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Hydroxyapatite Surface-Induced Peptide Folding

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Abstract: Herein, we describe the design and surface-binding characterization of a de novo designed peptide, JAK1, which undergoes surface-induced folding at the hydroxyapatite (HA)-solution interface. JAK1 is designed to be unstructured in buffered saline solution, yet undergo HA-induced folding that is largely governed by the periodic positioning of γ-carboxyglutamic acid (Gla) residues within the primary sequence of the peptide. Circular dichroism (CD) spectroscopy and analytical ultracentrifugation indicate that the peptide remains unfolded and monomeric in solution under normal physiological conditions; however, CD spectroscopy indicates that in the presence of hydroxyapatite, the peptide avidly binds to the mineral surface adopting a helical structure. Adsorption isotherms indicate nearly quantitative surface coverage and K_d = 310 nM for the peptide−surface binding event. X-ray photoelectron spectroscopy (XPS) coupled with the adsorption isotherm data suggests that JAK1 binds to HA, forming a self-limiting monolayer. This study demonstrates the feasibility of using HA surfaces to trigger the intramolecular folding of designed peptides and represents the initial stages of defining the design rules that allow HA-induced peptide folding.

Introduction

The function of most peptides is highly dependent on their ability to adopt a folded bioactive conformation.[1] However, peptides are typically conformationally mobile, and folded conformations are only realized after structural constraints, such as metal ion complexes[2−6] or disulfide bonds,[7−12] are imposed. In addition to typical structural constraints, surfaces provide a unique environment that can induce folding and stabilize selected conformations.[13−17] De novo peptide design can be used to help establish the rules by which different surfaces can trigger peptide folding. As a result of copious studies, it is now well-known that the amphiphilicity of a peptide's sequence can greatly influence its propensity to fold at a given surface.[18,19] For example, unfolded sequences with alternating hydrophobic residues having a periodicity of two (-[X-Hb-X-Hb]$_n$-) will often adopt β-strand and/or β-sheet structure at nonpolar surfaces such as an air−water interface.[20−24] Sequences that dispose their hydrophobic side chains with approximately 3.5 periodicity have a propensity to adopt helical structure at nonpolar surfaces.[18,25,26] In both of these examples, the side chains of the hydrophobic residues associate with the nonpolar surface, resulting in the conformational constraint of the backbone atoms of the intervening residues. With this mechanism, the surface imposes a folding preference on the naive sequence.

We are establishing design rules that allow peptides to fold at the surface of hydroxyapatite (HA), a common model of bone apatite. In this mechanism, peptide binding to the HA surface triggers the folding event. Herein, we describe the design of JAK1, a small peptide that is unstructured in solution under physiological buffer conditions (pH 7.4, 150 mM NaCl, 37 °C). However, when presented with an HA surface, JAK1 binds tightly to the calcium ions in the apatite lattice, adopting an α-helical conformation, Figure 1. This peptide offers high

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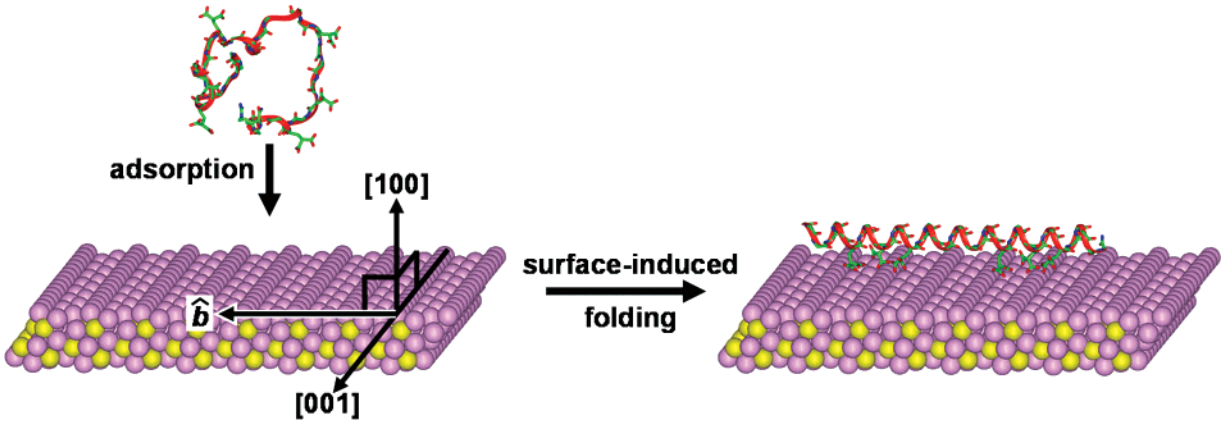

Figure 1. Model of surface-induced peptide folding. JAK1 (red ribbon) is designed to adopt a random coil conformation in solution; however, upon adsorption to the surface of HA, the peptide is designed to fold into an α-helix. This working model shows JAK1 binding to the (100) face of HA with the helical axis parallel to the crystallographic $\hat{b}$-axis. The HA lattice is represented as pink (calcium) and yellow (phosphorus) spheres where oxygen, hydrogen, and surface phosphorus atoms have been removed for clarity.

surface coverage, forming a self-limiting monolayer of helices at the HA surface.

Mineral surfaces such as HA represent a unique stimulus to trigger peptide folding.[27] The crystal lattice of HA ($Ca_{10}(PO_4)_6(OH)_2$) presents calcium ions in highly regular, repeating geometries that can be utilized for peptide binding.[28] Sequences that contain residue side chains that have an affinity for calcium such as aspartic and glutamic acid (carboxylates) or phosphorylated residues would be expected to bind to HA. For example, polyglutamic acid fusions have been used to adsorb peptides to HA surfaces.[29−31] In addition, Stayton has identified a fragment of statherin, an enamel binding protein, that binds to HA via phosphorylated serine residues at its N-terminus.[32,33] Last, although distinct from HA, an aspartate-rich helix that binds to calcite was designed by Laursen et al.[34]

Designing a peptide that folds at the surface of HA necessitates that one peptide sequence be developed that remains unfolded in solution under physiological conditions but adopts a stable, folded conformation upon binding to HA. We began the design of JAK1 by first considering the folded HA-bound state and generated a model of the hydroxyapatite lattice (Hyperchem, Hypercube) to target the (100) face for binding. Inspection of the (100) face of HA reveals that calcium ions are presented periodically along the crystallographic $\hat{b}$-axis at intervals that coincide with the distance between the repeating turns of an α-helix, Figures 1 and 2. Nature is cognizant of this coincidental spacing; the crystal structure of the bone matrix protein, osteocalcin, suggests that this protein binds calcium along one face of its N-terminal helix via γ-carboxyglutamic acid (Gla) residues that are located at its turns.[35] In addition, there is strong biophysical evidence that osteocalcin binds most avidly to the (100) face of HA when presented with alternate crystallographic faces.[36]

We designed a 36-residue peptide, JAK1, that when folded adopts an α-helix capable of presenting Gla side chains from one of its faces in an amphiphilic manner. The dicarboxy-containing side chain of Gla is a strong chelator of calcium and is found in naturally occurring proteins whose calcium binding activity is essential to their activity.[37−40] JAK1 has a total of six Gla residues, a set of three at the N-terminus of the helix at *i*, *i+4*, and *i+7* positions and a second set of three at the C-terminus with the same sequential arrangement. Molecular modeling (Insight, Accelyrs) suggests that each set of Gla residues presents its side chains spaced at 4.5−6.0 Å intervals in approximate register with the calcium ions contained along the $\hat{b}$-axis of the (100) face (Figure 2A, B**)** and that a minimum of 8 calcium ions are capable of forming Ca−O bonds with the Gla side-chain oxygens serving as bidentate ligands. Although the (100) face of HA serves as our initial target, the spacing of calcium ions contained in alternate faces is similar and may also support helical peptide binding.

The Gla side chains (Figure 2C) also play an important role in keeping JAK1 unfolded in the absence of the HA surface. Each side chain carries a formal −2 charge that must be accommodated on the same face of the helix if the peptide were to fold in the absence of calcium, resulting in a high density of similar charge. The energetically unfavorable interactions between Gla side chains should help keep the peptide unfolded in the absence of calcium. The choice and sequential positioning of the remaining residues is also important to ensure that JAK1 remains unstructured and monomeric in solution. Solution-phase peptide aggregation could lead to folding as is the case with many de novo designed amphiphilic peptides that are unstructured as monomers but self-assemble to form coiled-coil motifs; here, self-assembly is largely driven by the association of nonpolar residues that facially line each helix in the folded coiled

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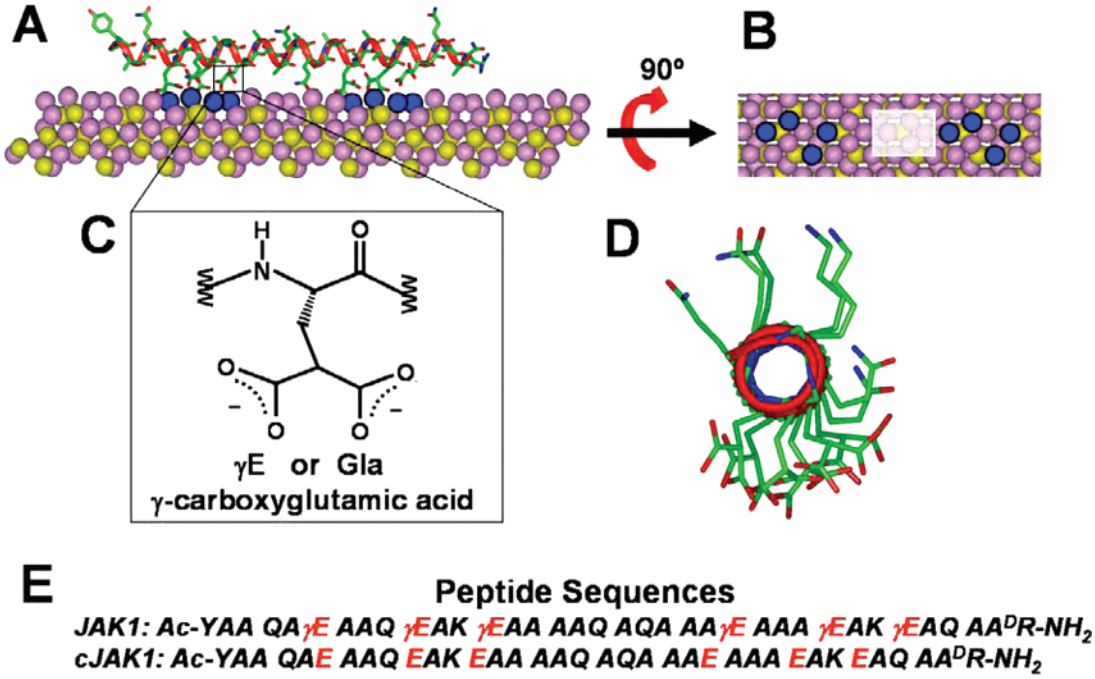

Figure 2. (A) Model of JAK1 bound to the (100) face of HA (side view from the (001) HA face). Calcium atoms in blue are predicted to bind to Gla residues based on modeling optimal Ca−O distances and angles. Lattice atoms positioned in front of the blue calcium atoms were removed for clarity. (B) Arrangement of Gla-bound calcium atoms (top view from (100) face). The gray box defines the HA unit cell. (C) Structure of Gla residue. (D) Axial view of JAK1 in folded state. (E) Sequences of JAK1 and cJAK1. γE is γ-carboxyglutamic acid.

coil.[41−46] For JAK1, aggregation-induced folding is designed against by incorporating polar glutamine (Gln)[47] and lysine residues on the face of the helix that is opposite the Gla residues, Figure 2D. Alanine (Ala) was chosen to occupy most of the remaining positions based on the extensive work of Baldwin et al. that shows that alanine-rich peptides of moderate length are largely helical at low temperature but unfold at physiological temperature (37 °C).[48,49] For Baldwin, decreasing temperature was used to induce folding; for JAK1, the surface of HA will be used. Last, a D-arginine was incorporated at the C-terminus of the peptide to further stabilize the HA-bound helix via a putative side chain−main chain capping interaction,[50] and tyrosine was placed at the N-terminus as a spectroscopic probe for concentration determination. These design attributes culminate in JAK1 whose sequence is shown in Figure 2E. We also prepared a control peptide to assess the importance of the Gla residues for HA binding, where all the Gla residues in JAK1 were replaced with glutamic acid (Glu), a structurally similar residue containing only one carboxylic acid group. JAK1 and the control peptide, cJAK1 were synthesized by Fmoc-based solid-phase peptide synthesis, purified to homogeneity via RP-HPLC, and characterized by ESI MS, see Supporting Information.

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Results and Discussion

Folding Response toward Soluble Calcium and Hydroxyapatite. Although JAK1 is designed to fold at the surface of HA, we first studied its calcium-dependent folding using freely soluble calcium in solution by circular dichroism (CD) spectroscopy. Solution-based experiments provide a rapid assessment of JAK1's folding propensity and allow the determination of the threshold concentration of free calcium that will initiate folding. This is important in that JAK1 should fold at the surface of the apatite lattice where the local calcium concentration is high and not in solution as a result of soluble calcium that has leached from the apatite or that is inherently present in the media. Figure 3A shows CD spectra for a 50 μM solution of JAK1 at pH 7.4 (50 mM BTP, 150 mM NaCl, 37 °C) as a function of added $CaCl_2$. Negative values of mean residue ellipticity at 208 and 222 nm indicate the formation of α-helical structure. In the absence of calcium, JAK1 is largely unstructured. Adding incremental amounts of $CaCl_2$ results in a spectrum consistent with α-helical secondary structure. JAK1 does not begin to fold until about 10 mM of $CaCl_2$ has been added, and maximal helicity is not reached until after the addition of at least 160 mM $CaCl_2$. These results indicate that JAK1 does not appreciably fold in response to low millimolar concentrations of soluble calcium. Therefore, soluble calcium leached from HA or the free calcium found in common buffers or biological fluids (e.g., in serum), should not induce folding. The inset in Figure 3A shows a similar titration for the control peptide, clearly indicating that the control is unable to undergo calcium-dependent folding even when presented with 200 mM $CaCl_2$; this strongly suggests that Gla-mediated calcium binding induces folding for JAK1.

HA surface-induced folding was assessed by measuring the CD of JAK1 bound to ceramic HA powder. In this CD slurry

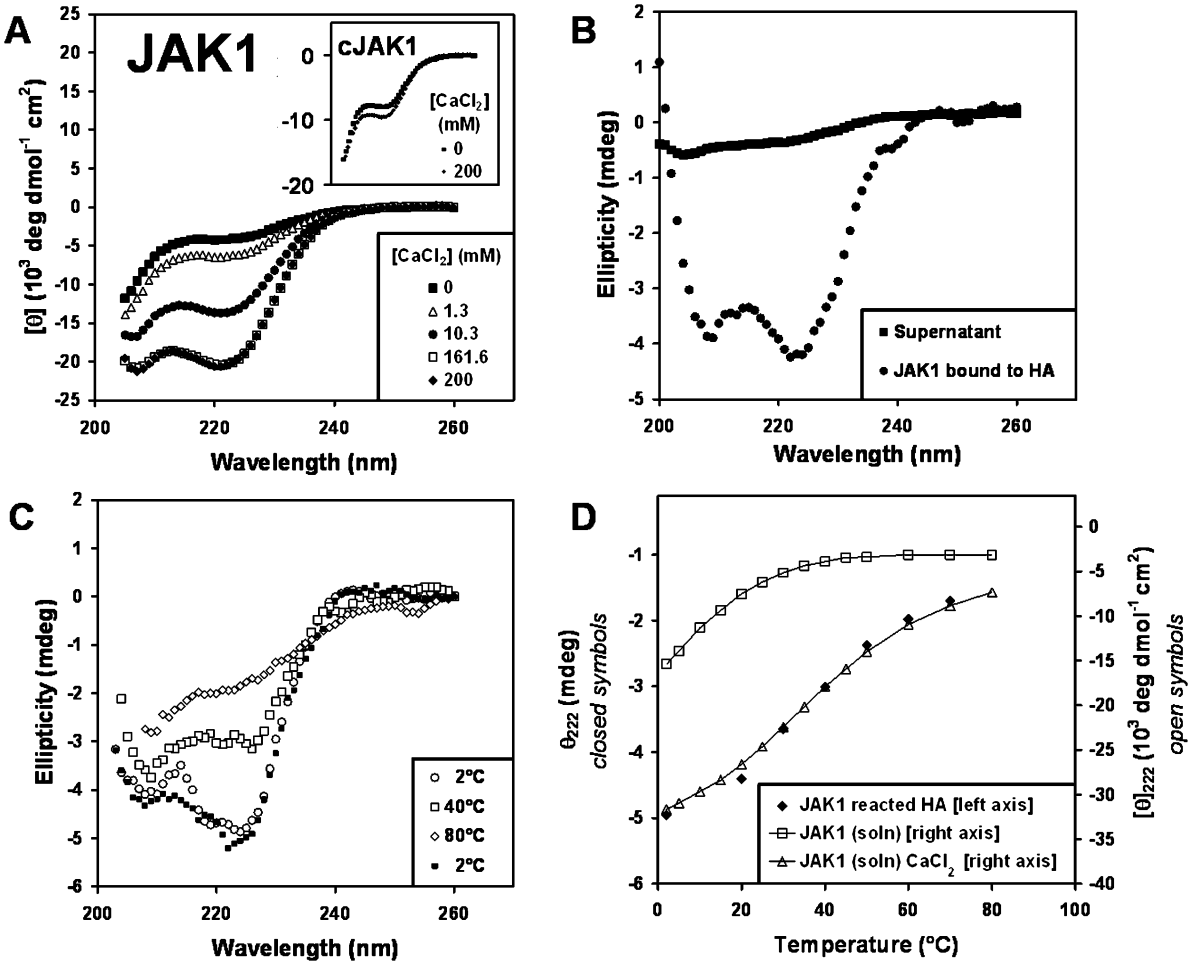

Figure 3. (A) Calcium-dependent CD spectra of 50 μM JAK1 and cJAK1 (inset) solutions (37 °C, pH 7.4, 50 mM BTP, 150 mM NaCl). (B) CD spectrum of JAK1 bound to ceramic powder HA (20 °C, pH 7.4, PBS). (C) Temperature-dependent CD spectra of JAK1 bound to HA (pH 7.4, PBS); the refolding curve is represented by the solid square symbols. (D) Ellipticity and mean residue ellipticity measured as a function of temperature for JAK1 bound to HA (left axis, closed symbols, pH 7.4, PBS) and in the absence and presence of 100 mM $CaCl_2$ (right axis, open symbols, pH 7.4, 50 mM BTP, 150 mM NaCl).

experiment, a low concentration of finely powdered HA (80 μm particle size) was used to limit light scattering, yet allow measurable signal with relatively low noise. Samples were prepared by incubating 10 mg of ceramic HA powder in 200 μL of a 1 mM JAK1 solution in phosphate buffer saline (PBS) at pH 7.4. After 10 min, peptide-coated HA was isolated by centrifugation, washed, and suspended in PBS, affording a 0.5 mg/mL slurry of HA. After spectra were collected, the slurry was centrifuged, and the spectrum of the resulting supernatant was recorded. The CD spectrum of JAK1-coated HA shows minima at 208 and 222 nm, which is indicative of α-helix formation (Figure 3B), and mirrors the results from the solution-phase CD experiments that demonstrate that JAK1 folds in the presence of high concentrations of soluble calcium. For the slurry experiment, raw ellipticity is reported as opposed to mean residue ellipticity since the concentration of adsorbed peptide is not known. The relatively featureless spectrum of the supernatant after HA removal demonstrates that there is no free peptide in solution, indicating that bound peptide does not desorb under these experimental conditions. The CD spectrum of uncoated HA (not shown) is similar to that of the supernatant. Although the data in Figure 3B is a strong indication that JAK1 adopts a helical conformation at the HA surface, thermal denaturation of the HA-bound peptide was attempted to assess whether the peptide could undergo a typical α-helix-to-random coil transition upon heating, Figure 3C. As the temperature is increased, JAK1 unfolds at the surface, adopting a random coil conformation. It is not known at this time whether the peptide desorbs to unfold or unfolds while still bound to the surface. However, cooling the slurry results in reversible helix formation; also the CD spectrum of the supernatant after refolding indicates that nearly all the peptide is bound to the HA surface. Figure 3D shows thermal denaturation data where the ellipticity at 222 nm for JAK1 bound to HA (left axis) and the mean residue ellipticity at 222 nm for soluble JAK1 in the absence and presence of 100 mM $CaCl_2$ (right axis) are measured as a function of temperature. It is evident that the helical conformation is stabilized either in the presence of high concentrations of soluble calcium (compare open symbol data) or in the presence of HA. More interesting, the increase in thermal stabilization of the peptide due to HA binding is exactly the same as that for peptide in solution at high $CaCl_2$ concentration, supporting the use of preliminary solution-based studies to investigate the propensity of designed peptides to fold at HA surfaces. Collectively, these studies show that JAK1 folds at the surface of HA and that the Gla residues most likely mediate binding.

Assessment of Hydroxyapatite Surface Coverage and Binding Affinity. X-ray photoelectron spectroscopy (XPS) was used to directly assess the binding of JAK1 and the control

Table 1. Relative Percentages of Carbon, Nitrogen, Oxygen, Calcium, and Phosphorus Calculated from High-Resolution XPS Spectra of HA Incubated in PBS Buffer, 1 mM JAK1, and 1 mM cJAK1 Solutions (37 °C, pH 7.4, PBS, 4 h)

HA incubated with:	% carbon	% nitrogen	% oxygen	% calcium	% phosphorus
buffer	4.5 ± 0.1	0.0 ± 0.1	51.7 ± 0.5	24.6 ± 0.2	18.9 ± 0.2
1 mM JAK1	12 ± 2	2.7 ± 0.7	46.2 ± 0.7	22.2 ± 0.8	16 ± 1
1 mM cJAK1 control	5.4 ± 0.4	0.0 ± 0.1	51.1 ± 0.5	24.7 ± 0.1	18.6 ± 0.7

peptide, cJAK1, to the surface of HA by measuring the relative amounts of each element found at the surface of peptide-treated HA. XPS analysis entails bombarding the HA sample surface with X-rays, resulting in the emission of core-level electrons (photoelectrons) from atoms which are present within a few nanometers of the HA surface. These photoelectrons exit the material with a kinetic energy that can be measured and used to determine the electron's binding energy in the atom from which it came. Nominal values of this binding energy indicate the element, while small shifts in this binding energy, so-called chemical shifts, indicate the chemical state of the atom, much like NMR chemical shifts. Two key pieces of information are extracted from this analysis: (1) the atomic percentages of each element (except hydrogen and helium) bound at the surface of a material and (2) quantitative information about the chemical states of each element present at the surface.

For these experiments, ceramic HA powder was incubated in buffer alone (control), in a solution of JAK1, or in a solution of cJAK1, all at pH 7.4 and 37 °C followed by thorough washing with water. Table 1 lists the atomic percentages of elements, common to both HA and peptide, that are found within ~2 nm of the surface. The surface of HA powder incubated in buffer alone contained primarily oxygen, calcium, and phosphorus, as expected for an exposed hydroxyapatite surface; in addition, a small carbon impurity was present even for well-washed HA, as is usually the case for non-carbonaceous powdered mineral surfaces examined by XPS. Importantly, only a negligible amount of nitrogen, an element inherent only to the peptide in these experiments, could be detected. After incubation with JAK1 under physiological conditions, the surface composition of the HA powder included a significant amount of carbon and nitrogen, attributable to the adsorption of JAK1 on the HA surface. Also, the percentages of oxygen, calcium, and phosphorus were slightly reduced. This is expected if JAK1 is forming a self-limiting monolayer whose height is defined by the diameter of the JAK1 helix (~1 nm); in this scenario, the peptide coating modestly attenuates detection of the underlying hydroxyapatite lattice. If JAK1 were forming a multilayered coating, XPS detection of HA-based elements would be strongly diminished. In contrast, the surface of HA powder incubated with cJAK1 displayed an elemental composition similar to that of the buffer control, indicating that this peptide, lacking the Gla residues, shows little propensity for HA surface adsorption.

High-resolution XPS spectra of the carbon 1*s* and nitrogen 1*s* regions are shown in Figure 4. The carbon spectrum exhibits the multicomponent peak shape expected for adsorbed peptide.[51] The C 1*s* peak was fit to four components for aliphatic (CH_2), phenolic/amine (C−O, C−N), amide (N−C═O), and carboxyl (O−C═O) carbons. The fitted percentages for each of these carbon species closely agreed with the hypothetical percentages (shown in parentheses below) determined from the molecular

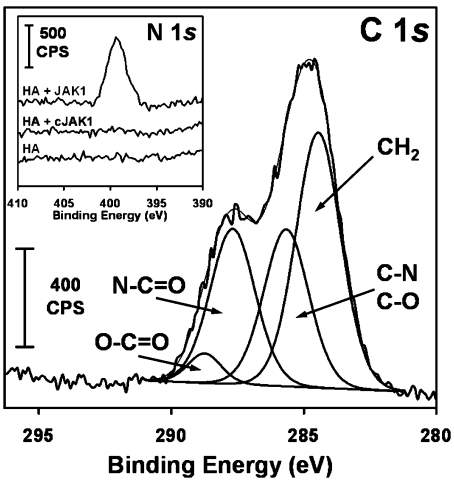

Figure 4. High-resolution X-ray photoelectron spectra of JAK1 adsorbed to HA (pH 7.4, 37 °C, PBS). Carbon 1*s* region was fit with four components; CH_2, C−O/C−N, N−C═O, O−C═O, and intensity indicated by the scale bar is 400 counts per second. (Inset) Nitrogen 1*s* region for HA incubated in PBS buffer, cJAK1, or JAK1 solutions, and scale bar indicates intensity of 500 counts per second.

formula of JAK1: CH_2: 43 ± 2% (38%); C−O and C−N: 25 ± 3% (26%); N−C═O: 27 ± 3% (28%); and O−C═O: 5 ± 2% (8%). The small difference between the aliphatic carbon values (43 vs 38%) is most likely due to the carbon impurities in the ceramic HA (see Supporting Information). The N 1*s* spectra (Figure 4 inset) shows definitive evidence for the presence of nitrogen on the HA surface after incubation with JAK1. No nitrogen was detected on the HA surface prior to incubation with JAK1 peptide; however, after incubation with JAK1, a clear nitrogen signal was evident, indicating $2.7 \pm 0.7\%$ nitrogen. The XPS spectrum of HA incubated in cJAK1 displayed no increase in nitrogen, demonstrating that the control peptide, cJAK1, which contains Glu in place of Gla, did not bind to the HA surface. In contrast, JAK1 bound to the HA surface under physiological conditions and did not desorb after vigorous washing in deionized water.

Quantitation of the absolute coverage of bound peptide was achieved by constructing adsorption isotherms for JAK1 and bovine serum albumin (BSA, positive control), Figure 5. Adsorption isotherms were collected in a manner similar to those used by Langmuir to examine monolayer adsorption of gases on surfaces. The classical Langmuir adsorption isotherm displays the dependence of surface coverage on the equilibrium pressure or solution concentration. The Langmuir model requires that the adsorption process be reversible, a characteristic that we have not yet rigorously tested for JAK1 adsorption to HA. Nevertheless, it is common to use Langmuir-type experiments to compare the binding of peptides and proteins to HA and to estimate maximal surface coverage, regardless of the reversibility of the process.[33,36,52,53] The data in Figure 5A were

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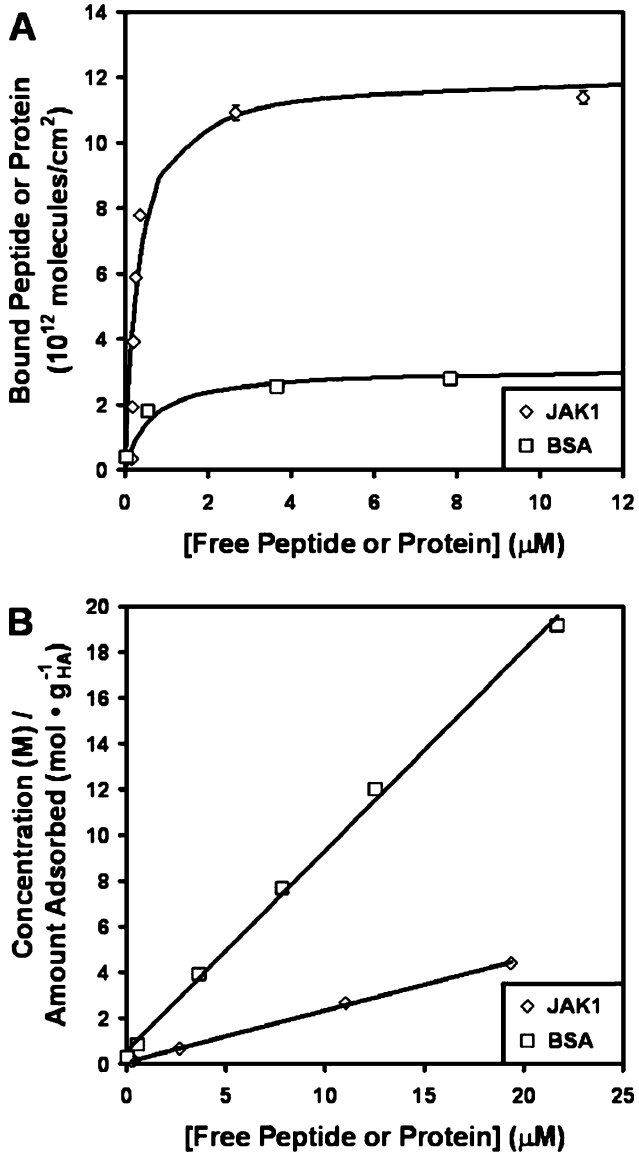

Figure 5. (A) Adsorption isotherms of JAK1 and bovine serum albumin (37 °C, pH 7.4, 50 mM HEPES, 150 mM NaCl). [Free Peptide or Protein] measured by fluorescence. Error bars for the BSA control are smaller than the symbol height. Maximum coverage (M) is $(12.1 \pm 0.5) \times 10^{12}$ molecules/cm^2 for JAK1 and $(3.1 \pm 0.5) \times 10^{12}$ molecules/cm^2 for BSA. (B) Linear plot of adsorption data according to eq 1. Dissociation constants (K_d) are 310 ± 160 nM for JAK1 and 630 ± 260 nM for BSA. Binding analysis performed in triplicate; error bars are smaller than the symbols. Errors in the binding constants are derived from the standard error in the least-squares determination of the slopes and intercepts of the fit data.

generated by incubating nanomolar to micromolar concentrations of JAK1 or BSA with known amounts of HA powder at pH 7.4, 37 °C, for 4 h. The HA powder containing bound peptide/protein was removed by centrifugation, and the concentration of peptide/protein remaining in the supernatant was determined by fluorescence. The amount of bound JAK1 or BSA was determined by difference and normalized to the total surface area of the HA powder, which was determined by nitrogen adsorption using the Brunauer−Emmett−Teller method in a separate experiment.[54] The maximum coverage of JAK1 on the HA surface was found to be $12.1 \pm 0.5 \times 10^{12}$ molecules/cm^2, corresponding to nearly 100% coverage based on the computationally measured footprint of one JAK1 helix. This data, taken together with the XPS data, strongly suggest that JAK1 forms a self-limiting monolayer. The binding of JAK1 was compared to that of bovine serum albumin ($3.1 \pm 0.5 \times 10^{12}$ molecules/cm^2) which is known to bind to HA with an affinity and coverage that is comparable to the HA-binding protein, osteocalcin.[54] The observed higher coverage for JAK1 compared to that for BSA is both promising and expected. It is expected that a larger number of smaller-sized peptide molecules can occupy a given area of HA surface, given the much larger molecular footprint of BSA. Importantly, the adsorption isotherm of the control peptide, cJAK1 (see Supporting Information), indicates that the control peptide does not significantly bind to HA even at high micromolar concentrations (800 μM), again demonstrating the importance of the Gla residue in HA−surface binding.

The affinity of JAK1 and BSA toward HA was calculated by fitting the adsorption isotherm data to a linear form of the Langmuir equation. For surfaces, the K_d is a measure of the desorption probability according to following equilibrium:

$$\mathrm{HA}_{\mathrm{SurfaceSite}} + \mathrm{JAK1}_{\mathrm{Solution}} \rightleftharpoons \mathrm{JAK1}_{\mathrm{Adsorbed}}$$

$$\frac{C}{m} = \frac{C}{M} + \frac{K_d}{M} \qquad (1)$$

Presenting the isotherm data linearly to show the concentration dependence of surface adsorption yields data that can be fit to determine K_d according to eq 1, where C is the concentration of peptide or protein in solution in equilibrium with the HA surface (mol/L), m is the amount of peptide or protein bound to the surface as a function of peptide/protein concentration (mol/g$_{HA}$), M is the maximum coverage of peptide or protein possible per gram of HA (mol/g$_{HA}$), and K_d is the dissociation constant (mol/L). Note that the units of M have been converted from molecules/cm^2 in panel A to mol/g$_{HA}$ in panel B by dividing M by Avogadro's number and multiplying by the specific surface area of HA previously determined by BET analysis.

Such an analysis is shown in Figure 5B for JAK1 and for the BSA positive control. From this plot the K_d for JAK1 adsorbed to HA was determined to be $K_d = 310 \pm 160$ nM. This value is approximately 50-fold better (i.e., stronger binding affinity) than has been measured for polyglutamic acid sequences on HA.[55] The K_d for BSA was determined to be $K_d = 630 \pm 260$ nM, approximately half the affinity of JAK1. This implies that on a per-residue basis, compared to BSA, JAK1 (with 36 residues) binds very strongly to HA. Although JAK1 was designed to bind at the (100) face of HA, the isotherm data indicating quantitative surface coverage of ceramic apatite suggests that additional exposed HA faces facilitate binding. This is expected since the ceramic apatite used in these studies (Bio-Rad Laboratories, Macro-Prep Ceramic Hydroxyapatite, type II, 80 μm diameter, 800−1000 Å pore size, cat. #158-826) is spherical and exposes many crystallographic faces. However, future experiments are aimed at assessing whether the (100) face is being selectively bound over others.

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Conclusion

JAK1, a de novo designed peptide capable of folding into a helical conformation at the surface of hydroxyapatite, is presented. This 36-residue peptide remains unfolded in aqueous buffer at pH 7.4, 150 mM NaCl, 37 °C until it binds to, and folds at, the surface of HA. CD spectroscopy confirms peptide folding at the HA surface, and XPS and adsorption isotherm data indicate that JAK1 binds with high affinity, most likely forming a self-limiting monolayer that offers quantitative coverage. The seminal feature of this peptide's design is that in the folded state, JAK1 contains six γ-carboxyglutamic acid (Gla) residues, each located on consecutive turns of the helix at its N- and C-termini. A control peptide, cJAK1, where the Gla residues are replaced with glutamic acid residues does not appreciably bind to, or fold at, the surface of HA, indicating that the dicarboxylated Gla residues in JAK1 facilitate HA binding and consequent folding. The surface-induced folding of JAK1 demonstrates that peptide design can be used to generate a sequence that adopts a predictable conformation in response to an inorganic surface.

Designing peptides that fold at surfaces could possibly lead to the temporal and spatial control of their function. The function of many peptides is highly dependent on their ability to adopt a folded bioactive conformation. Nature uses folding events to turn on biological activity by presenting stimuli to unstructured sequences that initiate folding and consequent function; in this way, temporal and spatial resolution of function is achieved by controlling when and where folding takes place within an organism. The implication of the surface-induced folding mechanism developed for JAK1 is that biological activity could potentially be turned on at the surface of HA by eventually linking function to the surface-induced folding event.

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Supporting Information Available: General methods for the synthesis and purification of JAK1 and cJAK1 peptides, CD experiments, sedimentation equilibrium analytical ultracentrifugation, X-ray photoelectron spectroscopy, Brunauer−Emmett−Teller surface area measurements, adsorption isotherms, and affinity determination and the associated spectra and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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