Dynamics of Retinal Studied by 2H NMR Relaxation Sheds New Light on Rhodopsin Activation

ARTICLE *in* BIOPHYSICAL JOURNAL · FEBRUARY 2009

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2008.12.2084

Andrey V Struts
The University of Arizona

Karina Martínez-Mayorga
Universidad Nacional Autónoma de México

Gilmar F Salgado
Institut Européen De Chimie Et Biologie

dynamic model, including a molecular order parameter scaling factor, gives good results only for moderately mobile peptides, while for high mobility cases the correct tilt is only obtained by re-introducing the explicit Gaussian fluctuations in the fitting functions.
In contrast, ^{15}N-NMR data appear to be less sensitive to rigid-body peptide motions, and PISEMA spectra can give correct orientations even for highly mobile peptides, and assuming a static model for the analysis. The differences are due to the different orientation of the tensors of ^{2}H- and ^{19}F-labels, placed on peptide side chains, compared to the orientation of the ^{15}N tensor, placed on amide backbone groups.
We conclude that dynamics should be included in the analysis of solid-state NMR data of membrane-bound peptides. Not only does this give more accurate orientations, but it can also provide information about the dynamics of the peptide.

2105-Pos Board B75
Dynamics of Retinal Studied by ^{2}H NMR Relaxation Sheds New Light on Rhodopsin Activation

Andrey V. Struts[1], Karina Martínez-Mayorga[2], Gilmar F.J. Salgado[3], Michael F. Brown[1].
[1]Department of Chemistry, University of Arizona, Tucson, AZ, USA,
[2]Torrey Pines Institute for Molecular Studies, Fort Pierce, FL, USA,
[3]Département de Chimie, école Normale Supérieure, Paris, France.

The dynamics of retinal ^{2}H-labeled at the C5-, C9-, and C13-methyl groups have been studied by solid-state deuterium NMR relaxation in the dark, meta I, and meta II states of the G protein-coupled receptor rhodopsin. Relaxation rates and quadrupolar splittings were interpreted in terms of axial rotation and off-axial motion of the methyl groups and revealed interactions between the retinal cofactor and the rhodopsin binding pocket. Surprisingly, in the dark state the crucial C9-methyl group is the most mobile despite its role in stabilizing the polyene chain. The C5-methyl group is slowest which is most likely due to interactions with Glu122 on helix 3. Dynamics of the ligand change significantly after light absorption. However, most of the changes occur between the dark and meta I states, and can be attributed to variations in intra-retinal interactions due to isomerization. Only small changes are observed upon transition from the meta I to meta II state where activation takes place. Overall, the dynamics of the C9- and C13-methyl groups in the meta I and meta II states indicate the absence of significant steric clashes of these groups with the surrounding amino acids. Even more surprising, there is little change in mobility of the β-ionone ring upon light activation. An activation mechanism based on the relaxation data is suggested which assumes that retinal is maintained in a similar environment, and does not experience significant reorientation or displacement upon transition from the pre-activated meta I to the active meta II state.

[1] G. F. J. Salgado *et al.* (2004) *Biochemistry* **43**, 12819.
[2] G. F. J. Salgado *et al.* (2006) *JACS* **128**, 11067.
[3] A. V. Struts *et al.* (2007) *J. Mol. Biol.* **372**, 50.

2106-Pos Board B76
Water Self-Diffusion in Cell Suspensions and Tissues: New PGSE NMR Protocols for Estimating Intracellular Diffusion, the Homogeneous Length Scale, and Membrane Permeability

Daniel Topgaard.
Lund University, Lund, Sweden.

Molecular transport by diffusion is a crucial process for the function of biological tissues.[1] By following the self-diffusion of molecules in a cellular system, information about structure and dynamics on the cellular scale can be obtained. PGSE NMR is a powerful method to non-invasively study molecular motion on the micrometer length scale and millisecond time scale.[2] Most of the present day PGSE NMR studies use the same basic experimental design as in the pioneering works of Stejskal and Tanner in the 60's.[3] Here we present new protocols specifically designed for estimating the diffusion in the intracellular medium and the cell membrane permeability for cell suspensions, and the length scale at which an inhomogeneous medium, such as brain tissue, start to appear homogeneous.[4] The new versions are based on a controlled use of deviations from the short gradient pulse approximation,[5] previously considered as an unwelcome experimental artifact, and multiple diffusion periods.[6] Implementation of the proposed protocols in the context of medical MRI is discussed.[7]

(1) Nicholson. *Rep. Prog. Phys.* **2001**, *64*, 815.
(2) Callaghan *Principles of Nuclear Magnetic Resonance Microscopy*; Clarendon Press: Oxford, 1991.
(3) Stejskal, Tanner. *J. Chem. Phys.* **1965**, *42*, 288.
(4) Åslund et al. *J. Phys. Chem. B* **2008**, *112*, 2782.
(5) Malmborg et al. *J. Magn. Reson.* **2006**, *180*, 280.
(6) Callaghan, Furó. *J. Chem. Phys.* **2004**, *120*, 4032.
(7) Le Bihan. *Nat. Rev. Neurosci.* **2003**, *4*, 469.

2107-Pos Board B77
Chemical Structure Effects on Bone Response to Mechanical Loading

Peizhi Zhu, Jiadi Xu, Michael D. Morris, Nadder Sahar, David H. Kohn, Ayyalusamy Ramamoorthy.
University of Michigan-Ann Arbor, Ann Arbor, MI, USA.

Using solid state NMR (SSNMR) we show that bone mineral and bone matrix both undergo measurable deformations in response to compressive loading. Using bovine cortical bone, load-induced changes in both protein conformation and mineral ion spacings are observed even under sub-physiological loads. Our finding that matrix distortion involves changes in the position of (proline) is not unexpected. Proline and hydroxyproline are the most abundant amino acids in X,Y positions of the repeat gly-X-Y unit of collagen. The local conformation is determined by enthalpic forces stabilizing hydroxyproline and hydrogen bonding stabilizing proline position. Mechanical forces would be expected to be greater, leading to some change in the local orientation. Substitution of another amino acid for glycine, as in most types of osteogenesis imperfecta and in other genetic defects, would have the effect of weakening the stabilizing forces on proline and hydroxyproline, thus allowing greater distortion of the collagen fibrils than would occur in normal bone. In turn, this weakness would contribute to the fragility of the tissue.

2108-Pos Board B78
Helicobacter Pylori: How is Adhesin BabA, a Blood Group Antigen Binding Membrane Protein, Involved in Bacterial Adherence?

Katja Petzold[1], Annelie Olofsson[1], Anna Arnqvist[1], Thomas Boren[1], **Gerhard Gröbner**[2], Juergen Schleucher[1].
[1]Medical Biochemistry, Umeï¿½ , Sweden, [2]Biophysical Chemistry, Umeï¿½ , Sweden.

The bacterium *Helicobacter pylori* is the causative agent for peptic ulcer disease. Bacterial adherence to the human gastric epithelial lining, a prerequisite for the pathological action of H. pylori, is caused by its outer membrane proteins. One of their most prominent members is the Lewis B binding adhesin, BabA which interacts with the bloodgroup antigen carbohydrate epitopes. To elucidate the structural basis of Lewis-b antigen recognition by BabA, STD (Saturation Transfer Difference) NMR experiments enabled the specific detection of *Helicobacter*-glycan interactions by using living *Helicobacter* cell suspensions and Lewis B blood group O determinant. In the NMR spectra, one can identify several carbohydrate segments which bind to BabA. This unique setup is ideal for continuing functional analyses of fully functional BabA adhesion protein in its native environment, the bacterial outer membrane.
Further work is using combined liquid/solid state ^{31}P NMR studies to elucidate the variation in membrane lipid compounds arising from outer membrane vesicles (OMV) which the bacterium produce to deliver bacterial virulence factors. Using tailored-made solution NMR (^{1}H, ^{31}P NMR, ^{1}H-^{13}C and ^{1}H-^{31}P correlation NMR spectroscopy) we could identify and quantify various lipids as a function of strain, clinical isolates, mutants and the different membranes. We observed marked differences in the phospholipid composition between inner (IM) and outer membrane (OM) as well as vesicles (OMV).

2109-Pos Board B79
Structure-Activity Relationships in Two Antimicrobial Peptides Based on Chemokine Helical Segments: RP-1 and IL-8α

Sarah Bourbigot, Valerie Booth.
Memorial University of Newfoundland, St John's, NL, Canada.

Antimicrobial peptides are naturally occurring molecules, part of the innate immune system, and are of high interest as novel antibiotic therapeutics given the increasing resistance of microbes to conventional antibiotics. RP-1 and IL-8α are 18 and 19 amino acid synthetic peptides that were designed based on the sequence of the C-terminal helical segments of two chemokines: platelet factor-4 and interleukin-8. In order to characterize structure-activity relationships and to understand the selectivity of these peptides for bacterial membranes, NMR was used to determine high-resolution structures of both peptides in complex with SDS and DPC micelles. Additionally, solid state NMR experiments in oriented lipid bilayers were performed to assess structure and orientation in a bilayer environment and to indicate the impact of the peptide on bilayer organization. Both peptides structure as amphipathic α-helices with hydrophobic residues on one side and polar and positively charged residues on the opposite side. RP-1 shows very subtle structural differences when in complex with SDS (anionic) versus DPC (zwitterionic) micelles. This suggests that its specificity for prokaryotic versus eukaryotic membranes does not derive from peptide structural differences in the two systems, but rather from differences in the details of the peptide-lipid interactions. The ^{2}H solid state NMR data are consistent with IL-8α associating peripherally with POPC bilayers, and penetrating deeper into POPC/POPG