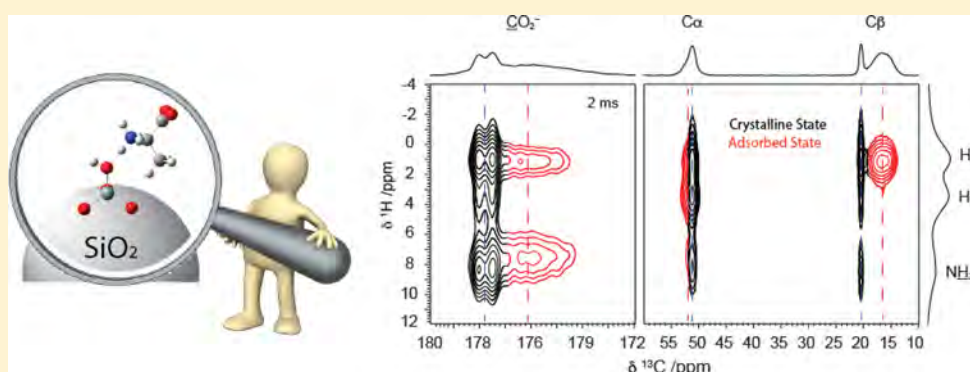


Alanine Adsorption and Thermal Condensation at the Interface of Fumed Silica Nanoparticles: A Solid-State NMR Investigation

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S Supporting Information



ABSTRACT: Investigating the interface between biomolecules and nanoparticles has attracted considerable attention in recent years since it has great significance in numerous fields including nanotechnology, biomineralization, cancer therapy, and origin of life. In this paper, we present a thorough solid-state NMR study on alanine adsorption and thermal condensation on fumed silica nanoparticles. The structure and dynamics at the interface between alanine and fumed silica nanoparticles were probed with a combination of ¹H, ¹³C, and ¹⁵N one- and two-dimensional (2D) magic angle spinning (MAS) solid-state NMR methods at different alanine surface coverages and hydration levels. It is illustrated that at high surface coverages both crystalline and adsorbed states of alanine exist in the samples while only adsorbed alanine is observed at low surface coverage (approximately a monolayer). At high hydration levels, the adsorbed alanine exhibits enhanced mobility, and both the carboxyl and amine group interact with mobile water molecules on the silica nanoparticle surface. At low hydration levels, the adsorbed alanine interacts with surface silanols via the protonated amine group and the carboxylate group. The thermal condensation of alanine on fumed silica nanoparticles was also investigated, and the results indicate that alanine can undergo thermal condensation at ~170 °C at the interface of fumed silica nanoparticles as confirmed by a battery of ¹³C and ¹⁵N 2D MAS solid-state NMR experiments. By combining the adsorption and thermal condensation results, a possible mechanism for the silica surface-catalyzed thermal condensation reaction of alanine is proposed.

INTRODUCTION

The interaction of biomolecules at the surface of inorganic materials has attracted considerable attention in recent years since it has a significant impact on drug delivery research, bionanotechnology, and bionanocomposite materials.^{1–9} Understanding the adsorption and binding mechanism of biomolecules at the biomolecule–inorganic interfaces is of major significance for developing novel bioinorganic composite systems for biomedical applications.^{9,10} Investigating the thermal condensation of biomolecular species on inorganic oxide surfaces is of great interest since mineral surfaces potentially served as catalysts for peptide bond formation in prebiotic chemistry and could have been involved in the synthesis of the first small oligopeptides.¹¹ Considerable studies have demonstrated peptide bond formation occurs at relatively

low temperatures in the absence of any activating agents when gently heating amino acids at the interface of inorganic oxides such as silica, alumina, and other minerals.^{12,13} Among all the bioinorganic systems, the amino acid/silica system is the simplest and most practical system and constitutes the first step in understanding more complicated bioinorganic systems such as peptide/silica and protein/silica systems.

Silica (SiO₂) is one of the most common inorganic materials on Earth and has been extensively investigated for decades due to its diverse properties such as polymorphism and biocompatibility. It has widespread applications ranging from

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structural materials to catalysis and as a medium for drug delivery.^{2,3,14–17} Fumed silica is one class of synthetic silica materials produced at high temperature by hydrolyzing silicon tetrachloride vapor in a flame followed by rapid quenching to room temperature.¹⁸ Because of this synthetic approach, the resulting silica particles exhibit a nonporous amorphous structure with nanoscale size and an extensively high surface area. The surface chemistry of fumed silica has been well investigated for decades because of the considerable utility of such high surface area amorphous materials in a range of applications.^{18–20}

Amino acids serve as the basic building blocks to form peptides and proteins. Understanding the amino acid/inorganic interface is thought to be one of the key steps for understanding how peptides and proteins bind and interact with inorganic surfaces.⁹ Furthermore, in the field of prebiotic chemistry research, it was proposed long ago and is continually investigated that mineral surfaces could have played a crucial role in catalyzing polymerization reactions of amino acids forming the first oligopeptides and proteins.^{11,13,21–25} Thus, understanding biocatalytic reactions at silica and other mineral surfaces may help unravel the mystery of the origin of life.¹¹

Solid-state NMR spectroscopy is a premier technique for elucidating molecular level detail regarding the interactions between biomolecules and inorganic surfaces.^{4,26–28} In the present work, the adsorption and thermal condensation of alanine on fumed silica nanoparticles surfaces are explored with solid-state NMR spectroscopy. Solid-state NMR spectroscopy is an excellent characterization method for obtaining structural information at a molecular level and is being increasingly used for monitoring surface chemistry.^{28–35} Considerable solid-state NMR studies on amino acid/silica systems including alanine/silica have been carried out. Ben Shir et al. investigated the binding and dynamics of glycine and alanine on SBA-15 and MCM-41 surfaces by using rotational-echo double-resonance^{36,37} (REDOR) NMR.^{31,32,35} They found that the adsorption of alanine on amorphous silica surfaces occurs via direct interaction of its charged amine group with silica surface silanols. Amitay-Rosen et al. applied ²H MAS NMR to understand the structural and dynamical state of water and alanine molecules adsorbed on SBA-15 surfaces at different degrees of surface hydration.²⁹ In the latter study, two distinct alanine populations were identified at the interface where one is dynamically undergoing exchange between bound and free states while the other is statically bonded to the surface. They also revealed that a small amount of interfacial water molecules made alanine exhibit a solution-like environment with isotropic mobility.

In the present work, a combination of ¹H high-speed MAS NMR spectroscopy, ¹H → ¹³C and ¹H → ¹⁵N cross-polarization magic-angle-spinning^{38,39} (CP-MAS), and two-dimensional (2D) ¹H–¹³C and ¹H–¹⁵N heteronuclear correlation (HETCOR) NMR spectroscopy is implemented to investigate the behavior of alanine molecules on fumed silica nanoparticles. The adsorption species of alanine on fumed silica surfaces were observed directly by CP-MAS NMR spectroscopy in contrast with previous studies for alanine adsorption on other silica surfaces.^{31,32} In the present study, the adsorption of alanine on the surfaces at high and low hydrated levels was thoroughly investigated, and a possible adsorption mechanism is proposed. Lastly, it is shown that alanine can undergo thermal condensation on fumed silica nanoparticles forming peptide bonds at ~170 °C. Based on the adsorption and thermal

condensation results, a mechanism for silica surface-catalyzed thermal condensation of amino acids is proposed where surface silanol groups are involved in the reaction.

■ EXPERIMENTAL SECTION

Materials. Fumed silica nanoparticles (~7 nm) with BET (Brunauer, Emmett, and Teller) surface area of 395 ± 25 m²/g and natural abundance pure L-alanine (99% purity) were purchased from Sigma-Aldrich. U- [¹³C/¹⁵N]-L-Alanine was purchased from Cambridge Isotopes, Inc. All materials were used as received and stable isotope enrichment levels of labeled compounds are 97–99%. In the following, silica or SiO₂ refers to fumed silica nanoparticles unless otherwise specified. Ala/SiO₂ and ¹³C/¹⁵N-Ala/SiO₂ will refer to natural abundance alanine and ¹³C/¹⁵N-labeled alanine adsorbed on fumed silica nanoparticles, respectively.

Adsorption of Alanine on Fumed Silica Nanoparticles. Fumed silica nanoparticles were initially heated to 500 °C overnight to remove impurities on the surface. In a typical adsorption procedure, 75 mg of fumed silica nanoparticles was mixed with L-alanine in 5.0 mL of DI water, and the mixture was then stirred at room temperature for over 3 h to ensure the adsorption reached equilibrium. Nanoparticles were then separated by centrifugation at 6000 rpm for 1 h and dried under vacuum at room temperature overnight. The samples prepared from solutions of various concentrations are noted as Ala/SiO₂-*x* M, where *x* refers to the alanine concentration in the adsorption solutions (in mol L⁻¹). In this work, ¹³C/¹⁵N-Ala/SiO₂-0.03 M and ¹³C/¹⁵N-Ala/SiO₂-0.10 M samples were prepared for solid-state NMR investigations. Briefly, two sets of 120.0 mg fumed silica nanoparticles were mixed with 21.6 and 72.0 mg of ¹³C/¹⁵N-L-alanine in 8.0 mL of DI water, and the pH values of both solutions were maintained at 6.7. The suspensions were then stirred for over 3 h to reach equilibrium. The two mixtures were then centrifuged at 6000 rpm for 1 h, and the remaining powders were allowed to vacuum-dry at room temperature overnight. The as-prepared samples were noted as “hydrated” samples. To prepare “dry” samples, the ¹³C/¹⁵N-Ala/SiO₂ samples were packed in NMR rotors and then further vacuum-dried with cap removed (0.001 mbar) for one month. The NMR investigations on dried samples were carried out immediately after drying. No obvious rehydration was found in this work during the course of solid-state NMR experiments, indicating that the rotor sealing is quite good.

Thermal Condensation of Alanine on Fumed Silica Nanoparticles. Solid-state NMR spectroscopy was used for studying alanine thermal condensation on fumed silica nanoparticles. ¹³C/¹⁵N-Ala/SiO₂-0.03 M was first packed in the rotor followed by incubation at 170 °C for 3 h with rotor cap removed. The rotor was then cooled down to room temperature and sealed for NMR characterization.

Thermal Analysis. Thermal gravimetric analysis (TGA) experiments were performed with a TA2910 (TA Instruments Inc.) instrument under dry N₂ flow (30 mL/min for furnace and 30 mL/min for balance). For each experiment, 7–10 mg of sample was used and a heating rate of 5 °C/min was applied. Before each experiment, the sample was kept under a nitrogen flow for 10 min to remove any physisorbed water and obtain a stable baseline.

Solid-State NMR Spectroscopy. Solid-state NMR spectra were collected on a Varian VNMRs 400 MHz spectrometer with a 1.6 mm triple-resonance probe operating in triple-resonance (¹H/¹³C/¹⁵N) mode at a MAS speed of 35 kHz. ¹H

high-speed MAS NMR data were collected with the DEPTH sequence,⁴⁰ $^1\text{H} \rightarrow ^{13}\text{C}$ and $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS experiments, two-dimensional (2D) ^1H - ^{13}C heteronuclear correlation (HETCOR), and ^1H - ^{15}N HETCOR NMR experiments were performed to characterize all samples. ^1H MAS NMR experiments were conducted with a $1.6 \mu\text{s}$ ^1H $\pi/2$ pulse, a recycle delay of 5.0 s, and a 10 kHz sweep width at a spinning speed of 35 kHz. The CP condition for $^1\text{H} \rightarrow ^{13}\text{C}$ CP-MAS NMR experiments consisted of a $1.6 \mu\text{s}$ ^1H $\pi/2$ pulse, followed by a 1.0 ms ramped (13%) ^1H spin-lock pulse of 75 kHz rf field strength. The experiments were performed with a 25 kHz sweep width, a recycle delay of 3.0 s, and a two-pulse phase-modulated⁴¹ (TPPM) ^1H decoupling level of 156 kHz for all samples. The CP condition for $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS NMR experiments consisted of a $1.6 \mu\text{s}$ ^1H $\pi/2$ pulse, followed by a 1.0 ms ramped (10%) ^1H spin-lock pulse of 95 kHz rf field strength. The experiments were performed with a 25 kHz sweep width, a recycle delay of 3.0 s, and a ^1H decoupling level of 156 kHz. A contact time of 1.0 ms was applied in $^1\text{H} \rightarrow ^{13}\text{C}$ and $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS NMR experiments for pure alanine and $^{13}\text{C}/^{15}\text{N}$ -Ala/ SiO_2 -0.10 M while a contact of 2.0 ms was applied for $^{13}\text{C}/^{15}\text{N}$ -Ala/ SiO_2 -0.03 M.

2D ^1H - ^{13}C HETCOR and 2D ^1H - ^{15}N HETCOR NMR experiments were carried out on two $^{13}\text{C}/^{15}\text{N}$ -Ala/ SiO_2 samples. The experimental CP condition and TPPM ^1H decoupling parameters for 2D HETCOR NMR experiments were identical to 1D CP-MAS NMR experiments with the exception of contact times. 2D ^1H - ^{13}C HETCOR NMR experiments were performed with contact times of 2.0 and 0.25 ms, a recycle delay of 3.0 s, sweep widths of 25 and 10 kHz for direct and indirect dimensions, respectively, and 32 complex t_1 points. 2D ^1H - ^{15}N HETCOR NMR experiments were performed with different contact times (0.25, 1, and 2 ms), a recycle delay of 3.0 s, a sweep width of 10 kHz for both dimensions, and 32 complex t_1 points.

In all experiments, the chemical shifts of ^1H , ^{13}C , and ^{15}N were indirectly referenced to adamantane ^1H (1.63 ppm), adamantane ^{13}C (38.6 ppm), and glycine ^{15}N (31.6 ppm), respectively.^{42,43} The line-broadening (lb) factor was set to 25 Hz for $^1\text{H} \rightarrow ^{13}\text{C}$ CP-MAS spectra, $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS spectra, 2D ^1H - ^{13}C HETCOR spectra, and 50 Hz for 2D ^1H - ^{15}N HETCOR spectra.

RESULTS AND DISCUSSION

Adsorption Behavior of Alanine on Fumed Silica Nanoparticles. The adsorption of alanine on silica surfaces has been well studied,^{29,31,44,45} and some factors such as initial concentration of alanine in the adsorption solution and pH values of adsorption solution have been shown to impact the amount of adsorbed alanine at the silica interface. In this paper, we use TGA to investigate the influence of initial aqueous alanine concentration on the content of adsorbed alanine at the surface of fumed silica nanoparticles, and the results are summarized in Table 1. The amount of adsorbed alanine is calculated from the TGA weight loss curves between 100 and 600 °C since the water contributes to the weight loss below 100 °C.²⁸ Figure 1 shows the amount of alanine adsorbed on fumed silica nanoparticles as a function of initial aqueous alanine concentration. With increasing the initial concentration, the amount of adsorbed alanine increases correspondingly. Furthermore, the adsorption of alanine fits well to a Langmuir isotherm in the low initial concentration range (<0.05 M). However, at high initial concentrations (>0.08 M), the

Table 1. Summary of TGA Results for Alanine Adsorption on Fumed Silica Nanoparticles

[alanine] (M)	weight loss (%)	adsorbed alanine (molecules/nm ²)
0.01	2.8	0.50 ± 0.03
0.03	4.2	0.76 ± 0.05
0.05	5.0	0.91 ± 0.06
0.08	7.0	1.31 ± 0.08
0.10	8.7	1.65 ± 0.10
0.15	10.5	2.02 ± 0.13

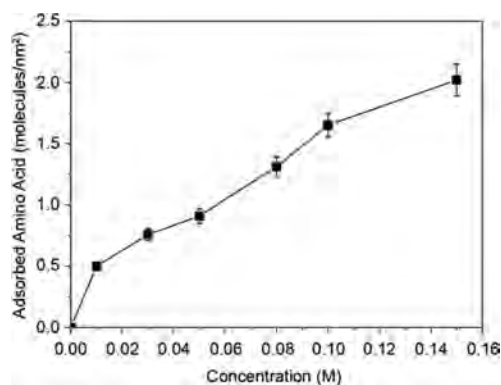


Figure 1. Amount of alanine adsorbed on fumed silica nanoparticles as a function of initial aqueous alanine concentration at room temperature and near neutral pH (pH = 6.7).

adsorption behavior is linear and cannot be properly fit to a Langmuir isotherm. This is probably due to the incomplete separation between liquid and solid phases or the formation of multilayers on the surface similar to previous observations for lysine adsorption on fumed silica nanoparticles.²⁸

Adsorption Mechanism of Alanine on Fumed Silica Nanoparticles. Solid-state NMR spectroscopy is a powerful tool for elucidating surface chemistry at nanomaterial interfaces. Here, a combination of solid-state NMR experiments is performed to investigate the adsorption mechanism of alanine on fumed silica nanoparticles surfaces. In previous studies, the protonation state of the amine group of alanine on mesoporous silica was investigated by using $^{15}\text{N}\{^1\text{H}\}$ separated local field⁴⁶ (SLF) experiments combined with PMLG-5 homonuclear decoupling.⁴⁷ These results indicated that the amine group of alanine was protonated (NH_3^+) with a ^1H resonance at ~ 7.4 ppm that facilitated the binding to silica surface groups.³¹ In this work, the bulk alanine and adsorbed alanine on fumed silica nanoparticles were found in zwitterionic forms since the ^1H resonance of NH_3^+ was clearly detected for all species with a similar chemical shift observed (Figure S1).³¹ Furthermore, two ^1H resonances were seen for methyl protons in the “hydrated” state: one at 1.4 ppm corresponding to alanine in an aqueous environment and one at 1.2 ppm corresponding to alanine interacting with the silica nanoparticles (Figure S1). This was confirmed in the “dry” state where the ^1H resonance at 1.4 ppm disappears presumably due to the restriction of reorientational motion of alanine at low hydration level.⁴⁸ The alanine molecules become less mobile when interacting with the dry nanoparticle surface. A ^1H resonance at 1.7 ppm was detected for dry samples that is due to the isolated silanol group on the fumed silica nanoparticle surface.¹⁸ This result is consistent with those obtained by Amitay-Rosen et al.²⁹

In Figures 2 and 3, the $^1\text{H} \rightarrow ^{13}\text{C}$ and $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS NMR spectra of bulk U- $^{13}\text{C}/^{15}\text{N}$ -L-alanine and $^{13}\text{C}/^{15}\text{N}$ -Ala/

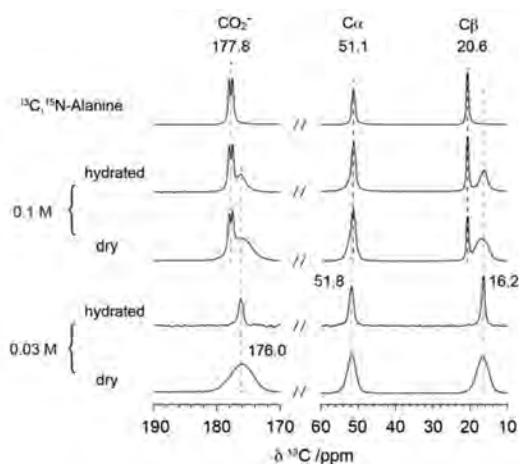


Figure 2. $^1\text{H} \rightarrow ^{13}\text{C}$ CP-MAS NMR spectra of U- $^{13}\text{C}/^{15}\text{N}$ -L-alanine, $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M (“hydrated” and “dry”), and $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M (“hydrated” and “dry”).

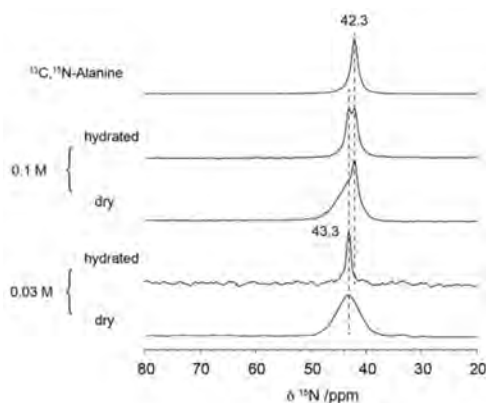


Figure 3. $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS NMR spectra of U- $^{13}\text{C}/^{15}\text{N}$ -L-alanine, $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M (“hydrated” and “dry”), and $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M (“hydrated” and “dry”).

SiO₂ samples are displayed with all peak assignments summarized in Table 2. Bulk U- $^{13}\text{C}/^{15}\text{N}$ -L-alanine is in the crystalline state exhibiting three sharp ^{13}C resonances for the carboxyl group (177.8 ppm), α -CH (51.1 ppm), and β -CH₃ (20.6 ppm) and one ^{15}N resonance for the NH₃⁺ (42.3 ppm) illustrating that alanine is in its zwitterionic form. The splitting for the carboxyl group is due to the ^{13}C - ^{13}C *J*-coupling with the neighboring α - ^{13}C H. These resonances were also detected for $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M, indicating crystalline alanine exists in the samples at this surface coverage. In addition to crystalline alanine, three unique ^{13}C resonances at 176.0, 51.8, and 16.2 ppm and one additional ^{15}N resonance are observed in

the spectra and are assigned to the carboxyl group, α -CH, β -CH₃, and NH₃⁺ of adsorbed alanine, respectively. The resonances broaden considerably following drying since the adsorbed alanine becomes more rigid. The broadening effect is due to chemical shift heterogeneity and illustrates that the alanine nanoparticle surface environment is disordered. The large change (4.4 ppm) in ^{13}C chemical shift to lower ppm observed for the β -CH₃ for adsorbed alanine is likely due to methyl packing differences between the crystalline and adsorbed forms. Chemical shifts to higher ppm are typically observed for more restricted methyl packing environments in alanine-containing peptides.⁴⁹ The methyl packing is expected to be less restrictive on the silica nanoparticle surface.

The crystalline alanine was found to disappear when lowering the concentration of alanine in the adsorption solution. In the $^1\text{H} \rightarrow ^{13}\text{C}$ and $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS NMR spectra of $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M, the characteristic resonances of crystalline alanine were not detected, and only adsorbed alanine was observed at the silica nanoparticle interface (see Figures 2 and 3). The ^{13}C and ^{15}N resonances of adsorbed alanine appeared at identical isotropic chemical shifts to those of $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M. Furthermore, the broadening effect of the adsorbed state was clearly observed for “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M, demonstrating the adsorbed species is highly disordered as evidenced by the heterogeneous line broadening. Based on results of $^1\text{H} \rightarrow ^{13}\text{C}$ CP-MAS NMR and $^1\text{H} \rightarrow ^{15}\text{N}$ CP-MAS NMR experiments, it is convincing to argue that at high surface coverage ($^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M) both crystalline and adsorbed states of alanine exist on the nanoparticle surfaces while only the adsorbed alanine state is present at low surface coverage ($^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M).

To further elucidate the interaction of alanine molecules with the surface groups on fumed silica nanoparticles, 2D ^1H - ^{13}C and ^1H - ^{15}N HETCOR NMR experiments were performed. The 2D ^1H - ^{13}C and ^1H - ^{15}N HETCOR NMR spectra of “hydrated” and “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M are shown in Figures 4 and 5 along with the ^1H traces for specific ^{13}C and ^{15}N chemical shifts. For both “hydrated” and “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M samples, the ^1H - ^{13}C and ^1H - ^{15}N correlations for crystalline alanine are identical. The ^{13}C resonances at 177.8, 51.1, and 20.6 ppm assigned to the carboxyl group, α -CH, and β -CH₃ of crystalline alanine clearly show three correlations with ^1H resonances at 0.9, 3.4, and 8.2 ppm corresponding to the β -CH₃, α -CH, and the NH₃⁺ in interaction with the carboxylate of a neighboring molecule through COO-HN hydrogen bonds in the crystalline state, respectively. The ^{15}N resonance at 42.3 ppm assigned to the NH₃⁺ of crystalline alanine also shows three correlations with these ^1H resonances in the two states. However, some differences for ^1H - ^{13}C and ^1H - ^{15}N correlations were found for adsorbed alanine after comparing the “hydrated” state and

Table 2. Summary of ^{13}C and ^{15}N Chemical Shifts of U- $^{13}\text{C}/^{15}\text{N}$ -L-Alanine and $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂ Samples^a

samples	^{13}C			^{15}N
	$-\text{CO}_2^-$	α -CH	β -CH ₃	NH ₃ ⁺
U- $^{13}\text{C}/^{15}\text{N}$ -L-alanine	177.8	51.1	20.6	42.3
$^{13}\text{C}/^{15}\text{N}$ -Ala/SiO ₂ -0.10 M (hydrated)	177.8, 176.3	51.1, 51.8	20.6, 16.2	42.3, 43.3
$^{13}\text{C}/^{15}\text{N}$ -Ala/SiO ₂ -0.10 M (dry)	177.8, 176.0	51.1, 51.8	20.6, 16.2	42.3, 43.3
$^{13}\text{C}/^{15}\text{N}$ -Ala/SiO ₂ -0.03 M (hydrated)	176.0	51.8	16.2	43.3
$^{13}\text{C}/^{15}\text{N}$ -Ala/SiO ₂ -0.03 M (dry)	176.0	51.8	16.2	43.3

^aChemical shifts are reported in ppm from TMS.

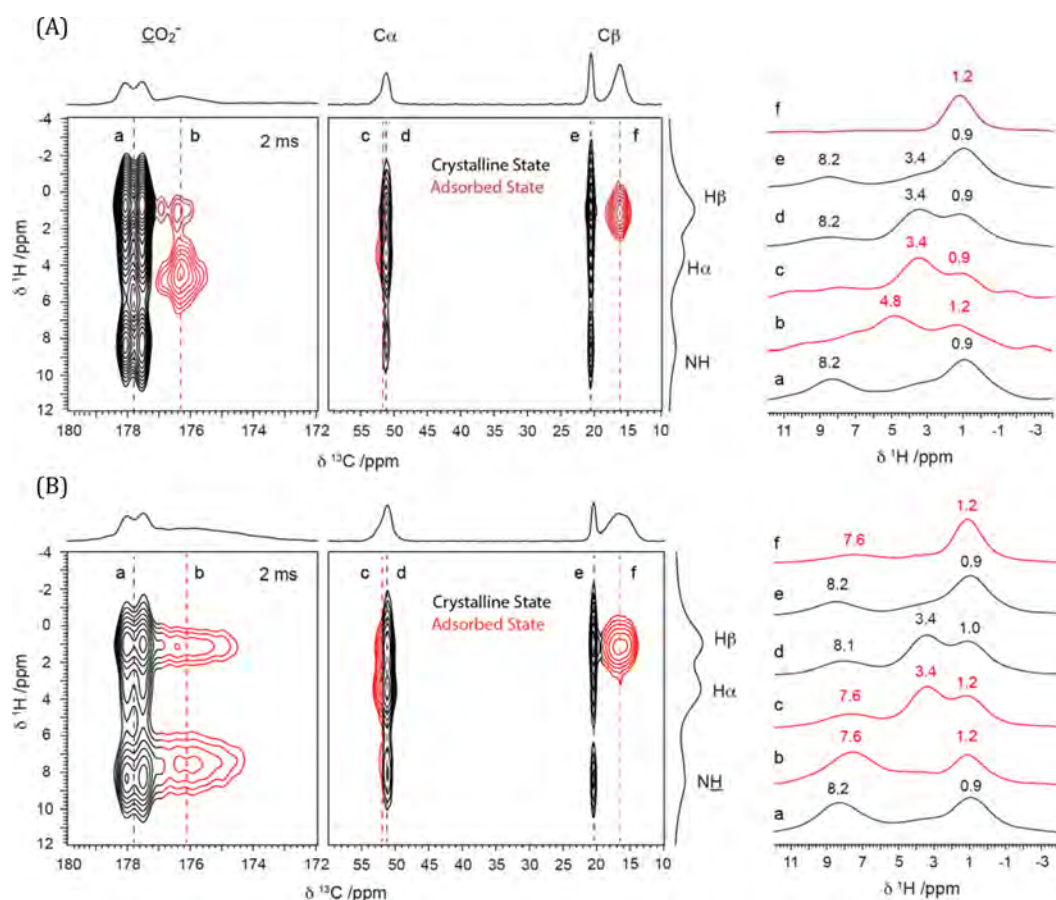


Figure 4. $^1\text{H} \rightarrow ^{13}\text{C}$ 2D-HETCOR MAS NMR spectra of (A) “hydrated” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M and (B) “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M. Experiments were conducted with a 2.0 ms contact time at 400 MHz and a MAS frequency of 35 kHz.

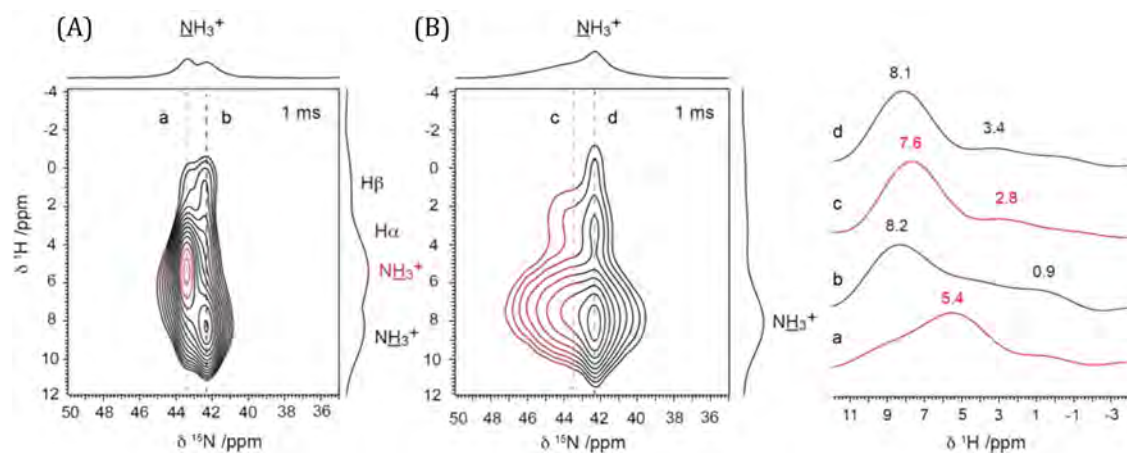


Figure 5. $^1\text{H} \rightarrow ^{15}\text{N}$ 2D-HETCOR MAS NMR spectra of (A) “hydrated” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M and (B) “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M. Experiments were conducted with a 1.0 ms contact time at 400 MHz and a MAS frequency of 35 kHz.

the “dry” state, indicating that the binding behaviors of alanine molecules on the surfaces are different in the two states. In the “hydrated” state, the ^{13}C resonance at 176.3 ppm assigned to the carboxyl group of adsorbed alanine shows two clear correlations with ^1H resonances at 4.8 and 1.2 ppm. The ^{15}N resonance at 43.3 ppm is assigned to the NH_3^+ of adsorbed alanine and shows one strong correlation with a ^1H resonance at 5.4 ppm. The ^1H resonance at 4.8 ppm is assigned to surface bound water whose behavior is similar to bulk, isotropic water, and the ^1H resonance at 1.2 ppm was assigned to $\beta\text{-CH}_3$ of

adsorbed alanine. The ^1H resonance at 5.4 ppm could be due to the interaction between water and NH_3^+ of free alanine that are not bound to the silica nanoparticle surface. These results indicate that the carboxyl group and the NH_3^+ of adsorbed alanine both interact with the interfacial water via a fast-exchange process in the “hydrated” state. After drying, the carboxyl group and the NH_3^+ of adsorbed alanine show distinguishable correlations with the ^1H resonance at 7.6 ppm that are assigned to the hydrogen bonding protons that interacts with surface silanol groups.^{32,33} This indicates that the

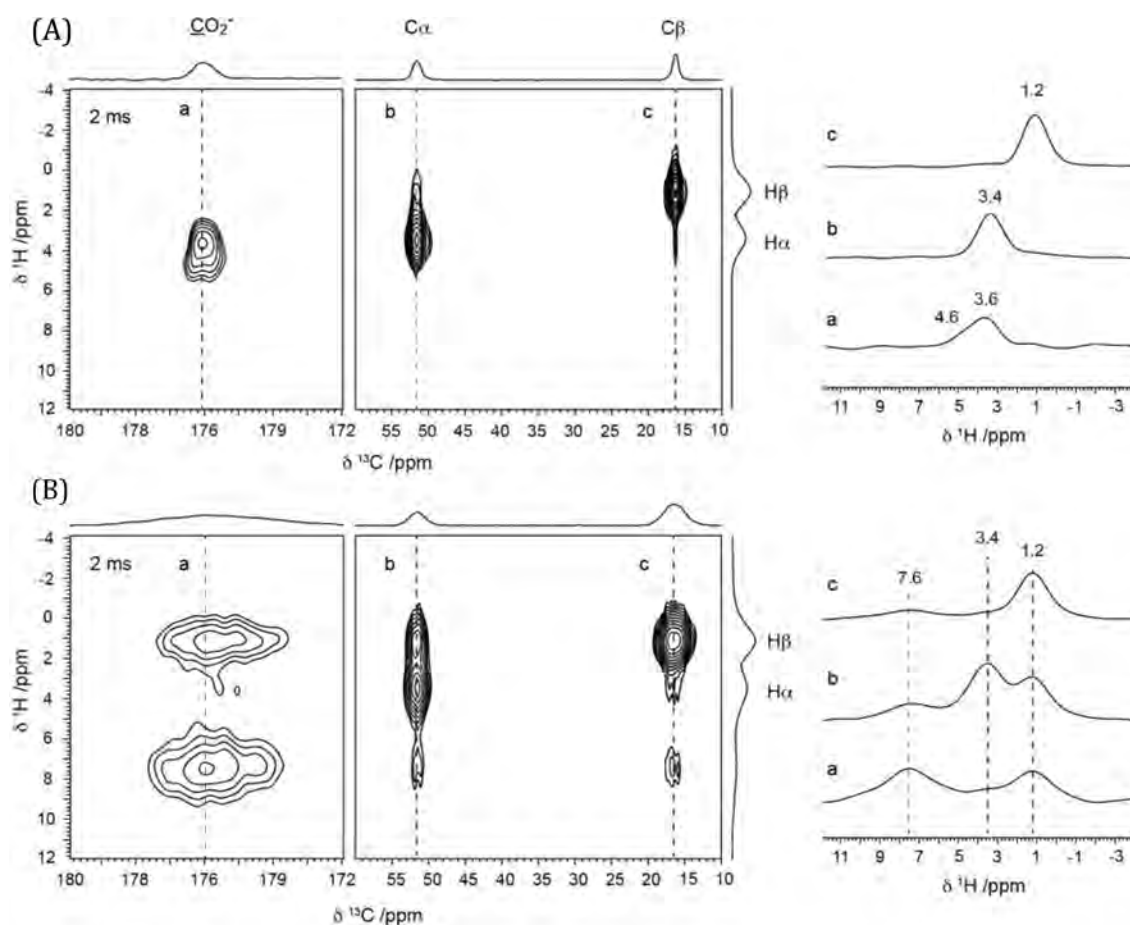


Figure 6. $^1\text{H} \rightarrow ^{13}\text{C}$ 2D-HETCOR MAS NMR spectra of (A) “hydrated” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M and (B) “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M. Experiments were conducted with a 2.0 ms contact time at 400 MHz with MAS frequency of 35 kHz.

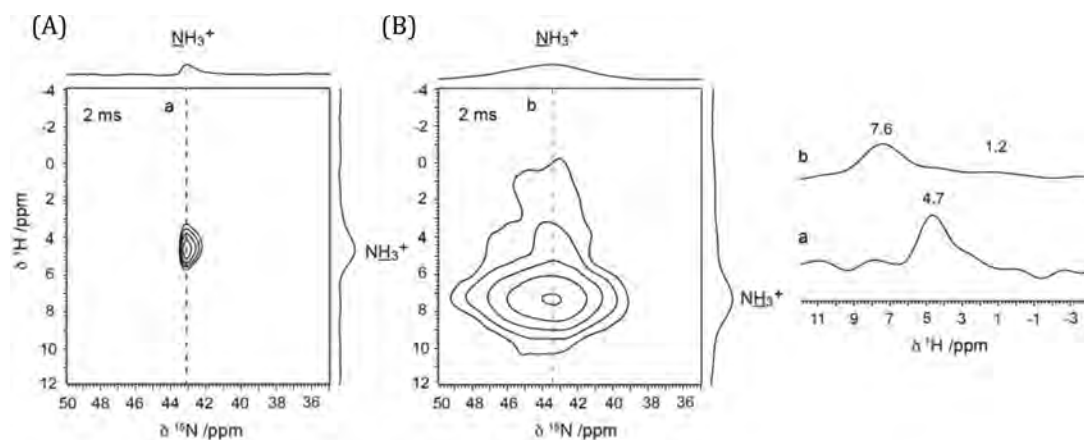


Figure 7. $^1\text{H} \rightarrow ^{15}\text{N}$ 2D-HETCOR MAS NMR spectra of (A) “hydrated” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M and (B) “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M. Experiments were conducted with a 2.0 ms contact time at 400 MHz with MAS frequency of 35 kHz.

protonated amine group and the carboxyl group likely interacts with silanol groups by forming hydrogen bonds on the silica nanoparticle surfaces in the “dry” state.

2D ^1H - ^{13}C and 2D ^1H - ^{15}N HETCOR NMR of the “hydrated” and “dry” $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M are shown in Figures 6 and 7. Since only adsorbed alanine was detected on surfaces for $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M, the ^1H - ^{13}C and ^1H - ^{15}N correlations shown in the spectra can offer more insight into the adsorbed state of alanine on fumed silica

nanoparticle surfaces. Similar to the findings for $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M, in the “hydrated” state, the carboxyl group and the NH_3^+ interact with interfacial water at ~ 4.6 ppm, indicating the alanine molecules are mobile interacting with free water at the interfaces. In the “dry” state, a clear strong ^1H contact is observed at ~ 7.6 ppm for the carboxyl group, indicating they interact with silanols. To further get a more detailed picture of the adsorption on surfaces, we performed HETCOR NMR experiments with variable mixing time, and the results are

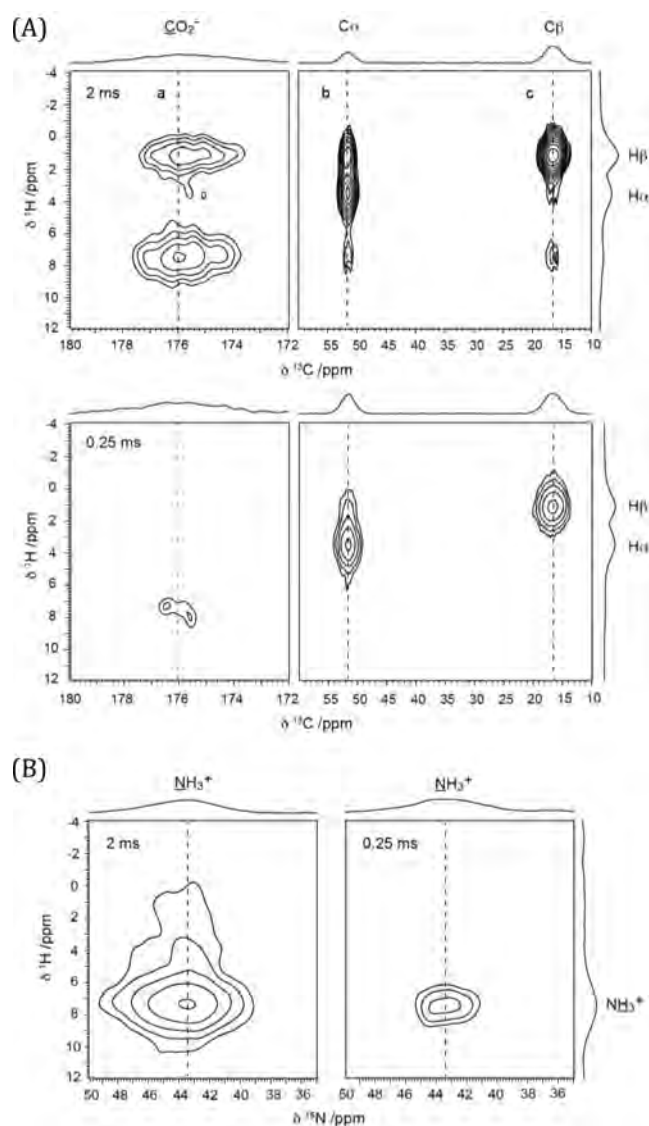


Figure 8. $^1\text{H} \rightarrow ^{13}\text{C}$ (A) and $^1\text{H} \rightarrow ^{15}\text{N}$ (B) 2D-HETCOR MAS NMR spectra of "dry" $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M with contact times of 2.0 and 0.25 ms. Experiments were conducted at 400 MHz with MAS frequency of 35 kHz.

shown in Figure 8. Since the 2D ^1H - ^{13}C and 2D ^1H - ^{15}N HETCOR NMR experiments are dipolar-based experiments, in which magnetization is transferred through space, long-range correlations can be detected by applying a longer CP contact time. With a mixing time of 2.0 ms, all ^1H - ^{13}C and ^1H - ^{15}N correlations can be seen clearly. However, applying a mixing time of 0.25 ms offers a chance to see only short-distance correlations, which makes it possible to determine the groups that interact with the NH_3^+ interacting at silanol groups (^1H resonance at ~ 7.6 ppm). Based on the 2D ^1H - ^{13}C and 2D ^1H - ^{15}N HETCOR NMR spectra with a mixing time of 0.25 ms, a weak correlation to ^1H resonance at 7.6 ppm was detected for the carboxyl group of alanine while a strong correlation to the same ^1H resonance (7.6 ppm) was seen clearly for the protonated amine groups of adsorbed alanine. This result provides convincing evidence that both protonated amine group and the carboxyl group of alanine interacts with the silanol group directly via hydrogen bonds at low hydration level (see Figure 9).

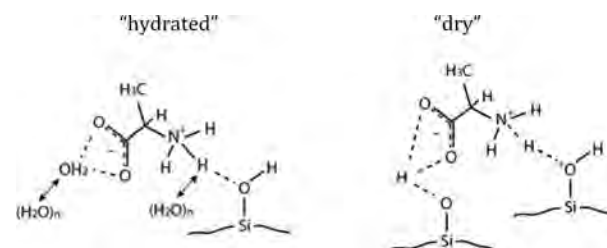


Figure 9. Schematic representation of alanine adsorption on fumed silica nanoparticles surfaces in "hydrated" and "dry" state.

Two Ala/SiO₂ samples with different surface coverages ratios ($^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.03 M and $^{13}\text{C}/^{15}\text{N}$ -Ala/SiO₂-0.10 M) were prepared and characterized in this work. At a high surface coverage (0.10 M), both crystalline and adsorbed states of alanine were observed on the fumed silica nanoparticle surfaces while only the adsorbed state was detected at a low surface coverage (0.03 M). Based on the 2D ^1H - ^{13}C and 2D ^1H - ^{15}N HETCOR NMR and ^1H MAS NMR results, the carboxyl group and protonated amine group of alanine were found to interact with interfacial water via a fast-exchange process in the "hydrated" state. After additional drying, adsorbed alanine molecules become more restricted and show strong interactions with the surface silanol groups via hydrogen bonding between silanol groups and the carboxyl groups and protonated amine groups. Furthermore, according to the variable mixing time HETCOR experiments, the carboxyl group is found to likely interact with the protonated amine group of neighboring alanine molecules and the surface silanol group. As a consequence, this result may provide some evidence for understanding the mechanism of thermal condensation reaction of amino acids forming peptide bonds catalyzed by the surfaces of silica nanoparticles as discussed below.

Thermal Condensation of Alanine on Fumed Silica Nanoparticles. Amino acids can undergo thermal condensation at the interface of inorganic materials such as silica, alumina, and other minerals.^{13,50–53} In this work, differential thermal gravimetric (DTG) analysis and solid-state NMR spectroscopy were used to investigate the thermal condensation of alanine at the interfaces of fumed silica nanoparticles. Figure 10 shows the DTG profiles of bulk alanine and Ala/SiO₂ as a function of alanine concentration. For bulk alanine, no thermal event occurs below 200 °C while one broad peak was detected around 270 °C with a shoulder at 250 °C. For Ala/SiO₂, a well-

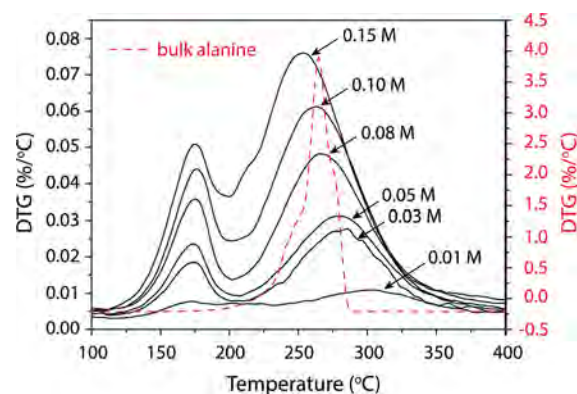


Figure 10. DTG curves of bulk alanine (dashed, red) and Ala/SiO₂ (solid, black) samples as a function of initial concentration of amino acids in the adsorption solutions.

distinguished peak was observed at 172 °C at a heating rate of 5 °C/min, which is due to the thermal condensation of alanine forming peptide bonds. The other peaks observed above 220 °C correspond to a complicated thermal degradation reactions with the elimination of NH₃ and CO₂ similar to bulk alanine and will not be further discussed in this paper.²² This result indicates that alanine can undergo thermal condensation on fumed silica nanoparticle surfaces. Furthermore, the temperature of thermal condensation was found to be independent of the alanine concentration (Table 3), indicating that the

Table 3. Summary of Amino Acids Adsorption and Condensation on Fumed Silica Nanoparticles from TGA Analysis

[alanine] (M)	DTG peak values (°C)		absorbed alanine (%)
	peak I	peak II	
0.01			2.8
0.03	171	280	4.2
0.05	171	279	5.0
0.08	172	269	7.0
0.10	173	263	8.7
0.15	171	253	10.5

adsorbed alanine is the main species contributing to the thermal condensation. Lowering the heating rate can drop the thermal condensation to even lower temperatures.¹³ The thermal condensation was detected ~149 °C at a heating rate of 1 °C/min (Figure S2) compared to 172 °C for the 5 °C/min heating rate.

¹H → ¹³C and ¹H → ¹⁵N CP-MAS NMR spectroscopy was performed on isotope-labeled samples following thermal condensation, and the results are shown in Figures 11 and

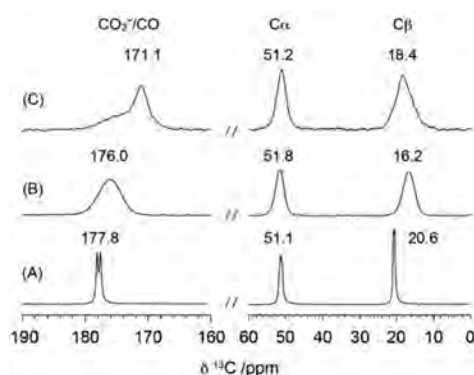


Figure 11. ¹H → ¹³C CP MAS spectra of (A) U-[¹³C/¹⁵N]-L-alanine, (B) ¹³C/¹⁵N-Ala/SiO₂-0.03 M, and (C) ¹³C/¹⁵N-Ala/SiO₂-0.03 M incubated at 170 °C for 3 h.

12, respectively. ¹³C/¹⁵N-Ala/SiO₂-0.03 M was incubated at 170 °C for 3 h, followed by solid-state NMR characterization. After incubation, the intensities of the ¹³C resonance at 176.0 ppm and ¹⁵N resonance at 43.3 ppm decreased, and two new resonances appear due to peptide bond formation during thermal condensation. The ¹³C resonance at 171.1 ppm and ¹⁵N resonance at 120.2 ppm were assigned to the carbonyl group and the amide group of the formed peptides, respectively. The ¹³C resonance of the carbonyl group is consistent with the ¹³C resonance of the carbonyl group of crystalline alanine dipeptide⁵⁴ and alanine anhydride (Figure S3), indicating the formation of a peptide bond. Furthermore,

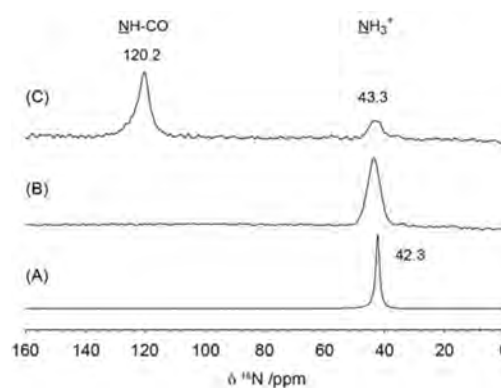


Figure 12. ¹H → ¹⁵N CP MAS spectra of (A) U-[¹³C/¹⁵N]-L-alanine, (B) ¹³C/¹⁵N-Ala/SiO₂-0.03 M, and (C) ¹³C/¹⁵N-Ala/SiO₂-0.03 M incubated at 170 °C for 3 h.

the intensity of the carboxyl group decreased after thermal condensation, showing a small broad peak overlaid with the strong carbonyl peak assigned to the peptide (Figure 11B,C). This indicates that a considerable fraction of adsorbed alanine is converted to a peptide product.

The amide group ¹⁵N chemical shift of the formed peptide shows ~3 ppm difference compared to the ¹⁵N resonance of the amide group for crystalline alanine dipeptide (~123 ppm).⁵⁴ However, it has a similar ¹⁵N resonance to the amide group of alanine residue in silk protein (~120 ppm) that is involved in strong hydrogen bonding.⁵⁵ This indicates that the formed peptide may interact with the silica surfaces via amide groups forming hydrogen bonds to the silica surface. Similar to discussion above from ¹³C CP-MAS NMR, a weak signal of ¹⁵N resonance for NH₃⁺ groups and a strong signal for peptide amide bond also supports that a considerable fraction adsorbed alanine is converted to peptide following thermal condensation. According to 2D ¹H-¹³C and 2D ¹H-¹⁵N HETCOR NMR spectra (Figure S4), the ¹H resonance of amide group was detected at 6.4 ppm while the ¹H resonances of α-CH and β-CH₃ are at 3.2 and 1.2 ppm, respectively. This smaller ¹H resonance of amide proton provides evidence that amide protons are involved in a hydrogen bond network with the silica surfaces.³³ Combined with DTG results, solid-state NMR results provide strong evidence that adsorbed alanine can undergo thermal condensation on the surfaces at ~170 °C, forming a peptide bond. This result indicates that the fumed silica nanoparticles surface can catalyze the thermal condensation reaction of alanine adsorbed at the interfaces. Furthermore, the mechanism of the surface-catalyzed reaction is probably correlated with the adsorption mechanism proposed above, where surface silanol groups play crucial roles interacting with both carboxyl groups and protonated amine groups of alanine molecules at the silica interface.

CONCLUSION

Alanine adsorption and thermal condensation on fumed silica nanoparticles were thoroughly investigated with ¹H, ¹³C, and ¹⁵N multinuclear, multidimensional MAS solid-state NMR spectroscopy. Three species of alanine were detected on fumed silica nanoparticles under different conditions: (1) crystalline and adsorbed alanine at high surface coverage; (2) freely rotating and reorientating adsorbed alanine interacting with mobile interfacial water at high hydration level; (3) reorientation restricted adsorbed alanine interacting with

surface silanol groups via the carboxyl group and protonated amine group (NH_3^+) under dry conditions at low surface coverage. Furthermore, DTG and solid-state NMR results show that the adsorbed alanine can undergo thermal condensation forming peptide bonds at $\sim 170^\circ\text{C}$ at the interface of fumed silica nanoparticles. These results provide some new insights for understanding the mechanism of surface-catalyzed thermal condensation reaction of amino acids at silica surfaces. Solid-state NMR shows great potential for obtaining structural information at the atomic/molecular level for surface chemistry research. Here we show one demonstration where advanced solid-state NMR techniques are able to elucidate the interfacial interactions as well as structural and reaction mechanism of surface bonded molecules for the alanine-fumed silica nanoparticle system. We continue to make use of these solid-state NMR techniques to further probe the interactions of other amino acid/inorganic interfaces and more complex system such as peptide/protein-inorganic systems and expect these approaches to provide powerful insight into the interaction between biomolecules and nanoparticle surfaces in the future.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.5b10236.

^1H MAS NMR spectra; DTG curves of Ala/ SiO_2 -0.03 M at two heating rates, 5 and $1^\circ\text{C}/\text{min}$; $^1\text{H} \rightarrow ^{13}\text{C}$ CP MAS spectra of U- $^{13}\text{C}/^{15}\text{N}$ -L-alanine, alanine anhydride, $^{13}\text{C}/^{15}\text{N}$ -Ala/ SiO_2 -0.03 M, and $^{13}\text{C}/^{15}\text{N}$ -Ala/ SiO_2 -0.03 M incubated at 170°C for 3 h; 2D $^1\text{H} \rightarrow ^{13}\text{C}$ and 2D $^1\text{H} \rightarrow ^{15}\text{N}$ HETCOR NMR spectra of $^{13}\text{C}/^{15}\text{N}$ -Ala/ SiO_2 -0.03 M incubated at 170°C for 3 h (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Ashley, C. E.; Carnes, E. C.; Phillips, G. K.; Padilla, D.; Durfee, P. N.; Brown, P. A.; Hanna, T. N.; Liu, J.; Phillips, B.; Carter, M. B.; et al. The Targeted Delivery of Multicomponent Cargos to Cancer Cells by Nanoporous Particle-Supported Lipid Bilayers. *Nat. Mater.* **2011**, *10*, 389–397.
- (2) Tarn, D.; Ashley, C. E.; Xue, M.; Carnes, E. C.; Zink, J. I.; Brinker, C. J. Mesoporous Silica Nanoparticle Nanocarriers: Biofunctionality and Biocompatibility. *Acc. Chem. Res.* **2013**, *46*, 792–801.
- (3) Hudson, S.; Cooney, J.; Magner, E. Proteins in Mesoporous Silicates. *Angew. Chem., Int. Ed.* **2008**, *47*, 8582–8594.
- (4) Gibson, J. M.; Popham, J. M.; Raghunathan, V.; Stayton, P. S.; Drobny, G. P. A Solid-State NMR Study of the Dynamics and Interactions of Phenylalanine Rings in a Statherin Fragment Bound to Hydroxyapatite Crystals. *J. Am. Chem. Soc.* **2006**, *128*, 5364–5370.
- (5) Mermut, O.; Phillips, D. C.; York, R. L.; McCrea, K. R.; Ward, R. S.; Somorjai, G. A. In Situ Adsorption Studies of a 14-Amino Acid

Leucine-Lysine Peptide Onto Hydrophobic Polystyrene and Hydrophilic Silica Surfaces Using Quartz Crystal Microbalance, Atomic Force Microscopy, and Sum Frequency Generation Vibrational Spectroscopy. *J. Am. Chem. Soc.* **2006**, *128*, 3598–3607.

(6) Somorjai, G. A.; Frei, H.; Park, J. Y. Advancing the Frontiers in Nanocatalysis, Biointerfaces, and Renewable Energy Conversion by Innovations of Surface Techniques. *J. Am. Chem. Soc.* **2009**, *131*, 16589–16605.

(7) Breen, N. F.; Weidner, T.; Li, K.; Castner, D. G.; Drobny, G. P. A Solid-State Deuterium NMR and Sum-Frequency Generation Study of the Side-Chain Dynamics of Peptides Adsorbed Onto Surfaces. *J. Am. Chem. Soc.* **2009**, *131*, 14148–14149.

(8) Emami, F. S.; Puddu, V.; Berry, R. J.; Varshney, V.; Patwardhan, S. V.; Perry, C. C.; Heinz, H. Prediction of Specific Biomolecule Adsorption on Silica Surfaces as a Function of pH and Particle Size. *Chem. Mater.* **2014**, *26*, 5725–5734.

(9) Patwardhan, S. V.; Emami, F. S.; Berry, R. J.; Jones, S. E.; Naik, R. R.; Deschaume, O.; Heinz, H.; Perry, C. C. Chemistry of Aqueous Silica Nanoparticle Surfaces and the Mechanism of Selective Peptide Adsorption. *J. Am. Chem. Soc.* **2012**, *134*, 6244–6256.

(10) Zhang, H.; Dunphy, D. R.; Jiang, X.; Meng, H.; Sun, B.; Tarn, D.; Xue, M.; Wang, X.; Lin, S.; Ji, Z.; et al. Processing Pathway Dependence of Amorphous Silica Nanoparticle Toxicity: Colloidal vs Pyrolytic. *J. Am. Chem. Soc.* **2012**, *134*, 15790–15804.

(11) Lambert, J. F. Adsorption and Polymerization of Amino Acids on Mineral Surfaces: a Review. *Origins Life Evol. Biospheres* **2008**, *38*, 211–242.

(12) Orgel, L. E. Polymerization on the Rocks: Theoretical Introduction. *Origins Life Evol. Biospheres* **1998**, *28*, 227–234.

(13) Lambert, J. F.; Jaber, M.; Georgelin, T.; Stievano, L. A Comparative Study of the Catalysis of Peptide Bond Formation by Oxide Surfaces. *Phys. Chem. Chem. Phys.* **2013**, *15*, 13371.

(14) Lee, C.-H.; Lin, T.-S.; Mou, C.-Y. Mesoporous Materials for Encapsulating Enzymes. *Nano Today* **2009**, *4*, 165–179.

(15) Lu, J.; Liong, M.; Zink, J. I.; Tamanoi, F. Mesoporous Silica Nanoparticles as a Delivery System for Hydrophobic Anticancer Drugs. *Small* **2007**, *3*, 1341–1346.

(16) Perry, C. C.; Li, X. Structural Studies of Gel Phases. Part 1. Infrared Spectroscopic Study of Silica Monoliths; the Effect of Thermal History on Structure. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 761.

(17) Perry, C. C.; Li, X. Structural Studies of Gel Phases. Part 2. Infrared Spectroscopic Study of Silica Monoliths; the Interaction of Water with Surface Species. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 3857.

(18) Liu, C. C.; Maciel, G. E. The Fumed Silica Surface: a Study by NMR. *J. Am. Chem. Soc.* **1996**, *118*, 5103–5119.

(19) Gun'ko, V. M.; Voronin, E. F.; Pakhlov, E. M.; Zarko, V. I.; Turov, V. V.; Guzenko, N. V.; Lebeda, R. Features of Fumed Silica Coverage with Silanes Having Three or Two Groups Reacting with the Surface. *Colloids Surf., A* **2000**, *166*, 187–201.

(20) Burneau, A.; Barres, O. Comparative Study of the Surface Hydroxyl Groups of Fumed and Precipitated Silicas. *Langmuir* **1990**, *6*, 1364–1372.

(21) Zaia, D. A. M. A Review of Adsorption of Amino Acids on Minerals: Was It Important for Origin of Life? *Amino Acids* **2004**, *27*, 113–118.

(22) Meng, M.; Stievano, L.; Lambert, J. F. Adsorption and Thermal Condensation Mechanisms of Amino Acids on Oxide Supports. 1. Glycine on Silica. *Langmuir* **2004**, *20*, 914–923.

(23) Bouchoucha, M.; Jaber, M.; Onfroy, T.; Lambert, J. F.; Xue, B. Glutamic Acid Adsorption and Transformations on Silica. *J. Phys. Chem. C* **2011**, *115*, 21813–21825.

(24) Costa, D.; Tougeri, A.; Tielens, F.; Gervais, C.; Stievano, L.; Lambert, J. F. DFT Study of the Adsorption of Microsolvated Glycine on a Hydrophilic Amorphous Silica Surface. *Phys. Chem. Chem. Phys.* **2008**, *10*, 6360–6368.

- (25) Nonella, M.; Seeger, S. Investigating Alanine–Silica Interaction by Means of First-Principles Molecular-Dynamics Simulations. *ChemPhysChem* **2008**, *9*, 414–421.
- (26) Shaw, W. J.; Long, J. R.; Campbell, A. A.; Stayton, P. S.; Drobny, G. P. A Solid State NMR Study of Dynamics in a Hydrated Salivary Peptide Adsorbed to Hydroxyapatite. *J. Am. Chem. Soc.* **2000**, *122*, 7118–7119.
- (27) Mirau, P. A.; Serres, J. L.; Lyons, M. The Structure and Dynamics of Poly(L-Lysine) in Templated Silica Nanocomposites. *Chem. Mater.* **2008**, *20*, 2218–2223.
- (28) Guo, C.; Holland, G. P. Investigating Lysine Adsorption on Fumed Silica Nanoparticles. *J. Phys. Chem. C* **2014**, *118*, 25792–25801.
- (29) Amitay-Rosen, T.; Kababya, S.; Vega, S. A Dynamic Magic Angle Spinning NMR Study of the Local Mobility of Alanine in an Aqueous Environment at the Inner Surface of Mesoporous Materials. *J. Phys. Chem. B* **2009**, *113*, 6267–6282.
- (30) Lopes, I.; Piao, L.; Stievano, L.; Lambert, J. F. Adsorption of Amino Acids on Oxide Supports: a Solid-State NMR Study of Glycine Adsorption on Silica and Alumina. *J. Phys. Chem. C* **2009**, *113*, 18163–18172.
- (31) Ben Shir, I.; Kababya, S.; Amitay-Rosen, T.; Balazs, Y. S.; Schmidt, A. Molecular Level Characterization of the Inorganic–Bioorganic Interface by Solid State NMR: Alanine on a Silica Surface, a Case Study. *J. Phys. Chem. B* **2010**, *114*, 5989–5996.
- (32) Ben Shir, I.; Kababya, S.; Schmidt, A. Binding Specificity of Amino Acids to Amorphous Silica Surfaces: Solid-State NMR of Glycine on SBA-15. *J. Phys. Chem. C* **2012**, *116*, 9691–9702.
- (33) Folliet, N.; Gervais, C.; Costa, D.; Laurent, G.; Babonneau, F.; Stievano, L.; Lambert, J. F.; Tielen, F. A Molecular Picture of the Adsorption of Glycine in Mesoporous Silica Through NMR Experiments Combined with DFT-D Calculations. *J. Phys. Chem. C* **2013**, *117*, 4104–4114.
- (34) Zhu, L.; Gu, Q.; Sun, P.; Chen, W.; Wang, X.; Xue, G. Characterization of the Mobility and Reactivity of Water Molecules on TiO₂ Nanoparticles by ¹H Solid-State Nuclear Magnetic Resonance. *ACS Appl. Mater. Interfaces* **2013**, *5*, 10352–10356.
- (35) Ben Shir, I.; Kababya, S.; Schmidt, A. Molecular Details of Amorphous Silica Surfaces Determine Binding Specificity to Small Amino Acids. *J. Phys. Chem. C* **2014**, *118*, 7901–7909.
- (36) Pan, Y.; Gullion, T.; Schaefer, J. Determination of C–N Internuclear Distances by Rotational-Echo Double-Resonance NMR of Solids. *J. Magn. Reson. (1969-1992)* **1990**, *90*, 330–340.
- (37) Gullion, T.; Schaefer, J. Rotational-Echo Double-Resonance NMR. *J. Magn. Reson. (1969-1992)* **1989**, *81*, 196–200.
- (38) Hartmann, S. R.; Hahn, E. L. Nuclear Double Resonance in the Rotating Frame. *Phys. Rev.* **1962**, *128*, 2042–2053.
- (39) Meier, B. H. Cross Polarization Under Fast Magic Angle Spinning: Thermodynamical Considerations. *Chem. Phys. Lett.* **1992**, *188*, 201–207.
- (40) Cory, D. G.; Ritchey, W. M. Suppression of Signals From the Probe in Bloch Decay Spectra. *J. Magn. Reson. (1969-1992)* **1988**, *80*, 128–132.
- (41) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. Heteronuclear Decoupling in Rotating Solids. *J. Chem. Phys.* **1995**, *103*, 6951–6958.
- (42) Hayashi, S.; Hayamizu, K. Chemical Shift Standards in High-Resolution Solid-State NMR (1) ¹³C, ²⁹Si, and ¹H Nuclei. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 685–687.
- (43) Hayashi, S.; Hayamizu, K. Chemical Shift Standards in High-Resolution Solid-State NMR (2) ¹⁵N Nuclei. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 688–690.
- (44) Rimola, A.; Sodupe, M.; Ugliengo, P. Affinity Scale for the Interaction of Amino Acids with Silica Surfaces. *J. Phys. Chem. C* **2009**, *113*, 5741–5750.
- (45) Gao, Q.; Xu, W.; Xu, Y.; Wu, D.; Sun, Y.; Deng, F.; Shen, W. Amino Acid Adsorption on Mesoporous Materials: Influence of Types of Amino Acids, Modification of Mesoporous Materials, and Solution Conditions. *J. Phys. Chem. B* **2008**, *112*, 2261–2267.
- (46) Hester, R. K.; Ackerman, J. L.; Neff, B. L.; Waugh, J. S. Separated Local Field Spectra in NMR: Determination of Structure of Solids. *Phys. Rev. Lett.* **1976**, *36*, 1081–1083.
- (47) Vinogradov, E.; Madhu, P. K.; Vega, S. High-Resolution Proton Solid-State NMR Spectroscopy by Phase-Modulated Lee–Goldburg Experiment. *Chem. Phys. Lett.* **1999**, *314*, 443–450.
- (48) Ben Shir, I.; Kababya, S.; Amitay-Rosen, T.; Balazs, Y. S.; Schmidt, A. Molecular Level Characterization of the Inorganic–Bioorganic Interface by Solid State NMR: Alanine on a Silica Surface, a Case Study. *J. Phys. Chem. B* **2010**, *114*, 5989–5996.
- (49) Asakura, T.; Okonogi, M.; Nakazawa, Y.; Yamauchi, K. Structural Analysis of Alanine Tripeptide with Antiparallel and Parallel B-Sheet Structures in Relation to the Analysis of Mixed B-Sheet Structures in SamiacynthiariciniSilk Protein Fiber Using Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* **2006**, *128*, 6231–6238.
- (50) Lambert, J. F.; Stievano, L.; Lopes, I.; Gharsallah, M.; Piao, L. The Fate of Amino Acids Adsorbed on Mineral Matter. *Planet. Space Sci.* **2009**, *57*, 460–467.
- (51) Rimola, A.; Tosoni, S.; Sodupe, M.; Ugliengo, P. Does Silica Surface Catalyze Peptide Bond Formation? New Insights From First-Principles Calculations. *ChemPhysChem* **2006**, *7*, 157–163.
- (52) Bujdak, J.; Rode, B. M. Silica, Alumina and Clay Catalyzed Peptide Bond Formation: Enhanced Efficiency of Alumina Catalyst. *Origins Life Evol. Biospheres* **1999**, *29*, 451–461.
- (53) Lahav, N.; White, D.; Chang, S. Peptide Formation in the Prebiotic Era: Thermal Condensation of Glycine in Fluctuating Clay Environment. *Science* **1978**, *201*, 67–69.
- (54) Bhate, M. P.; Woodard, J. C.; Mehta, M. A. Solvation and Hydrogen Bonding in Alanine- and Glycine-Containing Dipeptides Probed Using Solution- and Solid-State NMR Spectroscopy. *J. Am. Chem. Soc.* **2009**, *131*, 9579–9589.
- (55) Jenkins, J. E.; Creager, M. S.; Butler, E. B.; Lewis, R. V.; Yarger, J. L.; Holland, G. P. Solid-State NMR Evidence for Elastin-Like B-Turn Structure in Spider Dragline Silk. *Chem. Commun.* **2010**, *46*, 6714–6716.