The Structure and Dynamics of Poly(L-lysine) in Templated Silica Nanocomposites

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Biologically templated nanoparticles are of interest for a variety of biological applications and for controlling the porosity and other properties in nanocomposites. Solid-state NMR has been used to characterize polypeptide/silica composites templated from the poly(L-lysine) solutions in the α-helix, β-sheet, and random coil conformations. The results show that the poly(L-lysine) retains its solution conformation in the nanocomposites when templating from the α-helix or β-sheet conformation, but the random coil conformation is partially converted to the α-helical form during nanocomposite formation. The dynamics of poly(L-lysine) are restricted by incorporation into the composite compared to bulk poly(L-lysine) at the equivalent relative humidity. Bulk and film poly(L-lysine) samples undergo transitions from the random coil to the β-sheet to the α-helix conformation with increasing relative humidity, but these transitions are suppressed in the silica composite. {^{1}H-^{31}P} NMR studies show that inorganic phosphate is incorporated into the random coil-templated composite and that part of the phosphate is closely associated with the poly(L-lysine). The implications for biomimetic nanocomposites formation are considered.

Introduction

Biomimetic nanocomposites are of interest for encapsulating biologically active molecules, for controlling the porosity in nanocomposites, and for controlling the mechanical, electrical, magnetic, and optical properties of composite materials. Organic/inorganic composites are frequently difficult to prepare because the laboratory synthesis of the inorganic component often requires extreme reaction conditions (temperature, pH, etc.) that adversely affect the organic component. There are a number of examples in nature, including bones, teeth, shells, and diatoms, that show it is possible to produce exquisitely structured organic/inorganic composites at ambient temperature and pH.[1] Although interest in biomimetic composites has greatly expanded in recent years, fundamental questions remain about the mechanism of composite formation and the structure and organization of the composites.

In addition to composite materials design based on nature's template, biomimetic methods have more recently been expanded to produce materials not found in nature that are of great interest to materials scientists. There is interest not only in producing nanocomposites but also in using biological materials (peptides, proteins, nucleic acids, etc.) to template the formation of inorganic materials, including nanoparticles of silica,[2] metals,[3] and semiconductors.[4] A number of peptides have been discovered using phase-display technology that either bind to or template the formation of these materials.[3,4]

Silica nanocomposites are of interest for their biocompatibility and their mechanical[5] and optical properties.[6,7] Silica formation in diatoms is directed by proteins, but studies have shown that silica formation can also be directed by the sillafin-derived R5[2] and other peptides,[8] polyamino acids,[9] and synthetic polymers such as poly(allylamine).[10,11] Depending on the templating material and solution conditions, a variety of silica structures have been reported, including spheres,[8,12] hexagonal platelets, sheet-like and rope-dendritic structures.[10,11] Silica composites are also of interest for enzyme encapsulation, where it has been shown that encapsulation does not significantly decrease enzymatic activity, but greatly enhances the stability and shelf life.[13,14]

Poly(L-lysine) is of interest for silica composite formation because the long-chain polyamino acid can template silica formation under conditions where the polymer adopts the

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(1) Moss, S. C. In *Materials Research Society*; Moss, S. C., Ed.; Materials Research Society: Boston, MA, 2002; Vol. 711, p 364.

(2) Kroger, N.; Deutzmann, R.; Sumper, M. *Science* **1999**, *286*, 1129–1132.

(3) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169–172.

(4) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665–668.

(5) Woesz, A.; Weaver, J. C.; Kazanci, M.; Dauphin, Y.; Aizenberg, J.; Morse, D. E.; Fratzl, P. *J. Mater. Res.* **2006**, *21*, 2068–2078.

(6) Brott, L. L.; Naik, R. R.; Pikas, D. J.; Kirkpatrlck, S. M.; Tomlin, D. W.; Whitlock, P. W.; Clarsen, S. J.; Stone, M. O. *Nature* **2001**, *413*, 291–293.

(7) Sundar, V. C.; Yablon, A. D.; Grazul, J. L.; Ilan, M.; Aizenberg, J. *Nature* **2003**, *424*, 899–900.

(8) Naik, R. R.; Brott, L. L.; Clarson, S. J.; Stone, M. O. *J. Nanosci. Nanotechnol.* **2002**, *2*, 95–100.

(9) Coradin, T.; Durupthy, O.; Livage, J. *Langmuir* **2002**, *18*, 2331–2336.

(10) Patwardhan, S. V.; Mukherjee, N.; Clarson, S. J. *J. Inorg. Org. Polym.* **2001**, *11*, 117–121.

(11) Rodriguez, F.; Glawe, D. D.; Naik, R. R.; Hallinan, K. P.; Stone, M. O. *Biomacromolecules* **2004**, *5*, 261–265.

(12) Patwardhan, S. V.; Clarson, S. J. *Polym. Bull.* **2002**, *48*, 367–371.

(13) Luckarift, H. R.; Spain, J. C.; Naik, R. R.; Stone, M. O. *Nat. Biotechnol.* **2004**, *22*, 211–213.

(14) Luckarift, H. R.; Dickerson, M. B.; Sandhage, K. H.; Spain, J. C. *Small* **2006**, *2*, 640–643.

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α-helix, β-sheet, or random coil conformation.[15] The results show that the silica pore size depends on poly(L-lysine) secondary structure. The 1.5 nm pores observed for the composite templated from the α-helical conformation and the larger pores observed for the aggregated β-sheet conformation are consistent with entrapment of the solution conformation. These results, combined with FTIR data and electron micrographs, show that it is possible to prepare porous silica composites in which the pore size depends on the conformation of the templating polymer.

In these studies we have used solid-state NMR to determine the conformation of poly(L-lysine) in silica composites and to determine how encapsulation affects the conformational plasticity and the molecular dynamics of the backbone and side chain atoms. The results show that the α-helix and β-sheet conformations are maintained in templated silica composites, and that the random coil templated silica entraps a mixture of α-helical and random coil poly(L-lysine). The dynamics of the entrapped polymer are restricted compared to the bulk, and are insensitive to the relative humidity changes that drive the random coil to β-sheet to α-helix transformation in poly(L-lysine).[16]

Methods and Materials

Poly(L-lysine) hydrobromide with a molecular weights of 25,000 (degree of polymerization (DP = 122) and 93 000 (DP = 449) g/mol were obtained from Aldrich, along with the sodium phosphate, lithium chloride, sodium chloride, and potassium chloride used to prepare buffer solutions and saturated salt solutions for the humidity chambers. Tetraethyl orthosilicate (TEOS) and tetramethyl orthosilicate (TMOS) were also obtained from Aldrich and used without purification.

The silica composites were prepared using the published procedures of Hawkins,[15] Tomczak,[17] and their collaborators. For α-helical-templated silica, poly(L-lysine) (0.5 mg/mL) was dissolved in water at pH 11.2 and TEOS was added to a final concentration of 0.1 M. The pH was maintained at 11.2 by the addition of 0.1 N NaOH during nanocomposite formation (1–2 h). Poly(L-lysine) in the β-sheet conformation was obtained by heating the pH 11.2 poly(L-lysine) solution to 65 °C for 30 min to convert from the α-helix to β-sheet conformation.[18] Poly(L-lysine) retains the β-sheet conformation upon cooling, and the same procedure was used to prepare the β-sheet templated silica. Random-coil templated silica composites were fabricated by adding a freshly prepared solution of TMOS mixed with 1 mM HCl to a solution of poly(L-lysine) in 0.1 M sodium phosphate buffer at pH 7.5. The random coil templated composites were prepared at poly(L-lysine) concentrations of 0.5 and 10 mg/mL. The precipitates from the templating reactions were centrifuged, washed with DI water, dried, and stored at −20 °C until use. The relative humidity was controlled by equilibrating the samples over saturated salt solutions (LiCl, NaCl, or KCl) for 24 h to achieve the desired relative humidity (11, 75, or 84%).[19]

Table 1. Crbon Chemical Shifts (in ppm) for Poly(L-lysine) in the α-Helix, β-Sheet, and Random Coil Conformations

conformation	C=O	Cα	Cϵ	Cδ	Cβ	Cγ
α-helix	176.1	57.4	40.1	33.1	29.0	24.7
β-sheet	171.5	52.3	40.1	33.9	27.0	22.7
random Coil	174.7	54.4	40.5	30.1	27.5	23.1

The solid-state carbon and phosphorus and NMR spectra were acquired with cross polarization and magic-angle sample spinning on a Bruker 400 MHz or a Tecmag 500 MHz NMR spectrometer using either a 5 mm Doty or a 7 mm Bruker probe. In a typical experiment, the carbon and phosphorus pulse widths were set to 5 μs and a 50 kHz field was used for cross polarization and proton decoupling with two-pulse phase modulation.[20] The cross polarization contact times were set to 1 ms. The solid-state relaxation times were measured using the standard pulse sequences,[21,22] and the wide-line correlation (WISE)[23] spectra were acquired with a sweep width of 250 kHz in the proton dimension using time-proportional phase incrementation for quadrature detection in the indirectly detected dimension.[24] Lee−Goldburg cross-polarization was used to quench spin diffusion during cross-polarization in the 2D WISE experiments.[25,26]

Results

The carbon chemical shifts for polypeptides and proteins are sensitive to the secondary structure and can be used to monitor the conformation in nanocomposites.[27,28] This is illustrated in Table 1, which lists the carbon chemical shifts for poly(L-lysine) in the random coil, α-helix, and β-sheet conformations. The chemical shift of the carbonyl carbon is the most sensitive to conformation, and is observed at 176.1, 171.5, and 174.7 ppm in the α-helix, β-sheet, and random coil conformations. The chemical shifts for the Cα carbon are 57.4, 52.3, and 54.4 ppm for the same conformations.[27,28] The chemical shifts for the side chain carbons are insensitive to the conformation and are often not resolved in the solid state.

Poly(L-lysine) is often used as a model for proteins because it can adopt an α-helix, β-sheet, or random coil conformation in response to changes in the solution pH and temperature. It has long been known that poly(l-lysine) films undergo transitions from the random coil to the β-sheet to the α-helical conformations as the relative humidity is increased.[16] Figure 1 shows the effect of relative humidity on the solid-state carbon NMR spectra on poly(L-lysine).[29,30] The lines are relatively broad for the as-received samples (lyophilized, 0% RH), and the peaks for the Cβ, Cγ, and Cδ carbons are not resolved. The chemical shifts for the

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(15) Hawkins, K. M.; Wang, S. S.; Ford, D. M.; Shantz, D. F. *J. Am. Chem. Soc.* **2004**, *126*, 9112.

(16) Blout, E. R.; Henormant, H. *Nature* **1957**, *178*, 963–963.

(17) Tomczak, M. M.; Glawe, D. D.; Drummy, L.; Lawrence, C. G.; Stone, M. O.; Perry, C. C.; Pochan, D. J.; Demming, T. J.; Naik, R. R. *J. Am. Chem. Soc.* **2005**, *127*, 12577–12582.

(18) Greenfield, N.; Fasman, G. D. *Biochemistry* **1967**, *8*, 4118–4116.

(19) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*; CRC Press: Boca Raton, FL, 2006.

(20) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshimi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, *103*, 6951–6958.

(21) Mirau, P. *A Practical Guide to the NMR of Polymers*; John Wiley & Sons: Hoboken, NJ, 2004.

(22) Schmidt-Rohr, K.; Speiss, H. W. *Multidimensional Solid-State NMR and Polymers*; Academic Press: New York, 1994.

(23) Schmidt-Rohr, K.; Clauss, J.; Spiess, H. *Macromolecules* **1992**, *25*, 3273–3277.

(24) Marion, D.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967.

(25) Oliver, S. L.; Titman, J. J. *J. Magn. Reson.* **1999**, *140*, 235–241.

(26) Qiu, X.; Mirau, P. A. *J. Magn. Reson.* **2000**, *142*, 183–189.

(27) Fernandez, V. L.; Reimer, J. A.; Denn, M. M. *J. Am. Chem. Soc.* **1992**, *114*, 9634–9642.

(28) Kricheldorf, H. R.; Muller, D. *Macromolecules* **1983**, *16*, 615–623.

(29) Krushelnitzky, A.; Faizullin, D.; Reichert, D. *Biopolymers* **2003**, *73*, 1–15.

(30) Swanson, S. C.; Bryand, R. G. *Biopolymers* **1991**, *31*, 967–973.

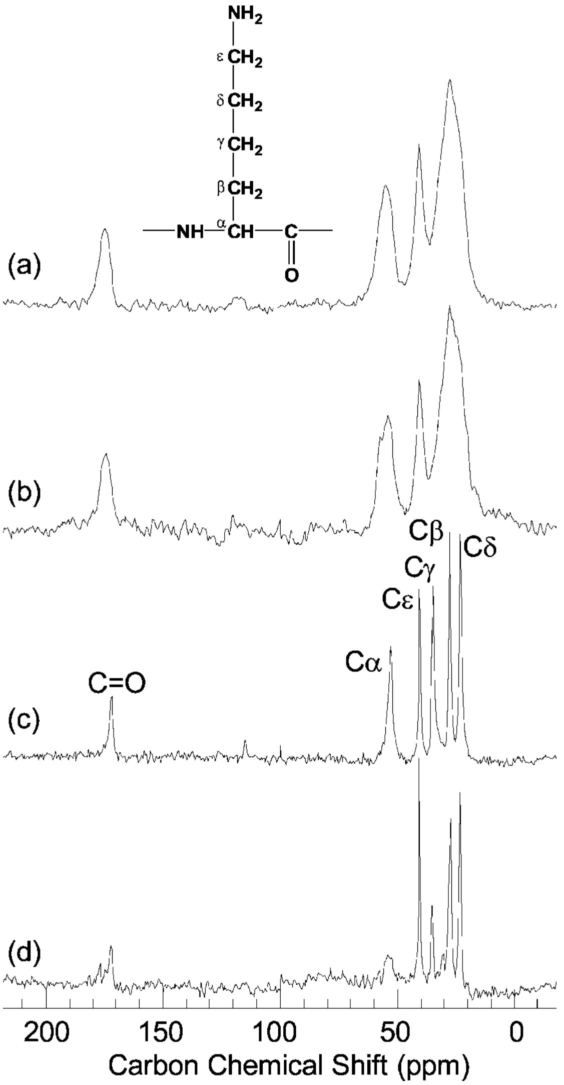

Figure 1. Solid-state carbon NMR spectra obtained with cross-polarization and magic-angle sample spinning for poly(L-lysine) at (a) 0, (b) 11, (c) 75, and (d) 84% relative humidity.

carbonyl and Cα carbons (174.2 and 54.9 ppm) are close to the values expected for the random coil conformation.

Large changes in the carbon chemical shifts and line widths are observed as the humidity is increased to 75%. All the lines are narrowed, but the side chain line widths are most strongly affected. The chemical shifts for the carbonyl and Cα carbons (172.3 and 53.0 ppm) are consistent with the formation of the *β*-sheet conformation. A second carbonyl peak is observed at 177.4 ppm with a relative humidity of 84% that is assigned to the α-helix conformation. At 84% relative humidity, the backbone and side chain atoms are very mobile and the signals are difficult to observe using cross polarization. These NMR data are consistent with the reported results on the effect of humidity on poly(L-lysine) studied by IR[16] and NMR.[29,30]

Templated silicas were prepared at pH 7.5 and 11.2, conditions that favor the formation of the random coil, α-helix, and *β*-sheet conformation of poly(L-lysine) in solution.[18] Poly(L-lysine) is highly charged at neutral pH, and the random coil conformation is observed, whereas the amino groups are neutralized at high pH (11.2), and an α-helical conformation is observed. Heating the high pH poly(L-lysine) solution for 30 min at 65 °C converts the α-helix into the *β*-sheet form, which is retained after the solution is cooled to ambient temperature.[18]

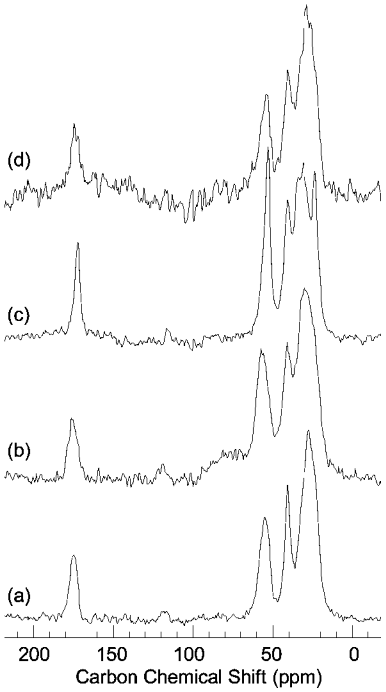

Figure 2. Solid-state carbon NMR spectra obtained with cross-polarization and magic-angle sample spinning for (a) oven-dried poly(L-lysine), (b) α-helical templated silica, (c) *β*-sheet templated silica, and (d) random coil templated silica.

Table 2. Solid-State Carbon Chemical Shifts (in ppm) for Poly(L-lysine) and Templated Silica Composites

sample	C=O	Cα	Cϵ	C*β*	C*δ*	C*γ*
poly(L-lysine)	174.2	54.9	41.0		28.1	
AHTS	176.0	57.2	41.0		29.9	
BSTS	172.8	53.5	40.6	33.2	31.3	23.9
RCTS	176.1, 174.2	54.4	40.6		29.0	

Figure 2 compares the solid-state carbon NMR spectra of poly(L-lysine) and the composites of random coil templated silica (RCTS), the *β*-sheet templated silica (BSTS) and the α-helix templated silica (AHTS). The silica samples were isolated by centrifugation and washed, so the carbon signals must arise from poly(L-lysine) associated with the silica particles. No differences are observed for samples prepared with 25 000 or 93 000 g/mol poly(L-lysine) samples.

The conformation of poly(L-lysine) in the silica composites can be evaluated from the carbonyl and Cα chemical shifts (Table 2). The carbonyl and Cα carbons are shifted to lower field in the AHTS, consistent with the encapsulation of α-helical poly(L-lysine). The chemical shifts for the BSTS are observed at higher field and are consistent with the encapsulation of the *β*-sheet conformation. The signals for the RCTS are broader and an expanded plot of the carbonyl region is shown in Figure 3 for the samples prepared using 0.5[15] and 10 mg/mL[17] poly(L-lysine) solutions. The spectra show that the RCTS composites contain a mixture of α-helix and random coil conformations. Although the intensities in the cross polarization spectra are not quantitative, it has been reported that the cross polarization dynamics of the α-helical and *β*-sheet conformations in polyamino acids are very

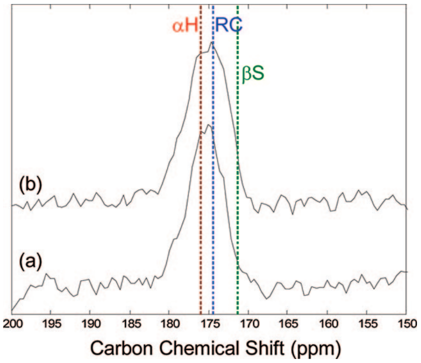

Figure 3. Carbonyl region of the solid-state carbon NMR spectra for the random coil templated silica prepared with (a) 0.5 and (b) 10 mg/mL poly(L-lysine). The chemical shift positions for the α-helix (αH), random coil (RC), and β-sheet (βS) conformations are shown.

similar,[28] suggesting that approximately equal amounts of the random coil and α-helix are entrapped in the silica composites.

Solid-state NMR methods have been used to measure the molecular dynamics of poly(L-lysine) and the composites through the carbon and proton relaxation times and the proton dipolar line widths.[21,22] The carbon relaxation parameter of most interest is the spin–lattice (T_1) relaxation time, which is sensitive to large amplitude motion near the carbon Larmour frequency (125 MHz). The carbon T_1 values are very long in rigid solids and are shortened considerably by molecular motions.[31] The relaxation times also depend on the number of nearby protons, so the Cα and Cβ relaxation times are expected to be much faster than the carbonyl relaxation times. The proton T_1's are sensitive to motions near 500 MHz, while the rotating-frame T_1's are sensitive to the molecular motions near the spin-locking frequency (50 kHz). The proton relaxation times are difficult to directly relate to molecular motions because the relaxation times for nearby protons are averaged by proton spin diffusion.[32,33]

The proton dipolar line widths for poly(L-lysine) have been measured indirectly through the carbon spectra using 2D wide line correlation spectroscopy (WISE)[23] with Lee–Goldburg spin-locking to prevent spin diffusion during the cross polarization period.[25,26] The proton dipolar line widths in rigid solids are on the order of 50 kHz and can be averaged by fast, large amplitude molecular motion.

Table 3. $^1H-^{13}C$ Relaxation Times and Dipolar Line Widths for Poly(L-lysine) at 0 and 75% relative humidity

parameter	%RH	C=O	Cα	Cϵ	Cβ−Cδ
T_1 (C) (s)	0	18.1	13.0	1.0	3.0
	75	16.1	3.9	0.24	0.27
T_1 (H) (s)	0		1.0	0.94	0.88
	75		0.58	0.57	0.88
$T_{1\rho}$ (H) (ms)	0		2.7	2.1	2.4
	75		2.6	2.4	2.6
$\Delta\upsilon_{1/2}$ (kHz)	0		49.2	21.9	38.0
	75		37.0	12.6	13.6

Table 4. Carbon Spin–Lattice Relaxation Times (s) for Poly(L-lysine) and Templated Silicas

sample	RH	C=O	Cα	Cϵ	Cδ
poly(L-lysine)	0	18.1	13.0	1.0	3.0
AHTS	0	34.7	24.8	3.5	7.2
	75		22.4	5.9	6.6
BSTS	0	24.3	13.9	2.6	2.0

Table 5. Dipolar Line Widths (kHz) for Poly(L-lysine) and Templated Silicas

sample	Cα	Cϵ	Cδ
poly(L-lysine)	49.2	28.7	38.0
AHTS	42.8	37.0	30.7
BSTS	43.3	12.6	13.1

Table 3 shows the effect of humidity on the relaxation times and line widths for bulk poly(L-lysine). An increase in humidity leads to a decrease in the carbon relaxation times for the protonated carbons in both the backbone and the side chain carbons. The relaxation time for the Cα carbon is quite long (13 s) for the oven-dried sample, suggesting that the mobility is greatly restricted at the low relative humidities that favor the random coil conformation. The relaxation times for the unresolved Cβ-Cδ carbons in the oven-dried sample are much shorter (3 s) than for the Cα, showing that the side chains experience substantial molecular motion on the ns-μs time scale relative to the backbone carbons. The shortest relaxation time is observed for the Cϵ carbon at 40 ppm. These data show that backbone is restricted in the dry state, but that side chain mobility increases with increasing distance from the backbone. No significant differences in the proton T_1 and $T_{1\rho}$ values are expected for the backbone and side chain atoms, because the relaxation times are averaged by proton spin diffusion.[32,33]

Large changes in the relaxation times are observed as the relative humidity is increased from 0 to 75% and the poly(L-lysine) is converted from the random coil to the β-sheet conformation. The Cα relaxation time is reduced from 13 to 3.9 s, whereas the relaxation times for the side chain carbons are reduced to less than 1 s. This shows that the rate and amplitude of local motions increase with humidity, which is consistent with previous studies of poly(L-lysine) dynamics.[29,30] The relaxation times for the side chain carbons are near the T_1 minimum, the value expected when the rotational correlation time for the C−H vector is near the inverse of the Larmour frequency (125 MHz).

A similar conclusion can be reached from an analysis of the proton line widths measured by 2D WISE NMR. The full-width at half-maximum ($\Delta\upsilon_{1/2}$) of the proton dipolar line width for the Cα carbon in oven-dried poly(L-lysine) is 49 kHz, near the value expected for a rigid solid. The line widths from the unresolved Cβ−Cγ carbons and the Cϵ carbons are substantially less (38 and 29 kHz), showing that fast, large amplitude molecular motion leads to partial averaging of the dipolar line shape. As with the carbon relaxation times, the proton line widths are greatly reduced in the samples exposed to 75% relative humidity.

We have evaluated the dynamics of poly(L-lysine) by comparing the carbon spin–lattice relaxation times of oven-dried poly(L-lysine) with the templated silica samples (Table 4). The longer relaxation times for the AHTS suggest that the polymer is held relatively rigidly in the silica matrix for

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(31) Komoroski, R. A. *High-Resolution NMR Spectroscopy of Synthetic Polymers in Bulk*; VCH Publishers: Dearfield Beach, FL, 1986; Vol. 7.

(32) McBrierty, V.; Douglass, D.; Kwei, T. *Macromolecules* **1978**, *11*, 1265–1267.

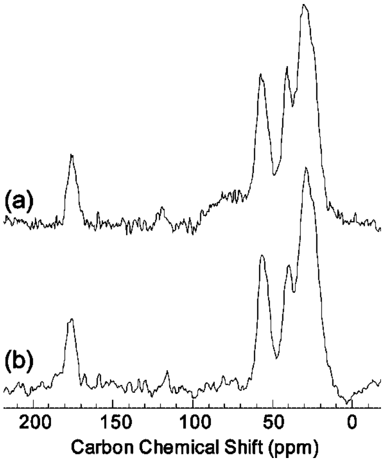

Figure 4. Solid-state ^{13}C NMR spectra of α-helix templated silica at (a) 0 and (b) 75% relative humidity.

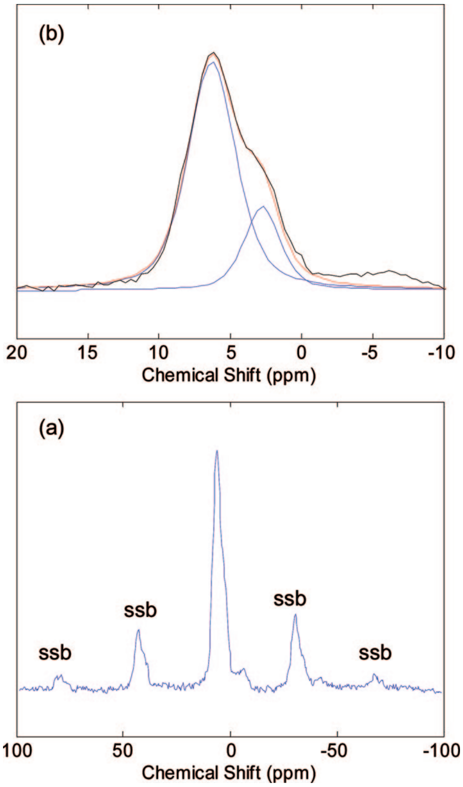

Figure 5. 202 MHz ^{31}P NMR spectra of random coil-templated silica. Part (a) shows the full spectrum, whereas part (b) shows the main peak (black) with the fitted spectrum (red) and the two component peaks (blue) at 6.7 and 2.3 ppm. The spinning sidebands are marked (ssb).

both the backbone and the side chain atoms. The relaxation times for the $C\alpha$, $C\epsilon$, and $C\gamma$ carbons in BSTS are similar to those observed in oven-dried poly(L-lysine). The effect of relative humidity was also studied for AHTS. The data in Table 4 show that increasing the humidity from 0 to 75% has a much smaller effect on the encapsulated poly(L-lysine) compared to the bulk material.

A similar conclusion is reached from the analysis of the proton line widths measured using 2D WISE NMR (Table 5). Broad proton lines are observed for all the resolved signals in AHTS. The line widths for the $C\alpha$ carbons in BSTS are similar to those observed for AHTS, but the side chains in BSTS are more mobile as judged by the narrower proton line widths observed for the $C\epsilon$ and $C\beta{-}C\delta$ carbons.

These and previous NMR studies[29,30] have shown that the structure and dynamics of bulk poly(L-lysine) is very sensitive to the humidity. Figure 4 compares the solid-state carbon spectra for oven-dried AHTS and the sample equilibrated for 24 h at 75% relative humidity. Within experimental error, no differences are detected in the chemical shifts and line widths as the relative humidity is increased from 0 to 75%. This can be directly contrasted with the behavior for poly(L-lysine) shown in Figure 1, where increasing humidity led the poly(L-lysine) signals to shift and narrow as the polymer undergoes a transition from a rigid random coil configuration to the more mobile β-sheet conformation. This shows that incorporation into the silica matrix prevents the transformation to the β-sheet conformation favored for bulk poly(L-lysine) under these conditions, and that the mobility is not significantly increased as the humidity increases.

It has been reported that silica templated from random coil poly(L-lysine) near neutral pH requires the presence of phosphate.[34] In silaffins from diatoms the phosphates are attached to the peptide chain mainly through modified serine residues, and it has been suggested that the zwitterionic nature of the peptide is important for the self-assembly that occurs prior to templating.[34] To access the role of phosphate in templating at neutral pH, we have measured the solid-state phosphorus NMR spectra of silica templated from random coil poly(L-lysine) in the presence of 0.1 M phosphate buffer. Figure 5a shows the 202 MHz ^{31}P NMR spectra obtained for RCTS with cross polarization and 7 kHz magic-angle sample spinning. In addition to the main peak near 7 ppm, several spinning side bands are observed, showing that the phosphorus is relatively rigid and has a large chemical shift anisotropy.[22] A closer examination of the center band (Figure 5b) shows that the main peak is composed of at least two peaks at 2.7 and 6.3 ppm. To establish the assignments for the two peaks, we measured the ^{31}P spin–lattice relaxation times and the proton T_1 and $T_{1\rho}$ relaxation times using ^{1}H$-^{31}$P cross polarization. The phosphorus T_1 relaxation can be fit to a single exponential with a relaxation time of 61.0 s, while the proton T_1 shows a single exponential relaxation time of 2.7 s. In contrast, the proton $T_{1\rho}$ was best fit with a double exponential with relaxation times of 0.94 ms (18% amplitude) and 32.7 ms (82% amplitude). Short relaxation times are expected for protons near the poly(L-lysine) (Table 1) as a consequence of proton spin diffusion, while long relaxation times are expected for the more rigid phosphorus in a silica matrix. Using these data we assign the smaller peak (2.7 ppm) to phosphorus atoms near the poly(L-lysine) and the larger peak to phosphorus atoms that are not in close proximity to the polymer.

Discussion

Silica nanocomposites are of interest for both biological and materials science applications. The templated synthesis

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(33) Schaefer, J.; Stejskal, E. O.; Buchdahl, R. *Macromolecules* **1977**, *10*, 384–405.

(34) Kroger, N.; Lorenz, S.; Brunner, E.; Sumper, M. *Science* **2002**, *298*, 584–586.

of inorganic/organic hybrids can lead to materials with improved mechanical[5] and optical propertiess,[6,7] as well as the encapsulation of enzymes and other biologically active materials.[13,14,35] Silica nanocomposites can be easily prepared on a benchtop with simple precursors (TMOS and TEOS) and commonly available peptides[8] and polymers,[12] but fundamental questions remain about the templating mechanism and the means by which the organic molecules are included and organized in the silica matrix.

These NMR studies have shown that the solution conformation of poly(L-lysine) used to template silica formation is retained in the composite. These data are consistent with the observations of Hawkins et al.[15] that the pore sizes in α-helical and β-sheet templated silicas reflect the dimensions of the α-helix and the aggregated β-sheet conformations. Although poly(L-lysine) maintains a random coil conformation in solution at neutral pH, the circular dichroism spectra show that long chain poly(L-lysine) (>100 residues) is converted to the α-helix in the presence of phosphate and silicic acid.[17] This observation is consistent with our observation that the silica prepared at pH 7.5 contains partially α-helical poly(L-lysine). We believe that the inclusion of random coil poly(L-lysine) is the result of nonspecific incorporation, since it has been shown that other molecules or nanoparticles present in the templating solution can be included in the inorganic matrix.[35]

The biomimetic studies have shown that silica-encapsulated enzymes retain their activity,[36] demonstrating that the silica is sufficiently porous to allow for the diffusion of substrates and products into and out of the silica matrix. With such porosity, we expect water molecules to easily penetrate the silica as the relative humidity is changed. The conformation of poly(L-lysine) is very sensitive to the humidity and undergoes a transformation from random coil to β-sheet to α-helix as the humidity increases.[16] These NMR studies show that this transformation is inhibited within the silica matrix. In addition, these and other NMR studies show that increasing relative humidity greatly increases the poly(L-lysine) mobility in bulk samples.[29,30] In contrast, these studies show that hydration effects on chain mobility are suppressed in the silica composites. Taken together these data show that poly(L-lysine) is rigidly held in the silica matrix and does not have sufficient mobility to undergo conformational transitions. The α-helix appears to be more tightly constrained by the silica matrix than the β-sheet, as judged by the dipolar line widths and relaxation times for the side chain atoms. Silica templating at neutral pH requires the presence of phosphate,[34] and the NMR studies show that part of the included phosphate is in close proximity to the poly(L-lysine).

Conclusion

Solid-state NMR has been used to study the structure and dynamics of bulk poly(L-lysine) and templated nanocomposites. The results show that the solution conformation is retained in the composite and that the molecular dynamics and conformational plasticity is greatly restricted by incorporation into the silica matrix. Studies are currently underway to determine if these conclusions can be generally applied to enzymes trapped within the silica matrix.

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(35) Naik, R. R.; Tomczak, M. M.; Luckarift, H. R.; Spain, J. C.; Stone, M. O. *Chem. Commun.* **2004**, 1684–1685.

(36) Luckarift, H. R.; Spain, J. C.; Naik, R. R.; Stone, M. O. *Nat. Biotechnol.* **2004**, *22*, 211.