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EPR Spectroscopic Studies on the Structural and Dynamic Properties of Human KCNE1 Membrane Protein in Lipid Bilayers

ARTICLE in BIOPHYSICAL JOURNAL · JANUARY 2010

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2009.12.2153

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EPR Spectroscopy

Alignment Studies Employing the Rigid TOAC Spin Label Utilizing Electron Paramagnetic Resonance (EPR)

Daniel J. Mayo, You Zhou, Gary A. Lorigan.

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For electron paramagnetic resonance (EPR) spectroscopic studies, the TOAC spin label offers the unique advantage over other conventional labels in that it reports accurate backbone motion and peptide dynamics due to its rigid nature. This label has become extremely important in EPR studies to study membrane protein topology and their associated dynamics. Additionally, some researches have also developed spectroscopic techniques using magnetically aligned (bicelles) and mechanically aligned (glass plates) lipid samples to extract additional information directly related to structural topology with respect to the membrane. Based upon the samples orientation, other anisotropic spectral parameters can also be determined. EPR spectroscopy offers a unique solution due to the fact it has a much higher sensitivity and also a different frequency domain than other conventional techniques. Thus, we have performed EPR alignment studies on two-model peptides magainin-2 and the M2δ subunit of the acetylcholine receptor. Both of these peptides have been well characterized and are 23 amino acids in length.

2060-Pos

Peldor Beyond Distances

Olav Schiemann.

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Structural Biology is engaging ever larger assemblies of biomacromolecules either isolated, embedded in membranes or in whole cells. Thus, biophysical methods are needed that access these architectures on the critical nanometer length scale in these environments. Electron Paramagnetic Resonance provides several tools to precisely and reliably measuring such these distance in the nanometer range in particular a method called Pulsed Electron-Electron Double Resonance (PELDOR). In this presentation, it will be shown that PELDOR yields not only distances and distance distribution but also full information about label orientation,² coupling mechanisms³ and that it can be used to count the monomers in aggregates. Examples will include covalently and non-covalently labelled duplex DNAs/RNAs, complex folds of RNAs and the 320 kDa membrane channel Wza⁵.

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2061-Pos

Increased Sensitivity and Range of Distance Measurements in Spin **Labeled Membrane Proteins**

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We report a significant methodological advance in the application of double electron-electron resonance (DEER) to spin-labeled membrane proteins. DEER is an unparalleled tool in structural biology yielding long range distance restraints that can be used to model protein folds, to define the nature of conformational changes and determine their amplitudes. Distances are obtained in native-like environments in the absence of conformational selectivity imposed by the crystal lattice and regardless of the molecular mass. However, the realization of these advantages in proteolipsomes has so far lead to significant reduction in the distance range and loss of sensitivity compromising experimental throughput. In the two-dimensional environment of a liposome, the background of intermolecular dipolar spin coupling leads to a strong decay that can obscure the contribution of intramolecular coupling rendering the DEER signals uninterpretable. We found that the combination of two emerging technologies, Q-band pulsed electron paramagnetic resonance and Nanodiscs phospholipid bilayers, overcome the factors limiting DEER sensitivity and distance range. Spin labeled mutants of the ABC transporter MsbA were functionally reconstituted into Nanodiscs at a ratio of one dimer per lipid bilayer. In comparison to proteolisposmes, DEER data from Nanodiscs have a linear baseline reflecting the three dimensional spatial distribution of MsbA. The order of magnitude increase in absolute sensitivity at Q-band microwave frequency is

critical given limited sample quantities and working concentrations in the 10-30 µM range. We took advantage of the higher throughput to demonstrate that the magnitude of the distance changes in the ATP hydrolysis cycle is not affected by the lipid headgroup. The advances described here set the stage for the use of DEER spectroscopy to analyze the conformational dynamics of eukaryotic membrane proteins.

2062-Pos

Novel Approaches for Distance Determination by EPR: Large Anisotropy, Fast Relaxing Paramagnetic Centres, Such as Fe(III), as Markers

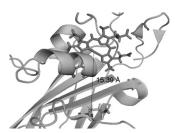
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Recent advances in structure determination of biomacromolecules have been achieved by pulsed EPR methods, such as double electron electron paramagnetic resonance (DEER or PELDOR), by which distances in the nano-meter range between two nitroxide spin labels or low-anisotropy paramagnetic metal ions are accessible. Ways to extend the existing methods are presented: One of them is a new pulse sequence improving the orientation selectivity of the method (os-DEER), which could be a step towards measuring shorter distances (Milikisyants et al., JMR 2008). High-anisotropy, fast relaxing paramagnetic centres had been off-limits for DEER. We show that distances between nitroxide spin labels and high-anisotropy paramagnetic centres are accessible with RIDME (Kulik et al. JMR 2002), if dead-time is avoided (Milikisyants JMR 2009). We show that thereby the dis-

tance between the low-spin Fe(III)ion and a nitroxide in a mutant of cytochrome f can be measured (see Fig.).

Given the prevalence of highanisotropy, fast relaxing centres in bio-macromolecules the method proposed extends the range of systems that can be accessed greatly, including protein-protein and protein-ligand



2063-Pos

EPR Spectroscopic Studies on the Structural and Dynamic Properties of **Human KCNE1 Membrane Protein in Lipid Bilayers**

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The KCNE1 membrane protein regulates KCNQ1, which forms the voltagegated potassium channel in the human heart. Mutations in these genes are responsible for the human genetic disease, long QT syndrome. However, the structure of KCNE1 in a lipid bilayer and biophysical basis for KCNE1's modulation of KCNQ1 are not completely understood. Recent research from Sanders group using solution NMR indicates the transmembrane domain of KCNE1 is a curved alpha-helix and is flanked by intra- and extracellular domains comprised of alpha-helices joined by flexible linkers (Kang et al., Biochemistry 2008 47:7999-8006). Site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy has emerged as a well-established method to study the structural properties of membrane proteins. Our objective is to use advanced EPR spectroscopic techniques including CW and pulsed EPR spectroscopy to obtain structural parameters of the KCNE1 protein in a bilayer. To date, we have successfully over-expressed KCNE1 in E. coli and reconstituted the protein into POPG/POPC lipid bilayers. Electrophysiological experiments confirm KCNE1 can co-assemble with the channel protein, KCNQ1 and are fully functional. Electrophysiological experiments further confirm that KCNE1 is inserted correctly into the lipid-bilayers. Additionally we have also demonstrated potential use of DEER (Double Electron-Electron Resonance) and CW-EPR power saturation experiments for distance and depth measurements of KCNE1 in lipid bilayers, respectively.

Structure and Dynamics of the Calcium Binding Domains of the Na/Ca Exchanger (NCX1.1) Determined by Site Directed Spin Labeling

Mrinalini Dixit, Sunghoon Kim, Eric J. Hustedt, Charles E. Cobb, Albert H. Beth.

Vanderbilt University, Nashville, TN, USA. The cardiac Na^+/Ca^{2+} exchanger (NCX1.1) serves as the primary means of Ca²⁺ extrusion from cardiomyocytes following the rise in intracellular Ca²