piperidine (TEMPO), or a five-membered pyrroline/pyrrolidine (proxyl) rings, or in an oxazolidinyl (doxyl) ring (Fig. 1a–d) [5,6,8,14].

For ESR studies of lipid aggregates, lipid membranes, and lipid/protein complexes appropriate spin-labels are the spin-labeled lipids, i.e., lipids to which the spin label group can be attached to the polarhead or is rigidly and stereospecifically bound to selected carbon atom position, C-*n*, along the hydrocarbon chain [5,6,19]. In Fig. 1e–g a polar-head labeled lipid and chain-labeled lipids are shown as an example. They are TEMPO-stearate, i.e., a stearic acid molecule in which the piperidine ring is on the polarhead, 5-stearic acid spin label (5-SASL), i.e., a stearic acid molecule in which the doxyl group is on the 5th carbon atom of the acyl chain, and 14-phosphatidylcholine spin-label (14-PCSL), i.e., a di-palmitoylphosphatidylcholine molecule bearing the nitroxide moiety on the 14th carbon atom of the *sn*-2 chain. To avoid spin–spin interaction, the spin-labeled lipids are introduced at very low concentration (typically 0.5–1 mol% of the total lipid concentration) in the basic lipid matrix. They are considered endogenous lipids that mix well with the parent lipids with the polarheads in register among them and the hydrocarbon tails in contact.

For ESR studies of proteins, spin-labels able to react towards specific groups of amino acid residues by forming a covalent bond are used. The features of the spin-labels depend on the functional group attached to the nitroxide ring. Maleimido and iodoacetamide spin-labels alkylate both sulphydryl and amino groups, MTSSL binds

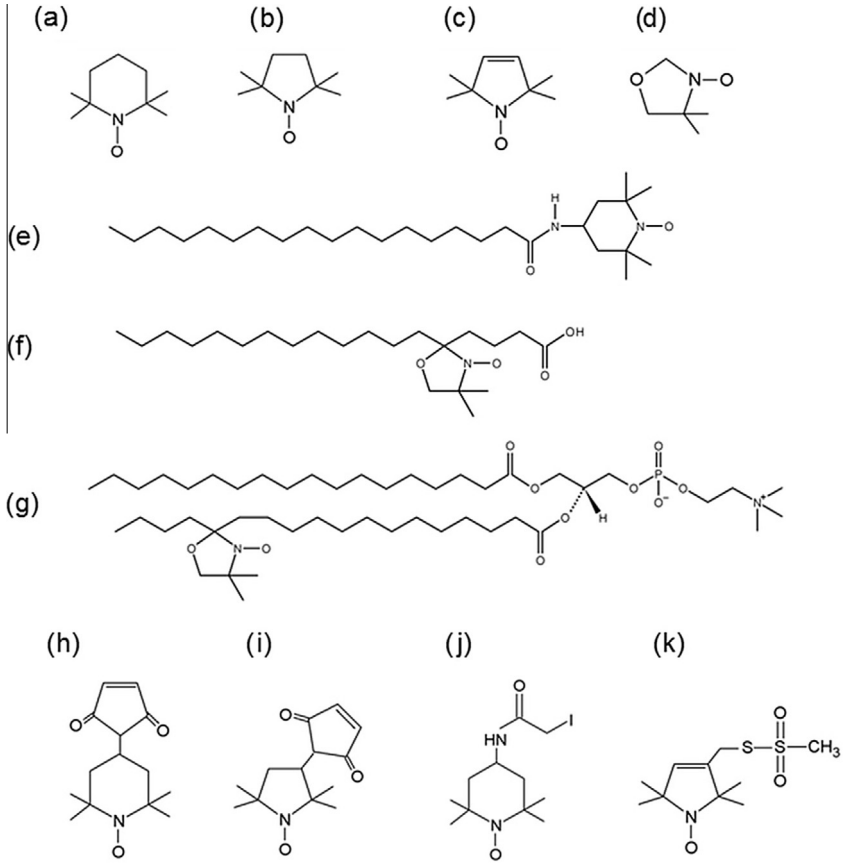


**Fig. 1.** Nitroxide radicals: (a) six-membered piperidine ring; (b) unsaturated five-membered pyrrolidine ring; (c) saturated pyrroline ring; (d) doxyl ring. Spin-labeled lipids: (e) 2,2,6,6-tetramethyl-piperidin-1-oxyl-4-yl octadecanoate, TEMPO-stearate; (f) 5-(4,4-dimethyloxazolidinyl-N-oxyl)stearic acid, 5-stearic acid spin label, 5-SASL; (g) 1-acyl-2-(*14*-(4,4-dimethyloxazolidinyl-N-oxyl)stearoyl)-sn-glycero-3-phosphocholine, 14-phosphatidylcholine spin label, 14-PCSL. Spin-labels used in covalent modification of proteins: (h) 4-maleimido-2,2,6,6-tetramethylpiperidine-1-oxyl, 6-MSL; (i) maleimide spin-label 3-maleimido-tetramethylpyrrolidine-1-oxyl, 5-MSL; (j) 4-(2-Iodoacetamido)-2,2,6,6-tetramethylpiperidine 1-oxyl; (k) 1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate spin label, MTSSL.

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