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Aluminum- and mild steel-binding peptides from phage display

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Abstract Using a phage library displaying random peptides of 12 amino acids on its surface, several peptides were found that bind to aluminum and mild steel. Like other metal-binding peptides, no obvious consensus motif has been found for these peptides. However, most of them are rich in hydroxyl-containing amino acids, serine or threonine, or contain histidine. For the aluminum-binding peptides, peptides with a higher number of hydroxyl-containing amino acids bind to the aluminum surface more tightly. For example, Val-Pro-Ser-Ser-Gly-Pro-Gln-Asp-Thr-Arg-Thr-Thr, which contains five hydroxyl-containing amino acid residues, was selected four-fold more frequently than a peptide containing only one serine, suggesting an important role for the hydroxyl-containing amino acids in the metal–peptide interaction.

Introduction

Beneficial biofilms can decrease corrosion (Potekhina et al. 1999) by removing oxygen at the metal surface (Jayaraman et al. 1997a, b, c). This protective effect was shown for mild steel (Jayaraman et al. 1997a, b, c), copper, and aluminum (Jayaraman et al. 1999c). Jayaraman et al. also used genetically engineered (Jayaraman et al. 1999b) and non-engineered biofilms (Jayaraman et al. 1999a) to generate antimicrobials in situ to inhibit the growth of sulfate-reducing bacteria (SRB) and hence decrease the corrosion rate of metals. Recently, Zuo and Wood (2004) used a gramicidin-S-producing *Bacillus brevis* biofilm to inhibit the corrosion caused by SRB and an iron-oxidizing bacterium simultaneously. In addition, Örnek et al. (2002a, b) showed that negatively charged corrosion inhibitors (e.g., polyaspartate, γ-polyglutamate) may be secreted by bacteria in protective biofilms to prevent metal corrosion. Building on these approaches, our goal here was to create metal-binding peptides that may be used to reduce corrosion, since corrosion causes an estimated US $276×10^9$ in damage in the United States, which is equivalent to 3.1% of the United States gross national product (Koch et al. 2001).

Phage display allows for the selection of peptide ligands from a vast random library that directly links the peptide phenotype with the DNA encoding the peptide (Manoutcharian et al. 2001). Perhaps the most attractive advantage of phage display is that novel peptides for specific targets may be found without knowing the peptide–target interaction (Scott and Smith 1990). This technique often employs the filamentous phage M13, which contains circular, single-stranded DNA wrapped in a long cylindrical envelope, around 900×7 nm (Wilson and Finlay 1998). The wall of the cylinder consists of about 2,700 copies of the pVIII major coat protein organized in a helix (Nam et al. 2004), and the caps at each end of the cylinder consist of four to five copies each of the minor coat proteins pIII, pVI, pVII, and pIX (Wilson and Finlay 1998). Among these coat proteins, the product of gene III, pIII, is responsible for infection of *Escherichia coli* containing the F-pilus (Smith 1985) and is the target for fusing external peptides, such as the random 12 amino acid peptides of the Ph.D.-12 phage display peptide library used in the present study.

Phage display has been used in medicine: for example, a library of antibodies has been generated that is not limited by the immune system (Hoogenboom and Chames 2000) and may be used to detect bacteria, spores, and viruses (Petrenko and Vodyanoy 2003). Phage display has also contributed to the successful generation of therapeutic agents (e.g., monoclonal antibodies) for clinical use (Manoutcharian et al. 2001). In other fields, phage-displayed peptides are used to direct the synthesis of inorganic materials such as semi-

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conducting materials, nanowires, liquid crystals, and magnetic materials (Mao et al. 2003; Flynn et al. 2003a, b).

Metal-binding peptides are frequently used as affinity tags to purify proteins through immobilized metal affinity chromatography (Beitle and Ataai 1993). Another important application for metal-binding peptides is heavy metal remediation, and cysteine-rich peptides are widely used to sequester Cd^{2+}, Cu^{2+}, Hg^{2+}, and Ag^{+} (Sousa et al. 1998). For example, metal-binding peptides with the repeated motif Cys-Gly-Cys-Cys-Gly have been expressed periplasmically in *E. coli* by fusing to the maltose-binding protein to enhance the uptake of cadmium and mercury (Pazirandeh et al. 1998), and cysteine-rich analogues of metal-chelating phytochelatin [$(Glu\text{-}Cys)_nGly$] have been displayed on the *E. coli* surface through fusion to the Lpp-OmpA system to accumulate cadmium (Bae et al. 2000). Another class of peptides widely used to chelate heavy metal ions is histidine-rich peptides (Xu and Lee 1999). *E. coli* expressing polyhistidine peptides on the cell surface through fusion to the outer membrane protein C shows increased adsorption of Cd^{2+} (Xu and Lee 1999) and polyhistidine peptides displayed on the surface of gram-positive *Staphylococcus xylosus* bind Ni^{2+} and Cd^{2+} (Samuelson et al. 2000).

Here, phage display was used to find novel peptides that bind to aluminum and mild steel and have potential for protecting these metals against corrosion. By forming a biofilm, bacteria can remain attached firmly to metal surfaces, and the expression of metal-binding peptides on the surface of such biofilm-forming bacteria may enhance the stability of the organic coating or provide a living paint.

Materials and methods

Metal coupon preparation

Aluminum 2024 coupons (UNS A92024, 1×1 cm, 1.2 mm thick) and mild steel 1010 coupons (UNS G10100, 1×1 cm, 1.2 mm thick) were cut from sheet stock (Yarde Metals, Bristol, Conn.), polished with 240 grit polishing paper (Buehler, Lake Bluff, Ill.) and rinsed with distilled water. The coupons were sterilized by autoclaving at 120°C for 20 min before the biopanning experiment.

Random peptide library, bacterium, and medium

The Ph.D.-12 phage display peptide library kit (E8110S; New England Biolabs, Beverly, Mass. contains 1.5×10^{13} plaque-forming units (pfu)/ml with a complexity of 2×10^{9} independent peptide sequences (the number of all possible random peptides of 12 amino acids is 4.1×10^{15}). Each phage contains five copies of the minor coat protein pIII, and each copy of pIII has a single peptide displayed at its N-terminus. Strain *E. coli* ER2738 containing the F^{+} Δ(*lacZ*)*M15* plasmid (New England Biolabs) was used to amplify the eluted phage (to make enough copies for the next round of biopanning). LB medium (Maniatis et al. 1982) was used to culture *E. coli*. To prevent contaminating phage from the environment interfering with DNA sequencing, eluted phage were plated on LB agar plates containing 50 μg/ml isopropyl β-D-thiogalactoside (IPTG) and 40 μg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal). The Ph.D.-12 phage library contains *lacZα* which can complement the defective *lacZ* gene on the F^{+} plasmid of the host, so they appear as blue plaques in the presence of IPTG and Xgal, while contaminating phage from the environment form white plaques.

Biopanning experiments

Biopanning was conducted following a modified protocol from New England Biolabs. An aliquot (10 μl) of the random peptide library was incubated with a metal coupon at 30°C for 1 h with gentle shaking in a microcentrifuge tube containing 1 ml of Väätänen nine salts solution (VNSS) which mimics a corrosive marine environment (Örnek et al. 2002b). To remove the unbound phage, the metal coupon was washed serially with several up–down movements, using 25 sterile glass tubes each containing 3 ml of TBST buffer [0.1% (v/v) Tween-20 in TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl)]. After the 25 washes, the bound phage was eluted with 1 ml of 0.2 M glycine-HCl (pH 2.2) containing 1 mg/ml bovine serum albumin (BSA), amplified using *E. coli* ER2738 to make more copies of each single phage, and then used in a second round of biopanning. After three rounds of biopanning (three rounds of 25 washes followed by amplification at the end of rounds one and two), the bound phage was eluted and DNA was sequenced as described by Canada et al. (2002) to identify the metal-binding peptides.

Selection of the strongest aluminum- and mild steel-binding peptides

To identify the strongest aluminum-binding peptides, the different phage identified after the three rounds of biopanning were mixed in equal amounts and incubated with an aluminum coupon. Then, the aluminum coupon was exposed to a more stringent treatment: sonication (FS3 sonicator, 50/60 Hz; Fisher, Hanover Park, Ill.) for 30 s instead of 25 washes. The phage displaying the strongest aluminum-binding peptides remained bound to the coupon during the sonication treatment, then were eluted, and the corresponding peptides were determined through DNA sequencing. The strongest mild steel-binding peptides were determined in a similar manner.

Results

During the course of the 25 washes of the metal coupons, progressively fewer loosely-bound phage were obtained in the wash buffer. For example, the phage titer for the aluminum-binding peptides after the first round of bio-

Table 1 Aluminum-binding (designated Al-S) and mild steel-binding (designated MS-S) peptide sequences selected by sonication from the mixture of phage found after three rounds of biopanning with 25 washes each (listed from N-terminus to C-terminus). A mixture of 15 purified phage bound to aluminum and 13 purified phage bound to mild steel identified after three rounds of biopanning was sonicated, and phage DNA from 12 Al-S and nine MS-S blue plaques was sequenced after elution, respectively. The frequency at which each peptide appeared in the group of sequenced plaques is indicated as "occurrence in sequenced plaques". Hydroxyl-containing amino acids are in bold, underlined and histidine is in italics. Note two wild-type phage without display of external peptides were also selected for the mild steel experiment

Peptide name	Peptide sequence	Occurrence in sequenced plaques	Number of serine and threonine residues
Al-S1	Val-Pro-**Ser**-**Ser**-Gly-Pro-Gln-Asp-**Thr**-Arg-**Thr**-**Thr**	4	5
Al-S2	Tyr-**Ser**-Pro-Asp-Pro-Arg-Pro-Trp-**Ser**-**Ser**-Arg-Tyr	3	3
Al-S3	**Thr**-Leu-Trp-**Ser**-Gln-Gly-Arg-**Ser**-Ala-Tyr-Pro-Val	2	3
Al-S4	Asn-Asn-Arg-Pro-Glu-Pro-**Ser**-Pro-Val-Val-Pro-*His*	1	1
Al-S5	**Ser**-Pro-Leu-Asp-Gly-Lys-Asn-Ile-Pro-Leu-Gly-*His*	1	1
Al-S6	Trp-Pro-Ala-Pro-Ala-Ile-Trp-*His*-Ala-Pro-**Thr**-Leu	1	1
MS-S1	Ala-**Thr**-Ile-*His*-Asp-Ala-Phe-Tyr-**Ser**-Ala-Pro-Glu	2	2
MS-S2	Asn-Leu-Asn-Pro-Asn-**Thr**-Ala-**Ser**-Ala-Met-*His*-Val	1	2
MS-S3	Asn-Leu-**Thr**-Ile-Ala-**Ser**-Tyr-Pro-**Ser**-Met-Val-Val	1	3
MS-S4	Gln-**Ser**-*His*-Tyr-Arg-*His*-Ile-**Ser**-Pro-Ala-Gln-Val	1	2
MS-S5	Gln-Met-Asp-Ile-**Ser**-Leu-Gly-Arg-Trp-**Ser**-**Ser**-Met	1	3
MS-S6	Tyr-Met-Lys-Gln-Ile-Pro-Ala-Gly-Arg-**Thr**-Asn-Pro	1	1
	No insertion	2	–

panning was 6.8×10^4 pfu/ml in wash 4, 900 pfu/ml in wash 10, and 200 pfu/ml in wash 16, and for washes 17–25, no loosely-bound phage was obtained. Thus, the final eluate (which contained 4.3×10^5 pfu in 1 ml elution buffer) after 25 washes contained only the tightly-bound phage from the metal coupon. The eluate from the first round of biopanning was amplified using *E. coli* ER2738 and then used as the input for the second round of biopanning. After three rounds of biopanning with 25 washes each, 15 phage (for aluminum-binding) and 12 phage (for mild steel-binding) in the eluate from the last round (without amplification) were randomly chosen for DNA sequencing, mixed in equal titers, and subjected to sonication to select the strongest-binding peptides. The results (Table 1) indicate that, although no obvious consensus motif was found for the aluminum-binding peptides, all the peptide sequences contained one to five amino acids with hydroxyl groups (i.e., serine or threonine). The more hydroxyl-containing residues present in the peptide, the more frequently the peptide was identified. Since there are 20 amino acids, the expected number of serines or threonines is 1.2 in a peptide with 12 amino acids. However, the most frequently identified aluminum-binding peptide, Al-S1 (Table 1), contains five serine or threonine residues, which is more than four times higher than expected if it were at random. Note that the aluminum-binding peptides containing one serine or threonine also contain a histidine residue that is frequently found in peptides that show binding specificity to Zn^{2+} (Matsubara et al. 2003), Cd^{2+} (Kotrba et al. 1999), Ni^{2+} (Samuelson et al. 2000), and Cu^{2+} (Patwardhan et al. 1997).

Similarly, the best mild steel-binding peptides (Table 1) are also rich in hydroxyl-containing amino acids (one to three), with some also containing histidine. Note that two wild-type phage were also selected for mild steel-binding, indicating that some non-specific binding occurred.

Discussion

Since metal-binding peptides have many functions [e.g., affinity chromatography to purify proteins (Beitle and Ataai 1993), bioremediation (Satoh et al. 1999; DeSilva et al. 2002), structural and catalytic functions in enzymes (Matsubara et al. 2003)], many studies on metal-binding peptides have been conducted which emphasize noble metals such as gold (Brown 1997), heavy metals such as cadmium and mercury (Brown 1997; Satoh et al. 1999; DeSilva et al. 2002; Matzapetakis et al. 2002), and metals which are physiologically important, such as zinc (Matsubara et al. 2003). However, the possibility of using metal-binding peptides as potential corrosion inhibitors has not been investigated. Our study is the first to find peptides that bind to common metals such as mild steel and aluminum, which are subject to severe corrosion. The random phage display peptide library is a powerful tool for searching for novel metal-binding peptides without knowing the basis for the metal–peptide interactions in advance.

Our finding that hydroxyl-containing amino acid residues predominate in the selected peptides that bind to aluminum and mild steel is consistent with the findings of other groups. Whaley et al. (2000), using a similar 12-amino-acid peptide library (i.e., random peptide library displayed on the

pIII coat protein of M13 phage), found that peptides recognizing GaAs crystals contained three to five serine or threonine residues. For example, one peptide that bound to GaAs showed the sequence Val-Thr-Ser-Pro-Asp-Ser-Thr-Thr-Gly-Ala-Met-Ala, which is similar to our best aluminum-binding peptide Al-S1 (Val-Pro-Ser-Ser-Gly-Pro-Gln-Asp-Thr-Arg-Thr-Thr) in that they are both rich in serine or threonine, although their primary sequences are different. Naik et al. (2002), also using the phage-displayed 12-amino-acid peptide library, found silver-binding peptides enriched with serine and threonine (e.g., Asn-Pro-Ser-Ser-Leu-Phe-Arg-Tyr-Leu-Pro-Ser-Asp). In addition, Brown (1997), using a peptide library fused to a surface protein (LamB) of *E. coli*, found that gold-binding peptides did not contain an obvious consensus motif, but contained five serine or threonine residues instead. These findings suggest the importance of the hydroxyl-containing amino acids in peptide–metal interactions.

At present, the exact mechanism by which peptides with serine and threonine bind to metals is not clear but may be probed using X-ray photoelectron spectroscopy to assess the phage–metal binding interaction and using atomic force microscopy (AFM) or transmission electron microscopy (TEM) to visualize phage binding to substrate (Whaley et al. 2000). In addition, pure peptides may be synthesized and electrospray ionization mass spectrometry (ESI-MS) may be used to clarify the specific metal–peptide interactions (Volz et al. 1998). Note that our preliminary corrosion tests using peptide Al-S1 (Table 1), that was either chemically synthesized and dissolved in VNSS medium (36 μg/ml) or secreted into VNSS medium from a genetically engineered bacterium that we constructed using host *B. subtilis* WB600, based on the method of Jayaraman et al. (1999b), did not lessen aluminum corrosion, probably because the concentration of the peptide was too low. To overcome this problem, an alternative technique is to use a surface expression system such as the Lpp-OmpA fusion system (Bae et al. 2000) to express the most promising peptides on the surface of biofilm-forming bacteria such as *E. coli*, and the genetically engineered bacteria may be assessed for their ability to lessen corrosion.

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References

Bae W, Chen W, Mulchandani A, Mehra RK (2000) Enhanced bioaccumulation of heavy metals by bacterial cells displaying synthetic phytochelatins. Biotechnol Bioeng 70:518–524

Beitle RR, Ataai MM (1993) One-step purification of a model periplasmic protein from inclusion bodies by its fusion to an effective metal-binding peptide. Biotechnol Prog 9:64–69

Brown S (1997) Metal-recognition by repeating polypeptides. Nat Biotechnol 15:269–272

Canada KA, Iwashita S, Shim H, Wood TK (2002) Directed evolution of toluene *ortho*-monooxygenase for enhanced 1-naphthol synthesis and chlorinated ethene degradation. J Bacteriol 184: 344–349

DeSilva TM, Veglia G, Porcelli F, Prantner AM, Opella SJ (2002) Selectivity in heavy metal-binding to peptides and proteins. Biopolymers 64:189–197

Flynn CE, Lee S-W, Peelle BR, Belcher AM (2003a) Viruses as vehicles for growth, organization and assembly of materials. Acta Mater 51:5867–5880

Flynn CE, Mao C, Hayhurst A, Williams JL, Georgiou G, Iverson B, Belcher AM (2003b) Synthesis and organization of nanoscale II–VI semiconductor materials using evolved peptide specificity and viral capsid assembly. J Mater Chem 13:2414–2421

Hoogenboom HR, Chames P (2000) Natural and designer binding sites made by phage display technology. Immunol Today 21: 371–378

Jayaraman A, Cheng ET, Earthman JC, Wood TK (1997a) Axenic aerobic biofilms inhibit corrosion of SAE 1018 steel through oxygen depletion. Appl Microbiol Biotechnol 48:11–17

Jayaraman A, Cheng ET, Earthman JC, Wood TK (1997b) Importance of biofilm formation for corrosion inhibition of SAE 1018 steel by axenic aerobic biofilms. J Ind Microbiol Biotechnol 18:396–401

Jayaraman A, Earthman JC, Wood TK (1997c) Corrosion inhibition by aerobic biofilms on SAE 1018 steel. Appl Microbiol Biotechnol 47:62–68

Jayaraman A, Hallock PJ, Carson RM, Lee C-C, Mansfeld FB, Wood TK (1999a) Inhibiting sulfate-reducing bacteria in biofilms on steel with antimicrobial peptides generated in situ. Appl Microbiol Biotechnol 52:267–275

Jayaraman A, Mansfeld FB, Wood TK (1999b) Inhibiting sulfate-reducing bacteria in biofilms by expressing the antimicrobial peptides indolicidin and bactenecin. J Ind Microbiol Biotechnol 22:167–175

Jayaraman A, Örnek D, Duarte DA, Lee C-C, Mansfeld FB, Wood TK (1999c) Axenic aerobic biofilms inhibit corrosion of copper and aluminum. Appl Microbiol Biotechnol 52:787–790

Koch GH, Brongers MPH, Thompson NG, Virmani YP, Payer JH (2001) Corrosion costs and preventive strategies in the United States. (Report FHWA-RD-01-156) FHWA, Washington, D.C.

Kotrba P, Delečková L, De Lorenzo V, Ruml T (1999) Enhanced bioaccumulation of heavy metal ions by bacterial cells due to surface display of short metal binding peptides. Appl Environ Microbiol 65:1092–1098

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Manoutcharian K, Gevorkian G, Cano A, Almagro JC (2001) Phage displayed biomolecules as preventive and therapeutic agents. Curr Pharm Biotechnol 2:217–223

Mao C, Flynn CE, Hayhurst A, Sweeney R, Qi J, Georgiou G, Iverson B, Belcher AM (2003) Viral assembly of oriented quantum dot nanowires. Proc Natl Acad Sci USA 100:6946–6951

Matsubara T, Hiura Y, Kawahito O, Yasuzawa M, Kawashiro K (2003) Selection of novel structural zinc sites from a random peptide library. FEBS Lett 555:317–321

Matzapetakis M, Farrer BT, Weng T-C, Hemmingsen L, Penner-Hahn JE, Pecoraro VL (2002) Comparison of the binding of cadmium(II), mercury(II), and arsenic(III) to the de novo designed peptides TRI L12C and TRI L16C. J Am Chem Soc 124:8042–8054

Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO (2002) Biomimetic synthesis and patterning of silver nanoparticles. Nat Mater 1:169–172

Nam KT, Peelle BR, Lee S-W, Belcher AM (2004) Genetically driven assembly of nanorings based on the M13 virus. Nano Lett 4:23–27

Örnek D, Jayaraman A, Syrett BC, Hsu C-H, Mansfeld FB, Wood TK (2002a) Pitting corrosion inhibition of aluminum 2024 by *Bacillus* biofilms secreting polyaspartate or γ-polyglutamate. Appl Microbiol Biotechnol 58:651–657

Örnek D, Wood TK, Hsu CH, Mansfeld F (2002b) Corrosion control using regenerative biofilms (CCURB) on brass in different media. Corros Sci 44:2291–2302

Patwardhan AV, Goud GN, Koepsel RR, Ataai MM (1997) Selection of optimum affinity tags from a phage-displayed peptide library. Application to immobilized copper(II) affinity chromatography. J Chromatogr A 787:91–100

Pazirandeh M, Wells BM, Ryan RL (1998) Development of bacterium-based heavy metal biosorbents: enhanced uptake of cadmium and mercury by *Escherichia coli* expressing a metal binding motif. Appl Environ Microbiol 64:4068–4072

Petrenko VA, Vodyanoy VJ (2003) Phage display for detection of biological threat agents. J Microbiol Methods 53:253–262

Potekhina JS, Sherisheva NG, Povetkina LP, Pospelov AP, Rakitina TA, Warnecke F, Gottschalk G (1999) Role of microorganisms in corrosion inhibition of metals in aquatic habitats. Appl Microbiol Biotechnol 52:639–646

Samuelson P, Wernérus H, Svedberg M, Ståhl S (2000) Staphylococcal surface display of metal-binding polyhistidyl peptides. Appl Environ Microbiol 66:1243–1248

Satoh M, Karaki E, Kakehashi M, Okazaki E, Gotoh T, Oyama Y (1999) Heavy-metal induced changes in nonproteinaceous thiol levels and heavy-metal binding peptide in *Tetraselmis tetrathele* (Prasinophyceae). J Phycol 35:989–994

Scott JK, Smith GP (1990) Searching for peptide ligands with an epitope library. Science 249:386–390

Smith GP (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 228:1315–1317

Sousa C, Kotrba P, Rumi T, Cebolla A, De Lorenzo V (1998) Metalloadsorption by *Escherichia coli* cells displaying yeast and mammalian metallothioneins anchored to the outer membrane protein LamB. J Bacteriol 180:2280–2284

Volz J, Bosch FU, Wunderlin M, Schuhmacher M, Melchers K, Bensch K, Steinhilber W, Schafer KP, Toth G, Penke B, Przybylski M (1998) Molecular characterization of metal-binding polypeptide domains by electrospray ionization mass spectrometry and metal chelate affinity chromatography. J Chromatogr A 800:29–37

Whaley SR, English DS, Hu EL, Barbara PF, Belcher AM (2000) Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly. Nature 405:665–668

Wilson DR, Finlay BB (1998) Phage display: applications, innovations, and issues in phage and host biology. Can J Microbiol 44:313–329

Xu Z, Lee SY (1999) Display of polyhistidine peptides on the *Escherichia coli* cell surface by using outer membrane protein C as an anchoring motif. Appl Environ Microbiol 65:5142–5147

Zuo R, Wood TK (2004) Inhibiting mild steel corrosion from sulfate-reducing and iron-oxidizing bacteria using gramicidin-S-producing biofilms. Appl Microbiol Biotechnol 65:747–753