Journal of Chromatography B 1073 (2018) 201–206

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Aptamer facilitated purification of functional proteins

Stanislav S. Beloborodov[1], Jiayin Bao[1], Svetlana M. Krylova, Agnesa Shala-Lawrence, Philip E. Johnson, Sergey N. Krylov*

Department of Chemistry and Centre for Research on Biomolecular Interactions, York University, Toronto, Ontario M3J 1P3, Canada

ARTICLE INFO

Keywords:
Aptamer-facilitated protein purification
Protein activity
AlkB
MutS
Demethylation assay
Mismatch-binding assay

ABSTRACT

DNA aptamers are attractive capture probes for affinity chromatography since, in contrast to antibodies, they can be chemically synthesized and, in contrast to tag-specific capture probes (such as Nickel-NTA or Glutathione), they can be used for purification of proteins free of genetic modifications (such as His or GST tags). Despite these attractive features of aptamers as capture probes, there are only a few reports on aptamer-based protein purification and none of them includes a test of the purified protein's activity, thus, leaving discouraging doubts about method's ability to purify proteins in their active state. The goal of this work was to prove that aptamers could facilitate isolation of active proteins. We refined a complete aptamer-based affinity purification procedure, which takes 4 h to complete. We further applied this procedure to purify two recombinant proteins, MutS and AlkB, from bacterial cell culture: 0.21 mg of 85%-pure AlkB from 4 mL of culture and 0.24 mg of 82%-pure MutS from 0.5 mL of culture. Finally, we proved protein activity by two capillary electrophoresis based assays: an enzymatic assay for AlkB and a DNA-binding assay for MutS. We suggest that in combination with aptamer selection for non-purified protein targets in crude cell lysate, aptamer-based purification provides a means of fast isolation of tag-free recombinant proteins in their native state without the use of antibodies.

1. Introduction

Purification of recombinant proteins is a key procedure in biochemical research [1] and development and production of biologics [2]. The gene, which encodes the protein, is cloned and over-expressed in cultured bacterial cells; the cells are lysed to obtain the cell lysate enriched with the target protein [3]. The target protein is then separated from the cell lysate based on its unique physicochemical properties such as charge [4], size [5], or affinity to a capture probe (such as Nickel-NTA or Glutathione) [6,7]. Affinity purification facilitates the highest degree of purity, as it relies on very selective interaction of the target protein with the capture probe. Affinity purification typically requires fusion of the target protein with an affinity tag (such as His or GST tag). An affinity tag may alter protein conformation and, thus, change its physicochemical properties [8], and its activity as a result. Although a tag can be enzymatically removed, the enzyme may cleave fragments of the target protein, rendering it inactive and causing additional contamination of the protein with tag-removal agents [9]. Therefore, purification of some proteins requires tag-less affinity techniques.

A commonly used tag-less purification method involves antibodies as capture probes that can specifically bind the target protein itself. However, the utility of this method is restricted by the limitations of antibodies. The development of antibodies is a lengthy and labor-intensive process that cannot be conducted *in vitro*. In addition, antibodies are very sensitive to temperature changes and undergo denaturation over the time. Hence, tag-less purification method would benefit from replacing antibodies with capture probes, which can be easily synthesized and are more stable.

Antibodies can be replaced with oligonucleotide aptamers, which are single-stranded DNA or RNA (ssDNA and ssRNA) that can selectively bind their target protein with high affinity and specificity [10]. Aptamers are usually selected from random-sequence DNA (or RNA) libraries *via* the SELEX (Systematic Evolution of Ligands by EXponential enrichment) process, which involves alternating steps of partitioning of protein-DNA complexes from unbound DNA and amplification of the aptamer-enriched DNA library by the polymerase chain reaction [11]. Aptamers are more stable than antibodies and can be synthesized *in vitro*. Aptamers can also be synthetically modified with a linker (such as an amine group, thiol group or biotin) [12], which allows their reliable non-covalent or covalent conjugation with the solid-phase substrate. The idea of aptamer facilitated protein purification was introduced in 1999 [13], and since then, a few proof-of-principle studies have been conducted [14–17]. To the best of our knowledge, protein's activity was

* Corresponding author.
E-mail address: skrylov@yorku.ca (S.N. Krylov).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jchromb.2017.12.024
Received 31 August 2017; Received in revised form 15 November 2017; Accepted 15 December 2017
Available online 16 December 2017
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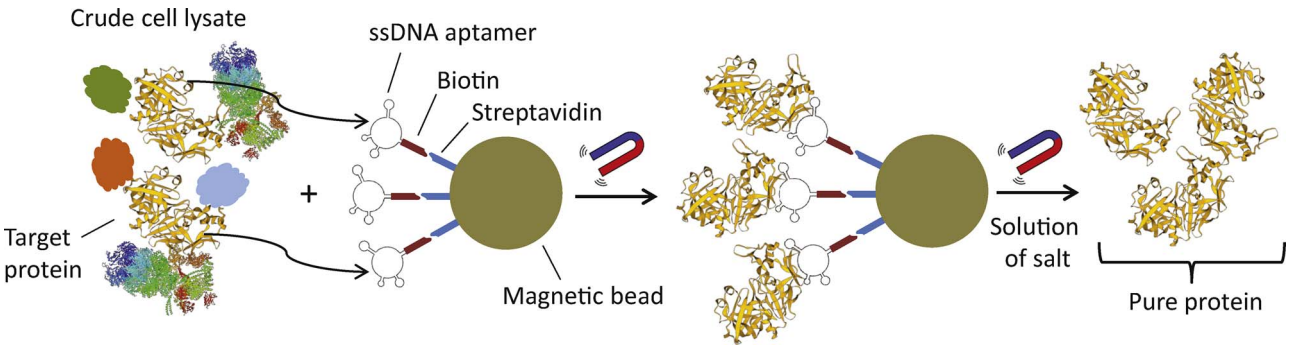

Fig. 1. Schematic illustration of aptamer-facilitated protein purification. Multiple copies of protein-specific ssDNA aptamer are attached to magnetic beads through the streptavidin-biotin bridge. The aptamers serve as capture probes for the target protein in a crude cell lysate. The beads with captured target protein are separated from the lysate by retaining the beads with a magnet and washing out the lysate. Finally, the target protein is eluted with phosphate-buffered saline (PBS) supplemented with an additional strong electrolyte (NaCl or $MgCl_2$) to increase the ionic strength.

not confirmed in any of the published studies. Also, some studies used aptamers that specifically bind His-tag [18,19] (thus, still requiring an affinity tag on a target protein) or involved the use of denaturing agents at the elution step [20] (thus potentially leading to protein inactivation). Therefore, our goal was to develop and validate a protocol for aptamer facilitated purification of tag-less proteins with preserved activity.

The developed purification procedure is applied to a crude bacterial cell lysate prepared by sonication and follows a general scheme shown in Fig. 1. The biotinylated aptamer is immobilized onto magnetic beads *via* streptavidin-biotin interaction. After immobilization, the beads are incubated with the cell lysate containing the target protein. During the incubation, the target protein binds to the immobilized aptamer. The unbound components of the lysate are then removed. The bound protein is eluted by a buffer containing a high concentration of a strong electrolyte (NaCl or $MgCl_2$) with physiological pH at +4 °C (the high ionic strength induces reversible structural alteration of DNA aptamer without denaturing the target protein). The procedure takes 4 h to complete.

The developed procedure was validated with the AlkB [21] and MutS [22] proteins. Both proteins are involved in DNA repair: AlkB (23.9 kDa) catalyzes dealkylation of ssDNA, and MutS (95 kDa) recognizes and binds to mismatches in double stranded DNA (dsDNA). Protein activity after purification was confirmed by two capillary electrophoresis based assays: an enzymatic assay for AlkB and a DNA-binding assay for MutS. These assays showed that both proteins retained their specific activity. Thus, our protocol can be used for isolation of the tag-less proteins in their active state and can also be combined with a previously-developed approach of aptamer selection for non-purified protein in a crude cell lysate [16]. This approach involves multiple rounds of the following alternating steps: incubation of the DNA library with the target-containing cell lysate, collection of the bound DNA, incubation of the collected DNA with target-free cell lysate and collection of the unbound DNA, followed by the PCR amplification and purification. The selected polyclonal aptamers can then be used for purification of the target protein.

2. Materials and methods

2.1. Chemicals and materials

E. coli strain MG1655 was kindly provided by Coli Genetic Stock Center (CT, USA). The pET-24a(+) vector and NovaBlue competent cells were purchased from EMD Millipore (PA, USA). *E. coli* BL21-Gold (DE3) competent cells were purchased from Agilent Technologies (CA, USA). A biotin labeled anti-AlkB aptamer [23] (biotin-AlkB-aptamer): 5′-/5BioTEG/CTC CTC TGA CTG TAA CCA CGT GCC TAG CGT TTC ATT GTC CCT TCT TAT TAG GTG ATA ATA GCA TAG GTA GTC CAG AAG CC-3′ and AlkB primers: forward 5′-GGTGGTCATATGTTGGATCTGTT TGCC-3′ and reverse 5′-GGTGGTGGATCCTTATTCTTTTTTACCTGC-3′ were custom-synthesized by Integrated DNA Technologies (IA, USA). The AlkB methylated substrate TTCmTTTTTTTTTTT3′-FAM (TTCm) was custom-synthesized by ATDBio (Southampton, UK). The restriction enzymes NdeI and BamHI, blue prestained protein standard, and T4 DNA ligase were purchased from New England Biolabs (MA, USA).

The pETMutS plasmid #13245 was obtained from Addgene (MA, USA). The *E. coli* Rossetta-gamiTM 2 (DE3) competent cells were purchased from EMD Millipore (PA, USA). A biotin labeled anti-MutS aptamer [24] (biotin-MutS-aptamer): 5′-/5BioTEG/CTT CTG CCC GCC TCC TTC CTG GTA AAG TCA TTA ATA GGT GTG GGG TGC CGG GCA TTT CGG AGA CGA GAT AGG CGG ACA CT -3′ and fluorescently-labeled dsDNA with a G-T mismatch (dsDNA-GT) with forward sequence of 5′-CTT CTG CCC GCC TCC TTC CTT CCA ACC TTC ATC GGC CAC CC-3′ (the mismatch is indicated with an underline) were custom-synthesized by Integrated DNA Technologies (IA, USA). Streptavidin-coupled magnetic beads (MagnaBind Streptavidin Beads) were purchased from Thermo Scientific (IL, USA). Mono Q anion exchange column (1 mL bed bolume) was purchased from Amersham (Amersham Pharmacia Biotech, currently GE Healthcare Life Sciences, IL, USA).

The isopropyl-β-D-thiogalactoside (IPTG) was purchased from Gold Biotechnology (MO, USA). A protease inhibitor cocktail (cOmplete™) was purchased from Roche (CA, USA). A bicinchoninic acid (BCA) assay kit was obtained from Pierce Biotechnology (IL, USA). A fused silica capillary (75 μm inner diameter, 360 μm outer diameter) was purchased from Molex (AZ, USA). All other chemicals were purchased from Sigma-Aldrich (ON, Canada). All solutions were prepared with deionized water filtered through a 0.22 μm Millipore filter (ON, Canada).

2.2. Immobilization of biotinylated aptamer on streptavidin-beads

To immobilize the aptamers on magnetic beads we took 1 mL of streptavidin-coated magnetic beads (which can bind 2 μg or 8.19 nanomoles of biotin) and washed them 3 times with deionized water. The washed beads were mixed with 0.8 mL deionized water and 105 μL of 100 μM aptamer-biotin solution. After a 2-h incubation at room temperature, the supernatant was removed and the beads were washed with deionized water three times and then resuspended in 0.5 mL of deionized water. The aptamer occupied 92% of the streptavidin molecules on the beads, which was determined by absorbance measurements of the aptamer-containing solution at 260 nm before and after the binding. In other words, 1 mL of the original beads suspension bound 7.53 nanomoles of biotin and aptamer, accordingly. A suspension of aptamer-free beads was prepared in a way similar to preparation of the suspension of aptamer-containing beads, but the aptamer solution was replaced with water at the mixing step and the final suspension was made in 0.5 mL of the resuspension buffer instead of deionized water.

2.3. Protein expression and preparation of cell lysate

AlkB was expressed as the following. The AlkB gene from *E. coli* strain MG1655 was amplified by PCR. The product of PCR amplification was then digested with NdeI and BamHI enzymes and cloned into the pET-24a(+) vector. The pET-24a(+)-AlkB vector was then transfected into NovaBlue cells, amplified, reextracted and purified by using the GenElute Plasmid Miniprep Kit. The purified plasmid was then transfected into *E. coli* BL21-Gold (DE3) competent cells. The culture was first grown in 25 mL Lysogeny Broth (LB) media with 50 μg/mL kanamycin at 37 °C overnight (at 250 rpm shaking). Then 1 mL of the culture was transferred to 100 mL of Terrific Broth (TB) media (with 50 μg/mL kanamycin) and was incubated with shaking at 250 rpm for 6 h at 37 °C until the Optical Density value of 0.8 was achieved at 600 nm (OD^{600}). Then, IPTG was added to 100 mL of the cell culture in LB media to achieve 1 mM final concentration, and the mixture was shaken overnight under room temperature at 250 rpm. Next, the cells were pelleted by centrifugation at ~5000 × *g* (Allegra 21R centrifuge with S4180 rotor (Beckman Coulter, ON, Canada)) for 30 min at 4 °C. The supernatant was removed, and the cells were resuspended in 10 mL of the resuspension buffer (100 mM 2-(N-morpholino)ethanesulfonic acid (MES), 1 mM Dithiothreitol (DTT) and 19 μL of protease P8340 for 10 mL solution, pH 5.8), and sonicated on ice with 15-s on/off intervals for 7 min at 20% of maximum power (Fisher Scientific, Sonic Dismembrator, Model 500). Next, the mixture was centrifuged for 30 min at ~20000 × *g* (Eppendorf 5417R centrifuge with F45-30-11 rotor (Fisher scientific, PA, USA)) at 4 °C. The supernatant was collected.

MutS was expressed in a similar way to that of AlkB expression [16,25]. The competent *E. coli* Rossetta-gami™ 2 (DE3) cells were transformed with the plasmid pETMutS. The transformed cells were grown in 1 L of LB medium in the presence of 50 μg/mL ampicillin at 37 °C with 250 rpm shaking to reach an OD^{600} value of 0.8. Protein expression was induced by adding IPTG to 1 mM in the cell culture. The culture was shaken overnight at room temperature at 250 rpm. The cells were pelleted by centrifugation at ~5000 × *g* (Allegra 21R centrifuge with S4180 rotor (Beckman Coulter, ON, Canada)) for 1 h at 4 °C. The cell pellet was resuspended in 10 mL Tris buffer (20 mM tris-HCl pH 7.4) that contained a cOmplete protease inhibitor cocktail tablet and sonicated on ice with 15-s on and 55-s off intervals for 4 min at 60% of maximum power of the sonicator. The cell debris was pelleted by centrifugation at 5000 × *g* for 1 h at 4 °C. The supernatant was incubated at 70 °C for 45 min followed by a new round of centrifugation for 1 h at 4 °C. For pre-affinity purification, the protein sample was applied onto a Mono Q anion exchange column, which was equilibrated with Tris buffer, and eluted with a linear gradient of 0.1–1.0 M NaCl at a flow rate of 1 mL/min. The MutS protein was eluted at approximately 0.4 M NaCl. Fractions containing MutS protein were pooled and dialyzed extensively against Tris buffer by using an Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-10 membrane (Millipore, Ireland).

2.4. Protein purification by aptamer-beads

In the case of AlkB purification, 4 mL of protein-containing supernatant was mixed with 0.5 mL of bead suspension (on ice) and incubated at continuous shaking for 2 h at 250 rpm. The beads were removed and sequentially incubated for 10 min with 1 mL of each of the following buffers: resuspension buffer, 0.5 M NaCl solution (in PBS) and 1 M NaCl solution in PBS. The eluates were analyzed with CE by using ProteomeLab SDS-MW Analysis Kit (Beckman-Coulter) according to the manufacturer's instructions. The resuspension buffer eluate contained traces of the cell lysate. The eluate of 0.5 M NaCl in PBS contained the largest amount of pure AlkB and then was subjected to a buffer exchange with AlkB protein storage buffer (50 mM Tris-HCl, 500 mM NaCl, 1 mM DTT, 0.02% NaN_3) by using Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-10 membrane (Millipore, Ireland). The eluate of 1 M NaCl in PBS contained only traces of AlkB and was not used. AlkB-containing cell supernatant was also used for the negative control experiments. In these experiments, 8 μL of the supernatant was mixed with 200 μL of the aptamer-free beads suspension and incubated on ice with continuous shaking at 250 rpm for 2 h. The same was done for 100, 50, 25 and 0 μL of the aptamer-free beads suspension with addition of an appropriate volume of the resuspension buffer to keep the final volume of the solution equal to 208 μL. The beads were then removed and the supernatant was analyzed with CE by using ProteomeLab SDS-MW Analysis Kit (Beckman-Coulter) according to the manufacturer's instructions.

For purification of MutS protein, 0.5 mL of MutS-protein-containing solution was incubated with 0.5 mL of aptamer-bead solution on ice for 1 h under gentle agitation. After the incubation, the beads were collected and the clear solution was removed. For MutS protein elution, the beads were sequentially incubated (10 min each) and eluted with 500 μL of 0.25, 0.5, 0.75, 1, 2 and 3 M $MgCl_2$ solution at room temperature. The fraction which eluted with 2 M $MgCl_2$ contained the largest amount of pure MutS and was further used. The eluted protein solutions were dialyzed extensively against Tris buffer (20 mM tris-HCl pH 7.4) by using Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-10 membrane (Millipore, Ireland). The concentrations of both AlkB and MutS were determined by using the BCA assay with bovine serum albumin (BSA) as a standard.

2.5. Capillary electrophoresis

CE experiments were carried out with a P/ACE MDQ instrument from SCIEX (Fullerton, CA, USA). Laser-induced fluorescence (LIF) detection with excitation at 488 nm and emission at 520 nm was used for AlkB enzymatic activity assay and dsDNA-MutS binding assay. Light-absorption detection at 214 nm was used for protein molecular weight analysis in the SDS-gel electrophoresis mode (SDS-MW). New capillaries were preconditioned by washing them with 10 capillary volumes of methanol. For CE experiments with LIF detection each run was followed by washing the capillary with 7 capillary volumes of each of 100 mM HCl, 100 mM NaOH, deionized water (dd), and running buffer. Capillary coolant temperature was kept at +25 °C. An electric field of 310 V/cm with a positive electrode at the injection end was used to run electrophoresis. Uncoated fused silica capillaries with a total length of 50 cm and a 40-cm distance from the inlet to the detection window and with inner diameter of 75 μm were used for detection of the output products of the AlkB enzymatic assay. Uncoated fused silica capillaries of a 30-cm total length and a 20-cm distance from the inlet to the detection window (50 μm inner diameter) were used for the MutS-DNA mismatch binding assay. In a case of SDS-MW analysis, the total length of capillaries was 30 cm (20 cm from the inlet to the detection window) and the inner diameter was 50 μm.

2.6. SDS-PAGE analysis

SDS-PAGE assay was conducted in the freshly made 12% polyacrylamide gel. Twenty microliters of MutS-containing solutions (cell lysate and MutS-containing eluates) were mixed with 80 μL of 4 × SDS-PAGE loading buffer (62.5 mM Tris, 10% Glycerol, 0.05% Bromphenol Blue, 2% SDS and 5% 2-mercaptoethanol) and 10 μL of each mixture was injected into the gel-plate vials. SDS-PAGE electrophoresis was conducted at 200 V for 50 min. Coommassie Blue dye was used for visualization of the protein bands.

2.7. AlkB enzymatic activity assay

The demethylation assay was carried out by mixing AlkB enzyme with the fluorescently labeled homopolymeric sequence TTCm at 20 and 200 nM final concentrations, respectively, in 50 mM Tris-HCl pH 7.5 buffer in the presence of 4 mM ascorbic acid, 0.16 mM α-

Ketoglutaric acid (2OG), 0.05 mM BSA, 2100 units of catalase and 0.08 mM Fe (II) sulphate. The aliquots of the mixture (15 µL each) were added to 5 µL of 20 mM EDTA (iron chelator) at different time intervals (0, 0.5, 1, and 2 min) and kept at 70 °C on a hot plate to denature the protein and stop the reaction. Each sample was then injected into the capillary by pressure pulse of 0.5 psi (3.45 kPa) for 6 s and analyzed with CE at an electric field of 300 V/cm. The methylated DNA (unreacted substrate) and demethylated DNA (product) were separated in a Borax buffer (20 mM Borax, 60 mM SDS, pH 8.2).

2.8. MutS-DNA mismatch binding assay based on plug–plug kinetic capillary electrophoresis (ppKCE)

A plug of 1 µM fluorescently-labeled dsDNA-GT solution was injected into the capillary by a 10 s pressure pulse of 0.5 psi (3.45 kPa). Next, a plug of the separation buffer was injected at 0.5 psi (3.45 kPa) for 30 s. Finally, the plug of 1.5 µM purified MutS protein solution was injected at 0.5 psi (3.45 kPa) for 10 s. The ends of the capillary were inserted into the inlet and outlet reservoirs, filled with the running buffer (50 mM Tris-HCl and 1 mM $MgCl_2$, pH 8.0) and electrophoretic separation was conducted at an electric field of 300 V/cm. The control experiments were done at the same temperatures by using a solution of dsDNA-GT void of MutS protein.

3. Results and discussion

3.1. AlkB expression and purification by aptamer-beads

The AlkB expression procedure was adapted from a previously developed protocol [25]. The main deviation from the protocol was the use of TB media instead of LB media. This replacement allowed us to increase the amount of the expressed protein. The crude AlkB-containing cell lysate was analyzed with SDS-MW in CE (Fig. 2, bottom trace). The AlkB peak was identified according to its molecular weight of 23.9 kDa, using molecular weight markers (Fig. 2, upper trace). Based on the area analysis it was established that AlkB is the most abundant protein (30% of the total protein yield). By using BCA, it was found that the total protein concentration in the lysate was equal to 20 mg/mL (± 10%) and the concentration of AlkB protein was approximately 6 mg/mL. Next, we applied the developed purification protocol to the crude cell lysate. By using 1 mL of aptamer-saturated bead suspension (containing 7.53 nanomoles of aptamer at 92% occupancy) we have obtained 0.21 mg of pure AlkB protein in a form of solution with the total volume of 70 µL. The purified eluate was analyzed by SDS-MW in CE (Fig. 2, middle trace). Based on the area analysis of peaks in this trace, it was established that the purity of AlkB protein was more than 85%. In theory, we could obtain only 0.18 mg of the protein with this amount of the aptamer, but due to non-specific binding of AlkB to the surface of the beads, we were able to extract more protein. The non-specific binding was confirmed in a series of negative control experiments with aptamer-free beads, which showed that these beads are capable of binding proteins in the cell lysate, but do not have specificity to any particular protein. According to SDS-MW electropherograms (not shown), the areas corresponding to all the proteins were gradually decreasing with increasing the number of beads in the system. It was found that approximately 200 µL of the aptamer-free beads suspension was sufficiant to bind all the proteins in 8 µL of the cell lysate.

3.2. AlkB activity assay

To confirm that the aptamer-purified AlkB protein retained its activity, we conducted a comparative demethylation assay. As a reference for comparison, we used another AlkB protein, which was purified by a previously published approach [25], based on cation exchange column chromatography. The methylated DNA substrate was incubated with AlkB protein in the presence of Fe (II) and 2OG as cofactors. The concentration of the DNA in a mixture was 10 times higher than the concentration of the protein. The demethylation reaction was terminated at different time intervals from reaction initiation. Then, CE was used to separate the methylated DNA substrate from the demethylated DNA product. As follows from the electropherograms (Fig. 3) the concentration of the methylated DNA is decreasing over the time, while the concentration of demethylated product becomes greater, which indicates that both aptamer-purified and cation-exchange chromatography-purified AlkB proteins were active. However, the AlkB that was purified by the aptamers led to an almost complete demethylation of the substrate in 1 min (Fig. 3B), while the reference protein demethylated less than a half of the substrate during the same time (Fig. 3A).

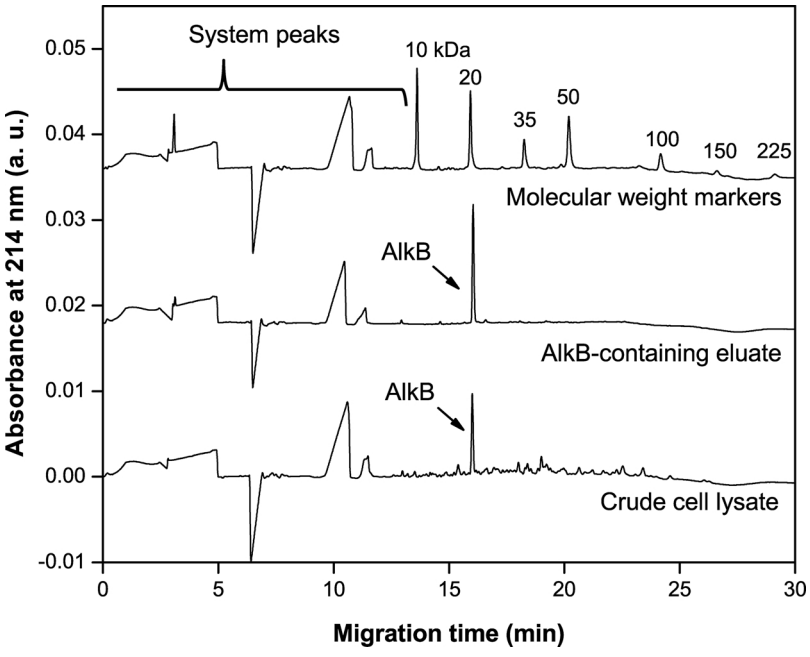

Fig. 2. CE based SDS-MW analysis of crude lysate and purified AlkB protein, confirming expression and purity of the product. The traces correspond to the following samples (from bottom to top): crude cell lysate, AlkB-containing eluate, and a mixture of molecular weight markers. The traces are vertically offset for clarity of presentation.

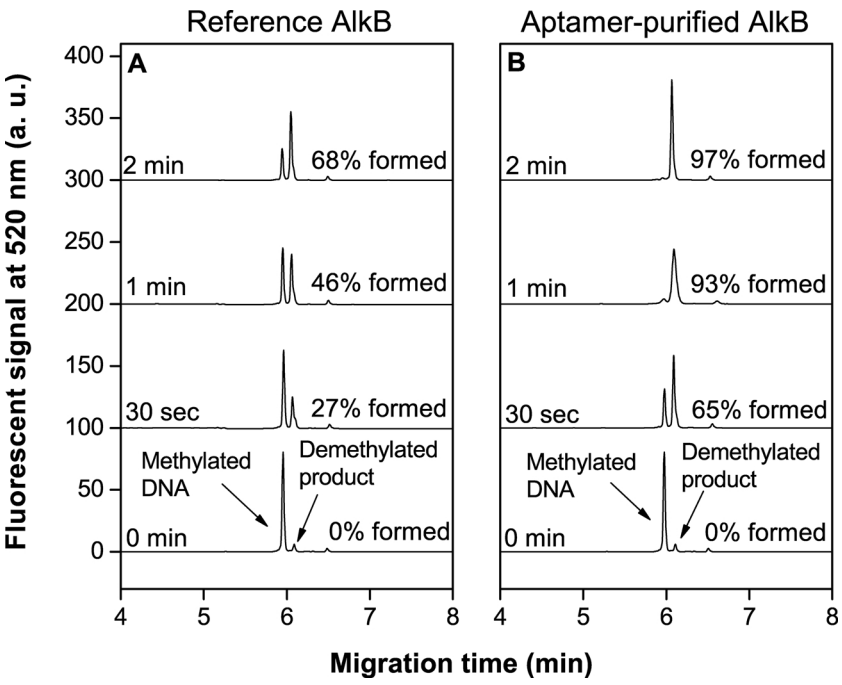

Fig. 3. Activity comparison of the reference AlkB (A) and aptamer-purified AlkB (B). The traces in each panel correspond to different reaction times ranging from 0 to 2 min. The percentage corresponds to relative amount of demethylated product formed by the time the reaction is stopped. The traces are offset vertically for clarity of presentation. The additive concentration of the methylated and demethylated DNA corresponds to 85 nM on all of the electropherograms.

3.3. MutS expression and purification by aptamer-beads

The MutS expression was induced with 1 mM IPTG, similar to induction of AlkB expression. The cell lysate before and after induction was analyzed by slab-gel SDS-PAGE (Fig. 4, lanes b, c). A large amount of undesirable non-target proteins was removed by heating the cell lysate at 70 °C for 45 min, consequently denaturing those proteins (Fig. 4, lane d); MutS was not affected as it is a temperature-stable protein. The soluble portion of heat treated lysate was loaded onto an anion exchange column. The anion exchange pre-purification was performed in order to minimize the nonspecific interactions between MutS and endogenous bacterial DNA (Fig. 4, lane e). The final step of protein isolation was done by incubating the MutS-containing eluate with aptamer-beads on ice for 1 h. The target protein was retained by aptamers on a surface of the beads, while the solution was removed. We eluted the bound protein with a high-ionic-strength solution of salt. We first tried the monovalent cation Na^+, however, the interaction between MutS and aptamer could not be disrupted even by 3 M NaCl. We then tested the divalent cation Mg^{2+} and found that 2 M $MgCl_2$ could elute 82%-pure MutS (based on the color densities of the bands in the SDS-PAGE slab). A total amount of obtained MutS was 0.24 mg (according to the BCA assay).

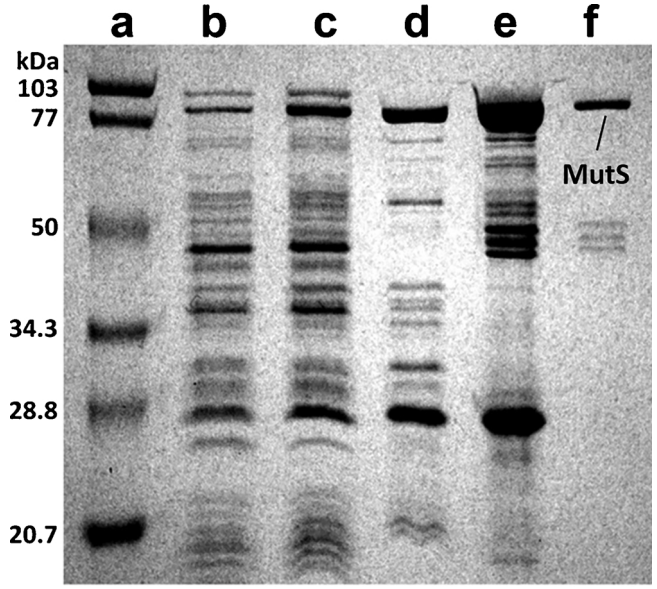

Fig. 4. Expression and aptamer facilitated purification of MutS protein. The lanes correspond to the following (from left to right): molecular weight markers (a), un-induced cell culture (b), IPTG-induced cell culture (c), temperature-treated supernatant (d), an eluate after anion exchange column purification (e) and MutS eluted by $MgCl_2$ (f).

3.4. MutS-DNA mismatch binding assay

The DNA-binding activity of purified MutS was assessed by a ppKCE-based binding assay. Short plugs of dsDNA-GT and MutS were sequentially injected one after another by low-pressure pulses. An electric field was then applied, causing movement of MutS with a faster velocity that dsDNA-GT. When passing through dsDNA-GT, MutS could form a complex with it. As separation continued, the zones of the complex and unbound dsDNA-GT and MutS protein were separated from each other (Fig. 5). Based on the area analysis, we found a K_D value of the complex of 326 ± 26 nM at 25 °C. The obtained value is within the range of previously reported K_D values, lying between 35 ± 6 nM (G in the mismatch is surrounded by G fragments only) [26] and 420 ± 30 nM (G in the mismatch is surrounded by AT fragments) [26]. Therefore, we conclude that aptamer-purified MutS protein retained its binding activity.

4. Conclusions

To conclude, we have successfully developed and validated an aptamer facilitated protein purification protocol. Using AlkB protein and MutS protein as two models, we demonstrated that our approach could be used to achieve fast and reliable purification of active proteins. To confirm the AlkB activity, we have conducted the DNA demethylation assay and to confirm the functional activity of MutS protein, we have conducted a CE-based dsDNA-GT binding assay. Both assays showed that proteins retained their respective activities. Thus, the developed protocol can be used for purification of tag-less proteins in their active state. It should also be noted that despite the approximate equality of the dissociation constants, K_d, for the utilized AlkB and MutS aptamers (20 and 15 nM, respectively), the optimum elution procedures were

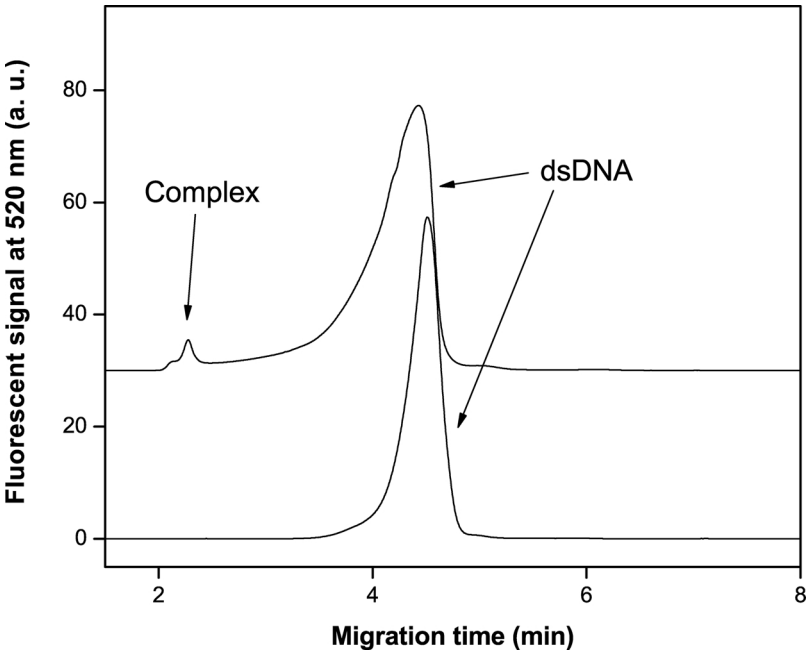

Fig. 5. CE-based activity assay of MutS protein, confirming the formation of the MutS-dsDNA-GT complex. The upper trace represent the electropherogram of dsDNA in the presence of MutS protein. The lower traces represents dsDNA-GT only. The traces are offset vertically for clarity of presentation (Leave just one temperature).

different for AlkB and MutS proteins. It indicates that each case of the aptamer-facilitated protein purification is unique and may require its own specific conditions, especially at the elution step.

Acknowledgement

This work was supported by the Natural Sciences and Engineering Research Council of Canada.

References

[1] T.K. Karikari, A. Turner, R. Stass, L.C.Y. Lee, B. Wilson, D.A. Nagel, E.J. Hill, K.G. Moffat, Expression and purification of tau protein and its frontotemporal dementia variants using a cleavable histidine tag, Protein Expr. Purif. 130 (2017) 44–54.
[2] A.F. Jozala, D.C. Geraldes, L.L. Tundisi, V.A. d. Feitosa, C.A. Breyer, S.L. Cardoso, P.G. Mazzola, L. d. Oliveira-Nascimento, C.O. d. Rangel-Yagui, P.O. d. Magalhães, M.A. d. Oliveira, A. Pessoa Jr, Biopharmaceuticals from microorganisms: from production to purification, Braz. J. Microbiol. 47 (2016) 51–63.
[3] I. Hunt, From gene to protein: a review of new and enabling technologies for multiparallel protein expression, Protein Expr. Purif. 40 (2005) 1–22.
[4] H.L. Knudsen, R.L. Fahrner, Y. Xu, L.A. Norling, G.S. Blank, Membrane ion-exchange chromatography for process-scale antibody purification, J. Chromatogr. A 907 (2001) 145–154.
[5] A. Goyon, A. Beck, O. Colas, K. Sandra, D. Guillarme, S. Fekete, Evaluation of size exclusion chromatography columns packed with sub-3 μm particles for the analysis of biopharmaceutical proteins, J. Chromatogr. A 1498 (2016) 80–89.
[6] C. Karakus, M. Uslu, D. Yazici, B.A. Salih, Evaluation of immobilized metal affinity chromatography kits for the purification of histidine-tagged recombinant CagA protein, J. Chromatogr. B 1021 (2016) 182–187.
[7] C. Scheich, V. Sievert, K. Büssow, An automated method for high-throughput protein purification applied to a comparison of His-tag and GST-tag affinity chromatography, BMC Biotechnol. 3 (2003) 12.
[8] J. Wu, M. Filutowicz, Hexahistidine (His_6)-tag dependent protein dimerization: a cautionary tale, Acta Biochim. Pol. 46 (1999) 591–599.
[9] J. Arnau, C. Lauritzen, G.E. Petersen, J. Pedersen, Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins, Protein Expr. Purif. 48 (2006) 1–13.
[10] M.V. Berezovski, M.U. Musheev, A.P. Drabovich, J.V. Jitkova, S.N. Krylov, Non-SELEX: selection of aptamers without intermediate amplification of candidate oligonucleotides, Nat. Protoc. 1 (2006) 1359–1369.
[11] M. Berezovski, A. Drabovich, S.M. Krylova, M. Musheev, V. Okhonin, A. Petrov, S.N. Krylov, Nonequilibrium capillary electrophoresis of equilibrium mixtures: a universal tool for development of aptamers, J. Am. Chem. Soc. 127 (2005) 3165–3171.
[12] S. Balamurugan, A. Obubuafo, S.A. Soper, D.A. Spivak, Surface immobilization methods for aptamer diagnostic applications, Anal. Bioanal. Chem. 390 (2008) 1009–1021.
[13] T.S. Romig, C. Bell, D.W. Drolet, Aptamer affinity chromatography: combinatorial chemistry applied to protein purification, J. Chromatogr. B Biomed. Sci. Appl. 731 (1999) 275–284.
[14] C. Forier, E. Boschetti, M. Ouhammouch, A. Cibiel, F. Ducongé, M. Nogré, M. Tellier, D. Bataille, N. Bihoreau, P. Santambien, S. Chtourou, G. Perret, DNA aptamer affinity ligands for highly selective purification of human plasma-related proteins from multiple sources, J. Chromatogr. 1489 (2017) 39–50.
[15] L. Qiao, B. Lv, X. Feng, C. Li, A new application of aptamer: one-step purification and immobilization of enzyme from cell lysates for biocatalysis, J. Biotechnol. 203 (2015) 68–76.
[16] S. Javaherian, M.U. Musheev, M. Kanoatov, M.V. Berezovski, S.N. Krylov, Selection of aptamers for a protein target in cell lysate and their application to protein purification, Nucleic Acids Res. 37 (2009) e62–e62.
[17] R. Ahirwar, P. Nahar, Development of an aptamer-affinity chromatography for efficient single step purification of Concanavalin A from *Canavalia ensiformis*, J. Chromatogr. B 997 (2015) 105–109.
[18] F. Bartnicki, E. Kowalska, K. Pels, W. Strzalka, Imidazole-free purification of His_3-tagged recombinant proteins usingss DNA aptamer-based affinity chromatography, J. Chromatogr. 1418 (2015) 130–139.
[19] J. Gädke, L. Kleinfeldt, C. Schubert, M. Rohde, R. Biedendieck, G. Garnweitner, R. Krull, *In situ* affinity purification of his-tagged protein A from *Bacillus megaterium* cultivation using recyclable superparamagnetic ironoxide nanoparticles, J. Biotechnol. 242 (2017) 55–63.
[20] S. Song, Y.S. Cho, S.J. Lee, S.S. Hah, Aptamer-based precipitation as an alternative to the conventional immunoprecipitation for purification of target proteins, Bull. Korean Chem. Soc. 35 (2014) 2666–2668.
[21] A.A. Karkhanina, J. Mecinović, M.U. Musheev, S.M. Krylova, A.P. Petrov, K.S. Hewitson, E. Flashman, C.J. Schofield, S.N. Krylov, Direct analysis of enzyme-catalyzed DNA demethylation, Anal. Chem. 81 (2009) 5871–5875.
[22] A.P. Drabovich, S.N. Krylov, Identification of base pairs in single-nucleotide polymorphisms by MutS protein-mediated capillary electrophoresis, Anal. Chem. 78 (2006) 2035–2038.
[23] S.M. Krylova, A.A. Karkhanina, M.U. Musheev, E.A.L. Bagg, C.J. Schofield, S.N. Krylov, DNA aptamers for as analytical tools for the quantitative analysis of DNA-dealkylating enzymes, Anal. Biochem. 414 (2011) 261–265.
[24] A. Drabovich, M. Berezovski, S.N. Krylov, Selection of smart aptamers by equilibrium capillary electrophoresis of equilibrium mixtures (ECEEM), J. Am. Chem. Soc. 127 (2005) 11224–11225.
[25] R.W. Welford, I. Schlemminger, L.A. McNeill, K.S. Hewitson, C.J. Schofield, The selectivity and inhibition of AlkB, J. Biol. Chem. 278 (2003) 10157–10161.
[26] F.S. Groothuizen, A. Fish, M.V. Petoukhov, A. Reumer, L. Manelyte, H.H. Winterwerp, M.G. Marinus, J.H. Lebbink, D.I. Svergun, P. Friedhoff, T.K. Sixma, Using stable MutS dimers and tetramers to quantitatively analyze DNA mismatch recognition and sliding clamp formation, Nucleic Acids Res. 41 (2013) 8166–8181.