Location of Cholic Acid Sequestered by Core−Shell Nanoparticles Using REDOR NMR

Hsien-Ming Kao,[†] Robert D. O'Connor, Anil K. Mehta, Haiyong Huang,[‡] Barbara Poliks,[§] Karen L. Wooley, and Jacob Schaefer*

Department of Chemistry, Washington University, St. Louis, Missouri 63130

Received August 15, 2000

ABSTRACT: Solid-state $^{13}C\{^{19}F\}$ rotational-echo double-resonance (REDOR) NMR was used to locate [24-^{13}C]cholic acid that had been absorbed by nanoparticles with a core−shell morphology. The nanoparticles were composed of poly(4-fluorostyrene)-*block*-poly(acrylic acid) copolymers in which 15% of the poly(acrylic acid) shell functionality had been cross-linked with 2,2′-(ethylenedioxy)bis(ethylamine). The REDOR data were consistent with no cholic acid in the core, approximately 28% located at or near the core−shell interface, and the remaining 72% of the cholic acid farther than 25 Å from the interface.

Introduction

Shell cross-linked (SCK) spherical nanoparticles with a core−shell morphology are created by cross-linking micelles formed from diblock copolymers.[1−4] Typically, the core is hydrophobic and the shell hydrophilic, but SCKs with hydrophilic or hollow cores have also been synthesized.[5−7] The size distribution of SCKs is usually narrow with a mean diameter between 10 and 100 nm. Larger diameters, on the order of 1 μm, have been synthesized.[8] The size distribution is dependent on the copolymer and micelle formation, both of which can be controlled.[9] Other routinely controlled properties include the surface functionality and the hydrophilicity, density, and porosity of the core and shell.[10] The ability to control these variables, and the mechanical stability of the SCKs, combine to make them attractive candidates for both biological and industrial use.[11]

One such use involves the sequestering of metabolites. The micelle-like structure of SCKs allows the absorption and sequestering of small molecules. A previous study has shown that the cross-link density of the shell is an important factor affecting the location of the small molecule in the SCK.[12] Generally, the nature of the core, shell, solution, and small molecule all affect the partitioning of guests in SCKs.

In this study, we are interested in the location of cholic acid, a cholesterol metabolite, which has been absorbed by SCKs synthesized from poly(4-fluorostyrene)-*block*-poly(acrylic acid) copolymers. We determine the location by measuring the dipolar couplings between the core fluorines and ^{13}C-labeled cholic acid using rotational-echo double-resonance (REDOR) solid-state NMR.[13,14] The dipolar coupling is then related to the internuclear separation and the location of the cholic acid.

Experiments

The anionic polymerization, micelle formation, and cross-linking with 2,2′-(ethylenedioxy)bis(ethylamine) to form SCKs were similar to those of previous reports.[15] The only notable differences were the substitution of 4-fluorostyrene for styrene and the hydrolysis of poly(*tert*-butyl acrylate) with HCl in *p*-dioxane instead of a *p*-toluenesulfonic acid in toluene.[16] The M_n and polydispersity of the poly(4-fluorostyrene) block of the copolymer were 13.8 kDa and 1.55, respectively (measured by GPC using polystyrene standards). On the basis of this M_n and the relative ratio of styrene to acrylic acid from ^{1}H NMR, the average numbers of 4-fluorostyrene and acrylic acid units per chain were 115 and 130, respectively. Loading of the SCKs was done by mixing 15 μmol of [24-^{13}C]cholic acid (Cambridge Isotopes) with 133 mL of an aqueous solution of SCK (0.86 mg/mL). After 2 weeks of stirring, the mixture was lyophilized and packed into 7.5 mm (o.d.) zirconia rotors for examination by solid-state NMR.

The $^{13}C\{^{19}F\}$ REDOR experiments were performed at 50 MHz for ^{13}C, at ambient temperature, 5 kHz magic-angle spinning, a single 10 μs ^{13}C π refocusing pulse, and 10 μs ^{19}F π pulses which were *xy*-8 phase cycled. Other experimental parameters are the same as previously reported.[17]

The positions of the ^{19}F nuclei relative to the ^{13}C are required to quantify the REDOR data. Although a random spherical distribution[12] of the ^{19}F nuclei could probably have been fit to the data, a molecular model of an SCK was developed to provide a more accurate ^{19}F density distribution and to help visualize the SCK. The modeling restraints were based on a spherical particle that had a core 11 nm in radius with a density of 1.1 g/cm^3 and a shell 3 nm thick with a density of 0.8 g/cm^3.[12] The dimensions of the core and shell were established by the composition and the overall size of the nanoparticle, as measured by atomic force microscopy.[15] With these parameters, a molecular model consisting of nine chains was developed using Insight II (MSI, San Diego, CA). The polymer chains consisted of a poly(4-fluorostyrene) block 120 units long connected by a diphenylethylene spacer to a poly(acrylic acid) block 136 units long. The chains were folded both manually and through simulated dynamics, which were followed by energy minimizations (cvff and cff91 force fields), until the appropriate bulk densities and particle radii were reached. After the structure matched the selected parameters, approximately 10 cross-links per chain (15%) were added. Finally, 12 cholic acid molecules were inserted, and the energy of the system was again minimized.

The dephasing, $S(t,r)$, as a function of the radial distance, r, of a ^{13}C label from the core−shell interface was calculated for each REDOR dipolar evolution time, t, using the ^{19}F

[†] Present address: Department of Chemistry, National Central University, Chung-Li, Taiwan 32054.
[‡] Present address: Union Carbide, Bound Brook, NJ 08805.
[§] Department of Physics, University of Binghamton, Binghamton, NY 13902.
* Corresponding author. Phone 314-935-6844; Fax 314-935-4481; E-mail schaefer@wuchem.wustl.edu.

10.1021/ma001429d CCC: $20.00
Published on Web 01/06/2001

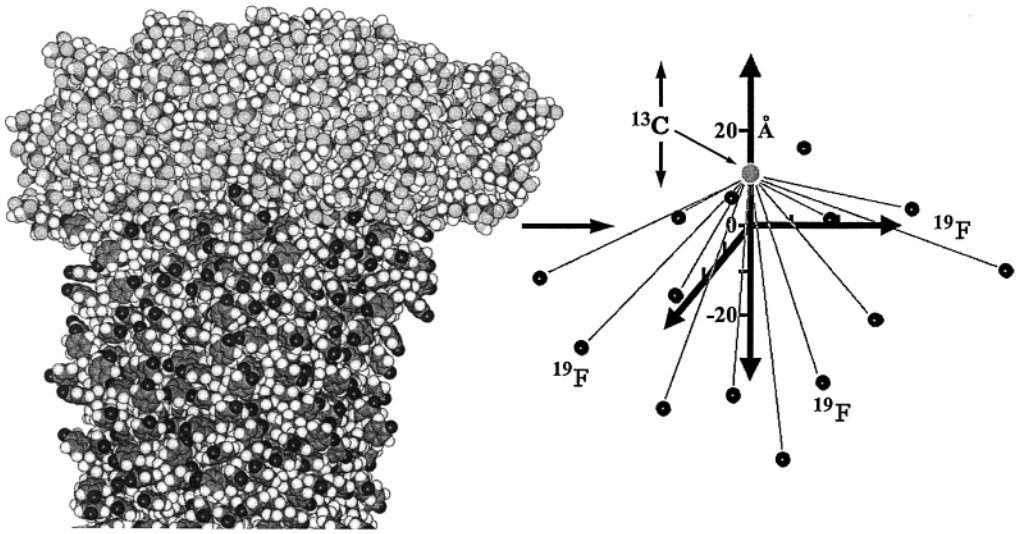

Figure 1. Molecular model (left) described in the text and a schematic representation of the REDOR calculation (right). The darkest circles in the model are the ^{19}F's of the core whose coordinates are extracted (right) for the REDOR calculation. Only about 50% of the length of the core is shown. The REDOR dephasing is calculated as a function of the position of a ^{13}C cholic acid nucleus on the *Z* axis which is normal to the core−shell interface. The lines between the ^{13}C and ^{19}F nuclei represent dipolar vectors which vary with the ^{13}C position.

coordinates from the above molecular model and eq 22 of ref 17. The calculation included *all* dipolar couplings between a ^{13}C label and ^{19}Fs that were within 30 Å of the interface (approximately 230). We define the interface as the average position of the last ^{19}F in the core or, essentially, the beginning of the diphenylethylene spacer. In the calculations, the ^{13}C position was varied along a normal to the core−shell interface, i.e., radially outward from the center of the nanoparticle as illustrated in Figure 1 (right). The dephasing for a distribution, $G(r;r_m,\sigma)$, of ^{13}C distances is

$$S(t;r_m,\sigma) = \int G(r;r_m,\sigma)\, S(t,r)\, dr \qquad (1)$$

where r_m and σ represent generalized mean and width parameters, respectively. Once $S(t,r)$ is known for each experimental t, it is straightforward to optimize the parameters of the distribution. For the cholic acid data, the parameters were optimized using a least-squares implementation of Mathematica's (Wolfram Research, Champaign, IL) nonlinear optimization procedure.

Results and Discussion

Figure 2 shows example spectra of the SCKs both with (left) and without (center and right) absorbed cholic acid. For all the spectra, the resonances between 100 and 165 ppm are from the aromatic core carbons and serve only to measure the relative amount of SCK in each sample. For the center and right spectra, the broad resonance around 180 ppm is from shell acrylic acid carbonyls, and for the left spectrum this resonance is from both the shell acrylic acid and [24-^{13}C]cholic acid carbons. Because the shell carbonyl and cholic acid resonances are unresolved, the cholic acid REDOR dephasing must be background-corrected as follows:

$$[\Delta S/S_0]^{\text{cholic}} = 1 - \frac{\Delta S^{\text{S/C}} - \Delta S^{\text{SCK}}}{S_0^{\text{S/C}} - S_0^{\text{SCK}}} \qquad (2)$$

where S_0 is the reference or full-echo, nondephased spectrum; S is the dephased spectrum; ΔS is difference between S_0 and S; and the superscripts SCK, S/C, and cholic denote quantities without cholic acid (SCK alone), with cholic acid (SCK and cholic acid), and without SCK (background corrected, cholic acid alone), respectively.

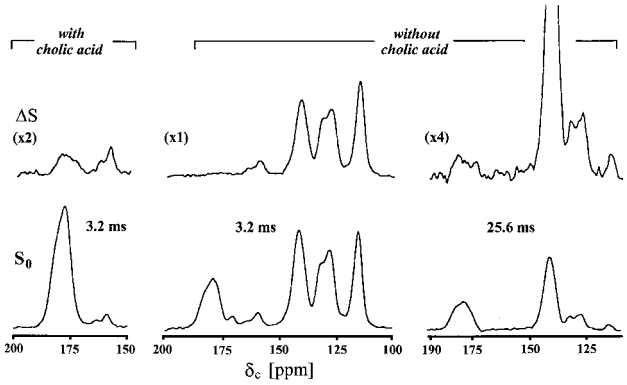

Figure 2. Carbonyl and aromatic carbon regions of the SCK ^{13}C{^{19}F} REDOR spectra with [24-^{13}C]cholic acid (left) and without cholic acid (center and right) for those values of the dipolar evolution time. The bottom full-echo spectra, S_0, were obtained without ^{19}F pulses and are used as references. The top row shows the corresponding difference spectra, ΔS, which represent the dephased intensity. The ΔS spectra were generated by subtracting the spectra obtained with ^{19}F pulses (i.e., with dipolar evolution) from the S_0 spectra (no dipolar evolution). Both the cholic acid S_0 and ΔS spectra must be corrected for contributions from the natural-abundance ^{13}C's of the shell acrylic acid carbonyls.

Figure 3 shows the background-corrected REDOR dephasing for bound cholic acid as a function of dephasing time. Qualitatively, the maximum dephasing (t = 28.8 ms) indicates that only approximately 30% of the cholic acid is close to the core−shell interface.

To quantify the location of the cholic acid, the REDOR data were fit to a distribution of ^{13}C distances extending radially outward from the core ^{19}Fs. Calculations based on unimodal distributions (Gaussian, linear, square, or exponential) could not reproduce the data. The solid line in Figure 3 is the calculated REDOR dephasing from a bimodal distribution in which one mode was Gaussian and the other was a delta function (i.e., intensity at a single distance). Figure 4 shows the ^{13}C radial distance distribution from this fit. The arrows on the line representing the 25 Å fraction indicate that the fit was insensitive to the shape of this mode as long as most of its intensity was beyond 25 Å. Essentially, the 25 Å

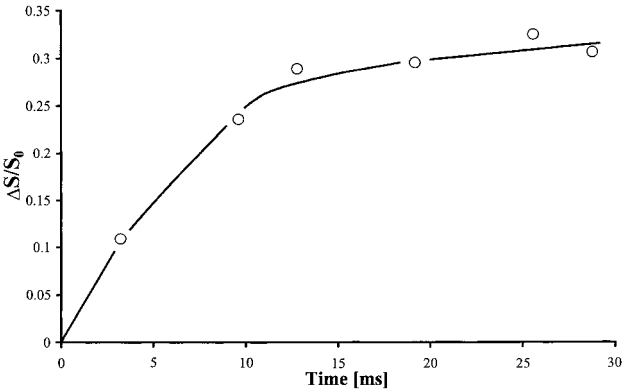

Figure 3. Background-corrected (eq 2 of the text) REDOR dephasing for [24-^{13}C]cholic acid absorbed by SCKs from aqueous solution (○). The solid line represents the calculated dephasing for a distribution of cholic acid distances from the core−shell interface.

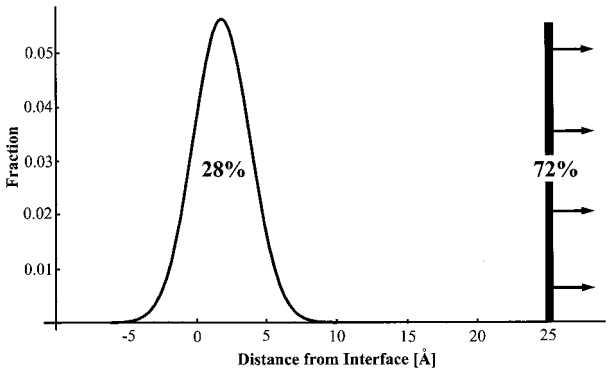

Figure 4. Distribution of distances of the cholic acid ^{13}C-labels from the core−shell interface resulting from the fit of the model of Figure 1 to the REDOR data of Figure 3. Approximately 28% of the [24-^{13}C]cholic acid labels are near the interface. The remaining 72% are *at least* 25 Å away.

distance represents the *closest* that *all* of this fraction could be to the interface. Some or most of this fraction could, in fact, be farther away. As stated earlier, the origin of the graph represents the average position of the last ^{19}F in the core, and the calculation included *all* of the dipolar couplings to the selected ^{19}F's that were within 30 Å of this origin. Including more distant ^{19}F's that were deeper inside the core in the calculation slightly decreased the width of the Gaussian mode but had no significant effect on its mean or weight. Because the ^{19}F density within the first few angstroms of the interface primarily governs the dephasing of the ^{13}C's at 25 Å, adding distant ^{19}F's had no effect on this fraction.

A surprising result of the model calculation was the 25 Å distance required to reproduce the experimental dephasing. Calculations with a fraction of ^{13}C's closer than 25 Å to the interface resulted in significantly more dephasing at longer times. Sensitivity to a distance this long is unusual for ^{13}C{^{19}F} REDOR and results from the presence of many ^{19}F dephasing centers. For example, for a ^{13}C label that is 20 Å from the interface of the SCK model, the calculated dephasing was 5% at 20 ms and 12% at 30 ms. By comparison, for an isolated ^{13}C−^{19}F pair with an internuclear separation of 20 Å (which corresponds to a dipolar coupling of 3.5 Hz), the dephasing is only $^1/_2$% at 20 ms and 1% at 30 ms.

The results of the fit indicate that 28% of the cholic acid is absorbed by the SCKs under the loading conditions used. The absorbed cholic acid is at or near the core−shell interface; none of it is in the core. The results also suggest that about 72% of the cholic acid is adsorbed on the surface of the SCKs or in the surrounding free volume between SCK particles. Future work will involve surface labels and could distinguish between these two possibilities.

Acknowledgment. This work was supported by the U.S. Army under Grant DAAH04-96-1-0158 (K.L.W.) and the NSF under Grant MCB9604860 (J.S.).

References and Notes

(1) Wooley, K. L. *Chem.—Eur. J.* **1997**, *3*, 1397.
(2) Thurmond, K. B. II; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1996**, *118*, 7239.
(3) Huang, H.; Remsen, E. E.; Wooley, K. *Chem. Commun.* **1998**, 1415.
(4) Wooley, K. L. *J. Polym. Sci. A*, submitted for publication.
(5) Huang, H.; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1999**, *121*, 3805.
(6) Zhang, Q.; Remsen, E.; Wooley, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 3642.
(7) Bütün, V.; Lowe, A. B.; Billingham, N. C.; Armes, S. P. *J. Am. Chem. Soc.* **1999**, *121*, 4288.
(8) Serizawa, T.; Takehara, S.; Akashi, M. *Macromolecules* **2000**, *33*, 1759.
(9) Thurmond, K. B. II; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *119*, 6656.
(10) Remsen, E. E.; Thurmond, K. B. II; Wooley, K. L. *Macromolecules* **1999**, *32*, 3685.
(11) Thurmond, K. B. II; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. *Nucleic Acids Res.* **1999**, *27*, 2966.
(12) Baugher, A. H.; Goetz, J. M.; McDowell, L. M.; Huang, H.; Wooley, K. L.; Schaefer, J. *Biophys. J.* **1998**, *75*, 2574.
(13) Gullion, T.; Schaefer, J. *Adv. Magn. Reson.* **1989**, *13*, 57.
(14) Gullion, T.; Schaefer, J. *J. Magn. Reson.* **1989**, *81*, 196.
(15) Huang, H.; Kowalewski, T.; Remsen, E. E.; Gertzmann, R.; Wooley, K. *J. Am. Chem. Soc.* **1997**, *119*, 11653.
(16) Chan, J.; Fox, S.; Kiserow, D.; Ramireddy, C.; Munk, P.; Webber, S. E. *Macromolecules* **1993**, *26*, 7016.
(17) Goetz, J. M.; Schaefer, J. *J. Magn. Reson.* **1997**, *127*, 147.

MA001429D