Aptamer-facilitated Protein Isolation from Cells (AptaPIC)

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ABSTRACT

Purification of proteins from cell lysate often represents a significant challenge. The most direct and efficient way of protein isolation is affinity purification. Unfortunately, this approach is limited by the lengthy and difficult process of development of classical affinity probes, such as antibodies. Production of antibodies requires the protein to be available in a purified form, but the purification of the protein requires the availability of antibodies. To resolve these problems, we introduce Aptamer-facilitated Protein Isolation from Cells (AptaPIC). This technology allows for generation of DNA aptamer affinity ligands for a protein target in a context of a crude cell lysate. This enables efficient, tag-free, affinity purification of target proteins which are not available in a pure form *a priori*, and for which no affinity ligands are available. We demonstrate the efficiency of the developed technique using two examples: MutS protein from *T. aquaticus* and human PDGF-B protein. AptaPIC has the potential to considerably speed up the purification of proteins and, thus, accelerate their structural and functional studies.

INTRODUCTION

Modes of affinity purification:

Affinity Tags:
- Established purification protocols and kits
- May cause changes in protein conformation, decreased expression levels, toxicity to host cells
- Difficult to cleave off without affecting protein

Antibodies:
- Superb specificity and affinity to target
- Expensive and have a short shelf life
- At development stage, require target protein in pure form

Aptamers:
- Superb specificity and affinity to target
- Inexpensive, easily synthesized and have a long shelf life
- Typically require target protein in pure form at development stage

Aptamers are DNA or RNA-based affinity ligands that are selected *in vitro* from diverse combinatorial libraries.

AptaPIC:
- Allows development of aptamers for a protein in crude cell lysate
- Facilitates affinity protein purification by the developed aptamers from the same lysate

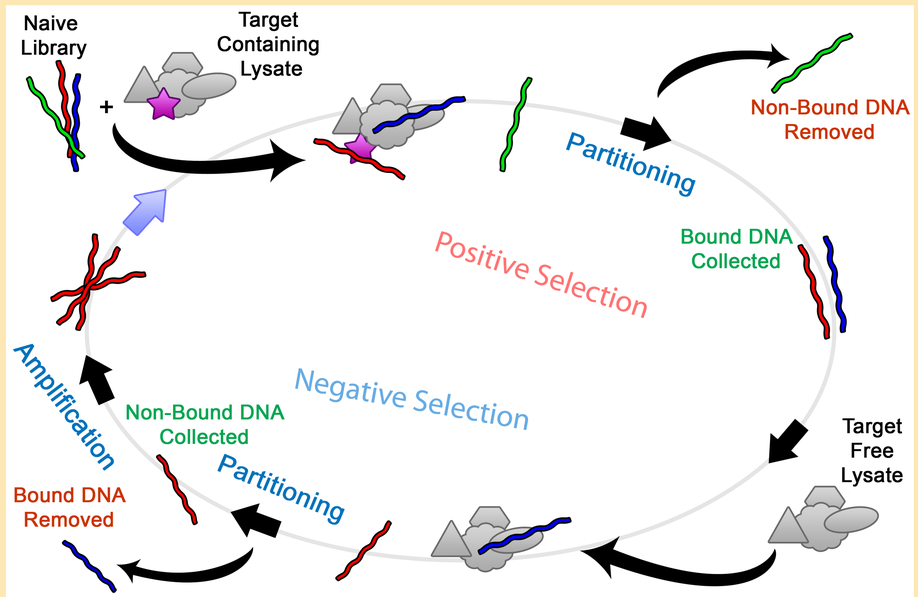

Fig. 1: Scematic representation of the aptamer selection step in AptaPIC

To make the generation of aptamers possible in the context of a cell lysate, AptaPIC integrates strategies that reduce protease- and nuclease-facilitated degradation of the target protein and the DNA ligands, respectively and reduce the negative effects of DNA-binding proteins on the efficiency of aptamer generation. These strategies include the use of background masking DNA sequences and a combination of protease inhibitors.

RESULTS

AptaPIC was used on two protein targets, MutS protein from *T. aquaticus* and human Platelet-derived Growth Factor chain B (PDGF-B). As proof of principle for each target, pure protein was added to *E. coli* cell lysate, at 5% of total protein for MutS and 10% for PDGF-B. Capillary electrophoresis (CE) was used as means of partitioning during aptamer selection step **(Fig. 2)**. Five rounds of aptamer selection were carried out for MutS protein and 3 rounds for PDGF-B. Affinity analysis showed significant improvements for each of the individual targets, but not for other components of the cell lysate **(Fig. 3)**.

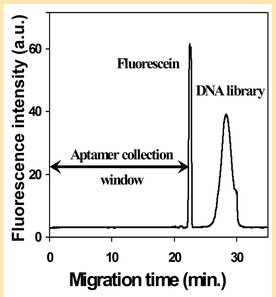

Fig. 2: Aptamer selection with CE partitioning

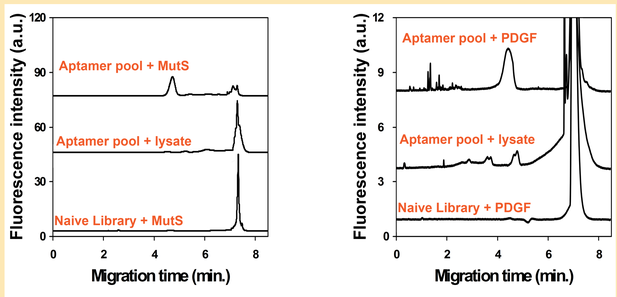

Fig. 3: Affinity and specificity of selected aptamer pools to respective protein targets and to target-free cell lysate

Aptamer Pool Target	Affinity of Naive Library	Affinity to Target	Affinity to Lysate
MutS	> 500 µM	60 nM	> 100 µM
PDGF-B	> 500 µM	4 µM	> 100 µM

Table 1. Comparison of EC_{50} values for aptamer-enriched DNA pools. EC_{50} values were calculated for a total protein concentration of 1 µM and DNA pools at the concentration of 200 nM.

Obtained aptamer pools were then biotinylated and used in isolation of the target protein from the cell lysate. Aptamers, along with bound targets, were precipitated out of the solution by streptavidin-coated magnetic beads. Cell lysate-containing supernatant was removed, and beads were washed multiple times. Protein targets were then eluted from the aptamer-bead complex by a sodium dodecyl sulfate (SDS)-containing buffer. SDS-PAGE analysis showed significant improvement in sample purity for both cases **(Fig. 4)**.

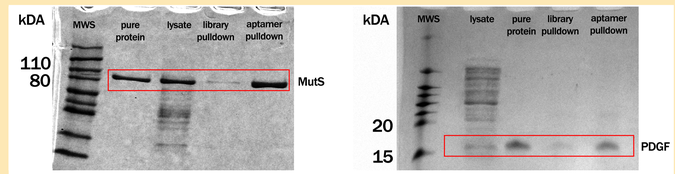

Fig. 4: SDS-PAGE analysis of aptamer-facilitated protein purification products

CONCLUSIONS

The results demonstrate that AptaPIC is a reliable method, which can be successfully applied to both DNA-binding proteins and to proteins that do not have an inherent DNA binding function. The aptamer-enriched DNA pools obtained through AptaPIC technique were comparable in affinity and specificity to aptamer-enriched pools obtained through a conventional aptamer selection approach. The success of the aptamer-mediated protein isolation procedure suggests that even an aptamer pool, developed within a small number of selection rounds and displaying a relatively low bulk affinity value towards it target protein, may possess sufficient specificity to allow for efficient purification of the target protein from cell lysate. While the aptamer pools obtained through AptaPIC may not possess sufficient affinity and specificity characteristics for analytical applications, they can be used as a starting point for subsequent aptamer selection steps for the pure target. AptaPIC approach has the potential to become a valuable tool for purification of newly-discovered proteins, for which no established isolation protocols are available.

REFERENCES

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