Biomolecular NMR

3253-Pos Board B358
The His-75-Asp-97 Cluster in Proteorhodopsin: A DNP and Solid-State NMR Study
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The proteorhodopsin (PR) family found in bacteria near the ocean's surface to high abundance consists of hundreds of PR molecules which are colour-tuned to their environment. The green absorbing species has been shown to act as a light-driven proton pump in vitro and in non native host organisms. In contrast to bacteriorhodopsin, the pKa of the primary proton acceptor Asp-97 is highly elevated and a single His is found close to the active site. Here, we report a complete biophysical study of this His-75, which is highly conserved within the PR family but not found in other retinal proteins. 13C-13C dipolar correlation experiments did show, that both side chains are close enough to enable the formation of a H-bond. In addition, 15N-MAS NMR provides evidence, that indeed a neutral, H-bonded His-Asp complex is formed in which the protonation is shifted to D97 explaining its high pKa. Using the sensitivity enhancement provided by dynamic nuclear polarisation, we were able characterise the active site and especially the H-bonding character of the His-Asp coupling in great detail. Our study is complemented by site-directed mutagenesis in combination with black lipid membrane measurements, ultrafast optical spectroscopy and flash photolysis. Our data show, that His-75 forms a pH dependent complex with Asp-97 but replacing His-75 with Met, Trp or Asn accelerates PR's photocycle and does not prevent proton transfer. This raises the question of the true function of PR in vivo.

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Structural Dynamics of Phosphorylated Pentameric Phospholamban in Lipid Membranes using a Combination of Solution and Solid-State NMR Spectroscopy
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Phospholamban (PLN) is a 52-residue membrane protein that regulates the cardiac sarcoplasmic reticulum Ca2+ATPase (SERCA). PLN is in equilibrium between monomeric and pentameric species. The monomer binds to and decreases the rate of Ca^{2+} transport of SERCA. This inhibitory action is relieved upon PLN phosphorylation at Serine-16 and Threonine-17. Here, we present the structural dynamics of fully phosphorylated pentameric PLN by a combination of solution and solid-state NMR spectroscopy. SDS-PAGE analysis revealed a higher thermal stability of the phosphorylated pentameric PLN (at Ser-16, Thr-17 and both) with respect to the unphosphorylated protein when reconstituted in different lipids and detergents.
Conventional T1, T2, and heteronuclear steady state NOEs experiments were used to probe fast (ps-ns) dynamics of the amide backbone upon phosphorylation at Ser-16, revealing a significant increase of the dynamics in this time scale. Slower (μs-ms) dynamics were probed by measuring rotating frame R (1rho) and R(2rho) dispersion curves using a new adiabatic irradiation scheme. The structural and topological effects of phosphorylation in pentameric PLN were investigated using solid-state NMR experiments on mechanically and magnetically oriented lipid systems. For the mechanically oriented samples, phosphorylated PLN was reconstituted in DOPC/DOPE lipid bilayers and uniaxially aligned between glass plates. For magnetically oriented samples, the protein was reconstituted in DMPC/Triton X-100 bicelles. Two-dimensional PISEMA and SAMPI4 experiments were used to determine the topology of PLN in lipid membranes. Chemical shifts anisotropy and dipolar coupling values were also used in combination with solution NMR restraints to determine a preliminary high-resolution structure of fully phosphorylated pentameric PLN.
These studies reveal residue-by-residue details of the pentameric PLN structure and topology upon phosphorylation.

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Solid State NMR Studies of Lung Surfactant Protein B Fragment, Mini-B, in Mechanically Oriented Lipid Bilayers
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Lung surfactant, a lipid protein complex, prevents lung collapse by lowering the surface tension at the alveolar air-water interface. Surfactant protein B (SP-B) is an essential component of lung surfactant and is indispensable for life. Mini-B is a two-helix fragment of lung surfactant protein SP-B which retains significant biological activity compare to the full-length protein SP-B. Solid-state ^{2}H-NMR was used to characterize mechanically oriented lipid bilayers composed of POPC-$_{d31}$/POPG or DPPC-$_{d62}$ doped BLES, with and without associated Mini-B. The spectra were interpreted with the help from orientational order parameter profiles. The results indicated that 1) the order parameters were greater when the number of plates were increased 2) DPPC-$_{d62}$ in BLES is more ordered than POPC-$_{d31}$/POPG and 3) Mini-B tends to disrupt the orientation of bilayers and reduce the order in the fraction of the sample that remain oriented. Even in the presence of Mini-B, the lipid bilayers could be well oriented by optimizing plate dimension, amount of sample between the plates and volume of the solvent used to dissolve the organic solvent. These well-oriented samples are being used in further studies of lipid headgroup orientation, as indicated by 31P NMR, as well as for studies of 15N-lebled peptides, to indicate the tilt angle of the peptide's helices within the bilayer.

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The Selectivity Filter of the hERG Channel - NMR Study of its Structure and Interaction with Membranes and Drugs Involved in the Long QT Syndrome
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The drug-induced (acquired) long QT syndrome (ALQTS) is a cardiac muscle dysfunction responsible for heart arrhythmia and failure. Virtually all cases of ALQTS are due to the blockage of the heart human ether-a-go-go-related-gene (hERG) potassium channels located in the myocardium cell membranes. The hERG channel is a unique member of the family of voltage-gated K^+ (K_v) channels because of its different selectivity filter (SF) signature and linker sequence between the last two pore helices (S5-S6) as compared to other bacterial, mammalian and *Drosophila* K_v channels. Recent work has shown that binding sites of LQTS-prone drugs could be found at the intracellular base of the SF. Considering the sequence particularities of the hERG channel's SF and the occurrence of potential drug binding sites, the objective of this work is to study the SF structure of the hERG channel and role in the ALQTS. Results obtained by liquid-state NMR experiments and circular dichroïsm suggest that the SF peptide has no defined secondary structure in water. However, changes in chemical shifts for the C-terminal end of the SF can be observed in the presence of a low concentration of K^+ ions (10mM), suggesting structural modifications. Using ^{2}H and ^{31}P solid-state NMR, we have investigated the interaction of the SF with model complex membranes and simple DMPC vesicles. The spectra reveal that both membrane systems are destabilized by the SF. Moreover, the presence of K^+ ions appears to reinforce this perturbing effect of the lipid bilayer. Our results, therefore, demonstrate a possible interaction for the hERG channel SF with the membrane environment. The interaction of bepridil, fluvoxamine and promethazine with the SF will also be discussed.

3257-Pos Board B362
Structural Studies of Mammalian Dynactin CAP-Gly Domain by Solid-State NMR
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Microtubules and their associated proteins play essential roles in a broad range of physiological functions, including cell migration, mitosis, polarization, differentiation, and vesicle and organelle transport. Dynactin, an activator and the dominant cofactor of dynein, is a large (1.2 mD) multisubunit complex that bridges vesicles to the MT network and thus is central to dynein mediated retrograde transport. Its central subunit, p150^{Glued}, binds to microtubules via the CAP-Gly domain that is thought to be critical for +MT tip localization. Mutations in the p150^{Glued} subunit of the CAP-Gly domain have been implicated in predominantly in neurological disorders including dSMBA and Perry's Syndrome. Despite its critical role, the mechanism by which the CAP-Gly domain recognizes microtubules remains largely unknown, particularly at the atomic level. Herein, we report our recent progress in structural studies of CAP-Gly domain of dynactin by using solid-state NMR. For tertiary structure calculation, we acquired numerous medium- and long-range distance restraints from the DARR and R2$_1^1$ spectra of the ^{13}C sparsely enriched CAP-Gly.

References
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Characterizing Recombinant Spider Wrapping Silk Monomers and Fibers by NMR and AFM
Marie-Laurence Tremblay, Lingling Xu, Paul X.-Q. Liu, Jan K. Rainey.
Spider silks are among the strongest and toughest naturally produced biomaterials. Despite these appealing properties, very little is known about the mechanisms by which these fibers form or about how fiber structure correlates to mechanical properties. Nuclear magnetic resonance spectroscopy (NMR) and far-ultraviolet circular dichroism (CD) spectroscopy were carried out in the solution-state on monomeric recombinant 13C and 15N labeled wrapping silk protein. This protein is a key constituent of egg case sacs, providing both flexibility and strength, produced in the aciniform spidroin with sequence