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A Solid-State Deuterium NMR and Sum-Frequency Generation Study of the Side-Chain Dynamics of Peptides Adsorbed onto Surfaces

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Development of biocompatible surfaces is a major focus of the materials and tissue-engineering communities,[1] particularly for using peptide or protein coatings in recreating a natural extracellular matrix to direct wound repair or tissue development and homeostasis.[2] Many of the current materials used in biomedical materials and tissue-engineering scaffolds are hydrophobic.[3] However, proteins often lose functionality when adsorbed onto hydrophobic surfaces, so a major concern in these applications is retention of native structure and/or dynamics. Deuterium solid-state nuclear magnetic resonance spectroscopy (NMR) is a versatile, nonperturbing probe for those parameters.[4]

Degrado and Lear first demonstrated synthetic peptides that adopt a chosen structure and orientation at interfaces using alternating periods of leucine (L) and lysine (K) residues.[5] At hydrophobic surfaces, rather than unfolding, these use a recognition method to stabilize a given secondary structure where the planar environment restricts the conformational degrees of freedom. Previously, we used NMR to explore a structural determination approach using one such 14-residue "LK" peptide, Ac−LKKLLKLLKKLLKL−OH (designated as LKα14), wherein dipolar recoupling and double-quantum NMR techniques were used to measure ϕ and ψ Ramachandran angles in adjacent carbonyl−carbonyl pairs, thus quantifying the local secondary structure of the peptide adsorbed on a hydrophobic polystyrene (PS) surface.[6]

The interactions of multiple amphiphilic peptides with PS and silica surfaces has also been studied by Somorjai and co-workers[7] using sum-frequency generation (SFG) vibrational spectroscopy. They demonstrated that LKα14 adsorbs onto PS with the leucine side chains alongside and lysine side chains opposite that surface.

Here we demonstrate how solid-state deuterium NMR can be used to site-specifically and quantifiably define the perturbations of amino acid side-chain dynamics induced by peptide adsorption onto a hydrophobic surface. In this initial study, we used a series of selectively deuterated LKα14 peptides, where [*isopropyl*-$^2H_7$]L-leucine (d_7Leu) (see Figure 1a) was incorporated at individual sites in the peptide's primary sequence.

Figure 2a shows the deuterium NMR spectrum of unadsorbed, lyophilized LKα14 with d_7Leu incorporated at position L8. Figure 2b−e displays spectra of PS-physisorbed and subsequently lyophilized LKα14 with d_7Leu incorporated selectively at L5, L8, L11, and L14. Figure 2f shows the spectrum of LKα14 with d_7Leu incorporated at L8 and adsorbed onto 14 nm diameter colloidal gold particles coated with a self-assembled monolayer (SAM) of carboxyl-terminated alkane thiolates, $HOOC(CH_2)_{16}SH$.

A striking aspect of the spectra in Figure 2 is that none resemble the deuterium NMR spectrum of polycrystalline d_7Leu (represented by a simulation in Figure 3a), which consists primarily of an axially symmetric, Pake-type powder pattern with an effective quadrupolar coupling constant (QCC) of ∼50 kHz, corresponding to the six deuterons of the rapidly rotating δ_1 and δ_2 CD_3 groups. The γ-methine deuteron generates a weaker pattern with a QCC of ∼170 kHz having low experimental sensitivity (not shown in Figure 3a).

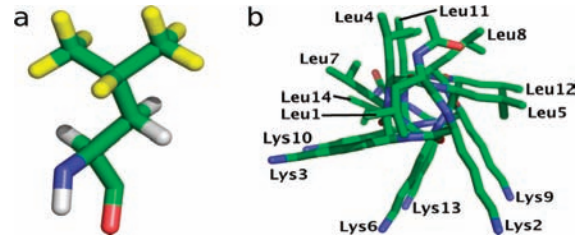

Figure 1. (a) Structure of d_7Leu, with deuterated sites highlighted in yellow. (b) End-on view of the LKα14 helical structure with residues labeled.

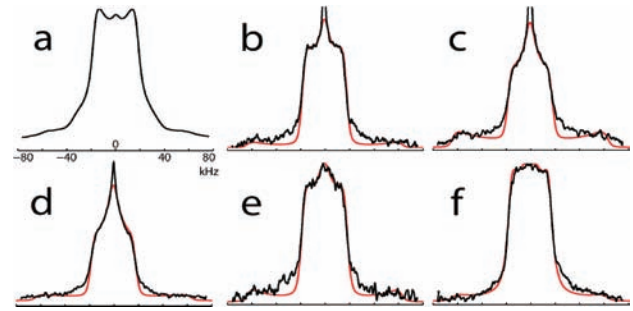

Figure 2. ^2H NMR spectra (black lines) and simulations (red lines, b−f only) of (a) free LKα14 labeled with d_7Leu at L8; polystyrene-adsorbed LKα14 samples labeled at (b) L5, (c) L8, (d) L11, and (e) L14; and (f) LKα14 deuterated at L8 and bound to carboxyl-functionalized gold nanoparticles.

Figure 2c represents PS-adsorbed LKα14 deuterated at L8, so any difference between Figure 2c and the spectrum of unbound LKα14 in Figure 2a is assumed to be due to the influence of the PS surface on the leucyl side-chain dynamics. Similarly, the spectral pattern variation observed in Figure 2b−e is presumably due to variation in the dynamics as a function of side-chain proximity to the PS surface. Finally, as Figure 2f represents the spectrum of LKα14 deuterated at L8 and bound to the surface of a carboxyl-terminated SAM on colloidal gold, the spectral pattern variation in Figure 2c,f is likely due to the differential impact on the leucyl side chain of adsorption onto a nonpolar PS surface versus adsorption onto the polar surface of the SAM.

The dynamics of leucyl side chains has been studied and quantified by Torchia and co-workers[8] in a ^2H NMR study of helical collagen fibrils with uniformly $^2H_{10}$-labeled L-leucine. The temperature variation of the deuterium line shape was modeled as variation in the rate of a two-site exchange between two conformations of the leucine side chain

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observed in crystallographic studies of leucine monomer and leucyl peptides.[9] To simulate the spectral patterns in Figure 2, we similarly assumed here an exchange between the two conformers shown in Figure 3b. The site exchange corresponds to an angular change of ~110° for the Cβ−Cγ bond axis. The rapid rotational motions of the CD_3 groups are treated simply with an effective QCC. The exchange rate and a priori conformer populations were varied in the simulations. In addition, to account for motion of the peptide chain itself, the Cα−Cβ bond was treated as moving on the surface of a cone.

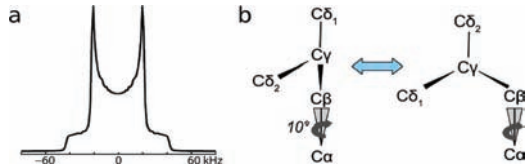

Figure 3. (a) Simulation of polycrystalline d_7Leu. (b) Two-site jump model with 10° half-angle cone motion used for the simulations.

Simulation results are shown with superimposed spectral data in Figure 2 and in Table 1. Although Figure 2b−e shows features of a dynamically induced $\eta = 1$ spectral powder pattern, a residual HDO signal limited our ability to exactly simulate the central feature of these spectra.

Table 1. Simulation Parameters for the Spectra Shown in Figure 2

	L5	L8(PS)	L11	L14	L8(SAM)
P_1/P_2	4/6	4/6	4/6	3.5/6.5	3/7
k_{ex} (s^{-1})	3×10^5	6×10^5	1×10^6	3×10^5	3×10^5
k_{cone} (s^{-1})	2×10^3	2×10^3	2×10^3	3×10^3	6×10^3
QCC_{eff} (kHz)	49	49	49	51	43

The simulation results shown in the table indicate that the rate constant for exchange between side-chain conformers, k_{ex}, is the model parameter that varies most significantly across the series Figure 2b−f: k_{ex} is greatest for L8 and L11, whose side chains are located closest to the PS surface, while k_{ex} for L5 and L14 are significantly smaller. There is much less variation in all of the other model parameters for the different leucine sites of LKα14 on PS. Interestingly, k_{ex} for L8 on a carboxyl-terminated SAM surface is identical to those for L5 and L14 and differs from that for L8 on PS by a factor of 2.

Verification of the peptide orientation on carboxylated surfaces was performed with SFG. Figure 4 presents spectra of a monolayer of carboxyl-terminated alkanethiolates (identical to those used in Figure 2e) on gold-coated CaF_2 windows before and after adsorption of LK peptides.

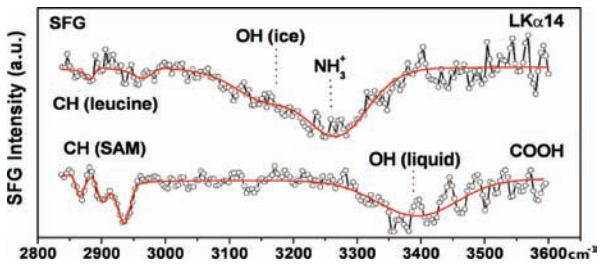

Figure 4. SFG spectra of COOH-functionalized gold surfaces alone (lower trace) and after adsorption of LKα14 (upper trace).

The spectrum of the bare SAM surface consists only of the methylene stretching modes at 2866 and 2933 cm^{-1}, with the carboxyl-adjacent methylene unit at 2901 cm^{-1} and loosely bound water near 3400 cm^{-1}.[10] Upon peptide adsorption, weak CH_3 resonances related to leucine side chains appear (2961 and 2880 cm^{-1}). These modes are phase-shifted by 180° with respect to the gold background (appearing as a dip in the spectrum), showing that the leucines are pointing away from the surface.[11] A band near 3200 cm^{-1} is related to tetrahedrally coordinated water molecules.[10] We assign the feature near 3265 cm^{-1} to ordered lysine NH^{3+} groups bound to the SAM substrate.

This study indicates that the dynamics of the isopropyl moiety of the leucine side chain is extremely sensitive to surface proximity and surface functionalization. The factor of 2−3 change in k_{ex} observed for the L8/L11 versus L5/L14 sites in LKα14 on PS surfaces is observed in collagen leucine line shapes only as a result of a 40 °C change in temperature.[8] The L8(SAM) data and SFG orientation data mutually demonstrate that a change in surface functionalization in turn changes the peptide orientation and consequently the surface proximities of amino acid side chains. The collagen study found the existence of a mobile interface between individual helical protein components of the fibers,[12] characterized in part by quantifying the dynamics of leucine side chains as a function of temperature. This study has used multiple, complementary spectroscopies to observe a similarly mobile interface between a peptide structure and bulk surfaces, with mobility quantifiable in terms of NMR exchange dynamics of leucine side chains with varying surface proximities, and suggests that the combination of NMR and SFG on such systems can provide significantly more information than either alone.

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Supporting Information Available: Details of sample preparation, NMR and SFG experiments, and fitting results for Figure 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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