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Opinion Article

Uses of Site-Directed Spin Labelling (SDSL) Electron Paramagnetic Resonance (EPR) Techniques to Study Membrane Proteins

Mary Schuster*

Department of Chemistry and Biochemistry, North Dakota State University, Fargo, USA

DESCRIPTION

The study of the structural and dynamic characteristics of membrane proteins in their natural context is made possible by the rapidly developing potent biophysical approach known as Site-Directed Spin Labelling (SDSL) in conjunction with Electron Paramagnetic Resonance (EPR) spectroscopy. Membrane proteins are essential for carrying out crucial tasks in a number of intricate biological systems necessary for the survival of living things. An overview of the most prominent SDSL EPR techniques will be given in this paper, along with examples of recent applications for researching relevant structural and dynamic features on membrane proteins.

Prior to recently, only metalloproteins with paramagnetic centres or enzymes with radical cofactors could be used for biological EPR. Most biological materials don't contain unpaired electrons, which would seem to limit the use of EPR techniques. EPR spectroscopy may now be used to almost any biological system because of molecular biology techniques that include stable radicals at precise sites on biological systems. These methods are referred to as spin labelling.

Site-Directed Spin Labelling (SDSL) is the process of adding unpaired electrons as spin labels to specified sites within biomolecules. In SDSL investigations, all naturally occurring, non-disulfide-bonded cysteines are eliminated by being swapped out for alanines or serine. Then, using site-directed mutagenesis, a distinctive cysteine residue is added to a recombinant protein, and after that, a sulfhydryl-specific nitroxide reagent is used to create a stable spin label side-chain.

EPR spectroscopy examines the microwave radiation absorption that results from an unpaired electron's energy splitting in a powerful magnetic field. As a result, an unpaired electron spin is necessary for an EPR active sample. One unpaired electron spin resting in a molecular orbital makes up the simplest EPR active system.

When compared to Nuclear Magnetic Resonance (NMR) spectroscopy, EPR spectroscopy has a sensitivity that is up to three orders of magnitude higher. Without the need for pricey isotopic labelling, it can be used with proteins of any size. It is unaffected by the sample's optical characteristics. In addition to proteins in solution, strongly packed membrane suspensions, tissue samples, ammonium sulfate-precipitated solids, and samples frozen and kept at cryogenic temperatures can all be used in EPR investigations. EPR tests can be carried out in low volume and concentration environments.

EPR spectroscopy can provide important structural and dynamic insights into both solution-bound and membrane-bound proteins that are extremely difficult to obtain using conventional biophysical techniques. The motion of the nitroxide side chain, the accessibility and polarity of the solvent, the intra- or intermolecular distances between two nitroxides, or the distance between a single nitroxide and another paramagnetic centre in the system are all structural and dynamic details that can be obtained by CW-EPR spectroscopy of spin labelled molecules. The structural characteristics of the protein can be analyzed at the backbone level of spatial resolution using the lineshape analysis of the EPR data for a number of spin labelled protein sequences.

Membrane proteins are crucial in various facets of biological functions because they exchange signals and physical materials across the membrane. 30% of sequenced genes are for membrane proteins. Many human dysfunctions, illnesses, and diseases are linked to gene mutations and membrane protein misfolding. Around 50% of all FDA-approved medications focus on membrane proteins. For the knowledge of intermolecular interactions, protein activities, and regulation, detailed structural and kinetic information for membrane proteins is essential. Despite the prevalence and obvious significance of membrane proteins, little is known about these systems. To ensure functional stability, membrane proteins can interact with a lipid bilayer in a variety of ways or orientations. The centre of the membrane bilayer may have bent or varying length helices of the proteins that interact with membranes. They might create reentrant loops, cross the membrane at various angles, or lay flat on the membrane surface.

Correspondence to: Mary Schuster, Department of Chemistry and Biochemistry, North Dakota State University, Fargo, USA, E-mail: mschuster@icloud.com

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The two most effective and well-liked biophysical methods for probing structural information on protein systems are X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy. Moreover, NMR spectroscopy is employed to gather dynamic data for a number of biological systems. Research on membrane proteins frequently combines SDSL and EPR spectroscopy. Membrane Protein Dynamics and Topology, Local Secondary Structure of Membrane Proteins, and SDSL Distance Measurement of Membrane Proteins are a few of its uses. In order to address important structural and dynamic concerns pertaining to biological systems, EPR spectroscopy, a technology with increasing popularity in structure biology, is used.

It can offer crucial details about complex biological systems that would be difficult or practically impossible to get using other biophysical techniques.