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Chromatographic biopanning for the selection of peptides with high specificity to Pb^{2+} from phage displayed peptide library

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ABSTRACT

Toxic heavy metal pollution is a global problem occurring in air, soil as well as water. There is a need for a more cost effective, renewable remediation technique, but most importantly, for a recovery method that is selective for one specific metal of concern. Phage display technology has been used as a powerful tool in the discovery of peptides capable of exhibiting specific affinity to various metals or metal ions. However, traditional phage display is mainly conducted in batch mode, resulting in only one equilibrium state hence low-efficiency selection. It is also unable to monitor the selection process in real time mode. In this study, phage display technique was incorporated with chromatography procedure with the use of a monolithic column, facilitating multiple phage-binding equilibrium states and online monitoring of the selection process in search of affinity peptides to Pb^{2+}. In total, 17 candidate peptides were found and their specificity toward Pb^{2+} was further investigated with bead-based enzyme immunoassay (EIA). A highly specific Pb^{2+} binding peptide ThrAsnThrLeuSerAsnAsn (TNTLSNN) was obtained. Based on our knowledge, this is the first report on a new chromatographic biopanning method coupled with monolithic column for the selection of metal ion specific binding peptides. It is expected that this monolith-based chromatographic biopanning will provide a promising approach for a high throughput screening of affinity peptides cognitive of a wide range of target species.

1. Introduction

Toxic heavy metals (Co, Cd, Pb, etc.) in air, soil and water are a growing threat to the environment and human health. There are hundreds of sources of heavy metal pollution, including the coal, natural gas, paper and chlor-alkali industries [1,2]. Unlike organic pollutants, heavy metals do not decay over a long time and thus pose a different kind of challenge for remediation. Removal of heavy metals is usually accomplished through pH neutralization followed by lime-mediated precipitation, peroxide addition, reverse osmosis, and/or ion exchange [3]. However, the liming process is often associated with some disadvantages (e.g. the use of a large amount of alkaline materials, and production of secondary wastes), necessitating highly regulated and costly disposal [1,4].

Currently, plants or microorganisms are used to remove some heavy metals such as mercury [5,6]. Plants which exhibit hyper accumulation can be used to remove heavy metals from soil by concentrating them in their biomass. This is used in the treatment of mining tailings and the vegetation is then incinerated to recover the heavy metals. As an alternative to these processes many researchers have developed ligands to detect and/or precipitate heavy metals from aqueous systems. Phage display is a selection technique where a library of peptides or protein variants is expressed on the phage surface, while the genetic material encoding each variant resides inside [7–9]. This allows rapid partitioning of phage particles based on their binding affinity to a given target molecule through an in vitro selection process called panning [10]. Nowadays, phage display of combinatorial peptide libraries has become one of the standard technologies for selecting peptides cognitive of specific molecules [11]. Nevertheless, the traditional phage display techniques, relying on batch equilibrium adsorption/desorption, have significant drawbacks in terms of selection efficiency and target binding avidity. This is mainly because most of the conventional

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biopanning procedures fractionate the binders from the rest in batch mode where only a single equilibrium stage separation is expected, often resulting in decreased binding efficacy of phage displayed peptides to target molecules hence repeated screening rounds. Besides, the screening processes are difficult to monitor in a real time mode, since phage particles are relatively large (e.g. 1 μm in length and less than 10 nm in diameter for M13 widely used in various biopanning procedures [12]) and thus difficult to be compatible with automated chromatography system (e.g. FPLC) typically operated with packed bed columns. Although chromatography technique has been used for phage binding test [13] following the screening process, no chromatographic procedure has been demonstrated in cooperative with phage display technique for the selection of specific affinity peptides toward target molecules.

The recent development of monolithic columns is one of the major breakthroughs in column technology. Monolithic columns are made of a single piece of porous cross-linked polymer or porous silica, thereby allowing for low column backpressure, high flow rates, faster transfer kinetics and passage of small particulates without clogging the columns. An extremely short column with large diameter channels (1500 μm) and low void volume gives an extraordinary resolution when separating proteins or other macromolecules such as DNA and viruses, while capacity remains high. The large through pore size and high porosities with a small diffusion path lead to high permeability with FPLC/HPLC instrumentation when compared to traditional columns with packed particles [14–17].

Lead, similar to heavy metal mercury, is a potent neurotoxin that accumulates in soft tissues and bone over time (especially in children) and causes systemic damage, blood and brain disorders [18,19]. Long-term exposure of lead can result in decreased functioning of the nervous system in adults. It may also cause weakness in fingers, wrists, or ankles [20]. In general, lead from wastewater can be precipitated or otherwise captured to give an insoluble form through adsorption or ion exchange [21–24]. However, the most widely used precipitation process is usually insufficient to reduce lead concentration to the level required by water quality standard [25]. The adsorption method involves the contact of the lead-containing water with a suitable adsorbent. Recently, there has been considerable interest in the use of relatively inexpensive agricultural sorbents which are capable of removing significant quantities of lead. For instance, a promising new method is to use living aquatic plants to absorb metal ion from water. It has been shown that aquatic plants are effective at separating metals from their surrounding waters [26]. This alternative process to physical and chemical based separation has been called biosorption, bioremoval, bioseparation or sometimes phytoremediation [27]. In addition, the use of biodegradable peptides as ligands to detect and/or precipitate heavy metals from aqueous systems also received much attention these days. Considering the high specificity and sensitivity of peptides (low K_d) toward target molecules, the use of peptide in sensor development is highly promising. In addition, though the usage of peptide per se on environmentally large scale for lead remediation may not be cost-effective, the use of M13 virus displaying the target cognitive peptide on the viral capsid as a biosorbent for Pb^{2+} would be economically viable [28]. The Pb^{2+} specific peptide can also be displayed as a part of surface proteins on various other microorganisms [28–31]. The biopanning in search of Pb^{2+} binders was first conducted in the conventional manner in our group, but it only gave peptides with high cross-binding affinities to other metal ions despite extensive negative selection. We thought that a more stringent negative screening strategy would be required to select peptide sequences specifically cognitive of Pb^{2+} without exhibiting cross-binding affinity to other metal ions.

In this study, a novel method based on chromatography technique for the selection of affinity peptides sequences specifically cognitive of Pb^{2+} was developed. Monolithic column with larger pore size which is sufficient for phage molecules to pass through was utilized. This chromatographic biopanning process enables multiple equilibrium stages for each round of screening, thereby greatly increasing the selection efficiency. In addition, the entire screening process can also be monitored online, providing an additional straightforward way to oversee the selection profile.

2. Experimental

2.1. Biological materials

A phage display peptide library for screening of disulfide bond constrained heptapeptide (Ph.D.—C7C™, Phage Display Peptide Library Kit, E8120S) was purchased from NEB (New England Biolabs, Beverly, MA). LB medium (10 g tryptone, 5 g yeast extract, 10 g NaCl in 1 L deionized water) supplemented with 12.5 mg/L tetracycline was used to grow *Escherichia coli* ER2738. 2 × YT medium (16 g tryptone, 10 g yeast extract, 5 g NaCl in 1 L deionized water, pH 7.0) containing 5 mM $MgCl_2$ was used in phage amplification.

2.2. Chromatographic biopanning

The chromatography system used was BioLogic DuoFlow Systems (760-2256, Bio-Rad) and the obtained data was processed with BioLogic DuoFlow Software Version 5.0. CIM® IDA monolithic column (217.3010, BIA Separations) was loaded with different types of metal ions (Pb^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} and Fe^{3+}) for chromatographic biopanning. Metal ion sources used in this study were $Pb(NO_3)_2$, $CuCl_2$, $NiCl_2$, $CoCl_2$ and $FeCl_3$, respectively. The final concentration of each metal ion solution was 50 mM. The monolithic column was loaded with 20 column volume (CV) of metal ion solution followed by 20 CV of Equilibration Buffer (0.1 M citrate–citric acid, 0.3 M NaCl, pH 5.4). All solutions were filtered through 0.22 μm filter prior to usage.

2.2.1. Pre-negative screening against uncharged monolithic column

A 100 μL sample of the phage library (~2×10^{12} virions, library complexity (unique clones) = 1.2×10^9 as claimed by NEB) was dissolved in 900 μL Equilibration Buffer and loaded to the uncharged monolithic column (1 mL bed volume, BV) in a total recycling mode. The flow rate was kept at 1 mL/min for 25 min. 2 mL of flowthrough was collected and 10 μL of which used for phage titering. Titration was conducted on agar plates supplemented with X-gal/IPTG. The rest of the phages were amplified by infecting *E. coli* ER2738 (phage library kit) in a 50 mL 2 × YT medium. The medium was incubated at 37 °C under vigorous shaking (250 rpm) for 4.5 h. The amplified phages were purified by precipitation with PEG/NaCl (20% (w/v) polyethylene glycol (PEG)-8000, 2.5 M NaCl). The amplified phages were then titered and adjusted to a desired concentration for the following positive screening. For phage titering, we used the plague forming unit assay as described by NEB manual.

2.2.2. Main biopanning (positive screening against Pb^{2+})

Three rounds of main biopanning were conducted against Pb^{2+} as a target. 100 μL of phage sample from pre-negative screening was dissolved in 900 μL Equilibration Buffer and loaded to the column pre-charged with Pb^{2+} at a flow rate of 0.5 mL/min. 50 mL of Washing Buffer 1 (0.1 M citrate–citric acid, 0.3 M NaCl, pH 5.4) was used to wash out any weakly bound phage at a flow rate of 2 mL/min. The elution was achieved by passing the column with 15 mL 1 M HCl. The flow rate was kept at