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Thermal Stabilization of DMPC/DHPC Bicelles by Addition of Cholesterol Sulfate

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Abstract: Doping DMPC/DHPC bicelles with cholesterol sulfate broadens the temperature range over which stable alignment occurs, forming an aligned phase at lower temperatures even with high lipid concentrations. Cholesterol sulfate appears to combine the advantages of cholesterol with those of charged amphiphiles, stabilizing the aligned phase and preventing precipitation. This allows NMR data for RDC and CSA protein structure constraints to be acquired at or below room temperature, an obvious advantage for solid-state and solution studies of heat-sensitive proteins.

Bicelles made from a mixture of short- and long-chain phospholipids (most commonly DMPC and DHPC)[1] are an important orienting medium in NMR studies of biomolecules. In this versatile medium, the lipid ratio and concentration can be adjusted to produce small, fast-tumbling bicelles with minimal impact on solution-state line widths or large, concentrated bicelles for solid state NMR.[2] Discoid or "Swiss cheese" bicelles contain a planar DMPC region where membrane proteins can insert, mimicking the bilayer of a biological membrane.[3] At lower concentrations, they induce weak alignment in soluble proteins, enabling measurements of residual dipolar couplings[4,5] and chemical shift offsets due to CSA,[6−8] from which distance and orientational constraints, respectively, can be extracted.

While a number of bicellar systems have been introduced, the original DMPC/DHPC mixture is the most commonly used and best characterized. DMPC/DHPC bicelles are limited by a narrow temperature range for optimal alignment (32−36 °C), which is unsuitable for heat-sensitive biomolecules. Strategies for increasing the temperature range and stabilizing the aligned phase include modification of the lipids[9,10] or addition of cholesterol (CH)[11,12] or charged amphiphiles.[13]

Here we show that doping DMPC/DHPC bicelles with cholesterol sulfate (CS), a minor component of mammalian membranes, increases the temperature range over which stable alignment occurs. This allows collection of NMR data at or below room temperature without requiring samples with very low bicelle concentrations, which are easily disrupted by introducing solutes. CS appears to combine the advantages of cholesterol with those of charged amphiphiles; it lowers the gel-to-liquid crystal phase transition temperature of the hydrocarbon chains and introduces repulsive interactions that prevent adjacent bicelles from adhering and precipitating.

In NMR analysis of membrane systems in general and bicelles in particular, the distinction between solid-state and solution experiments is rapidly disappearing, with many techniques applicable to both. Therefore we tested samples with ratios of DMPC/DHPC (q values) of 2.6 and 3.5, representing typical concentrations used for solution and solids experiments. NMR measurements of ^{2}H quadrupole splittings in D_2O were used to investigate the phase behavior of undoped, CH, and CS bicelles.

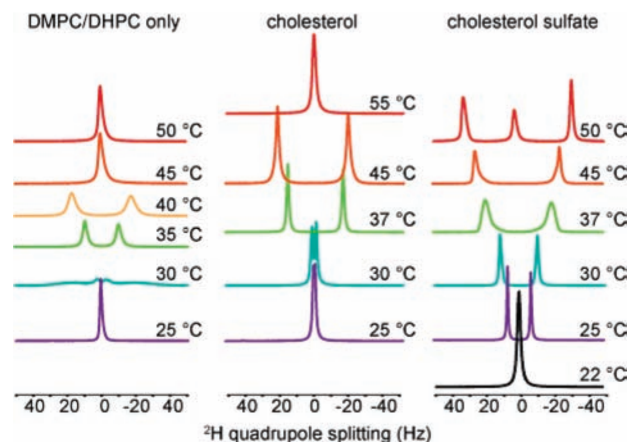

Figure 1. Comparison of deuterium quadrupole splitting in 10% D_2O/90% H_2O, $q = 2.6$ DMPC/DHPC bicelles of total lipid content 20% w/v using undoped bicelles and bicelles doped to 13.4% of the lipid content with either cholesterol (CH) or cholesterol sulfate (CS). Measurements were made in a 400 MHz Bruker DRX instrument. In this preparation, undoped bicelles have a narrow useful temperature range, CH extends it somewhat, and CS displays splittings at or below room temperature.

As illustrated in Figure 1, CS-doped bicelles were formed at a significantly lower temperature than CH-doped or undoped bicelles. In all three samples, isotropic phases are observed at low temperature. With increasing temperature, ^{2}H quadrupole splitting appears, indicating formation of the aligned phase. The splitting increases, consistent with growing bicelle size, until another phase transition yields a different isotropic phase. The CS sample forms a mixture of aligned and isotropic phases at 50 °C, as indicated by the presence of both the doublet and the isotropic peak.

Both CH and CS widen the temperature range of the desirable aligned phase and produce slightly larger splittings. However, CS stabilizes the aligned phase at room temperature even at this high concentration, in contrast to many previous bicelle compositions that required very low concentrations (∼3%) to achieve this result. This is useful because more concentrated bicelle solutions are more tolerant of introducing solutes and because it allows the possibility of increasing the alignment, if necessary, to measure RDCs from small or spherical molecules. In fact this type of measurement is likely to underestimate the alignment at low temperatures, since the ^{2}H quadrupole splitting has a stronger temperature dependence than the magnitude of the alignment tensor, probably because

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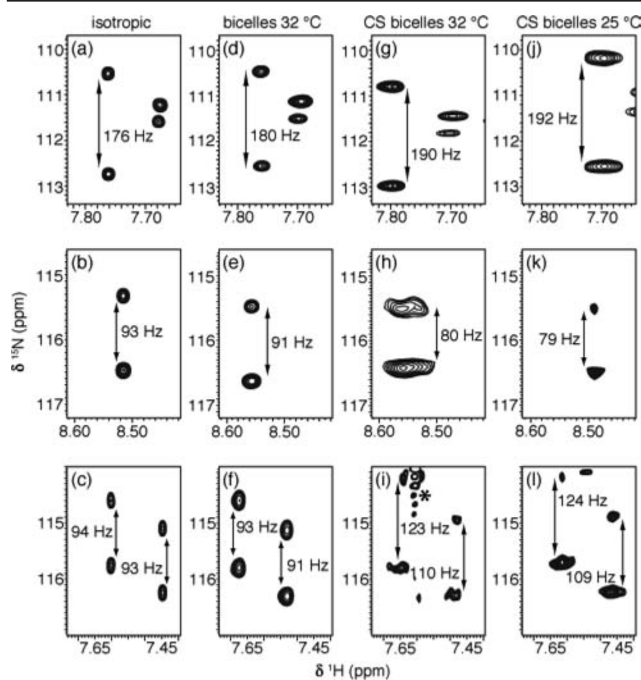

Figure 2. Representative RDCs for ubiquitin in DMPC/DHPC and DMPC/DHPC/CS bicelles. Expanded regions are from the IP spectra of ^{15}N-HSQC-IPAP data collected on an 800 MHz Varian Inova instrument. The left column shows the *J*-couplings for four residues in ubiquitin: (a) Gln 2 (side chain), (b) Asp 39, and (c) Ser 65 (left) and Lys 33 (right). Column two shows the splitting from the *J*-coupling + dipolar coupling (J+D) for the same residues in undoped bicelles at 32 °C (d, e, f). Columns three and four show $J+D$ in CS-doped bicelles at 32 °C (g, h, i) and 25 °C (j, k, l), respectively. RDCs are determined by subtracting the *J*-coupling from the total splitting values. The first row corresponds to the NH_2 group of the Gln 2 side chain: because this experiment gives a 1:0:1 triplet for an NH_2 spin system, the splittings shown for this residue are $2J$ for the first column and $2(J+D)$ for the rest. The asterisk in subfigure (i) indicates a truncation artifact extending down from a peak outside the frame.

increasing temperature causes small changes in the distribution of water molecules hydrating the bicelles.[14]

CS slightly increases the magnitudes of the splittings over the entire temperature range observed. In a similar set of experiments run with $q = 3.5$, 25% w/v bicelles, CS also raised the upper limit of the aligned phase temperature range from 40 °C in undoped and CH-doped bicelles to above 50 °C in CS-doped bicelles (Supporting Information). This result is also worthy of further exploration as a potential medium for investigating structure and dynamics of thermophilic proteins at biologically relevant temperatures.

To demonstrate the utility of CS-doped bicelles as a protein alignment medium, ^{15}N-labeled ubiquitin was added to both undoped and CS bicelles ($q = 3.5$, 13.6% w/v lipid, 0.3 mM ubiquitin). This composition was chosen because similar preparations have been used for MAS[15] and static[16] experiments on membrane proteins. RDCs and chemical shift anisotropies for ubiquitin have also been obtained using $q = 3.0$ bicelles, albeit at lower concentration.[17] Figure 2 shows representative data from ubiquitin in CS bicelles at 32 and 25 °C and in undoped bicelles at 32 °C, using standard ^{15}N-HSQC-IPAP NMR experiments.[18] The increased line widths in the CS bicelle spectra at 32 °C are due to this sample's stronger alignment, which increases contributions from both dipolar couplings and chemical shift anisotropy. At both temperatures investigated, the CS spectra show chemical shift differences that are significant but not large enough to interfere with resonance assignments, given sufficient spectral resolution.

Two features of the CS bicelles are promising for protein work. First, the stability of the protein sample itself was considerably higher in CS bicelles than in CH bicelles. The ubiquitin sample with CH-doped bicelles phase separated into an inhomogeneous mixture of phospholipids and protein after only a few hours at 32 °C: insufficient time to collect 2D data. This effect was also observed when identical bicelle samples were prepared with a different protein, HIV1-Nef_{NL4-3}; CH samples showed phase separation and protein precipitation after less than 48 h, while the sample made with CS is still intact after several weeks.

Equally important, RDCs comparable to those obtained at 32 °C in undoped bicelles were extracted from data taken at 25 °C in CS bicelles, on the order of 10 °C lower than the temperatures typically used in RDC experiments. Because many proteins are sensitive to thermal degradation, finding a medium that allows sample alignment at room temperature and below while providing longer-term sample stability potentially allows RDC measurements on a larger variety of proteins. CS bicelles may also be useful for X-ray crystallography; a previously reported method for crystallizing membrane proteins from bicelle solutions was limited by the high temperatures required.[19]

In summary, doping DMPC/DHPC bicelles with cholesterol sulfate allows measurement of RDCs at or below room temperature, even at high lipid concentrations. This can be used for solid-state and solution work on heat-sensitive biomolecules, particularly membrane proteins, which require high lipid concentrations to obtain sufficient protein in the sample.

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Supporting Information Available: Preparation protocols for bicelles and protein, additional representative RDC and ^{2}H data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sanders, C. R.; Schwonek, J. P. *Biochemistry* **1992**, *31*, 8898–8905.
(2) Marcotte, I.; Auger, M. *Concepts Magn. Reson. A* **2005**, *24A*, 17–37.
(3) Lee, D.; Walter, K.; Bruckner, A.-K.; Hilty, C.; Becker, S.; Griesinger, C. *J. Am. Chem. Soc.* **2008**, *130*, 13822–13823.
(4) Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111–1114.
(5) Tolman, J.; Prestegard, J. *J. Magn. Reson. B* **1996**, *112*, 269–274.
(6) Ottiger, M.; Tjandra, N.; Bax, A. *J. Am. Chem. Soc.* **1997**, *119*, 9825–9830.
(7) Prosser, R. S.; Hunt, S. A.; DiNatale, J. A.; Vold, R. R. *J. Am. Chem. Soc.* **1996**, *118*, 269–270.
(8) Kovacs, F.; Cross, T. *Biophys. J.* **1997**, *73*, 2511–2517.
(9) Wang, H.; Eberstadt, M.; Olejniczak, E. T.; Meadows, R. P.; Fesik, S. W. *J. Biomol. NMR* **1998**, *12*, 443–446.
(10) Triba, M. N.; Devaux, P. F.; Warschawski, D. E. *Biophys. J.* **2006**, *91*, 1357–1367.
(11) Sasaki, H,; Fukuzawa, S.; Kikuchi, J.; Yokoyama, S.; Hirota, H.; Tachibana, K. *Langmuir* **2003**, *19*, 9841–9844.
(12) Lu, J. X.; Caporini, M. A.; Lorigan, G. A. *J. Magn. Reson.* **2004**, *168*, 18–30.
(13) Losonczi, J. A.; Prestegard, J. H. *J. Biomol. NMR* **1998**, *12*, 447–451.
(14) Ottiger, M.; Bax, A. *J. Biomol. NMR* **1998**, *12*, 361–372.
(15) Xu, J.; Dürr, U. H.; Im, S.-C.; Gan, Z.; Waskell, L.; Ramamoorrthy, A. *Angew. Chem.* **2008**, *120*, 7982–7985.
(16) Park, S. H.; Prytulla, S.; DeAngelis, A. A.; Brown, J. M.; Kiefer, H.; Opella, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 7402–7403.
(17) Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 6836–6837.
(18) Ottiger, M.; Delaglio, F.; Bax, A. *J. Magn. Reson.* **1998**, *131*, 373–378.
(19) Faham, S.; Bowie, J. U. *J. Mol. Biol.* **2002**, *316*, 1–6.
(20) Ramirez, B. E.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 9106–9107.

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