

Studies of Dynamic Binding of Amino Acids to TiO₂ Nanoparticle Surfaces by Solution NMR and Molecular Dynamics Simulations

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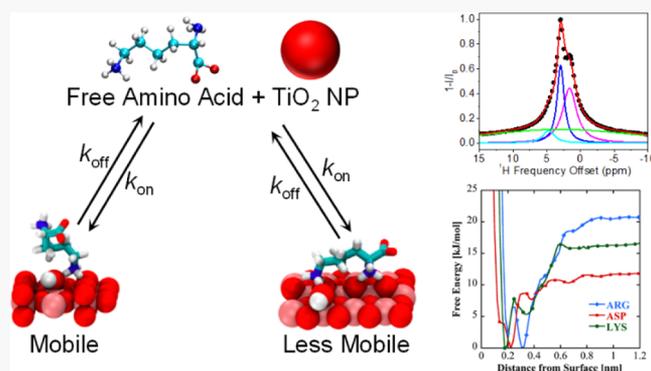


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ABSTRACT: Adsorption of biomolecules onto material surfaces involves a potentially complex mechanism where molecular species interact to varying degrees with a heterogeneous material surface. Surface adsorption studies by atomic force microscopy, sum frequency generation spectroscopy, and solid-state NMR detect the structures and interactions of biomolecular species that are bound to material surfaces, which, in the absence of a solid–liquid interface, do not exchange rapidly between surface-bound forms and free molecular species in bulk solution. Solution NMR has the potential to complement these techniques by detecting and studying transiently bound biomolecules at the liquid–solid interface. Herein, we show that dark-state exchange saturation transfer (DEST) NMR experiments on gel-stabilized TiO₂ nanoparticle (NP) samples detect several forms of biomolecular adsorption onto titanium(IV) oxide surfaces. Specifically, we use the DEST approach to study the interaction of amino acids arginine (Arg), lysine (Lys), leucine (Leu), alanine (Ala), and aspartic acid (Asp) with TiO₂ rutile NP surfaces. Whereas Leu, Ala, and Asp display only a single weakly interacting form in the presence of TiO₂ NPs, Arg and Lys displayed at least two distinct bound forms: a species that is surface bound and retains a degree of reorientational motion and a second more tightly bound form characterized by broadened DEST profiles upon the addition of TiO₂ NPs. Molecular dynamics simulations indicate different surface bound states for both Lys and Arg depending on the degree of TiO₂ surface hydroxylation but only a single bound state for Asp regardless of the degree of surface hydroxylation, in agreement with results obtained from the analysis of DEST profiles.



INTRODUCTION

The interaction of biomolecules with titanium(IV) oxide (TiO₂) nanoparticles (NPs) is a topic relevant to a variety of fields including medical and dental implants, biosensors, chromatography, and catalysis.^{1–12} Fundamental to our understanding of how biomolecules interact with TiO₂ NPs is the knowledge of the structures of these molecules at NP surfaces. However, detailed structural information of surface-adsorbed peptides is only now emerging,^{13–15} and the nature of protein–mineral surface interactions has yet to be clarified even for small monomeric amino acids and small mineral-binding peptides. The methods used to detect and characterize surface-bound molecular species include atomic force microscopy (AFM),^{16–18} sum frequency generation spectroscopy,^{19,20} solid-state NMR,²¹ and saturation transfer difference NMR,²² to name a few. These methods detect the presence of partly or entirely immobilized biomolecular species at material surfaces where the amino acid side chains provide points of surface contact.

The mechanism of surface adsorption of biomolecules onto TiO₂ NPs is complex and may involve, prior to final

attachment and immobilization on the surface, the formation of biomolecular species that interact with and are only partly immobilized near the NP surface. In addition, the material surface may be heterogeneous, resulting in a variation in binding affinity over the surface and in multiple forms of bound species. Because they are the monomeric constituents of proteins, adsorption of amino acids onto metallic and oxide surfaces has been widely studied. Although thermodynamic studies of lysine^{23,24} and histidine²⁵ adsorption onto TiO₂ NPs fitted data using a simple Langmuir model, which assumes a single affinity constant and a single independently bound form, spectroscopic studies have identified more complex scenarios for adsorption of some amino acids on TiO₂ NPs. An IR spectroscopic study of the binding of glutamic acid and

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aspartic acid to TiO₂ NPs found that while at all pH aspartic acid binds in a single form, glutamic acid binds in at least two forms.²⁶ A very recent thermodynamic study of the adsorption of L-amino acids onto TiO₂ NPs found that the Brunauer–Emmett–Teller (BET) model fits the binding of most amino acids to TiO₂, with the binding being endothermic and thus entropy driven.²⁷ In the same study, computations showed surface interactions via hydrogen bonding between the α -ammonium group and surface hydroxyl oxygens, while basic and acid amino acids can also interact with the surface via their side chains.

In view of the complex nature of amino acid and peptide adsorption onto TiO₂ NPs, it is useful to apply experimental techniques that can detect several types of adsorbed species under the same sample conditions. Dark-state exchange saturation transfer (DEST) NMR experiments have provided thermodynamic and kinetic information on the binding of small proteins to aggregates and large molecular machines.^{28–30} DEST relies on slow exchange of nuclear spins between sites with very different values of the transverse relaxation rate R_2 , as would occur, for example, when a freely tumbling molecular species in solution with a small R_2 , adsorbs onto a TiO₂ NP surface with the resulting immobilized species displaying a much larger R_2 . In addition to R_2 values for free and bound molecular species, simulation of the DEST saturation profile yields further information, including the relative populations of the free and adsorbed species, and the kinetic constants that quantify the rate of exchange between free and adsorbed species. In an initial demonstration of this approach, Egner et al.³¹ applied ¹H DEST and relaxation dispersion to the study of the adsorption of phenol and cholic acid onto cerium oxide NPs. By direct solution of the Bloch–McConnell equations and subsequent simulation of the ¹H DEST saturation profiles, Egner et al. showed that while cholic acid adsorbs from bulk solution to a weakly bound state that does not constitute a rigid adduct with the NP, phenol adsorption proceeds via an intermediate, weakly bound species to a state that is rigidly bound to the NP surface. Therefore, Egner et al.'s study afforded not only populations of free and bound species but also a kinetic mechanism for the adsorption process.

MD simulations have also provided valuable insights into the binding mechanism of amino acids and their analogues on the surface of TiO₂.^{32–36} Bowen et al. found that the adsorption of amino acids with polar side chains on a negative rutile (110) surface is a function of both backbone and side-chain binding.³² Walsh and co-workers studied the binding of arginine, aspartate, and lysine analogues on two variants of the rutile interface—negative and neutral; they report that the arginine analogue adsorbed the strongest to both interfaces, followed by the lysine and aspartate analogues.^{35,36} Recently, Schelokov et al. described the adsorption of amino acids on nanocrystalline anatase particles using QSPR and MD simulations; they found that the binding occurs primarily through the formation of two–three hydrogen bonds via side-chain or backbone groups that are charged.²⁷

In this paper, we apply ¹H DEST techniques to the study of the adsorption of small biomolecules, that is, amino acids, to TiO₂ rutile nanocrystals. Application of DEST methods to studying the binding of biomolecules to mineral surfaces has the same requirements detailed in the study of Egner et al.³¹ Namely, the NMR-visible molecules (i.e., the free, unbound molecules) and the NMR-invisible molecules (i.e., the surface-

bound molecules) have to remain homogeneously suspended in the NMR sample throughout the NMR measurement period. We followed the procedure described in Egner et al. and used 1 wt % agarose to prevent NP sedimentation. By introducing TiO₂ nanocrystals into agarose gel suspensions, we investigated the binding of arginine (Arg), lysine (Lys), leucine (Leu), aspartate (Asp), and alanine (Ala) amino acids. We chose amino acids as model systems for the initial study because they contain the same side-chain functional groups used by peptides and proteins to adsorb onto TiO₂ NP surfaces,^{16–18} yet their small size limits the number of surface-bound forms that may be present. Lorentzian deconvolution of ¹H DEST saturation profiles indicate for Arg and Lys the existence of multiple forms of adsorbed molecules, distinguished by differing degrees of residual molecular motion. Simulation of the ¹H DEST profiles by direct solution of the Bloch–McConnell equation provides quantitative information including relative populations of free and adsorbed species as well as kinetic constants that quantify the rate of exchange between free and adsorbed species. Finally, molecular dynamics (MD) simulations also demonstrate the existence of multiple binding states of Arg and Lys on rutile surfaces and investigate the role played by surface hydroxylation in mediating these interactions. Overall, this paper demonstrates how the application DEST NMR experiments and MD calculations in a concerted fashion can elucidate both thermodynamic and kinetic aspects of the adsorption of biomolecules at liquid–solid interfaces.

■ EXPERIMENTAL SECTION

Materials. Amino acids alanine, leucine, arginine, lysine, and aspartic acid were purchased from Sigma-Aldrich (St. Louis, MO) and used without purification. TiO₂ [product number 637262; rutile titanium(IV) oxide nanopowder with a reported particle size <100 nm] NPs were purchased from Sigma-Aldrich (St. Louis, MO) with a BET measured surface area of 28.6 m²/g. Agarose was purchased from Sigma Aldrich and used without further purification. D₂O was purchased from Sigma-Aldrich and used without further purification.

Preparation of NMR Samples. Amino acid (10 mM, Arg, Lys, Leu, Pro, Asp, and Ala) NMR samples were prepared in 20 mM phosphate buffer pD7 with 99.9% D₂O.

Samples without TiO₂ nanopowder in the presence of agarose gel were prepared by mixing 1% w/w agarose in 20 mM phosphate buffer (pD 7, 99.9% D₂O). The mixture was placed in a boiling water bath for 5 min and then removed to a block heater to cool. When the temperature equilibrated between 50 and 60 °C, amino acid solution was added, resulting in a final concentration of 10 mM. The warm solution was transferred to an NMR tube and allowed to cool at room temperature.

Samples that contained TiO₂ nanopowder in the presence of gel were prepared by mixing 1% w/w TiO₂ nanopowder and agarose in 20 mM phosphate buffer (pD 7, 99.9% D₂O). The mixture was vortex mixed and sonicated for 5 min and then placed in a boiling water bath for 5 min. The sample was then removed to a block heater and its internal temperature was allowed to equilibrate between 50 and 60 °C. The respective amino acid solution was added, resulting in a final concentration of 10 mM. The warm solution was transferred to an NMR tube and allowed to cool to room temperature.

NMR Spectroscopy. The NMR measurements were performed at 25 °C on a Bruker AVANCE III 700 MHz NMR instrument equipped with a 5 mm Broadband Observe (BBO) probe. ¹H-DEST experiments were measured at multiple saturation fields (50, 100, 150, 200, 250, and 300 Hz) using a ¹H DEST pulse scheme.³¹ 1D ¹H spectra were recorded in steps of 0.25 or 2.5 ppm with the position of the ¹H B₁ field ranging from –100 to +100 ppm, and an offset of –100 ppm was used for normalization. The saturation field was

applied for 1 s, with a repetition delay of 3 s. NMR spectra were processed using Topspin 4.0.2 and Mnova NMR (<http://mestrelab.com/software/mnova/nmr/>). The spectra were analyzed using the Bruker dynamics center (<https://www.bruker.com/products/mr/nmr/nmr-software/software/dynamics-center/overview.html>) and Mnova NMR.

Analysis of Data. Data were processed with Peak Analyzer in OriginPro (OriginLab Corporation, Northampton, MA, USA). Z-spectra (I/I_0) (or DEST profile) were normalized by the signal with RF irradiation at -100 ppm (I_0). For the conventional fitting method, multipool Lorentzian fitting of the Z-spectra was applied to estimate the DEST effects from different pools.^{37–39} Briefly, the inverted Z-spectra ($1 - I/I_0$) were fitted as the sum of multiple Lorentzian functions with the following equation

$$1 - \frac{I}{I_0} = \sum_{i=1}^N \frac{A_i}{1 + 4\left(\frac{\omega - \omega_i}{\sigma_i}\right)^2} \quad (1)$$

where ω is the frequency offset from the interest resonance and A_i , ω_i , and σ_i are the amplitude, frequency offset, and linewidth of the DEST peak for the i th proton pool, respectively. In the DEST phantom, we employed a four-pool Lorentzian model of magnetization transfer (MT) and nuclear Overhauser enhancement (NOE) effects from bound water and/or nearby hydrogens in molecules.^{40,41}

Global fitting was accomplished with a homogenous form of McConnell equations using Matlab code DESTfit (<https://spin.niddk.nih.gov/clore/Software/software.html>).²⁸ A single spin was assumed to be in exchange between an observable free state with low R_2 and two types of bound forms with larger R_2 values ($A \leftrightarrow$ the mixture of B and C).²⁸ The cross-relaxation between two spins was incorporated in the McConnell model: the cross-relaxation rate σ_A between H_1 (the observed signal) and H_2 (coupled with H_1 by cross-relaxation) in the free amino acid is assumed to be -0.5 s^{-1} , and the cross-relaxation rate σ_B between H_1 and H_2 of amino acid bound on particles is assumed to be -500 s^{-1} .²⁸

MD Calculations. The effects of molecular (nonhydroxylated TiO_2) and dissociated (hydroxylated TiO_2) surface water were investigated for two variants of the rutile (110) using the force field developed by Pěrdota et al.⁴² The surface dimensions are approximately $5.5 \times 5.3 \times 1.8 \text{ nm}^3$, and both surfaces are negatively charged with a charge density of -0.103 C/m^2 , corresponding to a pH of 8. Three amino acids were chosen for this study—aspargic acid (Asp), lysine (Lys), and arginine (Arg); in the pH range of 7.5–8.0, the charges of the amino acid side chains are -1 , $+1$, and $+1$, respectively. To remain comparable with the ^1H NMR experiment, amino acid termini have a deprotonated carboxylate group and a protonated amine group. The amino acids were modeled using the CHARMM36 forcefield.⁴³ A water slab 8 nm thick consisting of ~ 7500 molecules of SPC/E water was added above the surface. System equilibration was carried out using a Donadio–Bussi–Parrinello⁴⁴ and a Parrinello–Rahman⁴⁵ barostat to maintain a temperature and pressure of 300 K and 1 bar, respectively. After equilibrating the system at 1 bar and 300 K, well-tempered metadynamics⁴⁶ was employed to calculate binding free-energy profiles by biasing the vertical distance of the amino acid from the surface (see the Supporting Information for details). Simulations were performed using GROMACS 5.1.2,⁴⁷ along with the PLUMED plugin⁴⁸ for enhanced sampling.

RESULTS AND DISCUSSION

^1H DEST NMR Studies of the Interactions of Amino Acids Arg, Lys, Asp, Leu, and Ala with TiO_2 NPs in Agarose Gels. The addition of TiO_2 (1 wt %) to a 10 mM amino acid solution in 99.9% D_2O resulted in extensive line broadening of the NMR resonances in all cases (Figures 1 and S1), thus indicating that Arg, Lys, Asp, Leu, and Ala all interact with TiO_2 and exchange between a free and a bound state. A lesser degree of peak broadening is observed upon the addition

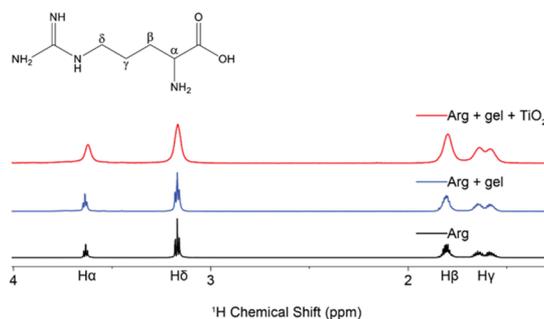


Figure 1. ^1H proton spectra of arginine neat (black), suspended in agarose gel (blue), and exposed to TiO_2 NPs in the presence of agarose gel (red). Addition of TiO_2 to the sample creates distinct peak broadening not observed in the other two spectra, indicative of interactions with a slow tumbling object.

of agarose gel to the sample, indicating that the amino acids are weakly interacting with the matrix, but they retain the ability to diffuse and tumble freely.

In DEST NMR experiments, slow exchange, by inference contact with NP surfaces, is indicated by broadening of the DEST saturation profile upon the addition of agarose gel and TiO_2 NPs. Figure 2 shows the ^1H DEST profiles of side-chain protons for five monomeric amino acids (Arg, Lys, Leu, Asp, and Ala): (1) free in solution (10 mM), (2) in the presence of agarose gel, and (3) in the presence of TiO_2 NPs and agarose gel. These amino acids were chosen for their appearance in the hexamer peptide TBP-6, which has been shown to bind strongly to rutile TiO_2 .¹⁶ Mutation studies of the peptide have suggested that the three polar residues (Arg, Lys, and Asp) are involved in surface adhesion, while further studies have suggested that nonpolar residues may also play a role in surface interactions.^{32–36} In the absence of TiO_2 NPs, the DEST saturation profiles for $\text{H}\epsilon$ of Lys (Figure 2b) and $\text{H}\delta$ of Arg (Figure 2a) are narrow and confined to <1 ppm region about the respective resonance frequencies. In both cases, there is a slight broadening of the saturation profile upon the addition of agarose gel (Figure 2a,b), indicating weak interactions of these basic amino acids with the gel. Upon the addition of TiO_2 NPs, the saturation profiles of both Arg and Lys become broadened and display broad “wings”, indicative of slow exchange between the free state of the amino acid and a “dark”, that is, surface-bound state, with a very large R_2 . The larger broadening of the Arg DEST profile upon TiO_2 addition suggests that Arg has a greater affinity of binding to the NPs than Lys.

Figure 2c–e shows analogous DEST saturation profiles for $\text{H}\beta$ of Asp, $\text{H}\delta$ of Leu, and $\text{H}\beta$ of Ala, respectively. In the three cases, there is no broadening of the saturation profile upon the addition of agarose gel (Figure 2c,d), indicating no interactions of these amino acids with the matrix. Interestingly, the Asp monomer and the nonpolar amino acids do not show broad “wings” in their DEST profiles upon the addition of TiO_2 NPs, indicating that these individual amino acid monomers retain considerable reorientational degrees of freedom upon the addition of TiO_2 NPs and as a result have much smaller R_2 values than is the case with Arg and Lys.

In the case of Arg and Lys, the ^1H DEST profiles in the presence of TiO_2 NPs are asymmetric, indicating the presence of spectral features in addition to the direct saturation lines. Information on the origins of the broad asymmetric DEST profiles for Arg (Figure 3) and Lys (Figure S2) may be

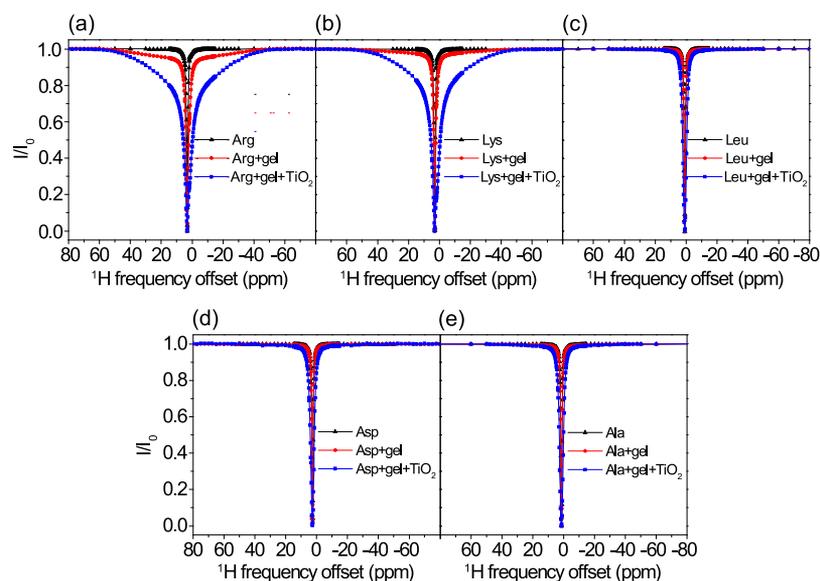


Figure 2. ^1H DEST profiles for $\text{H}\delta$ of 10 mM Arg (a), $\text{H}\epsilon$ of 10 mM Lys (b), $\text{H}\delta$ of 10 mM Leu (c), $\text{H}\beta$ of Asp (d), and $\text{H}\beta$ of 10 mM Ala (e) neat (black), with 1% w/w agarose gel (red) and with 1% w/w agarose gel and 1% w/w TiO_2 NPs (blue). Slight peak broadening of the DEST profile upon the addition of agarose gel for Arg and Lys (a,b) indicates a weak interaction between the agarose gel and the amino acids, and broadening upon the addition to TiO_2 indicates an interaction with the oxide. In all figures, $B_1 = 300$ Hz.

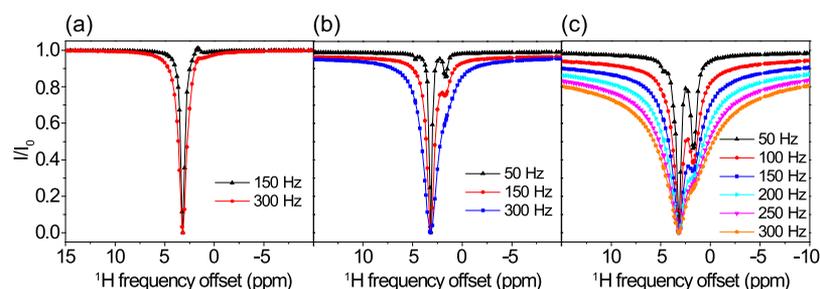


Figure 3. ^1H DEST profiles for $\text{H}\delta$ of 10 mM Arg in the absence of gel (a), in the presence of gel (b), and in the presence of gel and 1 wt % TiO_2 (c).

obtained by a study of the DEST profiles as a function of ^1H B_1 field strength. Figure 3 shows ^1H DEST profiles for the $\text{H}\delta$ proton of Arg: (a) free in 10 mM solution; (b) 10 mM concentration in agarose gel; and (c) 10 mM concentration in agarose gel and in the presence of TiO_2 NPs, for B_1 saturation fields ranging from 50 to 300 Hz. In all three figures, a partial source of the asymmetry is traced in part to a line at a chemical shift of 1.68 ppm. In Figure 3a, this is the small feature out of phase with the direct saturation line, while in Figure 3b,c, the line at 1.68 ppm is in phase with the direct saturation line and is much more intense. This 1.68 ppm line is therefore an NOE to neighboring $\text{H}\beta$ and $\text{H}\gamma$ protons. The fact that the NOE inverts from positive to negative upon the addition of the gel indicates a slowing of molecular reorientations, and the increase in the NOE intensity upon the addition of TiO_2 NPs indicates a further slowing of molecular reorientations because of strong interactions between the amino acid and the TiO_2 NP surface.

From the line shapes in Figure 3c, at least four components to the ^1H DEST saturation profile are discernible: (1) the direct $\text{H}\delta$ saturation component at 3.16 ppm, (2) the aforementioned NOE to neighboring side-chain protons at 1.68 ppm, (3) a component at about 4.73 ppm which is the NOE to the protons of surface-adsorbed water on TiO_2 particles, and (4) a broad component most clearly observable

in the 300 Hz profile. In the DEST saturation profile of the $\text{H}\delta$ proton of Arg, this broad component is centered at about 3.16 ppm and corresponds to a surface-immobilized molecular species. This broad component and strong NOE peak is also observed in the ^1H DEST profile for the $\text{H}\epsilon$ proton of Lys (Figures 2b and S2) but not in the DEST profiles for the side-chain protons in Leu, Asp, or Ala (see Figures 2c–e and S3–S5).

To confirm the assumption that ^1H DEST profiles for Arg in Figure 3 are interpreted in terms of four spectral components, inverted ^1H DEST saturation profiles of Arg (i.e. $1 - I/I_0$) were fitted to eq 1 for $B_1 = 50$ –300 Hz, as described in the data analysis section (Figure 4a–c and Tables S1–S3). It was assumed that the chemical shifts ω_i were constant for all B_1 values, while A_i and σ_i were varied. As expected, the intensity of the NOE peaks and broad ‘wing’ components are enhanced with increasing B_1 saturation power, although the NOE peak intensities shows relatively less sensitivity to B_1 saturation power than the broad ‘wing’ components (Tables S1–S3). Several conclusions can be drawn from inspection of the fitted data. First, the good agreement between the fits and the data validates the four-component assumption. Second, the direct saturation line is in phase with the NOEs, that is, the NOEs are negative. This indicates that Arg is tumbling slowly because of interactions with the NP surface. Third, the negative NOE to

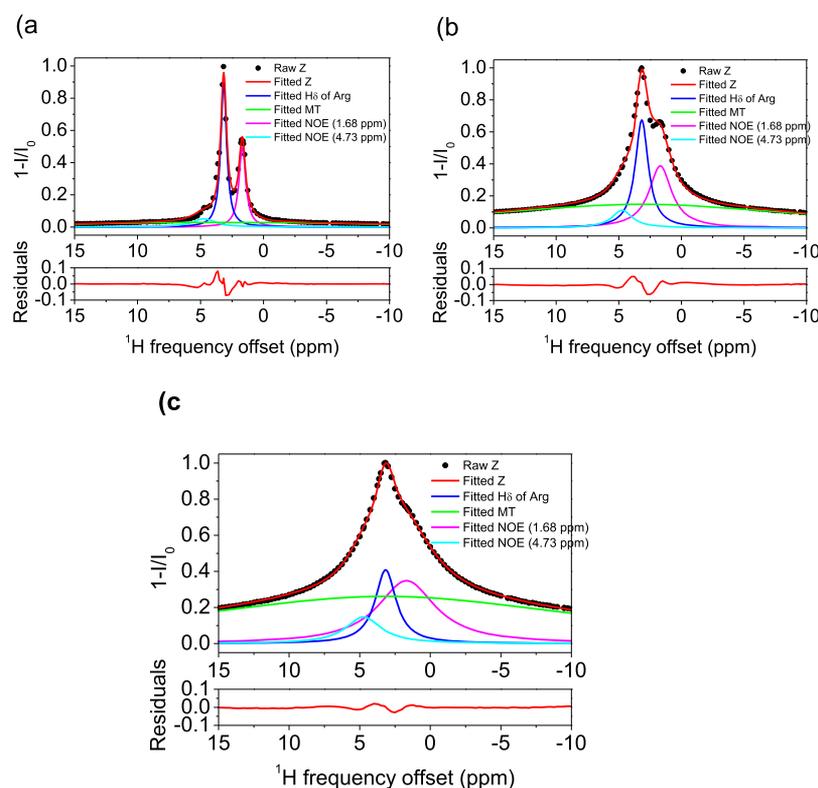


Figure 4. Fitted ^1H DEST profiles for $\text{H}\delta$ of 10 mM Arg in the presence of gel and 1 wt % TiO_2 at 50 (a), 150 (b), and 300 Hz (c). Z-spectra were fitted as the sum of multiple Lorentzian functions. The black dots show the raw data, the red (—) line is the fit of the raw Z function, the dark blue (—) line is the fitted $\text{H}\delta$ of arginine centered at 3.16 ppm, the green (—) line is the fitted MT signal or the strongly bound, immobilized species, the purple (—) line is the NOE centered at 1.68 ppm, and the cyan (—) line is the NOE centered at 4.73 ppm. Further discussion of the fitting can be found in the text.

water is likely not due to MT to bulk water but rather involves water that is bound to the TiO_2 NP surface. Finally, these simulations indicate the presence of two bound forms of Arg: a weakly bound form which undergoes slow reorientational motions and is closely associated with surface water molecules and an immobilized form. A similar analysis has also been performed on the ^1H DEST profile for the $\text{H}\epsilon$ proton of Lys with similar results (Figure S6).

In contrast to Lys and Arg, the DEST profiles for the side-chain protons of Asp, Leu, and Ala lack the broad component feature (Figure 2c,d, and e) and NOE intensities to proximal protons are not observed or are much weaker than is the case for protons in Arg and Lys (Figures S3–S5). These data indicate that Asp, Leu, and Ala do not have multiple forms of surface-attached species, these amino acids do not display a strongly surface-attached, immobilized form, and reorientational motions are much faster than is the case for Lys and Arg, indicating much weaker interactions with the TiO_2 NP surface.

To obtain surface adsorption/desorption rates, transverse relaxation R_2 rates and the populations of the bulk solution versus adsorbed species, DEST saturation profiles were simulated by numerical solution of the Bloch–McConnell equations.⁴⁹ The multi-Lorentzian fittings to the inverted DEST saturation profiles for 10 mM Arg in the presence of TiO_2 NPs indicate the presence of at least two adsorbed forms of Arg. The simplest kinetic scheme consistent with the simulated profiles in Figure 4 is shown in Figure 5. Figure 5 is based on a model where free amino acid in bulk solution is in exchange with two physically distinct adsorbed forms: a partially mobile bound form and an immobilized bound form.

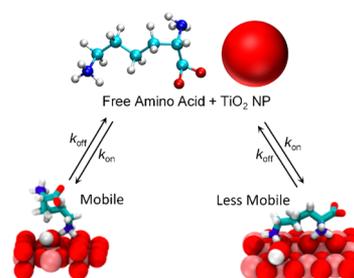


Figure 5. Kinetic scheme for adsorption of free amino acid onto TiO_2 NPs. The amino acid in bulk solution adsorbs onto the TiO_2 surface in two forms: a partially mobile form and a form that is strongly held on the surface and is more extensively immobilized.

Adsorption of the amino acid in bulk solution to these bound forms is characterized by the kinetic constant k_{on} . Desorption from the surface to bulk solution is characterized by k_{off} , respectively. Exchange between the surface-bound forms was excluded from this model.

^1H DEST profiles of 10 mM Arg in 1 wt % TiO_2 and 1 wt % agarose at a proton Larmor frequency of 700 MHz were simulated using the program DESTfit according to protocols described in detail in refs.^{28,50} As expected from the multi-Lorentzian fits in Figure 4, the DEST profile for 10 mM Arg is fitted best by a model involving exchange between the amino acid in bulk solution A and two adsorbed forms B and C. The best-fit model is shown in Figures 6 and S7 and corresponds to the pseudo-two site exchange described in refs.^{28,50} where A exchanges with a single kinetic off rate k_{off} between a mixture

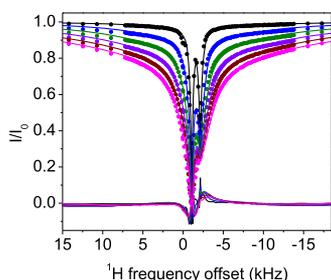


Figure 6. ^1H DEST profiles for $\text{H}\delta$ of 10 mM Arg in the presence of 1 wt % TiO_2 and 1 wt % agarose on a 700 MHz spectrometer with different B_1 saturation fields 50, 100, 150, 200, 250, and 300 Hz, and global fitting with a homogeneous form of McConnell equations using Matlab code DESTfit,^{28,50} with a single spin in exchange between an observable free state A with low R_2 and two bound states (B and C) with larger R_2 values ($A \rightleftharpoons$ the mixture of B and C). The cross-relaxation rate σ_A between $\text{H}\delta$ (the observed signal) and $\text{H}\gamma$ (coupled with $\text{H}\delta$ by cross-relaxation) in free Arginine is assumed to be -0.5 s^{-1} , and cross-relaxation rate σ_B between $\text{H}\delta$ and $\text{H}\gamma$ of Arginine bound on particles is assumed to be -500 s^{-1} , the output of global fitting: R_2 (strong binding) = $38,785 \pm 119 \text{ s}^{-1}$, R_2 (weak binding) = $784 \pm 2 \text{ s}^{-1}$ with population weights of 0.296 and 0.704, respectively, $k_{\text{off}} = 36.9 \pm 0.1 \text{ s}^{-1}$, $k_{\text{on}} = 2.9 \pm 0.0 \text{ s}^{-1}$, total population of binding state = 0.073, population of free state = 0.927, population of strong binding state = 0.022, population of weak binding state = 0.051.

of B and C forms. Notably, a two-site exchange model where A exchanges with a single bound form did not fit the data well. This case is included in the Supporting Information (Figure S8). A similar analysis of the ^1H DEST profile for Lys adsorbed onto TiO_2 NPs is also included in the Supporting Information (Figure S9).

MD Simulations. To investigate the nature and origins of structural diversity of adsorbed amino acids at liquid– TiO_2 NP interfaces and the role played by surface-adsorbed water and surface hydroxyl groups in molecular adsorption, metadynamics was used to compute the binding free energy as a function of amino acid center-of-mass distance from the nonhydroxylated (Figure 7a) and hydroxylated (Figure 8a) surfaces for Arg and Lys, both of which show broadened DEST profiles in the presence of TiO_2 NPs, as well as Asp, which does not show a broadened DEST profile in the presence of TiO_2 NPs. The calculation of binding free energy is described in the Supporting Information. On both surfaces, we see that

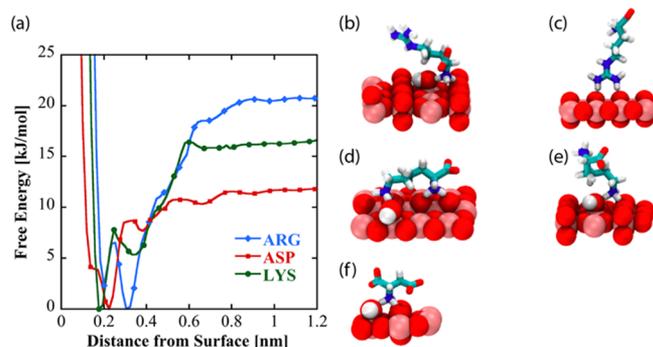


Figure 7. (a) Free-energy profiles as a function of amino acid distance from the low-hydroxylated surface of TiO_2 . Dominant binding conformations represented by these curves are shown for arginine (b,c), lysine (d,e), and aspartic acid (f) on the low-hydroxylated surface.

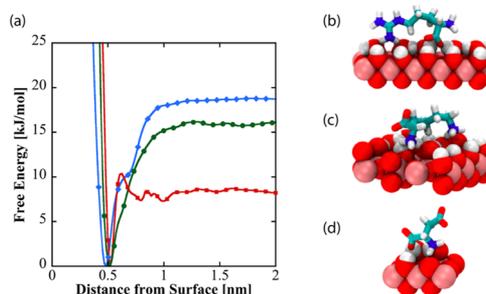


Figure 8. (a) Free energy as a function of amino acid distance from the hydroxylated surface of TiO_2 . Dominant binding conformations are shown for arginine (b), lysine (c), and aspartate (d) on the hydroxylated surface. Note: the peaks in Asp binding profile after 0.5 nm are within the thermal fluctuation (2.5 kJ/mol) at 300 K and do not indicate a different binding mode.

Arg is the strongest binder, followed by Lys and finally Asp. On the nonhydroxylated surface (Figure 7a), there are two free-energy minima for Lys and Arg, whereas Asp only shows a single minimum. Interestingly, on the hydroxylated surfaces, although the binding free energies for Arg and Lys are comparable to the nonhydroxylated surface, there is only a single minimum in the binding free-energy profile. Asp binds with a lower free energy on the hydroxylated surface compared to the nonhydroxylated surface. The binding free energies for Arg, Lys, and Asp on the nonhydroxylated and hydroxylated surfaces are given in Table 1.

Table 1. Binding Free Energies for the Three Amino Acids on the Two Surfaces in kJ/mol^a

	binding free energy (kJ/mol) non-hydroxylated surface		binding free energy (kJ/mol) hydroxylated surface
	mode 1 (flat)	mode 2 (extended)	
arginine	−17	−20	−17
lysine	−15	−10	−15
aspartate	−10		−6

^aThe binding modes indicate binding poses (flat vs extended) on the surface.

To understand the structural diversity of the amino acids on the surface, bound structures corresponding to the minima in the free energies for Arg (Figures 7b,c and 8b), Lys (Figures 7d,e and 8c), and Asp (Figures 7f and 8d) are shown. On the nonhydroxylated surface, Arg and Lys adopt distinct flat (Figure 7b,d, respectively) and extended (Figure 7c,e, respectively) conformations. The flat conformation for both (Figure 7b,d) is mediated by the binding of the N-terminus, whereas the extended conformation occurs through the side-chain binding. On the hydroxylated surface, both amino acids adopt a flat conformation, mediated by the side chain and C-terminus. This is in overall agreement with the features of the free-energy curve in Figure 8. In contrast, Asp adopts a single conformation on both hydroxylated and nonhydroxylated surfaces. There is also good agreement with prior simulation studies, which report that binding occurs through backbone and side-chain associations,⁵⁴ as well as the fact that arginine binding is the strongest.^{32,33}

Binding of Amino Acids with TiO_2 . Study of the binding of amino acids to inorganic oxide NP surfaces, and TiO_2 NP

surfaces in particular, is motivated by the fact that amino acids serve as models for the binding of more complicated polypeptides which are believed to interact with TiO₂ surface hydroxyl groups via amino acid side chains. Numerous experimental studies, cited above, have characterized the binding affinity of amino acids to TiO₂ NP surfaces. The present DEST NMR study, accompanied by a theoretical analysis of binding structures, characterizes not only the binding affinity of five amino acids (Arg, Lys, Leu, Asp, Ala) to TiO₂ NP surfaces but also the kinetics of binding. This is accomplished by simulation of the ¹H DEST saturation profiles through solution of the Bloch–McConnell equations, which yields *R*₂ values and populations as well as the kinetic constants that quantify exchange between the various free and bound species.

The kinetic information afforded by DEST simulations is useful for interpreting both the relationship between the free and multiple bound states and the degree to which interactions with the surface immobilizes the various bound molecular forms, where the transverse relaxation rate increases with the degree of immobilization of the molecule on the surface. It is interesting to compare the results of the DEST study to earlier studies of amino acids bound to TiO₂ surfaces and how kinetic information enhances our view of the binding mechanism. The ¹H DEST NMR studies indicate that the amino acids Leu, Ala, and Asp have only a single partly mobile bound form in exchange with the freely tumbling amino acid in bulk solution. The Asp result is in accord with an earlier reflectance IR study which detected at least two bound forms of glutamic acid (Glu) on TiO₂ surfaces but a single bound form for Asp.²⁶ The fact that Asp is weakly adsorbed onto the TiO₂ NP surface is indicated by its narrow DEST profile, which indicates the occurrence of reorientational motion even in the bound form. This weak binding is also in accord with the fact that at pD 7 the net charge on Asp is negative and TiO₂ has a negative surface charge.

From ¹H DEST profiles of side-chain protons however, both Arg and Lys have at least two bound forms on TiO₂ NP surfaces, which display in both cases varying degrees of reorientational motion, as indicated by very different *R*₂ values. An interesting conclusion of the DEST study of Arg involves the relationship between these weakly and strongly bound forms. In the DEST study of cholic acid and phenol to cerium oxide particles by Egner et al.,³¹ analysis of DEST saturation profiles for cholic acid indicated only a single weakly bound form. However, similar DEST studies of phenol indicated two bound forms: a weakly bound form displaying residual reorientational motion and a strongly bound form with a larger *R*₂ relaxation rate, indicating a greater degree of surface immobilization, a similar conclusion to that drawn by our DEST studies of Lys and Arg on TiO₂ NP surfaces. However, the kinetic information derived from Egner et al.'s study affords a different view of the binding mechanism than is given by the present study of amino acid binding to TiO₂ NPs. With reference to the kinetic scheme in Figure 5, Egner et al. found a best fit to their DEST profile, assuming that phenol does not directly attach from the bulk solution, where it freely reorients, to the surface, where it is in an immobilized state. Egner et al. also found a finite rate of exchange between the weakly and strongly bound forms, indicating that phenol binds strongly to cerium oxide NPs via a weakly bound intermediate, which retains some reorientational degrees of freedom.

The DEST analysis of amino acid attachment to TiO₂ NPs presented in this paper indicates a different mechanism of binding between basic amino acids Lys and Arg and rutile TiO₂ NPs than occurs between phenol and cerium oxide NPs. Again with reference to Figure 5, a model that best fits DEST profiles assumes for both Lys and Arg the absence of exchange between the weakly and strongly bound species and that Lys and Arg in solution interact directly with NP surfaces to form populations of weakly and strongly bound forms.

Because exchange between the two bound forms of Lys/Arg is absent in the best-fit model to the DEST data, we cannot propose that the partially mobile forms of Lys or Arg are binding intermediates. Therefore, we turn to the surface chemistry of TiO₂ as a possible source of variation in binding. It has long been known that dissociative adsorption of water onto rutile surfaces results in the formation of surface hydroxyl groups.^{51,52} Dissociative adsorption of water and subsequent formation of surface hydroxyl groups is known to be face sensitive.⁵³ The surface chemistry observed for TiO₂ NPs is also known to vary with crystal face and surface area.⁵⁴ Therefore, the occurrence of bound Lys and Arg amino acids with different *R*₂ values may arise when amino acids attach to NP faces with different surface hydroxyl densities, resulting in different degrees of immobilization.

MD simulations were used to assess the degree to which the variation of surface water and surface hydroxyl groups on the TiO₂ NP surface might account for these multiple bound forms of Lys and Arg. MD simulations in this paper treated two extreme cases: the complete absence and presence of surface hydroxyl groups. Under these conditions, good qualitative agreement was achieved with ¹H DEST data. For example, on nonhydroxylated surfaces, Arg was found to occur in two forms distinguished by two modes of surface attachment: (1) attachment via the guanidinium group and (2) attachment via the amino group. In each case, the opposite end of the amino acid was free to undergo restricted reorientational motions. We propose that these two forms would contribute to the so-called partly mobile bound form observed as a relatively narrow component of the DEST profile of the Hδ Arg proton. However, on hydroxylated TiO₂ surfaces, Arg is attached via both its side chain and its amino group, essentially immobilizing the molecule or restricting its motions to a much greater degree than is observed on nonhydroxylated surfaces. This would account for the broad components observed in the ¹H DEST profiles of Arg and Lys.

The binding mode of Arg on TiO₂ predicted by MD calculations can be further studied and confirmed experimentally by the analysis of relaxation data. For example, the binding mode of phenol on CeO₂ NPs at atomic resolution was studied based on the analysis of ¹³C *R*₂ and *R*₁ of phenol in the bound state.⁵⁵ Assuming that the bound molecule undergoes rapid restricted rotation on the surface, the ¹³C *R*₂ of phenol bound on CeO₂ NPs shows a dependence on the angle θ between the C–H bond vector and the axis of rotation, with maxima in *R*₂ occurring when the bond vector is parallel ($\theta = 0^\circ$) or antiparallel ($\theta = 180^\circ$) with the axis of rotation and minimum at angles $\theta = 54.74$ and 125.26° . In contrast, the ¹³C *R*₂ of phenol bound with Pt/CeO₂ is independent of the angle between the C–H bond vector and the axis of rotation and indicates that the phenol molecule is rigidly associated with Pt/CeO₂ particles. It will be interesting to similarly observe the position-specific relaxation of ¹³C–¹H vectors in Arg bound on TiO₂ by solution ¹³C NMR to determine the orientation of

Arg on the TiO₂ surface at atomic resolution. The results of such a study would further enhance the synergism between solution NMR and computational methods.

CONCLUSIONS

This paper describes an application of ¹H DEST NMR techniques and MD simulations to the study of the kinetics and thermodynamics of biomolecular adsorption onto rutile TiO₂ surfaces. In this work, basic amino acids are observed by ¹H DEST experiments to adsorb onto rutile TiO₂ surfaces in multiple forms distinguished by varying degrees of mobility on the surface. MD simulations indicate that variation in surface hydroxyl group density may be partly responsible for these observations.

Although this study focused on the adsorption of monomeric amino acids onto TiO₂ NP surfaces, ¹H DEST will be useful in general for studying the effect of surface heterogeneity in the binding of larger peptides and proteins to inorganic oxide surfaces. For example, a widely studied surface-binding peptide is the 12 amino acid peptide aptamer, that is, TBP-1 (RKLDPAPGMHTW) developed by Sano and co-workers used the peptide phage display methodology, which electrostatically interacts with the oxidized surface of Ti.^{16–18} The N-terminal hexapeptide RKLPGA is sufficient for TiO₂. Even in the case of this relatively small peptide, diverse views have been reported for its interactions with TiO₂ surfaces. Sano and co-workers proposed that the positively charged side chain of R₁ binds to acidic (–O[–]) hydroxyl sites, while the negatively charged side chain of D5 binds to basic (–OH₂⁺) hydroxyl sites.¹⁶ This binding model was supported by a subsequent adhesion force analysis using AFM.¹⁸ In contrast, an NMR study by Suzuki et al. of TBP-6 bound to TiO₂ NPs concluded that the peptide interacts with the NP surface via the side chains of R₁ and K₂, while the C-terminal amino acids do not display interactions with the surface.⁵⁶

Based on the results of this study, it is possible that the results of both of these studies are valid. The structure observed by Suzuki et al. may be a partly mobile form with a small R₂, while the fully immobilized or dark state form reported by Sano and co-workers possesses a much larger R₂. This situation, which may result from the heterogeneity of the NP surface chemistry, could be identified by a ¹H DEST study. We will report such a study in the near future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.langmuir.0c01256>.

Well-tempered metadynamics; ¹H NMR spectra of 10 mM amino acids lysine, leucine, aspartic acid, and alanine, in the absence of gel, in the presence of gel, and in the presence of gel and 1 wt % TiO₂; ¹H DEST profiles for Hε of 10 mM lysine, leucine, aspartate, and alanine in the absence of gel, in the presence of gel, and in the presence of gel and 1 wt % TiO₂; deconvoluted ¹H DEST profiles for Hε of 10 mM Lys as a function of B₁ field; simulations of Lys and Arg ¹H DEST profiles using global fitting with a homogenous form of McConnell equations using DESTfit; fitting parameters for Z-spectra of Arg and Lys; summary of kinetic parameters for the binding of 10 mM Arg and Lys with

TiO₂ using DESTfit; and simulation setup for Arg, Lys, and Asp on the four titania surfaces (PDF)

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Notes

The authors declare no competing financial interest.

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