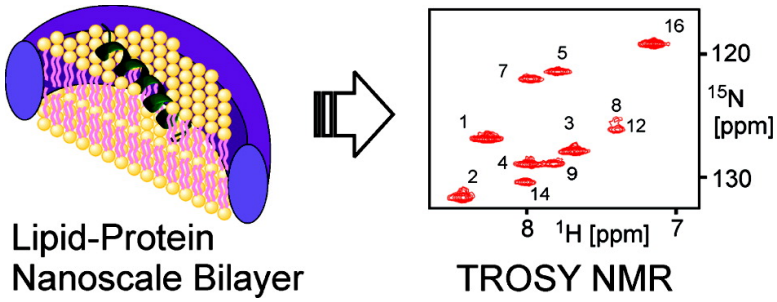
Communication

Lipid–Protein Nanoscale Bilayers: A Versatile Medium for NMR Investigations of Membrane Proteins and Membrane-Active Peptides

Ekaterina N. Lyukmanova, Zakhar O. Shenkarev, Alexander S. Paramonov, Alexander G. Sobol, Tatiana V. Ovchinnikova, Vladimir V. Chupin, Mikhail P. Kirpichnikov, Marcel J. J. Blommers, and Alexander S. Arseniev

J. Am. Chem. Soc., **2008**, 130 (7), 2140-2141 • DOI: 10.1021/ja0777988



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 01/30/2008

Lipid−Protein Nanoscale Bilayers: A Versatile Medium for NMR Investigations of Membrane Proteins and Membrane-Active Peptides

Ekaterina N. Lyukmanova,[#] Zakhar O. Shenkarev,[#] Alexander S. Paramonov,[#] Alexander G. Sobol,[#] Tatiana V. Ovchinnikova,[#] Vladimir V. Chupin,[#] Mikhail P. Kirpichnikov,[#] Marcel J. J. Blommers,[§] and Alexander S. Arseniev*[,#]

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 16/10 Miklukho-Maklaya Street, 117997 Moscow, Russia, and Novartis Institutes for BioMedical Research, CH-4002 Basel, Switzerland

Received October 10, 2007; E-mail: aars@nmr.ru

NMR structural investigations of water-soluble proteins are well established in biological and biochemical research.[1] The recent developments in solution-state NMR methodology and in isotope-labeling strategies significantly extend the applicability of these methods for large proteins and supramolecular complexes. Uniform side-chain deuteration and relaxation-optimized NMR experiments (TROSY, CRINEPT) permit the spatial-structure determination of water-soluble proteins with a molecular weight (MW) up to 100 kDa and investigation of protein−protein complexes with a MW up to 1000 kDa.[2] Unfortunately, this rapid progress does not significantly facilitate the studies of other biologically important class of proteins, the membrane ones.[3]

The main stumbling stone in the NMR investigation of membrane proteins is a search for suitable membrane mimetic environment for protein solubilization.[3,4] In the ideal membrane mimetic media, proteins should retain their native structure and should provide high quality NMR spectra in terms of signal dispersion and line-width. The commonly used media suffer from a number of serious shortcomings. Organic solvents are isotropic in nature and cannot stand as a suitable model for biological membranes which have a water/lipid interface.[3a] On the other hand, such membrane mimetics as detergent micelles and small lipid/detergent bicelles display a significant curvature of the water/lipid interface[3,4] which can seriously deteriorate the structure of the embedded protein.[5] The lability of the detergent complexes also enhances the dynamics in the helical membrane proteins that often makes transmembrane fragments inaccessible for NMR investigations.[3,4]

In search of alternative media for high-resolution NMR studies of membrane proteins, we concentrated our attention on reconstituted high-density lipoprotein particles (rHDLs).[6,7] These particles are composed of a patch of planar lipid bilayer (∼160 lipid molecules) surrounded by a dimer of apolipoprotein A-I (apoA-I). The reconstitution of rHDL particles in vitro was for the first time described in 1982,[6a] but recently this reconstitution system has been elegantly adapted by S.G. Sligar and co-workers to incorporate various membrane proteins.[7a] rHDL has a disclike shape with diameter of about 10−12 nm and thickness of ∼ 4 nm, the same thickness as measured for a biological relevant lipid membrane.[6,7] The theoretical calculation of the rotational diffusion tensor for rHDL with dimensions 10 nm × 4 nm indicates rather isotropic motions of this particle.[8] Calculated rotational correlation times (τA, τB, and τC) at a temperature of 303 K are 78, 80, and 85 ns, respectively. That corresponds to an isotropic rotation of a globular protein with a MW of about 200 kDa, and a protein/rHDL complex should have similar relaxation properties and comparable line-widths ($\Delta\nu$) of the NMR signals. Thus, the NMR spectra of membrane proteins reconstructed into rHDL should be observable using the TROSY technique and modern high-field NMR spectrometers. In principle, the rHDLs could provide superior membrane mimicking characteristics and enhanced sample stability as compared with conventional membrane mimetics, therefore they may be a very attractive environment for NMR investigations of membrane proteins.

To test the applicability of high-resolution NMR methods for studies of membrane proteins and membrane-active peptides incorporated into rHDL particles, the uniformly ^{15}N-labeled peptide Antiamoebin-I (Aam-I, one membrane associated helix) was used. Aam-I (Ace-Phe^{1}-Aib−Aib-Aib-Iva-Gly-Leu-Aib^{8}-Aib-Hyp-Gln-Iva-Hyp-Aib-Pro-Phl^{16}) is a membrane-active peptaibol antibiotic isolated from fungal species belonging to the *Emericellopsis* genus.[9] Previous studies showed that Aam-I is insoluble in water, displays fast conformational exchange between several conformations in isotropic membrane mimetic (methanol), and adopts a right-handed helical conformation upon binding to the surface of DMPC/DHPC bicelles.[9]

The attempts to incorporate Aam-I into rHDL particles following published protocols[7a] were unsuccessful and therefore an alternative protocol was developed. Small volumes of uniformly ^{15}N-labeled Aam-I solubilized in methanol were added to the preparations of empty rHDL containing zwitterionic (DOPC, DPPC, or POPC) or anionic (DOPG) lipids (total amount of added methanol was less then 5%). After that, the samples composed from zwitterionic lipids became turbid and a significant amount of the peptide precipitated. At the same time, the sample of Aam-I with DOPG/rHDL was visually transparent and clear from precipitates. This sample exhibits a ^{1}H-^{15}N correlation spectrum which is very similar to the Aam-I spectrum in neutral DMPC/DHPC bicelles (Figure 1AB) and slightly different from spectra in anionic micelles (LMPG, SDS, see Supporting Information). All expected 13 backbone HN resonances were observed in this spectrum, but the signals were significantly broader (^{1}H $\Delta\nu \approx 40-60$ Hz in rHDL versus ∼30 Hz in bicelles). Dynamic light scattering measurements indicated that the size of the rHDL particles did not change upon Aam-I incorporation. The measured hydrodynamic radius (4.4 nm) agrees well with theoretical expectation for the disclike rHDL particle with dimensions of 10 × 4 nm.[8] However, the ^{1}H line-width of Aam-I resonances corresponds to the overall rotation of the peptide with correlation time not greater than 50 ns, indicating the presence of additional peptide motions within the Aam-I/rHDL complex.

The observed preferential binding of the neutral Aam-I to the DOPG/rHDL particles indicates that the choice of lipids plays an important role to the insertion of Aam-I in the lipid bilayer and that the optimization of phospholipid rHDL composition is required for NMR studies of membrane proteins and membrane-active

[#] Russian Academy of Sciences.
[§] Novartis Institutes for BioMedical Research.

10.1021/ja0777988 CCC: $40.75

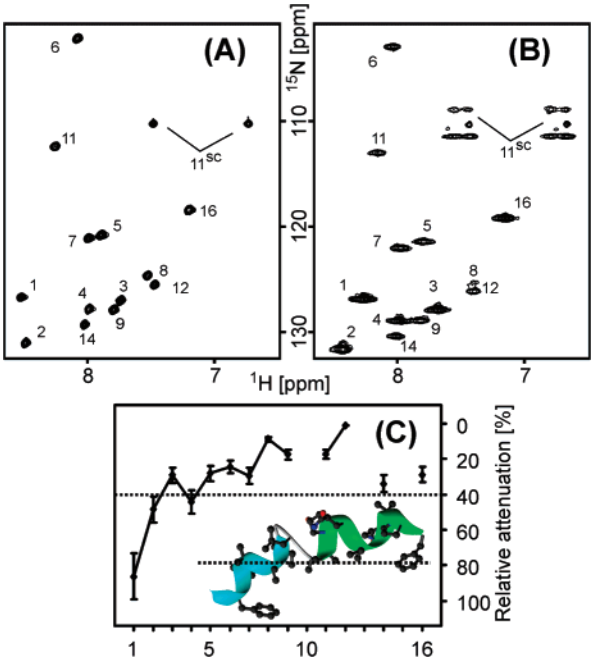


Figure 1. (A) 2D ^{15}N-HSQC spectrum of Aam-I solubilized in DMPC/DHPC bicelles. (B) 2D ^{15}N-TROSY spectrum of Aam-I/DOPG/rHDL complexes. (C) Relative attenuation (%) of intensity of cross-peaks in the TROSY spectrum of the Aam-I/DOPG/rHDL complexes by the 16-doxylstearate. (Insert) Topology of the Aam-I binding to the fragment of DOPG bilayer in the rHDL particle. The horizontal dashed line arbitrary divides Aam-I residues in two categories: strongly and weakly attenuated. The ribbon, representing the N-terminal part that immerses deeper into the membrane is colored cyan, whereas, the ribbon of the C-terminal part is colored green.

peptides. Interestingly, the outer membrane of bacterial cells (the natural target of antiamoebin action) also contains a large amount of anionic lipids. The close similarity of Aam-I spectra in anionic rHDLs and in neutral DMPC/DHPC bicelles indicates that the peptide in complex with rHDL adopts a right-handed helical conformation very similar to conformation observed in lipid membranes.[9] In contrast to that, the solubilization of Aam-I in anionic detergents (LMPG, SDS) possibly leads to distortion of the peptide structure.

To give an example of structural information that can be gained from protein/rHDL complexes, we investigated the topology of Aam-I in the lipid bilayer of rHDL using the well-established lipid-soluble relaxation probe technique.[10a] This method has been successfully applied to peptide/micelle and protein/micelle complexes.[10] The 16-doxylstearate relaxation probe (spin label) was added to the sample of Aam-I/DOPG/rHDL in a drop of methanol to a final concentration of one relaxation probe per rHDL particle. The incorporation of the probe into the lipid bilayer inside the rHDL led to the specific attenuation of the Aam-I signals in the TROSY spectra (Figure 1C). The analysis of the obtained attenuation pattern indicates the peripheral mode of the Aam-I binding to the membrane surface. At the same time, due to the bend in the Aam-I helix, the N-terminus of the peptide immerses slightly deeper into the membrane interior as compared to other parts of the molecule.

The obtained NMR data indicate that rHDL particles are suitable media for investigation of membrane proteins and membrane-active peptides by means of high-resolution NMR. The advantages of this medium are the controlled size of the particles, isotropic hydrodynamic properties and enhanced stability. In contrast to the detergent containing complexes (micelles and bicelles), the rHDLs allow a change of the sample buffer by dialysis and can be easily diluted or concentrated. Moreover, the integral membrane proteins trapped into rHDLs are protected from unfavorable intramolecular interactions.[7a] The rHDL particles contain a section of lipid bilayer with packing properties identical to that of liposomes.[7b] The phospholipid composition of rHDLs can be easily varied to better mimic the properties of different biological membranes. The major shortcoming of the proposed system is the relatively broad linewidth of the signals. The present communication gives an example of a membrane associated peptide, which is possibly involved in a fast rotation relative to rHDL bilayer normal. In the case of integral membrane proteins, twice larger ^{1}H $\Delta\nu$ values are expected. This problem can be significantly alleviated by using deuterated proteins and phospholipids for the preparation of rHDL particles.

In summary, the here presented study of the membrane bound peptide Aam-I reconstituted in rHDL particles, shows considerable promise for future studies of membrane proteins by solution-state NMR. To the best of our knowledge, this is the first application of rHDL in the field of the high-resolution NMR spectroscopy.

Acknowledgment. This work was financially supported by the Russian Federal Agency for Science and Innovations and the Russian Foundation for Basic Research.

Supporting Information Available: The experimental details of the protocol for rHDL formation and the comparison of Aam-I NMR spectra in different membrane mimetics. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Wuthrich, K. N*MR of Proteins and Nucleic Acids*; John Wiley and Sons: New York, 1986. (b) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Skelton, N. J.; Rance, M. *Protein NMR Spectroscopy: Principles and Practice*, 2nd ed.; Academic Press: San Diego, CA, 2006.

(2) (a) Pervushin, K.; Riek, R.; Wider, G.; Wüthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12366−12371. (b) Riek, R.; Fiaux, J.; Bertlesen, E. B.; Horwich, A. L.; Wuthrich, K. *J. Am. Chem. Soc.* **2002**, *124*, 12144−12153. (c) Tugarinov, V.; Choy, W. Y.; Orekhov, V. Y.; Kay, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 622−627. (d) Horst, R.; Bertelsen, E. B.; Fiaux, J.; Wider, G.; Horwich, A. L.; Wuthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12748−12753.

(3) (a) Sanders, C. R.; Sonnichsen, F. *Magn. Reson. Chem.* **2006**, *44*, S24−S40. (b) Fernandez, C.; Wuthrich, K. *FEBS Lett.* **2003**, *555*, 144−150.

(4) (a) Page, R. C.; Moore, J. D.; Nguyen, H. B.; Sharma, M.; Chase, R.; Gao, F. P.; Mobley, C. K.; Sanders, C. R.; Ma, L.; Sonnichsen, F. D.; Lee, S.; Howell, S. C.; Opella, S. J.; Cross, T. A. *J. Struct. Funct. Genomics* **2006**, *7*, 51−64. (b) Krueger-Koplin, R. D.; Sorgen, P. L.; Krueger-Koplin, S. T.; Rivera-Torres, I. O.; Cahill, S. M.; Hicks, D. B.; Grinius, L.; Krulwich, T. A.; Girvin, M. E. *J. Biomol. NMR* **2004**, *28*, 43−57.

(5) Chou, J. J.; Kaufman, J. D.; Stahl, S. J.; Wingfield, P. T.; Bax, A. *J. Am. Chem. Soc.* **2002**, *124*, 2450−2451.

(6) (a) Matz, C. E.; Jonas, A. *J. Biol. Chem.* **1982**, *257*, 4535−4540. (b) Li, L.; Chen, J.; Mishra, V. K.; Kurtz, J. A.; Cao, D.; Klon, A. E.; Harvey, S. C.; Anantharamaiah, G. M.; Segrest, J. P. *J. Mol. Biol.* **2004**, *343*, 1293−1311. (c) Wu, Z.; Wagner, M. A.; Zheng, L.; Parks, J. S.; Shy, J. M.; Smith, J. D.; Gogonea, V.; Hazen, S. L. *Nat. Struct. Mol. Biol.* **2007**, *14*, 861−868.

(7) (a) Nath, A.; Atkins, W. M.; Sligar, S. G. *Biochemistry*. **2007**, *46*, 2059−2069. (b) Denisov, I. G.; McLean, M. A.; Shaw, A. W.; Grinkova, Y. V.; Sligar, S. G. *J. Phys. Chem. B* **2005**, *109*, 15580−15588.

(8) The numerical calculations of the hydrodynamic properties of the rHDL particle were done using equations from: Ortega, A.; de la Torre, J. G. *J. Chem. Phys.* **2003**, *119*, 9914−9919.

(9) Shenkarev, Z. O.; Paramonov, A. S.; Nadezhdin, K. D.; Bocharov, E. V.; Kudelina, I. A.; Skladnev, D. A.; Tagaev, A. A.; Yakimenko, Z. A.; Ovchinnikova, T. V.; Arseniev, A. S. *Chem. Biodivers.* **2007**, *4*, 1219−1242.

(10) (a) Brown, L. R.; Bosch, C.; Wuthrich, K. *Biochim. Biophys. Acta* **1981**, *642*, 296−312. (b) Hilty, C.; Wider, G.; Fernandez, C.; Wuthrich, K. *Chembiochem* **2004**, *5*, 467−473. (c) Papavoine, C. H.; Konings, R. N.; Hilbers, C. W.; van de Ven, F. J. *Biochemistry* **1994**, *33*, 12990−12997.

JA0777988