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Large Protein Complexes with Extreme Rotational Correlation Times Investigated in Solution by Magic-Angle-Spinning NMR Spectroscopy

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Solution-state NMR spectroscopy has been very successful in the past in characterizing supramolecular protein assemblies (with molecular weights of up to 1 MDa)[1,2] through the use of TROSY-type techniques.[3] However, even at very high magnetic fields, low molecular tumbling rates limit the applicability of solution-state NMR spectroscopy in the investigation of these systems. The problem that large rotational correlation times impose on the experimental spectral quality can potentially be overcome by encapsulating a protein in a reversed micelle dissolved in a low-viscosity fluid.[4] Alternatively, the viscosity of the solution might as well be increased to the static limit to solve the relaxation problem. Magic-angle-spinning (MAS) NMR spectroscopy can then be beneficially employed to average coherent anisotropic interactions, as overall motional reorientation is absent. MAS solid-state NMR has become a powerful tool for the structural investigation of biomolecules at atomic resolution. Especially membrane proteins, amyloidogenic peptides/proteins, and also large protein complexes benefit from solid-state NMR methods.[5−7] On the other hand, the necessity to crystallize or precipitate the biological molecule of interest is often a bottleneck. Interaction studies with potential binding partners are often prohibitive, when ligands do not coprecipitate with the target molecule. In addition, crystal-packing artifacts can result in misinterpretation of structural and dynamic data. A protein in solution lacking molecular tumbling would be ideally suited to circumvent the limitations of the conventional solution- and solid-state approaches.

We therefore suggest suppression of rotational diffusion of proteins in solution as an approach for studying large protein complexes by MAS NMR spectroscopy. Accordingly, the scheme is coined FROSTY (*f*reezing *r*otational diffusion *o*f protein *s*olutions at low *t*emperature and high viscosit*y*) NMR spectroscopy. Freeze-trapped protein solutions have been studied previously using solid-state NMR to investigate protein folding intermediates[8] and an antifreeze protein.[9] The experiments reported here were performed on a nonfrozen protein solution, in which only the molecular tumbling of the target protein was restricted by adding glycerol and employing low temperature and high protein concentrations.

As a model system, we used the 20 kDa small heat shock protein (sHSP) αB-Crystallin (αB), which assembles in vivo into high-molecular-weight complexes of roughly 600 kDa.[10] As a member of the sHSP family, αB exhibits chaperone-like activity that prevents unfolded proteins from amorphous or amyloidogenic aggregation.[11−14] Because of its polydispersity, accessing high-resolution structural information has been challenging to date.[15] Recently, a 3D reconstruction of the αB assembly by electron microscopy (EM) was reported,[16] and high-quality MAS solid-state NMR spectra were obtained for PEG-precipitated human αB.[7]

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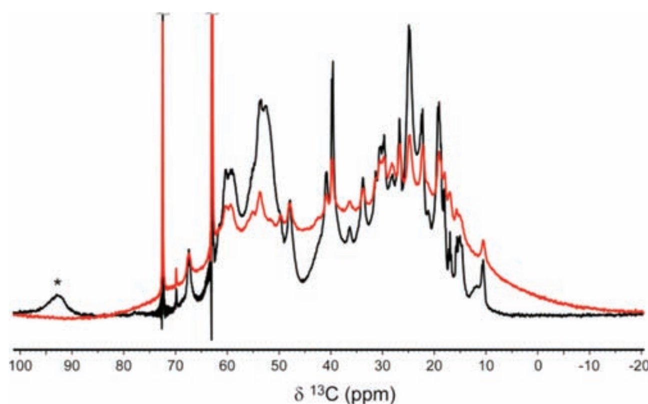

Figure 1. ^{13}C direct excitation spectra of a solution of ^{13}C,^{15}N-αB recorded with 12 kHz MAS (black) and under static conditions (red). The aliphatic region of the spectrum is shown. A carbonyl spinning sideband is marked with an asterisk. Truncated resonances originate from glycerol.

To circumvent the precipitation procedure, we prepared human wild-type ^{13}C,^{15}N-αB (at an approximate oligomer concentration of 170 μM) in TBS buffer containing 20% v/v glycerol. This water-clear solution was used directly for MAS NMR experiments. The ^{13}C direct excitation spectra of αB at −10 °C (effective temperature) and spinning frequencies ω_R of 0 and 12 kHz are shown in Figure 1. The line width of the water resonance was quantified at each temperature to monitor the freezing process. Sample spinning at 12 kHz improved the spectral resolution significantly, since anisotropic interactions were efficiently averaged to yield narrow resonance lines.

To further investigate αB dynamics, we performed cross-polarization (CP) buildup experiments, incrementing the contact time of the Hartmann−Hahn transfer from 0 to 1.5 ms. Figure 2 shows that with ^{1}H,^{13}C CP, the ^{13}C signal intensities build up within 75 to 90 μs. The magnetization transfer to the carbonyl resonances is significantly slower (∼500 μs), as they are not directly bound to a proton. A comparison between precipitated and soluble αB revealed similar buildup rates, and this was also found with respect to ^{15}N,^{13}C cross-polarization (see the Supporting Information). This behavior is expected for rigid solids and demonstrates that αB must exhibit extremely slow molecular tumbling under the employed experimental conditions.

For a detailed comparison of the solution-state-like sample and the precipitate, 2D ^{13}C−^{13}C proton-driven spin diffusion (PDSD) spectra were acquired (τ_{mix} = 15 ms). Figure 3 presents selected regions of the correlation spectrum (the full spectrum as well as temperature-dependent spectra are shown in the Supporting Information). We found only marginal differences between the two spectra, indicating that the αB structure is basically the same in both preparations.

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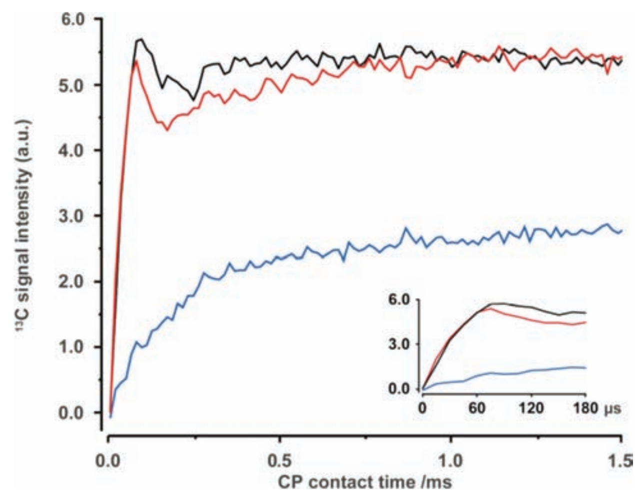

Figure 2. ^{1}H,^{13}C CP buildup curves for (blue) carbonyl, (red) C_α, and (black) methyl resonances at an effective temperature of −10 °C. The Hartmann−Hahn contact time was increased from 0 to 1.5 ms in steps of 15 μs. Spectra were recorded in a pseudo-2D fashion. The inset focuses on the first 180 μs of the CP buildup.

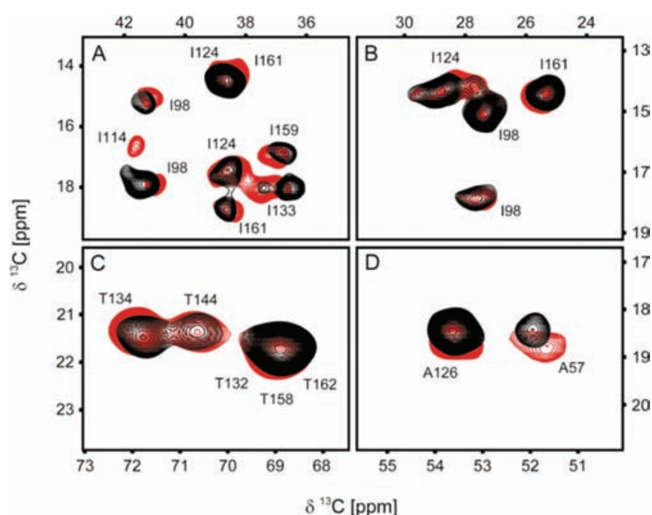

Figure 3. Selected regions of 2D ^{13}C−^{13}C PDSD spectra recorded for (red) PEG-precipitated αB and (black) αB in solution. Panels correspond to (A) Ile Cβ−Cδ/γ, (B) Ile Cδ−Cγ, (C) Thr Cβ−Cγ, and (D) Ala Cα−Cβ correlations. Assignments were taken from precipitated αB.[7]

To achieve efficient averaging of anisotropic interactions by MAS, rotational diffusion must be slow relative to the MAS rotor period $\tau_R = \omega_R^{-1}$ (80 μs). Accordingly, the rotational correlation time τ_c of αB must be larger than one rotor period (i.e., $\tau_c \geq \tau_R$). Using the Stokes−Einstein−Debye equation (see the Supporting Information), we estimate the correlation time τ_c of αB to be on the order of 13 μs in TBS buffer in the presence of 20% glycerol at a temperature of −10 °C. This approximation assumes that αB adopts a spherical structure. The hydrodynamic radius r_H of αB determined using dynamic light scattering (DLS) was on the order of 9 nm (see the Supporting Information), corresponding to an estimated molecular weight of ∼580 kDa. This is consistent with the results of gel-filtration experiments (595 kDa, data not shown) and previous studies.[17,18] The estimated correlation time appears to be shorter than the time required to fulfill the conditions for efficient averaging of anisotropic interactions by MAS. Presumably, molecular crowding due to the high protein concentration induces nonlinear effects in the Stokes−Einstein relation and yields a longer-than-expected correlation time. αB protein−protein interactions might play a crucial role in vivo, as it is known that αB oligomers can adopt chainlike clusters in EM images after extraction from eye lenses.[19] In human eye lenses, crystallins make up 90% of the total protein mass, with concentrations of up to 400 mg/mL.[20] α-Crystallins, comprising αA and αB, contribute ∼30% to this protein mass. This dense packing and the short-range spatial order of lens-specific proteins seem to be responsible for the high refractive index and the transparency of the eye lens.[20] In this respect, the high protein concentration used seems to mimic the native system rather well.

The results reported here demonstrate the feasibility of applying MAS NMR spectroscopy to biomolecules in solution. αB as a model system fulfills the main requirement for this approach, i.e., an extremely long rotational correlation time essential for magic-angle spinning. We believe that the suggested approach is generally applicable for large protein complexes. The rotational correlation time can be adapted to the individual biological system by adjusting the protein concentration, the sample temperature, and the viscosity of the solution (e.g., by adding glycerol). The presence of glycerol in turn allows lower temperatures, further damping the molecular tumbling rate. In light of the progress made in NMR hardware performance, the required rotational correlation times can be reduced by faster MAS rates. The need for very high protein concentrations might be overcome in the future by the use of ^{1}H detection.[21] We therefore propose FROSTY MAS NMR spectroscopy as a tool for overcoming protein size limitations of solution-state NMR without the necessity of precipitation procedures required for conventional solid-state NMR spectroscopy.

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Supporting Information Available: Protein expression/purification, sample preparation, comparison of CP buildup rates, temperature-dependent PDSD spectra, DLS data, and estimation of τ_c. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Fiaux, J.; Bertelsen, E. B.; Horwich, A. L.; Wüthrich, K. *Nature* **2002**, *418*, 207.
(2) Sprangers, R.; Kay, L. E. *Nature* **2007**, *445*, 618.
(3) Pervushin, K.; Riek, R.; Wider, G.; Wüthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12366.
(4) Wand, A. J.; Ehrhardt, M. R.; Flynn, P. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15299.
(5) McDermott, A. *Annu. Rev. Biophys.* **2009**, *38*, 385.
(6) Tycko, R. *Quart. Rev. Biophys.* **2006**, *39*, 1–55.
(7) Jehle, S.; van Rossum, B.; Stout, J. R.; Noguchi, S. M.; Faelber, K.; Rehbein, K.; Oschkinat, H.; Klevit, R. E.; Rajagopal, P. *J. Mol. Biol.* **2009**, *385*, 1481.
(8) Havlin, R. H.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3284.
(9) Siemer, A. B.; McDermott, A. E. *J. Am. Chem. Soc.* **2008**, *130*, 17394.
(10) Merck, K. B.; Groenen, P. J. T. A.; Voorter, C. E. M.; Dehaardhoekman, W. A.; Horwitz, J.; Bloemendal, H.; de Jong, W. W. *J. Biol. Chem.* **1993**, *268*, 1046.
(11) Horwitz, J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10449.
(12) Narayanan, S.; Kamps, B.; Boelens, W. C.; Reif, B. *FEBS Lett.* **2006**, *580*, 5941.
(13) Raman, B.; Ban, T.; Sakai, M.; Pasta, S.; Ramakrishna, T.; Naiki, H.; Goto, Y.; Rao, C. M. *Biochem. J.* **2005**, *392*, 573.
(14) Stege, G. J. J.; Renkawek, K.; Overkamp, P. S. G.; Verschuure, P.; van Rijk, A. F.; Reijnen-Aalbers, A.; Boelens, W. C.; Bosman, G. J. C. G. M.; de Jong, W. W. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 152.
(15) Haley, D. A.; Horwitz, J.; Stewart, P. L. *J. Mol. Biol.* **1998**, *277*, 27.
(16) Peschek, J.; Braun, N.; Franzmann, T. M.; Georgalis, Y.; Haslbeck, M.; Weinkauf, S.; Buchner, J. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 13272.
(17) Horwitz, J. *Exp. Eye Res.* **2003**, *76*, 145.
(18) Aquilina, J. A.; Benesch, J. L. P.; Ding, L. L.; Yaron, O.; Horwitz, J.; Robinson, C. V. *J. Biol. Chem.* **2004**, *279*, 28675.
(19) Schurtenberger, P.; Augusteyn, R. C. *Biopolymers* **1991**, *31*, 1229.
(20) Delaye, M.; Tardieu, A. *Nature* **1983**, *302*, 415.
(21) Chevelkov, V.; Rehbein, K.; Diehl, A.; Reif, B. *Angew. Chem., Int. Ed.* **2006**, *45*, 3878.

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