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IDENTIFICATION OF GOLD BINDING PEPTIDES USING A PHAGE DISPLAY PEPTIDE LIBRARY

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ABSTRACT

Micron gold particles were used in this study to assess potential protein-metal interactions. A total of three rounds of biopanning with the gold particles were carried out using a phage display peptide library. In this system, the random 12-residue peptides are displayed at the N-terminus of pIII gene of M13mp19 phage. Peptides that bind to gold particles with high affinity were amplified after each round of the biopanning process. After 3 rounds of biopanning selection, five clones were isolated and the affinities of the clones were estimated and confirmed by ELISA analysis. The deduced peptide sequence, VGAHYNYGVIML was obtained through translation of DNA sequencing data. Overall, the ELISA results showed strong binding affinity of this peptide towards gold.

Keywords: Gold, phage display, peptide, biopanning.

INTRODUCTION

Gold has several properties that have made it useful to mankind over the years. The deposits of gold are of two basic types: hydrothermal veins, associated with quartz and pyrite; and placer deposits which are related to multistage mineralization, derived from the erosion of gold-bearing rocks and appear in alluvium and stream beds. Theories contend that gold was carried to the surface from the earth's mantle in partial solid solution and precipitated in the later stage. However, the origin of enriched veins is still not fully known (Burnett, 1997; Youngson and Craw, 1999). Usually, the amount of gold is so minute that the cost of extracting it would exceed its value. Gold found in veins and alluvial deposits is often separated from rocks and ore by mining and panning operations. The gold is then recovered from its ores by cyaniding, amalgamating and smelting process (Green and Russell, 1997). In addition, some genera of bacteria have been used in biomineralization, including *Acidothiobacillus* (previously known as *Thiobacillus*), *Leptospirillum*, *Acidiphilium*, *Sulfobacillus*, *Ferroplasma*, *Sulfolobus, Metallosphaera* and *Acidianus*. These bacteria are highly acid-tolerant and are able to use reduced sulphur compounds and/or ferric (Fe^{2+}) ion as its electron source. This characteristic is important since both of these molecules are readily available in the form of iron pyrite in most ore deposits (Brierley, 1995). Generally, gold-binding peptides/proteins could be obtained through the *E. coli* cell surface display system and some of the properties of the gold-binding polypeptides were observed (Brown, 1997). The effects of gold-binding proteins on the morphology of gold particles were characterized using the well-known Faraday technique (Sarikaya *et al*., 2003).

Recent development of a molecular biomimetic approach in nanotechnology involves genetically engineered proteins or polypeptides that are specific to inorganic surfaces, which could then be used as linkers for self-assembly of materials with regulated organization and specific functions. In addition, combinatorial genetic techniques including phage display have made isolation of simple peptides with specific binding affinity to inorganic materials possible (Tamerler *et al*., 2003). In this context, the sequences of the amino acids and their overall composition play a vital role in the physical conformation of the engineered polypeptides. The inorganic material can then be recognized physically or chemically through its surface composition, morphology, structure or crystallography (Tamerler *et al*., 2003). This specific protein/inorganic interaction have played a significant role in the biological control over materials formation during biomineralization.

Phage display technology involves the generation of peptide libraries and the binding of heterogenous mixture of phage clones to the desired substrate. In general, the single-stranded genome of filamentous phage encodes 3 classes of proteins including the replication, morphogenetic and structural proteins. For phage display technology, the foreign DNA insert is being cloned into the unique cloning site(s) between the signal peptide and the wild type sequence of the phages which allow the deduced amino acids to be displayed on their pIII or pVIII

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proteins. During biopanning approach, phages display peptides will bind to its specific immobilized substrates on a solid support. The phages that strongly bind to the target substrates are retained, whereas the unbound phages will be washed off. The bound phages can then be recovered from the immobilized substrates by elution with harsh wash at low pH which will disrupt the strong binding between the phages and substrates. Subsequently, the recovered phages are propagated in *E. coli* (Parmley and Smith, 1988). This process can be repeated several times in order to enrich the phage pool in favour of the selected clones. Finally, DNA sequencing is carried out on these selected clones to identify the peptides that bind specifically to the selected substrates (Tamerler *et al*., 2003). Overall, this is the approach used to screen for gold-binding peptides in this study.

MATERIALS AND METHODS

Immobilization of the target molecules

Prior to immobilization on a solid phase, the 0.6 micron gold particles (Bio-Rad Laboratories Ltd., USA) and the 0.7 micron tungsten particles (negative control) were first treated with absolute ethanol. The precipitated particles were then collected via centrifugation. Subsequently, 1 ml of ultra pure water was added to these particles. At the same time, 500 µl of blocking buffer was coated onto one of the well of a 96 well flat bottom microtiter plate, and allowed to incubate overnight at 4^{o}C.

On the following day, the blocking buffer in the well was discarded and washed with PBS-T (0.1% Tween 20) for 6 times. The treated gold and tungsten particles, which were pre-resuspended in absolute ethanol, were then added into the particular well and thoroughly covered the well surface. The ethanol was then removed from the well by pipetting and the remaining was left to evaporate.

Affinity selection of the phage display library

The peptide library used in this research is a dodecapeptide Library containing vector M13KE fused with 12-mers random peptide (New England Biolabs, USA). A total of 10 µl of phage (original library) were diluted with 100 µl of PBS-T. All diluted phage were then added to the gold-coated well and incubated at room temperature. After that, the phages were removed and the well was washed thoroughly with PBS-T. A 100 µl of trypsin (10 mg/ml) was then added to the gold-binding phages (in the well) and incubated at 37^{o}C. The gold-binding phage eluates were collected and amplified through reinfection on early-log phase *E. coli* in LB broth. Subsequently, the amplified phages were precipitated out by polyethylene glycol/NaCl. These amplified phage eluates were used as input phages for the second round of biopanning and subsequently the eluates collected from this round of biopanning were then used for the third round of biopanning. Phage titering was carried out after each round of biopanning to estimate the concentration of bound/amplified phage.

After a third round of biopanning, a serial dilution ranging from 10^1 to 10^5 was carried out on the unamplified phages. Subsequently, these diluted phages were transferred to the *E. coli* culture, and the infected *E. coli* culture was then spread on LB agar plates containing IPTG and X-Gal for incubation. On the following day, five plaques were randomly selected from the plate for further analysis.

Phage ELISA detection

Prior to ELISA detection, 200 µl of blocking buffer was coated on the well surface of 15 separate wells in a microtiter plate. Two hundred µl of PBS was also dispensed into 5 separate wells in the same microtiter plate to serve as negative controls. Next, the samples (gold particles in absolute ethanol) were added into five separate wells. Similarly, the negative controls (tungsten particles in absolute ethanol) were added into another five wells. The samples and controls must cover the well surfaces thoroughly. The ethanol in the wells was removed by pipetting and the remaining was left to evaporate, giving a dry layer of gold and tungsten particles in the wells.

After coating the wells, each of the selected amplified phage suspensions was diluted in an equal amount of PBS-T (0.5 % Tween 20). Two hundred µl of each diluted phage solution was dispensed into three different target wells, i.e., gold-coated, tungsten-coated and PBS-coated. After incubation, the wells were washed carefully with PBS-T for three times. A 200 µl of diluted horseradish peroxidase-conjugated anti-M13 antibody was dispersed into each well and allowed to incubate at room temperature for 1 hour. The solution in the wells was then discarded and washed carefully with PBS-T for three times. Following this, 200 µl of 5-Amino-2, 3-Dihydro-1, 4-Phthalazinedione substrate with 0.34 µl of 30 % H_2O_2 was added into each well and incubated in dark for 10 min. Finally, the color development in each well was detected and the absorbance was read at 415 nm by using an ELISA reader (Power Wave X340).

DNA sequencing

Minipreparations for plasmid DNA was performed to obtain the M13 phagemid DNA. The bacterial pellet was collected by centrifugation and resuspended in 200 µl of alkaline lysis solution. RNase A was then added and incubated at 37 ^{0}C for 3 hours. A 600 µl of phenol was added and the aqueous layer was transferred to a new tube. Subsequently, an equal volume of chloroform was added and the aqueous layer was retrieved to a new tube. A 25 µl of 5 M NaCl and 625 µl of isopropanol were added and incubated on ice for 20 min. The precipitated

Table 1. The initial input phages, output eluates and the percentage of recovery in each round of biopanning.

Biopanning	Input ($\times 10^9$ pfu)	Eluted ($\times 10^5$ pfu)	Recovery ($\times 10^{-3}$ %)
1st round	1500	74	0.49
2nd round	18	5.5	3.1
3rd round	1.32	35	265

Table 2. Data collected using Power Wave X340 ELISA reader.

	G1	G2	G3	G4	G5	Average
Gold	0.900	0.912	0.929	0.915	0.966	0.924
Tungsten	0.061	0.071	0.059	0.064	0.077	0.066
PBS	0.058	0.067	0.072	0.070	0.092	0.072

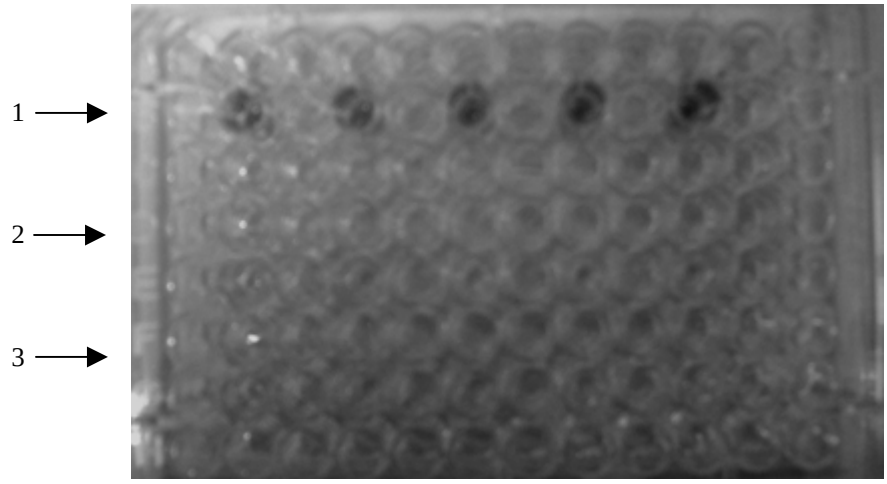

Fig. 1. Microtiter plate containing samples with developed results after ELISA. Lane 1: Gold-coated wells; Lane 2: Tungsten-coated wells; Lane 3: PBS-coated wells.

Nucleotide Sequence

pIII leader sequence *Kpn* I

TTA TTC GCA ATT CCT TTA GTG GTA CCT TTC TAT TCT CAC TCT **GTT GGG GCG CAT TAT**
V G A H Y

Eag I

AAT TAT GGT GTT ATT ATG CTT GGT GGA GGT TCG GCC GAA ACT GTT GAA A*GT TGT*
N Y G V I M L

*TTA GCA AAA TCC CAT AC*A GAA AAT TCA TGG ACT AAC GTC GAA AGC GCA

–28 sequencing primer

Fig. 2. The nucleotide and deduced amino acid sequence of the gold-binding peptide. The DNA sequence and the deduced amino acid sequence of the gold-binding peptide are shown in bold.

DNA was rinsed with 1 ml of 70% ethanol. The DNA pellet was then recovered through centrifugation and dried using a DNA concentrator. Finally, the DNA was dissolved in 50µl of deionized water for storage at -20°C. The DNA concentration and purity were determined by using a spectrophotometer prior to sending for sequencing. DNA sequencing was carried out by Research Biolabs Technologies Pte. Ltd. using the -96 gIII (5'-CCCTCATAGTTAGCGTAACG-3') primer.

RESULTS

After each round of biopanning, the phage plaque forming unit (pfu) was determined by a serial dilution, followed by plating on LB agar plates containing IPTG and X-Gal. In this study, the number of pfu in the eluates decreased after the second round of biopanning. However, the total number of phages recovered increased after the third round of biopanning. Even though the yield of target

phages obtained from each round of biopanning was inconsistent, there was an overall increase in the percentage of recovery of target phages (Table 1). Table 1 shows the initial input phages for gold particle reaction and the output phage after the biopanning processes. The yield of pfu after each round of biopanning is expressed as a percentage of recovery of the initial input.

A total of five plaques were randomly selected from a lawn of infected *E. coli* culture for subsequent ELISA analysis and DNA sequencing. In ELISA detection, the binding affinities of these five clones were estimated using HRP-conjugated anti-M13 antibody as the probe. The reaction between HRP and its substrate, ABTS, gave a blue color complex in the gold-coated wells, whilst no changes were observed in the negative control wells (tungsten- and PBS-coated) (Fig. 1). An ELISA reader was used to analyze the microtiter plate and the absorbance readings measured at 415 nm as shown in Table 2. The gold samples demonstrated an average absorbance reading of 0.924, whilst the absorbance value for tungsten and PBS were 0.066 and 0.072, respectively (Table 2). Finally, DNA sequencing was carried out on all the phagemid M13 DNA, in order to obtain the desired gold-binding peptide sequence. The consensus sequences of the 12-mer peptide, VGAHYNYGVIML are similar for all the clones as shown in bold in fig. 2.

DISCUSSION

In phage display technology, a total of at least three rounds panning has been recommended for the selection of good binders (Scott and Smith, 1990). The specifically bound phages obtained from initial panning are enriched through these repetitions of biopanning step. During the interval of each round of biopanning, an intermediate amplification step is introduced in order to increase the final yield of phage clones. This affinity enrichment step is essential in a phage display system where the final yield of phage clones can be increased and the possible loss of rare clones can be prevented (Parmley and Smith, 1988). In this study, the overall increment of the target phage yield after three rounds of biopanning was low. However, the combination of high-detergent washes and intermediate amplification steps were able to provide sufficient individual phage clones for subsequent analysis.

The results of ELISA detection for binding of the gold particles had demonstrated an average absorbance reading of 0.924, whilst the absorbance value for negative controls, i.e., binding to tungsten and PBS were 0.066 and 0.072 respectively. The significant absorbance ratio of more than twelve fold revealed that the five selected phage clones possess a strong specific binding affinity to gold. Tungsten, which serves as negative control in this study acts as a relative metal comparison to assess the metal specificity of the phage display system. In this case, the affinity and specificity of the selected gold-binding phages were assured as there was also no binding of the selected phage clones against the PBS solution (negative control) observed.

Following DNA sequencing, the deduced amino acid sequence of gold-binding peptide, VGAHYNYGVIML obtained from all the selected M13 phagemids were identical. The location of this consensus sequence suggested that the random peptide is still accessible for foreign peptide binding, even though the random peptide region is in proximate to the glycine short spacer (Devlin *et al*., 1990). A total of eight amino acid residues of gold-binding peptides obtained in this study were found similar to those previously obtained by using the *E. coli* cell surface display approach (Brown *et al*., 2000; Braun *et al*., 2002; Tamerler *et al*., 2003). In addition, methionine, which is one of the residues commonly present in all the gold-binding proteins, was observed in the selected gold-binding peptides (Braun *et al*., 2002). These results showed that the biopanning of phage display library was successfully carried out in this study and it may then serve as a promising approach in further analyzing the properties of peptide-gold interactions. Furthermore, the gold-binding peptide sequences demonstrated in this study may serve as a useful starting point for future investigation, such as structural and functional analysis in order to improve the binding affinity. The binding affinity of these selected peptides needs to be further analyzed for more detailed characterization of peptide-gold interactions. This can be achieved by synthesizing the particular peptide followed by subsequent testing of its binding affinity on different types of metal other than gold. In depth, alanine-scanning mutagenesis can also be used to determine the specific amino acid residues that are responsible for the binding to gold particles. More potential gold-binding peptides need to be isolated to quantitatively produce an established linkage between the ligand and the gold surface in various aspects, i.e., morphology, size, crystallography and stereochemistry. In 2003, Tamerler and his collaborators had improved the stability and binding affinity of the gold-binding peptides by repeating the binding sequence proportionately three times. It was observed that the binding property of the repeated peptide was retained and its affinity to gold had increased with the increase in number of sequence repeats Braun *et al*. (2002). The integration of computerized bioinformatics in the analysis of DNA sequencing data will also enhance the activity, stability and specificity of phage display approach in determining the desired gold-binding peptide sequences (Thor and Terryberry, 2004). To date, progress has been made towards the determining of protein structure on the solid surface. The use of radioisotope labeling, antibody or epitope binding, surfactant elutability and catalytic activity provide information on the polypeptides changing state on the solid surface (Gray, 2004).

CONCLUSION

In this study, the biopanning process with gold particles was successfully carried out with the determination of the desired gold-binding peptide sequence, VGAHYNYGVIML from a few selected M13 phagemids. The binding affinity of the selected gold-binding peptide to gold particles was also shown. In future, binding specificity of the peptide towards gold will be determined using other metals as controls.

REFERENCES

Braun, R., Sarikaya, M. and Schulten, K. 2002. Genetically engineered gold-binding polypeptides: Structure prediction and molecular dynamics. Journal of Biomaterial Science. 13:747-757.

Brierley, CL. 1995. Bacterial oxidation. Engineering and Mining Journal. 196:42-44.

Brown, S. 1997. Metal recognition by repeating polypeptides. Nature Biotechnology. 15: 269-272.

Brown, S., Sarikaya, M. and Johnson, E. 2000. A genetic analysis of crystal growth. Journal of Molecular Biology. 299:725-735.

Burnett, R. 1997. Gold in Namibia. International Liaison Group on Gold. Mineralisation Newsletter. 25.

Devlin, JJ., Panganiban, LC. and Devlin, PE. 1990. Random peptide libraries: A source of specific protein binding molecules. Science. 249:404-406.

Gray, JJ. 2004. The interaction of proteins with solid surfaces. Current Opinion in Structural Biology. 14:110-115.

Green, T. and Russell, D. 1997. The gold companion: The A-Z of mining, marketing and technology. Rosendale Press. URL: http://www.gold.org/discover/knowledge/index.html [Last accessed January 2005]

Parmley, SF. and Smith, GP. 1988. Antibody-selectable filamentous fd phage vectors: affinity purification of target genes. Gene. 73:305-318.

Sarikaya, M., Tamerler, C., Jen, AK., Schulten, K. and Baneyx, F. 2003. Molecular biomimetics: nanotechnology through biology, Nature Materials. 2.

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

Tamerler, C., Dincer, S., Heidel, D., Zareie, MH. and Sarikaya, M. 2003. Biomimetic multifunctional molecular coatings using engineered proteins. Progress in Organic Coatings. 47:267-274.

Thor, G. and Terryberry, J. 2004. Peptide mimetics. Drug and Market Development. 15:558-566.

Youngson, JH. and Craw, D. 1999. Variation in Placer Style, Gold Morphology, and Gold Particle Behavior Down Gravel Bed-Load Rivers: An Example from the hotover/Arrow-Kawarau-Clutha River System, Otago, New Zealand. Economic Geology. 94:615-634.