EPR Spectroscopy

2059-Pos

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

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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

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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).[1] 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 mechanisms[3] and that it can be used to count the monomers in aggregates.[4] Examples will include covalently and non-covalently labelled duplex DNAs/RNAs, complex folds of RNAs and the 320 kDa membrane channel Wza[5].

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2. O. Schiemann, P. Cekan, D. Margraf, T. F. Prisner, S.T. Sigurdsson, *Angew. Chem. Int. Ed. Engl.* **2009**, 121, 3342.
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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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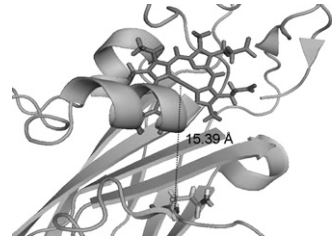
Recent advances in structure determination of biomacromolecules have been achieved by pulsed EPR methods, such as double electron electron paramagnetic resonance (DEER or PELDOR), by which distances in the nano-meter range between two nitroxide spin labels or low-anisotropy paramagnetic metal ions are 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 distance 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 high-anisotropy, 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 interactions.

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 voltage-gated 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.

2064-Pos

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

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

during contraction. The exchanger is regulated by binding of Ca^{2+} to the intracellular domain. This domain is composed of an α-catenin-like domain (CLD) that connects two structurally homologous Ca^{2+} binding domains (CBD1 and CBD2) to the transmembrane domain of the exchanger. NMR and X-ray crystallographic studies have provided structures for the isolated CBD1 and CBD2 domains and have suggested how Ca^{2+} binding alters their structures and motional dynamics. It remains unknown how Ca^{2+} binding to the intact Ca^{2+} sensor signals the transmembrane domain to regulate exchanger activity. We have used site directed spin labeling to address this question. Conventional EPR experiments have shown that: 1) residues in, or near, the Ca^{2+} binding loops of CBD1 and CBD2 show decreased mobility upon Ca^{2+} binding; and 2) residues in the β-sandwich regions are insensitive to Ca^{2+} binding. Double Electron Electron Resonance (DEER) measurements on doubly labeled constructs revealed that: 1) the structure of the β-sandwich domains of CBD1 and CBD2 are not altered upon Ca^{2+} binding; 2) CBD1 and CBD2 do not lie lengthwise antiparallel in close proximity but rather residues in the distal ends that connect to the CLD are greater than 60 Å apart; and 3) residues nearer to the apex of the Ca^{2+} sensor are in close enough proximity to be measured by DEER and these distances are sensitive to Ca^{2+} binding. These studies support recent SAXS studies by Hilge et al. (PNAS 106:14333-8, 2009) and provide additional insight into a structural rearrangement of the intact Ca^{2+} sensor that may be involved in regulation of Na^{+}/Ca^{2+} exchange.

2065-Pos

Structure of the CDB3 - ankD34 Complex from Site - Directed Spin - Labeling Studies

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The association between the cytoplasmic domain of band 3 (CDB3) and ankyrinR forms a critical link between the lipid bilayer of the erythrocyte membrane and its underlying spectrin cytoskeleton. This interaction is responsible for the remarkable mechanical stability of the erythrocyte membrane that is essential for the durability of the erythrocyte. While the structures of CDB3 [1] and ankD34 (repeats 13-24 from full length ankyrinR) [2] have been determined by X-ray crystallography, the structure of the CDB3-ankD34 complex has not been established. Using distance constraints from site-directed spin labeling (SDSL) and DEER spectroscopy, we propose a new structural model of CDB3-ankD34 complex modeled assuming rigid-body docking between the two proteins combined with rigorous modeling of the spin label. Unexpectedly, the new model generated by Rosetta docking calculations and filtered through multiple DEER distance constraints shows features which are quite different from the previously proposed docking model. The binding interface of CDB3 is widely scattered over its peripheral surface but the β6-β7 hairpin loop makes no direct contact with ankD34. Second, the binding interface of ankD34 resides on the opposite side of β-hairpin loops from the concave groove. The validity of our current model is also supported by a series of SDSL and cross-linking experiments where the binding interface of ankD34 was mapped by the model-guided scanning of a series of surface sites on ankD34. Supported by NIH P01 GM080513.

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[2] P. Michaely et al., *EMBO J.*, 21, 6387 (2002)

2066-Pos

Structural Origins of Nitroxide EPR Spectra in a β-Barrel Membrane Protein

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Site-directed spin labeling is a powerful tool for studying structure and dynamics in proteins, due to its ability to bypass several fundamental limitations suffered by methodologies such as NMR and x-ray crystallography. The utility of this technique, however, hinges on our ability to reliably interpret EPR lineshapes of spin-labeled proteins, so that spectral features may be unambiguously associated with their structural origins. In the present work, X-ray crystallography has been combined with mutagenesis and a quantitative analysis of EPR spectra to examine for the first time the origins of spectra from a β-barrel membrane protein, BtuB. The hydrocarbon-exposed residue T156C was spin-labeled and gave rise to a two-component EPR spectrum, corresponding to two conformers of the spin-labeled side chain. Quantitative lineshape analysis revealed a dominant population of highly (spatially) ordered yet mobile nitroxide, and a second population of weakly ordered yet immobile nitroxide. EPR spectra show that single mutations to nearest-neighbor residues affect the ordering and or equilibrium of label rotamers, however these changes are small in each case. In the 2.6Å crystal structure of spin-labeled BtuB, the likely source of weak pairwise interaction with nearest-neighbors is attributed to the extent of barrel curvature, β-strand twist, and direction of strand tilt. It is postulated that residues Q158, L160 (periplasmic loop), V166, and L168 (hydrogen-bonded neighbor) may cooperatively stabilize the nitroxide spin label by forming a hydrophobic pocket. This approach is being applied to an additional hydrocarbon-exposed site on BtuB which exhibits a different degree of strand tilt and twist.

2067-Pos

Characterization of the L511P and D512G Mutations in the MsbA Lipid Flippase

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MsbA is a 65kDa lipid flippase found in the inner membrane of Gram-negative bacteria such as E. coli and S. typhimurium. As a member of the ABC transporter superfamily, MsbA contains two nucleotide binding domains and two transmembrane domains, one from each of its two monomers. ABC transporters transport a diverse group of substrates from lipids to antibiotics and their dysfunction contributes to a number of human pathologies including cystic fibrosis. As an essential protein in E. coli, the deletion or dysfunction of MsbA results in the toxic accumulation of lipid A in the inner membrane resulting in membrane instability and cell death. The L511P and D512G mutations have been previously identified through mutational analysis as dysfunctional nucleotide binding domain mutations specific to MsbA and were suggested to have a lower affinity for ATP. To further understand the cause of dysfunction in these point mutations, in vivo growth assays, in vitro ATPase activity assays, DEER and CW EPR spectroscopy studies throughout the ATP hydrolysis cycle were conducted. L511P and D512G were each paired with nine different reporter residues, each in or near an important conserved nucleotide binding domain motif and compared to the reporter residues alone. To identify the stage in the ATP hydrolysis cycle in which the L511P and D512G mutations are dysfunctional, the local tertiary interactions before, during, and after ATP hydrolysis were monitored by EPR spectroscopy at each stage of the ATP hydrolysis cycle.

2068-Pos

Free Radical Generation and Electron Flux in Mitochondrial Fe-S Centers During Cardiac Injury; Changes with Mitochondrial Protective Drug Ranolazine

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Some TCA cycle enzymes, like aconitase, are more susceptible to ischemia reperfusion (IR) injury. Ranolazine (RAN) is cardioprotective against IR injury. It is a late Na^{+} current blocker that may also limit lipid peroxidation and complex I activity. It is unknown if RAN alters the redox state of Fe-S clusters or free radical generation (FRG) to underlie its protection. Here we examined how IR injury affects FRG and Fe-S clusters of aconitase and succinate dehydrogenase, using electron paramagnetic resonance (EPR), and if RAN alters these effects. Guinea pig hearts (n 8) were isolated and perfused with Krebs Ringer buffer and exposed to: a) control, b) 30 min global ischemia, c) 10 μM RAN for 10 min just before ischemia, or d) ischemia and 10 min reperfusion. Hearts were immediately ground in liquid N_2 and packed into EPR tubes. We examined changes in signal intensity in liquid He (10°K) of assigned g 2.016 (aconitase 3Fe-4S), g 1.93 (succinate dehydrogenase 2Fe-2S), g 2.006 (free radical), and g 6.0 (Fe group of cytochrome c). Versus time control (100%), the signal for aconitase Fe-S at the end of ischemia was 46%, suggesting oxidative damage; this was partially restored by 10 min reperfusion to 91% and after I+RAN treatment to 55% of control. Signal intensity for succinate dehydrogenase was unaltered by IR or RAN+IR. The presumptive ubisemiquinone radical signal increased 19% after ischemia, suggesting increased FRG, but only by 4% at 10 min reperfusion. I+RAN treatment decreased the signal by 19%. The signal for cytochrome c (g 6.0) increased 730% after IR, but was only 81% after I+RAN. These data suggest that RAN treatment partially restores electron flow through some Fe-S centers and reduces FRG, which may partially underlie its cardioprotective effects.

2069-Pos

Structural Analysis of the Membrane Docking Geometry of PI(3,4,5)P3-Specific GRP1-PH Domain Via Site-Directed Spin Labeling

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Peripheral membrane binding proteins play critical roles in dynamic cell signaling processes that occur at membrane surfaces. Many of these signaling proteins contain membrane targeting domains that act to mediate signal dependent membrane localization for proper enzyme function. Phosphoinositide-specific