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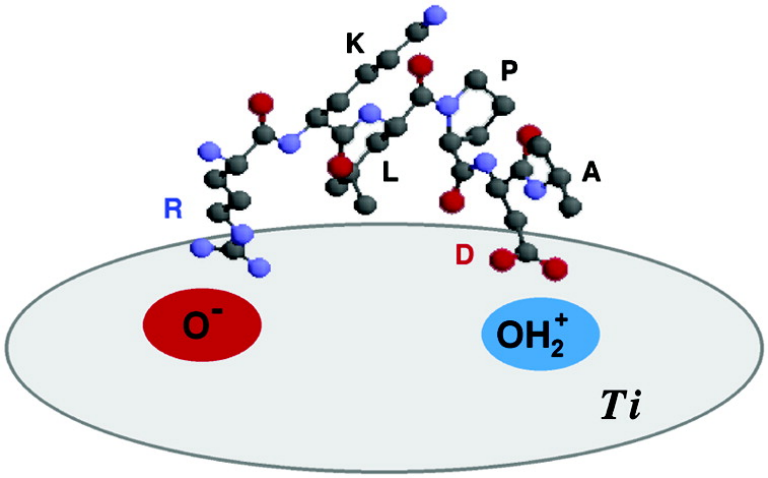

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A Hexapeptide Motif that Electrostatically Binds to the Surface of Titanium

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With its light weight and toughness, titanium (Ti) holds a special place among the biomaterials used for medical implants. But although it is now routinely used for dental implants and artificial joints, there are still problems with its biocompatibility.[1,2] With the long-term goal of developing artificial proteins that improve the biocompatibility of materials, we set out to isolate peptide motifs that bind to the surface of Ti. Here, we describe our use of peptide phage display methodology[3] to isolate a peptide aptamer that electrostatically interacts with the amphoteric surface of Ti.

We used a peptide phage library that displays a linear 12-mer peptide with a diversity of $\sim 2.7 \times 10^9$ (Ph.D.-12 Phage Display Peptide Library Kit, New England Biolabs) that is now routinely used for isolating peptide aptamers against inorganic materials.[4] After three rounds of panning procedures carried out against Ti particles (Supporting Information), we observed that 33 of 43 phages displayed on their surfaces peptides having the identical sequence (Figure 1A and Supporting Information). We confirmed that, after cloning, the predominant phage retained the ability to bind to a Ti particle but did not bind to bovine serum albumin (BSA), which was used as a blocking agent in the panning experiments (data not shown). We named the peptide sequence Ti-12-3-1 and the cloned phage displaying it ϕTi-12-3-1. Cloned phages other than ϕTi-12-3-1 did not show significant binding (Supporting Information).

ϕTi-12-3-1 bound to the surface of Ti particles at a density of 5×10^4 pfu/mm^2, whereas phages displaying unrelated peptides or no peptides bound at densities of $(1-3) \times 10^2$ pfu/mm^2. Notably, the binding of ϕTi-12-3-1 was unaffected by increases in the ionic strength of the buffer (Supporting Information), indicating hydrophobic interaction was not a major factor in the binding.

The adsorption of ϕTi-12-3-1 to the surface of a Ti particle was further characterized using a quartz crystal microbalance and energy dissipation (QCM-D, Q-Sense), which measures changes in the frequency (Δf) and energy dissipation (ΔD) of Ti-deposited quartz crystal.[5] Figure 1b shows the changes in Δf and ΔD elicited by nonspecific adsorption of BSA, being washed with buffer, and the binding of peptide phages. A marked increase in ΔD was observed upon adsorption of ϕTi-12-3-1, whereas little change was observed upon absorption of a control phage. The significant increase of ΔD suggests that only the very end of the ϕTi-12-3-1 particle (where the Ti-12-3-1 peptide is displayed) interacted with the Ti sensor, thereby augmenting the viscoelasticity of the sensor's surface; the remainder of the phage particle (6.5 nm $\times$ 930 nm) did not interact with the Ti sensor (Figure 1c).[6]

To identify the amino acid residues involved in the binding of the peptide to the Ti and to gain additional insight into the mode of the binding, we constructed a group of 11 alanine (whose side chain is an uncharged methyl group) substitution mutants and investigated their effects on phage (Figure 2). We found that by changing the charged side chain of the arginine at position 1 (R1A), the aspartic acid at position 5 (D5A) or especially the proline at

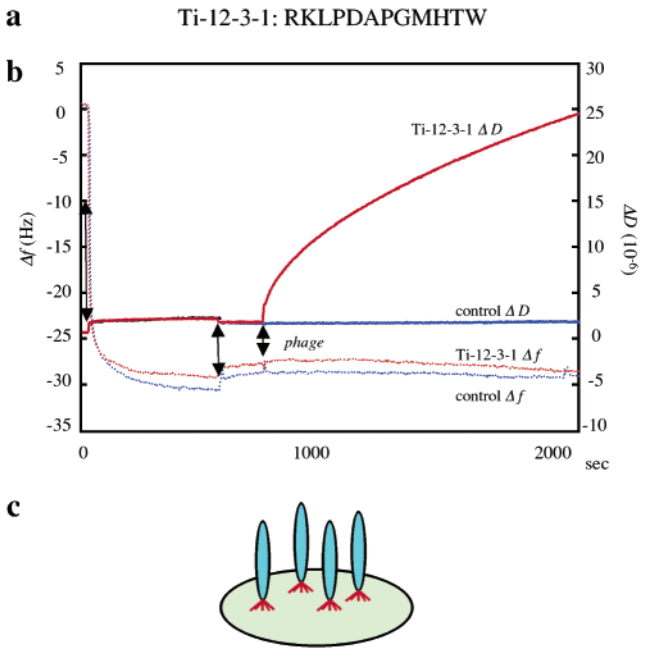

Figure 1. (a) Amino acid sequence of Ti-12-3-1. (b) Changes in frequency (Δf; left axis) and dissipation (ΔD; right axis) as a function of time during incubation of ϕTi-12-3-1 and the control phage (which displays no peptide). Ti sensors were equilibrated in 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl at 24.7 °C. BSA was used as a blocking reagent at a concentration of 0.1% (w/v), and phages were added to a concentration of 10^{11} plaque-forming unit (pfu)/mL. In this case, the Sauerbrey relation (a linear correlation between a decrease in resonant frequency and an increase in mass on the electrodes) was not applicable.[6] We were therefore unable to quantitate the amount of phage bound to the Ti sensor. (c) Schematic drawing of the mode of ϕTi-12-3-1 binding to a Ti sensor.

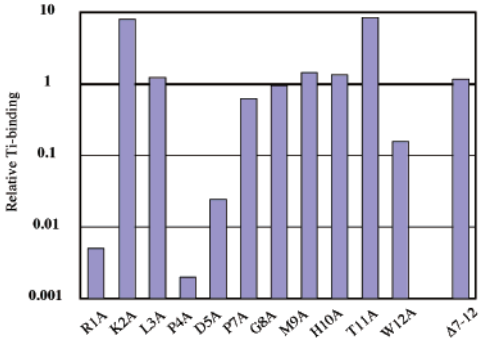

Figure 2. Effects of alanine substitutions and a deletion on the affinity of Ti-12-3-1 binding to Ti. Relative ϕTi-12-3-1 binding is shown.

position 4 (P4A) markedly impaired binding to Ti particles (Figure 2). Changing any of the other eight residues tested had little or no effect on binding.

This mutational analysis indicated a major role for the N-terminal region of Ti-12-3-1 in Ti recognition, suggesting, in fact, that the

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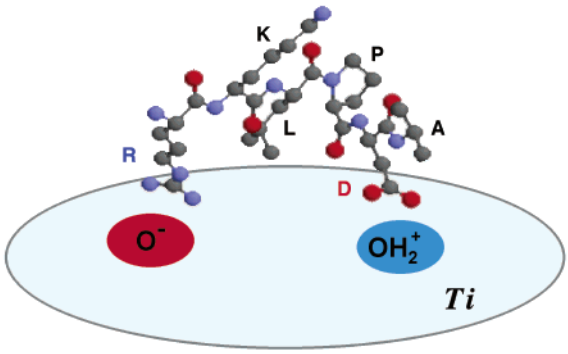

Figure 3. Model of the RKLPDA peptide binding to the surface of titanium.

C-terminal half of the peptide is dispensable for the binding. To confirm this finding, we evaluated the binding of a variant, Δ7-12, in which the C-terminal half of Ti-12-3-1, extending from the proline at position 7 to the tryptophan at position 12, was deleted (Figure 2). We found that the phage Δ7-12 deletion mutant retained the ability to bind to Ti particles and concluded that the N-terminal hexapeptide, RKLPDA, was sufficient for Ti binding. We also constructed an alanine insertion mutant (ϕTi A-12-3-1) in which an extra alanine residue was added at the N-terminus of Ti-12-3-1 to assess the contribution of the primary amine of R1 to the binding. We found that ϕTi A-12-3-1 efficiently bound to Ti particles and concluded that R1 of Ti-12-3-1 does not necessarily need to be located at the N-terminus (Supporting Information).

It is well-known that in a biological environment the surface of metallic Ti is covered by an oxide film composed of amorphous and nonstoichiometric TiO_2.[7] The surface of this oxide film is believed to display many oxygen atoms from hydroxyl groups and to have a net negative charge.[8] Indeed, at pH 7.5, positively charged lysine residues accumulated on the surface of TiO_2 through electrostatic interaction.[9] Interestingly, the surface adsorbed a negatively charged aspartic acid residue as well,[10] indicating the oxide film of Ti has an amphoteric nature composed of $-Ti-OH_2^+$ and $-Ti-O^-$.[11] The fact that treating Ti particles with H_2O_2, which increases the number of hydroxyl groups ($-OH_2^+$ or $-O^-$, or both) on their surfaces,[12] enhanced the binding of ϕTi-12-3-1 (Supporting Information), implying that hydroxyl groups are the target of the phage recognition. Given the amphoteric nature of the surface of Ti, our mutational analysis of ϕTi-12-3-1 suggests that the hexapeptide motif, RKLPDA, binds to the surface of Ti as follows (Figure 3): (i) interaction with the particle causes the hexapeptide to kink at the cis-peptide bond of P4, directing R1 and D5 to the same surface; (ii) a Lewis base in the side chain of R1 electrostatically interacts with $-Ti-O^-$; and (iii) a Lewis acid in the side chain of D5 electrostatically interacts with $-Ti-OH_2^+$. The model can explain why R1 and D5, but not K2, were important for the binding (Figure 2). The fact that trans−cis isomerization of P4 would be expected to proceed slowly under physiological conditions[13] most likely explains the slower rate of phage binding to the Ti sensor seen in Figure 1b.

The binding of biomolecules, e.g., osteogenic BMPs, to the surface of implants is critical for their proper function. However, the absorbance of proteins on the surface of metallic materials largely relies on nonspecific hydrophobic interactions,[14] which generally result in destruction of the protein structure and, thus, inactivation of the bound biomolecules. Moreover, hydrophobic interactions are generally irreversible, and it has been pointed out that controlled binding and release of biomolecules to implant surfaces is critical for the development of intelligent implant materials.[2] The success of the panning procedures in the present study clearly indicates that ϕTi-12-3-1 bound to the surface of a Ti particle can be released by acid treatment, indicating the binding to be reversible. Using the isolated hexapeptide motif, we are now exploring artificial proteins that bring active biological functions, including osteogenesis, to the surface of Ti.

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Supporting Information Available: Panning experiments, effect of ionic strength, H_2O_2 treatment, and alanine insertion mutants (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sykaras, N.; Iacopino, A. M.; Marker, V. A.; Triplett, R. G.; Woody, R. D. *Int. J. Oral Maxillofac. Implants* **2000**, *15*, 675−690.
(2) Puleo, D. A.; Nanci, A. *Biomaterials* **1999**, *20*, 2311−2321.
(3) (a) Scott, J. K.; Smith, G. P. *Science* **1990**, *249*, 386−390. (b) Zwick, M. B.; Shen, J. Q.; Scott, J. K. *Curr. Opin. Biotechnol.* **1998,** *9*, 427−436.
(4) (a) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665−668. (b) Lee, S. W.; Mao, C.; Flynn, C. E.; Belcher, A. M. *Science* **2002**, *296*, 892−895. (c) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169−172. (d) Wang, S.; Humphreys, E.; Chung, S.-Y.; Delduco, D. F.; Lustig, S. R.; Wang, H.; Parker, K. N.; Rizzo, N. W.; Subramoney, S.; Chiang, Y.-M.; Jagota, A. *Nat. Mater.* **2003**, *2*, 196−200. (e) Zhu, J.; Kase, D.; Shiba, K.; Kasuya, D.; Yudasaka, M.; Iijima, S. *Nano Lett.* **2003**, *3*, 1033−1036.
(5) Rodahl, M.; Höök, F.; Fredriksson, C.; Keller, C. A.; Krozer, A.; Brzezinski, P.; Voinova, M.; Kasemo, B. *Faraday Discuss.* **1997**, *107*.
(6) Höök, F.; Kasemo, B. *Anal. Chem.* **2001**, *73*, 5796−5804.
(7) Hanawa, T. *Corros. Rev.* **2003**, *21*, 161−181.
(8) Kosmulski, M. *Adv. Colloid Interface Sci.* **2002**, *99*, 255−264.
(9) Roddick-Lanzilotta, A. D.; Connor, P. A.; McQuillan, J. A. *Langmuir* **1998**, *14*, 6479−6484.
(10) Roddick-Lanzilotta, A. D.; McQuillan, J. A. *J. Colloid Interface Sci.* **2000**, *227*, 48−54.
(11) Jones, F. H. *Surf. Sci. Rep.* **2001**, *42*, 75−205.
(12) Tengvall, P.; Lundstrom, I.; Sjoqvist, L.; Elwing, H.; Bjursten, L. M. *Biomaterials* **1989**, *10*, 166−175.
(13) Brandts, J. F.; Halvorson, R. L.; Brennan, M. *Biochemistry* **1975**, *14*, 4953−4963.
(14) Norde, W.; Haynes, C. A. In *Interfacial Phenomena and Bioproducts*; Brash, J. L., Wojciechowski, P. W., Eds.; Dekker: New York, 1996; pp 123−144.

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