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A new application of aptamer: One-step purification and immobilization of enzyme from cell lysates for biocatalysis

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ABSTRACT

Aptamers are nucleic acid-based high affinity ligands that are able to capture their corresponding target through molecular recognition. In this study, several DNA aptamers with high affinity and specificity for β-glucuronidases (PGUS-E) were obtained by our modified SELEX method. Among them, Apt5 and Apt9 were selected as representatives and covalently linked to magnetic beads, respectively. The aptamer-modified magnetic beads were characterized and successfully applied to one-step purification and immobilization of PGUS-E from the complex cell lysates. By conveniently adjusting the pH and ion strength, the PGUS-E purities reached 84% for Apt5-modified beads and 88% for Apt9-modified beads. Moreover, the maximum PGUS-E capturing capacity of the Apt5 and Apt9 modified magnetic beads were found to be 31.75 μg/mg and 32.95 μg/mg, respectively. The immobilized PGUS-E on aptamer-based magnetic beads showed good reusability, and the conversion of glycyrrhizin still remained more than 70% after 7 cycles. In addition, the aptamer-modified beads support can be easily regenerated, and the conversion rate of glycyrrhizin (GL) was still 62% after the 7th cycle of regeneration. This investigation can be easily extended to other enzyme systems and may help open a generic route to develop a novel enzyme immobilization technology for biocatalysis based on aptamer.

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1. Introduction

In the drive towards green processes, sustainable biocatalysis is increasingly being regarded as a competitive and cost-effective technology for the manufacture of fine chemicals, pharmaceuticals and agrochemical intermediates. Naturally, enzymes have recently been more frequently used in industrial applications because of their properties of high efficiency and specificity (Adrio and Demain, 2014). For practical and economical reasons, reusability of enzymes in the industrial applications needs to be considered and this is often achieved by using immobilization technologies (Jia et al., 2014; Song et al., 2014), which allows to separate the enzymes from the reaction milieu easily and to simplify the recovery of products. However, traditional enzyme immobilization methods such as covalent binding, entrapment (encapsulation) and cross-linking have some drawbacks, including mass-transfer limitations, uncontrolled enzyme orientation and the massive losses of enzyme activity due to the use of organic reagents or harsh immobilization environment (Brady and Jordaan, 2009). Importantly, prior enzyme purification is necessary to avoid undesired side reactions catalyzed by contaminant enzymes in crude preparations, but this will cause some problems in practical application such as time-consuming, high cost and enzyme inactivation. Therefore, to overcome these drawbacks, it would be great to integrate the purification and immobilization steps by designing a highly selective immobilization process.

In the current study, the immobilized metal affinity chromatography (IMAC) (Cassimjee et al., 2011) and immunoaffinity chromatography (Garrido-Medina et al., 2014; Pascal and Swapan, 1990) were popular methods for possibly coupling the purification and immobilization steps. But typically in biocatalysis applications, the protein tag dependences and toxicity of metal ion to proteins in IMAC, and the instability and high-cost of immunoprotein have restricted their further use. However, the characteristics of aptamers attracted our attention and brought us a new inspiration. Aptamers are oligonucleic acid or peptide molecules that exhibit high affinity and specificity to their corresponding target (Nimjee et al., 2005). They range in size from approximately 6–40 kDa and sometimes have complex three-dimensional structures, formed by a combination of Watson-Crick and noncanonical intramolecular interactions. The aptamers can be recognized by various target

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molecules, including small chemical molecules, nucleic acids, proteins to protein complexes, entire cells and even microorganisms (Cox et al., 2002). Regarding their use in affinity-based purification or immobilization, aptamers offer several advantages over antibodies or proteins in general including increased stability against high temperature and extreme pH (Cho et al., 2013). As known to all, when the target protein has an extremely low abundance or has similar properties like molecular weight, hydrophobicity or isoelectric point with other contaminants in the mixture, it is very difficult to get a satisfying purification using conventional methods such as the isoelectric point, sodium dodecyl sulfate, gel permeation chromatography and so on. However, according to the high specificity and affinity of aptamers, it is very easy to enrich these target proteins and separate them from contaminants. Furthermore, aptamers have no immunogenicity. They are synthesized chemically and different modifications can be fused at defined positions within the oligonucleotide sequence enabling direct immobilization of the aptamer to a solid support in a controlled orientation. However, it is a challenge to couple the enzyme purification and immobilization in one step for biocatalysis based on aptamer, because the purification and immobilization must be matched with the subsequent catalytic reaction conditions and there have been no reports about this so far.

Aptamers are generally generated by a process that combines combinatorial chemistry within vitro evolution (Strehlitz and Stoltenburg, 2008), known as SELEX (systematic evolution of ligands by exponential enrichment) (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990). Some high-throughput methods for aptamer production have been published in recent years, but most of them require customized robotics not easily available to many laboratories and could not produce aptamers against large numbers of diverse targets. Therefore, an efficient and inexpensive method applicable to most ordinary laboratory needs to be established and improved.

In our previous work, β-glucuronidases (PGUS-E) from *Penicillium purpurogenum* Li-3 (Feng et al., 2006) was overexpressed in *Escherichia coli* BL21 and used to catalyze the hydrolysis of glycyrrhizin (GL) into glycyrrhetinic acid monoglucuronide (GAMG) and glycyrrhetinic acid (GA) (Zou et al., 2013a). GAMG and GA are very promising fine chemicals with multi-pharmacological activity such as anti-inflammation, antivirus, anti-tumor, immunomodulating and so on. Therefore, this study focused on using this biocatalysis process as a model to prove our concept of one-step purification and immobilization based on aptamer.

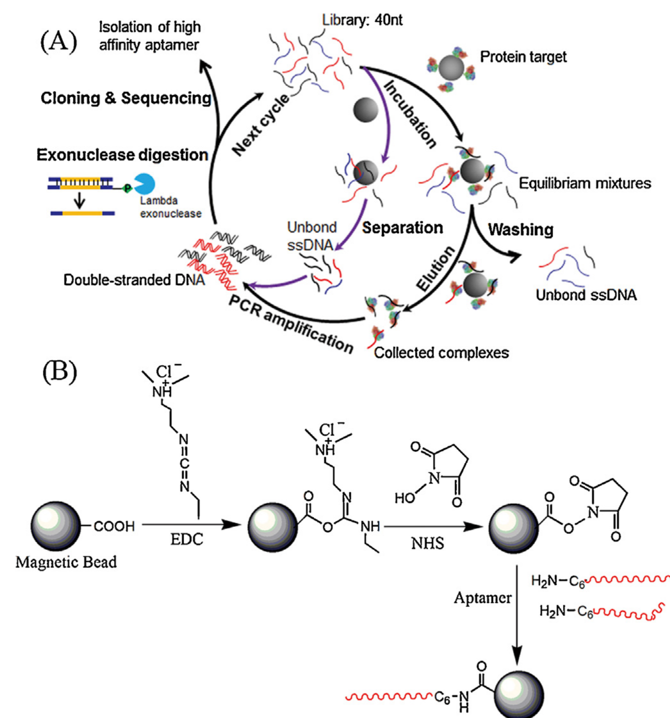

Fig. 1. (A) Scheme for the systematic evolution of ligands by exponential (SELEX) enrichment process. The counter selection process was indicated by the purple arrow. (B) The scheme of the covalent coupling of the carboxyl groups on the surface of the magnetic beads with the primary amino groups on the ssDNA.

2. Materials and methods

2.1. In vitro selection of aptamers for PGUS-E

Firstly, PGUS-E was immobilized on Ni-NTA magnetic beads for the fast in vitro selection of aptamers. The details for the immobilization can be seen in SI. PGUS-E-specific aptamers were selected via an affinity selection procedure, as illustrated in Fig. 1A. For each SELEX round, the ssDNA library was incubated with bead-bound PGUS-E in molar excess so the target-binding oligonucleotides were competitively separated from those that bound the target with lower affinity or physical absorption. The amount of beads, the incubation time of target-coated beads with ssDNA, and the wash times were chosen as control variables to accelerate the screening efficiency. In the initial round of selection, 100 μl ssDNA library was diluted by twice with 100 μl PBS in a PCR tube, heated to 95 °C for 5 min and immediately cooled at 4 °C for 15 min. The resultant material was added to 100 μl PGUS-E-coated magnetic beads and incubated at room temperature for 1 h with tilting and rotation. The beads were separated by a magnet and washed three times with 500 μl PBS-T to elute unbound oligonucleotides. The protein and aptamer complexes were eluted from the Ni-NTA magnetic beads with 50 μl 20 mM Tris buffer (pH 7.5) containing 500 mM imidazole, and then transferred to PCR tubes for amplification. As a result, dsDNA fragments with a phosphate group modification at the complementary strands were produced. The 200 μl PCR products were then purified by the NucleoSpin® Gel and PCR Clean-up kit.

The obtained dsDNA product was further selectively digested into ssDNA by lambda exonuclease (Marimuthu et al., 2012). Finally, this new ssDNA pool (50 μl) was diluted with 50 μl PBS, heated to 95 °C for 5 min, and then immediately placed at 4 °C until the next round of SELEX. For further rounds of selection, the amount of PGUS-E-coated beads, incubation time and wash times were respectively reduced to 50 μl, 30 min, 6 times (rounds 2–6) and subsequently 25 μl, 15 min, 9 times (rounds 7–12) in the incubation step. Considering the optimal catalytic temperature of PGUS-E, the incubation temperature in the rounds of 13–18 was increased to 40 °C for the sake of screening aptamer that could still capture PGUS-E tightly in the catalytic reactions. Similarly, the amount of PGUS-E-coated beads, incubation time and wash times were minished to 10 μl, 10 min, 9 times (rounds 13–15) and 5 μl, 5 min, 12 times (rounds 16–18).

To exclude those ssDNA that bound with Ni-NTA magnetic beads rather than PGUS-E, counter selection was performed by using beads without PGUS-E in the rounds 3, 6, 9, 12, and 15. The preprocessed Ni-NTA magnetic beads were incubated with the ssDNA pool for 30 min with rotation. After magnetic separation, the supernatant was used for the next round selection. Finally, the recovered ssDNA pool from the 12th and 18th SELEX round were amplified with unlabeled primers and cloned into the vector pMD-19T (TaKaRa) for sequencing. Prediction of shapes with the lowest free energy and secondary structure analysis of the sequences were executed by using the free-energy minimization algorithm according to Zuker (2003) via the Internet tool Mfold (Andersen, 2010).

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