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Application of streptavidin mass spectrometric immunoassay tips for immunoaffinity based antibody phage display panning

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ABSTRACT

Antibody phage display panning involves the enrichment of antibodies against specific targets by affinity. In recent years, several new methods for panning have been introduced to accommodate the growing application of antibody phage display. The present work is concerned with the application of streptavidin mass spectrometry immunoassay (MSIA™) Disposable Automation Research Tips (D.A.R.T's®) for antibody phage display. The system was initially designed to isolate antigens by affinity selection for mass spectrometry analysis. The streptavidin MSIA™ D.A.R.T's® system allows for easy attachment of biotinylated target antigens on the solid surface for presentation to the phage library. As proof-of-concept, a domain antibody library was passed through the tips attached with the Hemolysin E antigen. After binding and washing, the bound phages were eluted via standard acid dissociation and the phages were rescued for subsequent panning rounds. Polyclonal enrichment was observed for three rounds of panning with five monoclonal domain antibodies identified. The proposed method allows for a convenient, rapid and semi-automated alternative to conventional antibody panning strategies.

1. Introduction

The mass spectrometry immunoassay (MSIA™) system is based on the principles of affinity separation of antibodies and antigen for mass spectrophotometry (MS) analysis (Nelson et al., 1995; Parker et al., 2010). The efficiency of this technique was first demonstrated with the capture and concentration of antigen to overcome signal repression of analytes for MS. MSIA™ has been used for fast, selective and quantitative screening of human blood for the presence of myotoxin *a*, and Mojave toxin from the venoms of *Crotalus viridis viridis*, and *Crotalus scutulatus scutulatus* (Nelson et al., 1995). Antibodies are bound to the modified matrix of the distal tips to facilitate selective protein capture to enrich and purify the target protein for subsequent MS analysis (Niederkofler et al., 2001; Niederkofler et al., 2008; Lopez et al., 2010). MSIA™ Streptavidin D.A.R.T's® (Disposable Automation Research Tips) are MSIA™ tips that contain covalently linked streptavidin to the porous monolithic solid support as a capture molecule. The streptavidin molecules introduced to the D.A.R.T's® MSIA™ platform functions for easy capture of biotinylated targets due the strong streptavidin affinity to biotin.

MSIA™ Streptavidin D.A.R.T's® takes advantage of the high affinity between the biotin and streptavidin tetramer for rapid, specific and strong capture of target molecules (Diamandis and Christopoulos, 1991). The conventional application of MSIA™ Streptavidin D.A.R.T's® platform which employs biotin-conjugated antibody to capture and enrich endogenous or clinically relevant antigens is done for rapid detection and analysis by MS. The MSIA™ tips serve as a convenient solid phase for target presentation for accessible target-ligand interaction (Niederkofler et al., 2008; Parker et al., 2010). As the design is based on the pipette tip, the physical motion of liquid movement through the tips allows a higher interaction of molecules to the solid phase to be achieved. The pipetting motion allows for all conventional stages of work to take place including incubation, washing and elution. The packing material of the tips also allows large amounts of protein to be captured for presentation. Subsequently, the MSIA™ tips are rinsed to remove any unbound antibodies and non-specific proteins while bound antibodies are eluted using suitable elution buffer. This allows for simultaneous target protein concentration and purification for downstream applications (Kiernan et al., 2002; Kiernan et al., 2003).

Phage display technology allows the physical display of peptides or proteins on the surface of bacteriophage (Smith, 1985). Antibody phage display panning involves the attachment of target proteins to solid phase for binding with specific antibodies displayed by

Abbreviations: dAb, domain antibody; D.A.R.T's, Disposable Automation Research Tips; HlyE, Hemolysin E; MS, mass spectrophotometry; MSIA, mass spectrometry immunoassay; scFv, single-chain variable fragment; Ub, ubiquitin.

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bacteriophages (Hairul Bahara et al., 2013; Hammers and Stanley, 2014). This is commonly done using high protein binding microtiter plates (Krebs et al., 2001), immunotubes (De Kruif et al., 1995), magnetic particles (Walter et al., 2001) and even an affinity column (Noppe et al., 2009) to physically present target antigens for binding with antibody presenting phages. The steps involved in conventional panning are similar to the processes involved in the MSIA™ protocol with an initial incubation step followed by incubation, washing and final elution (Coomber, 2002). The basic principle of antibody phage display panning is similar to that of MSIA™ with selection and concentration of antibodies by affinity pressure.

Here, we propose a new method for antibody phage display panning using the MSIA™ technology. This MSIA™ protocol was modified and customized to allow for antibody phage display panning based on conventional panning methods (Hammers and Stanley, 2014). The initial target binding is carried out to physically attach the target antigen on the MSIA™ tips. The incubation step allows for the antibody phage library to bind to the target molecule. The wash step will remove any unbound phages followed by the final elution. The elution step of MSIA™ will allow for phage recovery from each round of panning. Bound phages are subsequently amplified and used for subsequent panning rounds in order to obtain clonal enrichment. A proof-of-concept experiment using synthetic single-domain antibody (dAb) phage library for panning to generate monoclonal antibodies against the target antigen is performed. The proposed method provides an attractive alternative for rapid recombinant antibody development.

2. Materials and methods

2.1. Materials

Chloramphenicol and isopropyl β-D-1-thiogalactopyranoside (IPTG) were purchased from Calbiochem (San Diego, California). Glucose and polyethylene glycol 6000 were obtained from R&M chemicals (Essex, UK). Bovine serum albumin (BSA) was purchased from Nacalai Tesque (Kyoto, Japan). Ampicillin was obtained from Fisher Scientific (Pittsburgh, Pa.). Kanamycin and 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Amresco (OH, USA). 2YT was obtained from Merck (Darmstadt, Germany). *Escherichia coli* strain BL21 (DE3) competent cell was purchased from Stratagen (California, USA). TG1 and XL1-Blue competent cells were obtained from Agilent Technologies (Santa Clara, CA). M13KO7 helper phage was purchased from NEB (MA, USA). Helper plasmid pRARE3 encoding the biotin ligase and rare tRNAs together with the pRSET-BH6 plasmid were obtained from Dr. Zoltan Konthur, Max Planck Institute for Molecular Genetics (Berlin, Germany). Anti-M13 horseradish peroxidise (HRP)-conjugated monoclonal antibody was purchased from GE healthcare (NJ, USA). MSIA™ Streptavidin D.A.R.T's® and Finnpipette™ Novus i Electronic 12-channel Pipette were obtained from Thermo scientific (USA). QIAprep Spin Miniprep Kit was purchased from Qiagen (CA, USA). Costar flat bottom high protein binding microtiter plate was purchased from Corning (NY, USA) and BluElf prestained protein ladder was obtained from GeneDireX. Horseradish peroxidase-anti-c-Myc antibody was purchased from Abcam (MA, USA).

2.2. Expression of biotinylated antigens

For the production of biotinylated antigens, the *E. coli* strain BL21 (DE3) was transformed with the vector pRSET-BH6 and the helper plasmid, pRARE. The hemolysin E and ubiquitin genes in pRSET-BH6 allows for N-terminal fusion of the avi-tag. The transformed cells were grown at 37 °C to $OD_{600nm} = 0.6$ in 2YT broth containing 100 μg/ml ampicillin, 17 μg/ml chloramphenicol and 0.1% glucose. The T7 promoter was induced by 1 mM IPTG and further expressed overnight (o/n) at 25 °C with 160 rpm agitation. At the end of protein induction, cells were resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) with 20 mg/ml lysozyme. The cells were then chilled on ice for 30 min and the lysate was sonicated for 3 min continuously on ice. Subsequently, cell lysate was centrifuged and the supernatant containing the soluble proteins was collected. IMAC purification was performed for both recombinant antigens using the 1 ml Ni-NTA Agarose fast flow column (GE healthcare, Uppsala, Sweden) according to the manufacturer's instructions. Purified fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

2.3. Preparation of antibody phage library

An in-house synthetic domain antibody phagemid library stock (Hairul Bahara et al., 2015) with library size of 10^9 in XL1-Blue was inoculated into 500 ml 2YT broth containing ampicillin and 2% glucose with initial OD_{600nm} around 0.1–0.2. The cells were further grown to $OD_{600nm} = 0.5$ and co-infected with M13KO7 helper phage at 37 °C, static for 30 min. Subsequently, the culture was centrifuged and resuspended with 500 ml 2YT containing ampicilin, 60μg/ml kanamycin and 0.1% glucose. The library phage was propagated at 30 °C, 180 rpm for overnight. The culture was then centrifuged and the phage-containing supernatants were collected and precipitated by incubation with 20% (wt/vol) polyethylene glycol 6000 for 1 h at 4 °C. The supernatant was removed by centrifugation and the phage pellet was suspended in 1 ml phosphate buffer saline (PBS, pH 7.4) and stored at 4 °C.

2.4. MSIA™ streptavidin D.A.R.T's® loading of biotinylated antigen

MSIA™ Streptavidin D.A.R.T's® were mounted to a Finnpipette™ Novus i Electronic 12-channel Pipette for antigen binding. Biotinylated recombinant antigen at 100 μg in bicarbonate buffer (0.1 M $NaHCO_3$, pH 8.6) was loaded in MSIA™ Streptavidin D.A.R.T's® by continuous aspiration and dispensing. The electronic pipette program was set for 999 cycles with a moderate speed (Speed Setting 5). Then, the MSIA™ Streptavidin D.A.R.T's® were washed twice (20 cycles, Speed Setting 8) with PBS-T (PBS in 0.5% Tween 20) and finally (20 cycles, Speed Setting 8) with PBS. The antigen captured MSIA™ tip is ready for use.

2.5. MSIA™ streptavidin D.A.R.T's® antibody phage library panning

The recombinant Ubiquitin (rUb) antigen coupled tip was first used to enrich monoclonal antibodies by affinity selection before it was used for panning and recombinant Hemolysin E (rHlyE) and MSIA™ Streptavidin D.A.R.T's® were also passed through non-specific anti-rUb monoclonal phage for background system control. Then, in the panning process, the antigen-coupled tip, i,e. rHlyE coupled tip was blocked with 3% skimmed milk in PBS-T (PTM) (500 cycles, speed setting 5). A total of 10^{12} phage particles of the antibody library was pre-incubated with PTM before used for panning. Antibody phage capture was done by performing repetitive pipetting with a fixed volume of 150 μl, with 999 cycles repeat and a speed setting of 5. Then, the MSIA™ Streptavidin D.A.R.T's® were rinsed 5 rounds with PBS-T and 5 rounds with PBS. Each wash cycle constitutes 20 cycles of aspirating and dispensing with a faster flow (Speed Setting 8). The bound phages were eluted by using 100 μl of 0.2 M glycine-HCl, pH 2.2 with 300 cycles of aspiration and dispensing with a slow speed (Speed Setting 3). The eluted fraction is then immediately neutralized with 1 M Tris HCl, pH 9.1. The eluted phages were used to infect an exponentially growing TG1 culture (OD_{600} of 0.5) for phage rescue and phage titer was also done by serial dilution. The culture was spun down and resuspended with 20 ml 2YT containing ampicilin. The cells were further grown for 3.5 h and infected with helper phage, M13KO7 for 30 min at 37 °C. Packaging and amplification of successive enriched phage was done by culturing the cells (30 °C, 180 rpm) o/n with ampicillin and kanamycin. The culture was then