Selective preference of engineered peptides for aluminum alloy

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Bacterial cell surface display technology offers a powerful tool for the discovery and study of peptide–material interactions offering the potential toward tailoring the interaction and tuning the properties of multicomponent materials for future military and commercial systems. With bacterial cell surface display technology, a large diverse library containing billions of peptide material variants is encoded directly into the bacterial DNA, resulting in a self-sustaining and replicating population that can easily be propagated without requiring elution from the target. Recently, this method was extended to genetically engineered peptides for inorganics: specifically to a bulk aluminum alloy. In this paper, we further investigate the binding interaction of this peptide material including a study of relative binding to aluminum, glass, copper and brass. The surface display peptide exhibited more than 2 order of magnitude greater binding to the aluminum alloy compared to the glass sample, and little to no interaction with copper and brass. These results highlight, for the first time, the potential for selection interaction of genetically engineered peptides using the eCPX construct with inorganic materials and open the possibility for biohybrid materials and systems of the future.

1. Introduction

Biological materials demonstrate a level of complexity and functionality not yet seen in engineered materials. Such materials are made from molecular building blocks with a highly controlled assembly from the nano- to macroscale.^{1–3} Insight from an understanding of biological materials and the principles that govern their synthesis, as well as the integration of their components and assembly processes, are pivotal for the development of biohybrid materials in modern engineered material systems. Currently, engineered materials are constructed using a combination of techniques, including melting and solidifying, thermomechanical treatments and solution/vacuum deposition and growth.⁴ Alternative biocompatible methods for building biohybrid materials are needed to advance the next generation of materials. This may be accomplished through a bioinspired, bottom-up molecular assembly utilizing the recognition and binding properties of peptides to inorganic surfaces. There are currently a number of peptides published for binding to metals (Au, Ag, Pt, Pb),^{1,5–7} metal oxides (ZnO, Al₂O₃, SiO₂),^{8–11} semiconductors (GaN, GaAs, Cu₂O, TiO₂, InN, ZnS),^{12–16} polymers,¹⁷ carbon nanotubes¹⁸ and other functional materials (hydroxyapatite, mica, graphite, calcite, sapphire)^{1,19–22} and have primarily been discovered through biopanning of combinatorial peptide libraries. These genetically engineered peptides for inorganics (GEPI) have been successfully

used in nanoparticle immobilization on inorganic substrates,²³ synthesis of inorganics,⁷ biomineralization,²⁴ as well as surface modification and functionalization.^{17,18}

Combinatorial peptide libraries consist of a large peptide population, containing the same number of amino acids, but differing in sequence, displayed on the outside surface of a virus (phage display), bacteria (cell-surface display) or fungi (yeast display). The inorganic material of interest is incubated with the library and then biopanned or mined for specific sequences that bind to the inorganic material.¹ Several different types of cell-surface display libraries have been developed for peptide biopanning.^{25,26} In the studies reported herein, the *Escherichia coli* eCPX display system was utilized. Briefly, the natural *E. coli* outer membrane protein, OmpX, was altered and evolved into eCPX such that the N terminus contains an unconstrained 15 amino acid peptide library and the C terminus contains the P2X tag to monitor expression.^{27–30} We have previously utilized this library to discover peptide binders for several protein targets, such as Anthrax toxin component protective antigen^{30,31} and the *Staphylococcal enterotoxin B*,³² as well as peptide binders for bulk aluminum.^{10,33} We found that *E. coli* displaying peptide DBAD1 bound well to the surface of bulk aluminum, which is expected to possess a natural oxide (alumina layer). Moreover, through a

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Level of specificity	Example	Reference
Disparate materials	Specificity of peptides for gold and silica	36
	Specificity of ZnO and CuO ₂ peptide binders for target metal over gold	13
Similar materials	Gold binding peptide discrimination between gold and platinum	23
	CuO ₂ binding peptide discrimination between CuO ₂ and Cu(OH) ₂	13
Structural differences for same material	Peptide binding discrimination between Pt(111) and Pt(100)	37
	Peptide binding discrimination between ZnO(001) and ZnO(1010)	39

Table 1. Inorganic binding peptide specificity

Figure 1. Relative interaction of DBAD1 (blue) and a negative control displaying the entire scaffold except 15 residue-binding sequence. A comparison measured through an inorganic recovery assay shows relative interaction with aluminum, glass, copper and brass

combination of sequence analysis, modeling and simulation, we showed evidence that the DBAD1 peptide possesses a high helical propensity due to the presence of alanine residues which facilitate the presentation of hydroxyl-containing residues for interaction with the bulk aluminum surface.

GEPI polypeptides are distinguished from other polypeptides, including other peptide binders developed from combinatorial libraries, in that their amino acid sequences facilitate a specific and selective binding to an inorganic surface. The surface may be highly defined (e.g. a single crystal) or ambiguous (e.g. powders or bulk materials), and the peptide specificity arises from a combination of chemical³⁴ (hydrogen bonds, polarity, charge effects) and structural³⁵ (size, morphology) recognition mechanisms. It is through these mechanisms that peptide specificity is achieved for very disparate materials, in which the chemical and structural

properties are vastly different; as well as for very similar materials that are chemically similar, but only slightly structurally different (Table 1). Peptide specificity is critical for the development of hybrid materials and bio-nanotechnology. It allows peptides to be targeted to a material and assembly via that peptide to be controlled. For example, Hnilova *et al.*³⁶ used the specificity of gold- and silica-binding peptides to create patterns for simultaneous co-assembly of two or more inorganic nano-entities and/or molecular probes onto a surface. Streptavidin was immobilized onto gold via a biotin-conjugated gold-binding peptide, and green fluorophore molecules were immobilized onto silica glass simultaneously. Achieving target specificity for GEPI applied to a noble metal templated glass, with two very different inorganic surfaces, does not require a high degree of specificity. However, other peptides have been shown to have a very high degree of both chemical and structural recognition. In fact, one of the first reports of GEPI material specificity was the demonstration that a phage-derived peptide could differentiate between the crystal facets of GaAs(100) and the surfaces of GaAs(111)A and GaAs(111)B, and Si(100).¹⁶ However, the peptide bound equally to InP(100), a surface that is isostructural with GaAs(100). Other examples of GEPI that are able to recognize specific structural characteristics of the target material include Pt³⁷, graphite³⁸ and ZnO.³⁹ In addition to structural-level differentiation, peptides have been developed with high chemical specificity. Schembri *et al.*⁴⁰ identified a motif that bound PbO₂ and CoO, but not Cr₂O₃ or MnO₂. Additionally, Okochi *et al.*⁴¹ found that a designed ZnO-binding peptide could preferentially bind the target oxide (ZnO) over other metal oxides tested (ZrO₂, Al₂O₃ and SnO₂). These studies demonstrated that a peptide could be developed with the capability of discriminating between various metal oxides. Similar degree of specificity was found for a CuO₂-binding peptide developed by Thai *et al.*¹³ which demonstrated a preference for its oxide target over the hydroxylated form. One of the advantages of GEPI discovery from a combinatorial library is that target specificity can be increased by modifying

the biopanning process, such as adding a counter selection step,⁴² further genetically engineering (directed evolution) or chemically modifying the peptide. Currently, *de novo* peptide design for inorganics is not possible; however, studies continue to elucidate the binding mechanisms to enable such technology in the future.

In this report, we investigated the binding specificity of bulk aluminum peptide, DBAD1, for bulk aluminum, glass, copper and brass. The number of binders recovered from each metal target was determined using the method previously described¹⁰ and normalized for the surface area of the target. Cells displaying the scaffold only (no peptide) were included in the studies as a negative control. Figure 1 shows that *E. coli* cells expressing the DBAD1 peptide on the cell surface bound best to bulk aluminum, as previously shown.¹⁰

It was also demonstrated that DBAD1 did not bind strongly to glass, copper or brass. There was almost a three order of magnitude difference in cell binding between aluminum and glass. Remarkably, there was little to no binding to copper and brass. These results, combined with the little to no binding exhibited by the negative control, strongly indicate that cell adhesion was likely due to the presence of the DBAD1 peptide. Due to the selectivity of DBAD1 to bulk aluminum over copper or brass, this peptide can be used to address the assembly of other proteins and materials using an aluminum pattern. By combining aluminum and copper into a patterned substrate and employing previously discovered copper binding peptides, a dual co-assembly technique is now possible using two common metals.

2. Conclusions

This work is the first investigation of the selectivity of the DBAD1 peptide for an aluminum alloy/alumina surface. Despite the fact that the discovery process did not introduce a strong selective stringency (e.g. a negative selection against other metal oxide systems), a preference for bulk alumina over other materials is clearly present. A nearly three-order greater interaction in recovered cells displaying the DBAD1 peptide was observed for aluminum, over the next highest investigated, glass. Remarkably, there was no binding to both copper and brass. The negative control, displaying the entire display scaffold without peptide insert, showed little to no interaction, indicating that the binding interaction is directed by the peptide displayed on the surface.

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