DNA Aptamers: Discovery and Affinity Purification of Proteins

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1 Introduction

Current immuno-affinity chromatographic methods enable target proteins to be isolated, purified, detected, identified, and quantified. However, the success of these methods depends upon the availability of a high quality antibody, the development of which is non-trivial and costly. An aptamer is a functional nucleic acid that exhibits high affinity and specificity to its target (Bunka & Stockley, 2006; Ellington & Szostak, 1990; Robertson & Joyce, 1990; Tuerk & Gold, 1990). In contrast to antibodies, aptamers are discovered and developed without the extensive time and financial investments or ethical concerns of animal husbandry (Iliuk et al., 2011; Tombelli et al., 2005). In this chapter, the discovery of an aptamer by a systematic evolution of ligands by exponential enrichment (SELEX) method used in our laboratory (DeGrasse, 2012) is detailed. Further, a method to utilize the successful aptamer in an aptamer isolation protocol is provided. Finally, using an aptamer (APT\textsuperscript{SEB1}) specific to staphylococcal enterotoxin B (SEB), the general aptamer isolation protocol is applied to the isolation of SEB from a mixture of closely related enterotoxins using non-fat dry milk as a representative food matrix.

2 Methodology

2.1 Preparation of Target-Coupled Paramagnetic Beads

2.1.1 Coupling the Target Protein to Paramagnetic Beads Using the Dynabeads® Antibody Coupling Kit

1. Suspend 60 mg of freeze dried Dynal M-270 epoxy coated paramagnetic beads in 2 ml dimethyl formamide.
2. Transfer 100 µl of M-270 epoxy coated beads (30 mg/ml) to a screw cap vial.
3. Place the vial onto a magnet (e.g., DynaMag™-2 Magnet, Life Technologies) and allow beads to collect to the side of tube (about 1 minute). Aspirate the supernatant.
4. Wash the beads by adding 1 ml buffer C1 (buffers C1, C2, HB, LB, and SB are components of the Dynabeads antibody coupling kit; Life Technologies). Mix by gentle pipetting.
5. Place the vial onto a magnet and allow beads to collect to the side of tube. Aspirate the supernatant.
6. To the beads, add 120 µl of buffer C1 and mix.
7. Add 30 µg of target protein.
8. Add 150 µl of buffer C2 and mix.
9. Incubate with end-over-end rotation overnight at 37 °C.
10. The next day, spin down the solution briefly, and then place the vial onto a magnet and allow beads to collect to the side of tube. Aspirate the supernatant.
11. Wash the beads sequentially with the following buffers:
   a. 800 µl of buffer HB
b. 800 µl of buffer LB

c. 800 µl of buffer SB

d. 800 µl of buffer SB

e. 800 µl of buffer SB. Incubate with end-over-end rotation for 15 minutes.

f. Note: After each wash, place the vial onto a magnet and allow beads to collect to the side of tube. Aspirate the supernatant.

12. Resuspend the beads in 300 µl PBS-T (10 mM phosphate buffer, 2.7 mM KCl, 140 mM NaCl, 0.05% Tween, pH 7.4, Sigma, St. Louis, MO) and store at 4 °C. The final concentration of beads is 10 µg/µl, or 6.7 x 10^5 beads/µl.

13. Note: Uncoupled beads for counter-selection are produced in a similar manner, but without a ligand.

2.1.2 Preparation of Uncoupled Paramagnetic Beads Using the Dynabeads® Antibody Coupling Kit

1. Suspend 60 mg of freeze dried Dynal M-270 epoxy coated paramagnetic beads in 2 ml dimethyl formamide.

2. Transfer 100 µl of M-270 epoxy coated beads (30 mg/ml) to a screw cap vial.

3. Place the vial onto a magnet (e.g., DynaMag™-2 Magnet, Life Technologies) and allow beads to collect to the side of tube (about 1 minute). Aspirate the supernatant.

4. Wash the beads by adding 1 ml buffer C1 (buffers C1, C2, HB, LB, and SB are components of the Dynabeads antibody coupling kit; Life Technologies). Mix by gentle pipetting.

5. Place the vial onto a magnet and allow beads to collect to the side of tube. Aspirate the supernatant.

6. To the beads, add 150 µl of buffer C1 and mix.

7. Add 150 µl of buffer C2 and mix.

8. Incubate with end-over-end rotation overnight at 37 °C.

9. The next day, spin down the solution briefly, and then place the vial onto a magnet and allow beads to collect to the side of tube. Aspirate the supernatant.

10. Wash the beads sequentially with the following buffers:

    a. 800 µl of buffer HB

    b. 800 µl of buffer LB

    c. 800 µl of buffer SB

    d. 800 µl of buffer SB

    e. 800 µl of buffer SB. Incubate with end-over-end rotation for 15 minutes.
f. Note: After each wash, place the vial onto a magnet and allow beads to collect to the side of tube. Aspirate the supernatant.

11. Resuspend the beads in 300 µl PBS-T (10 mM phosphate buffer, 2.7 mM KCl, 140 mM NaCl, 0.05% Tween, pH 7.4, Sigma, St. Louis, MO) and store at 4 °C. The final concentration of beads is 10 µg/µl, or 6.7 x 10^5 beads/µl.

2.1.3 Bead Coupling Validation via Mass Spectrometry

1. Simultaneously analyze pure standard (positive control), uncoupled beads (negative control), and coupled beads for comparison.

2. Transfer 40 µl of coupled beads and uncoupled beads into separate vials.
   a. Wash the beads three times with 100 µl of 50 mM ammonium bicarbonate.

3. Transfer 1 µl of 1 µg/µl standard to another vial.

4. To each vial, add 25 µl of 50 mM ammonium bicarbonate, heat at 99 °C for 5 minutes, and then cool on ice. Spin down.

5. Add 25 µl of 50 mM ammonium bicarbonate/18% acetonitrile.

6. Add 2 µl of 0.5 µg/µl trypsin (Promega, Madison, WI).

7. Incubate for 4 hours at 60 °C with agitation.

8. Quench the proteolysis with 5.2 µl of 10% acetic acid.

9. Analyze via any appropriate LC-MS/MS method.

2.2 SELEX

The general schematic of the SELEX protocol is outlined in Figure 1. The DNA used for the SELEX protocol is listed in Table 1.

2.2.1 Round 1

1. Prepare the library by diluting 50 µl of 100 µM library stock (5 nmoles) into 450 µL PBS-T. Incubate at 95 °C for 5 minutes and then cool on ice until needed.

2. Prepare target-coupled Dynabeads. Transfer 20 µl of the protein-coupled beads to a microcentrifuge vial and remove the supernatant. Wash the beads twice in 500 µl PBS-T and resuspend the beads in 1 ml PBS-T.

3. Mix in a 50 ml centrifuge tube in the following order:
   a. 48.5 ml of PBS-T,
   b. 1 ml of washed protein-conjugated beads in PBS-T,
   c. 50 µl of 1 mg/ml bovine serum albumin (Sigma-Aldrich),
   d. 5 µl of 1 mg/ml poly(deoxyinosinic-deoxycytidylic) acid (Sigma-Aldrich), mix the solution, and
   e. 500 µl of heated/cooled library solution.
**Table 1:** DNA used for SELEX

| Library | 5’ – GGT ATT GAG GGT CGC ATC N$_{40}$ GAT GGC TCT AAC TCT CCT CT |
| Forward Primer | 5’ – GGT ATT GAG GGT CGC ATC |
| Reverse Primer | 5’ – AGA GGA GAG TTA GAG CCA TC |
| Biotinylated Reverse Primer | 5’ – [Biotin]AGA GGA GAG TTA GAG CCA TC |

**Figure 1:** Schematic outlining the SELEX protocol.

4. Selection: Incubate with rotation at room temperature for 30 minutes.

5. Collect beads by placing the centrifuge tube onto a large magnet for 20 minutes and remove all but ~1.0 ml of supernatant.

6. Resuspend the beads and transfer the mixture to a microcentrifuge tube, place onto a magnet, and then remove the remaining supernatant.

7. Wash the beads once with 1 ml of PBS-T and remove the supernatant.

8. PCR amplification step 1. Add:
   a. 22.5 μl of nuclease-free water to beads in microcentrifuge tube and transfer entire volume to PCR tube,
b. 2.5 µl of 10 µM forward and biotinylated reverse primer mix,
c. 25 µl of AmpliTaq Gold Fast PCR Master Mix (Life Technologies),
d. PCR reaction:
   i. 95 °C for 10 minutes
   ii. 15 cycles of
      1. 96 °C for 3 seconds
      2. 56 °C for 3 seconds
      3. 68 °C for 5 seconds
   iii. 72 °C for 10 seconds
   iv. Hold at 4 °C.

9. Transfer the supernatant (not the beads) to a fresh PCR tube.

10. PCR amplification step 2. Add to four PCR tubes:
    a. 21.5 µl of nuclease-free water,
    b. 1 µl of PCR product from step 1,
    c. 2.5 µl of 10 µM forward and biotinylated reverse primer mix,
    d. 25 µl of AmpliTaq Gold Fast PCR Master Mix (Life Technologies),
    e. PCR reaction:
       i. 95 °C for 10 minutes
       ii. 35 cycles of
          1. 96 °C for 3 seconds
          2. 56 °C for 3 seconds
          3. 68 °C for 5 seconds
       iii. 72 °C for 10 seconds
       iv. Hold at 4 °C.
    f. Pool the contents of the four PCR tubes.
    g. Test product on an agarose gel (e.g., E-Gel® 4% pre-cast high-resolution agarose gel, Life Technologies) for size and quality.

11. Purify and denature dsDNA
    a. Transfer 100 µl of streptavidin-coupled Dynabeads (Life Technologies, 1 mg) to a microcentrifuge tube and remove supernatant. Wash twice with 1 ml of PBS-T and remove the final wash.
b. To 135 µl PCR product, add 34.5 µl of 5 M NaCl and mix. Freeze remainder of PCR products as an archive.

c. Add the DNA to the streptavidin-coupled Dynabeads and incubate for 10 minutes, with rotation at room temperature.

d. Remove the supernatant and then wash the beads three times with 1 ml PBS-T and then remove the supernatant.

e. Denature the dsDNA to liberate ssDNA aptamer candidates by adding 50 µl of freshly prepared 100 mM NaOH to the washed beads.

f. Incubate for 5 minutes, place onto a magnet and retain the supernatant.

12. Transfer supernatant to a fresh vial containing 850 µl of PBS-T and 100 µl of 0.1 M monobasic phosphate buffer (Test pH beforehand to ensure a solution pH of ~7.5).

13. Heat the mixture to 95 °C for 5 minutes, then cool on ice.

### 2.2.2 Round 2

1. Prepare target-coupled Dynabeads. Transfer 20 µl of the protein-coupled beads to a microcentrifuge vial and remove the supernatant. Wash the beads twice in 500 µl of PBS-T and resuspend the beads in 1 ml of PBS-T.

2. Add the ssDNA product from Section 2.2.1, step 13, to the coupled beads.

3. Selection: Incubate with rotation at room temperature for 10 minutes.

4. Wash the beads three times with 1 ml of PBS-T and remove the supernatant.

5. PCR amplification. Add:

   a. 22.5 µl of nuclease-free water to beads in microcentrifuge tube and transfer entire volume to PCR tube,

   b. 2.5 µl of 10 µM forward and biotinylated reverse primer mix,

   c. 25 µl of AmpliTaq Gold Fast PCR Master Mix (Life Technologies),

   d. PCR reaction:

      i. 95 °C for 10 minutes

      ii. 35 cycles of

         1. 96 °C for 3 seconds
         2. 56 °C for 3 seconds
         3. 68 °C for 5 seconds

      iii. 72 °C for 10 seconds

      iv. Hold at 4 °C.

   e. Test product on an agarose gel for size and quality.
6. Purify and denature dsDNA
   a. Transfer 100 µl of streptavidin-coupled Dynabeads (1 mg) to a microcentrifuge tube and remove supernatant. Wash twice with 1 ml of PBS-T and remove the final wash.
   b. To 45 µl PCR product, add 11.5 µl of 5 M NaCl and mix. Freeze remainder of PCR products as an archive.
   c. Add the DNA to the streptavidin-coupled Dynabeads and incubate for 10 minutes, with rotation at room temperature.
   d. Remove the supernatant and then wash the beads three times with 1 ml of PBS-T and then remove the supernatant.
   e. Denature the dsDNA to liberate ssDNA aptamer candidates by adding 50 µl of freshly prepared 100 mM NaOH to the washed beads.
   f. Incubate for 5 minutes, place onto a magnet and retain the supernatant.

7. Transfer supernatant to a fresh vial containing 850 µl of PBS-T and 100 µl of 0.1 M monobasic phosphate buffer (Test pH beforehand to ensure a solution pH of ~7.5).

8. Heat the mixture to 95 °C for 5 minutes, then cool on ice.

### 2.2.3 Rounds 3-14

<table>
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<th>Round</th>
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<th>Incubation Time</th>
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<tbody>
<tr>
<td>3</td>
<td>0</td>
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<td>10 min</td>
</tr>
<tr>
<td>4-6</td>
<td>50 µl</td>
<td>10 µl</td>
<td>10 min</td>
</tr>
<tr>
<td>7-9</td>
<td>50 µl</td>
<td>3 µl</td>
<td>10 min</td>
</tr>
<tr>
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<td>50 µl</td>
<td>1 µl</td>
<td>10 min</td>
</tr>
<tr>
<td>12-14</td>
<td>50 µl</td>
<td>1 µl</td>
<td>None</td>
</tr>
</tbody>
</table>

Table 2: The amount of selection beads and incubation is reduced as SELEX progresses in order to the increase selection constraints.

1. Prepare the coupled beads:
   a. Selection beads: transfer X µl of protein-conjugated beads to DNA microcentrifuge tube and remove supernatant (See Table 2, where X is the volume indicated in the column entitled “Protein-conjugated Selection Beads”). Wash the beads twice in 500 µl of PBS-T.
   b. Counter Selection: transfer 50 µl of blank/negative conjugated (Counter Selection) beads to DNA microcentrifuge tube and remove supernatant. Wash the beads twice in 500 µl of PBS-T.

2. Add the ssDNA product from the previous round to the counter-selection beads.

3. Selection: Incubate with rotation at room temperature for 10 minutes.
4. Transfer supernatant to protein-conjugated beads and incubate for the appropriate amount of time (see Table 2) at room temperature.

5. Wash the beads five times with 1 ml of PBS-T and remove the supernatant.

6. PCR amplification. Add:
   a. 22.5 µl of nuclease-free water to beads in microcentrifuge tube and transfer entire volume to PCR tube,
   b. 2.5 µl of 10 µM forward and biotinylated reverse primer mix,
   c. 25 µl of AmpliTaq Gold Fast PCR Master Mix (Life Technologies),
   d. PCR reaction:
      i. 95 °C for 10 minutes
      ii. 35 cycles of
         1. 96 °C for 3 seconds
         2. 56 °C for 3 seconds
         3. 68 °C for 5 seconds
      iii. 72 °C for 10 seconds
      iv. Hold at 4 °C.
   e. Test product on a gel for size and quality.

7. Purify and denature dsDNA
   a. Transfer 100 µl of streptavidin-coupled Dynabeads (1 mg) to a microcentrifuge tube and remove supernatant. Wash twice with 1 ml PBS-T and remove the final wash.
   b. To 45 µl PCR product, add 11.5 µl of 5 M NaCl and mix. Freeze remainder of PCR products as an archive.
   c. Add the DNA to the streptavidin-coupled Dynabeads and incubate for 10 minutes, with rotation at room temperature.
   d. Remove the supernatant and then wash the beads three times with 1 ml of PBS-T and then remove the supernatant.
   e. Denature the dsDNA to liberate ssDNA aptamer candidates by adding 50 µl of fresh 100 mM NaOH to the washed beads.
   f. Incubate for 5 minutes, place onto a magnet and retain the supernatant.

8. Transfer supernatant to a fresh vial containing 850 µl of PBS-T and 100 µl of 0.1 M monobasic phosphate buffer (Test pH beforehand to ensure a solution pH of ~7.5).

9. Heat the mixture to 95 °C for 5 minutes, then cool on ice.
2.2.4 DNA Sequencing

1. After the final round, insert the PCR product into a cloning plasmid, such as the TOPO® TA cloning vector (Life Technologies) according to manufacturer instructions.

2. Insert the cloning plasmid into a suitable strain of E. coli, such as One Shot® Top10 E. coli (Life Technologies) using the appropriate transformation protocol.

3. Plate the transformed bacteria and grow overnight on pre-warmed (37 °C) LB agar plates containing the appropriate antibiotics.

4. At least 50 individual colonies should be selected for sequencing. In this work, the bacterial colonies were submitted for rolling circle amplification and Sanger sequencing (GENEWIZ, South Plainfield, NJ).

5. The returned sequences should be edited to remove known plasmid and primer regions, assessed for quality (i.e., proper length and sequence confidence), and then aligned with Geneious 5.5 and ClustalW2.

6. Correctly-sized sequences with the greatest number of copies should be assessed for binding using the aptamer isolation assay described in Section 2.3.

2.3 Aptamer Isolation Assay

Following the selection of an aptamer, the goal is to then use the aptamer to isolate the target from a complex mixture.

1. For each experiment, generate 1 µg of modified (5' NH3-C6 linker) aptamer using PCR (same reaction conditions as above).

2. Purify the PCR product by ethanol precipitation:
   a. Transfer the PCR product to a microcentrifuge tube.
   b. Adjust the MgCl2 concentration to 0.01 M.
   c. Add ammonium acetate to a final concentration of 2.5 M.
   d. Add 2 volumes of ice-cold ethanol.
   e. Incubate the solution at – 20 °C for one hour.
   f. Recover the DNA by centrifugation at maximum speed for 30 minutes at 4 °C.
   g. Remove supernatant.
   h. Carefully add 800 µl of 70% ethanol and centrifuge for 5 minutes at 4 °C.
   i. Remove supernatant and allow the pellet to dry.
   j. Resuspend in 30 µl of 10 mM Tris, pH 7.5.

3. Couple the dsDNA to Dynal M-270 epoxy coated paramagnetic beads:
   a. Suspend 60 mg of freeze dried Dynal M-270 epoxy coated paramagnetic beads in 2 ml dimethyl formamide.
b. Transfer 10 µl of M-270 epoxy coated beads (30 mg/ml) into a screw cap vial.

c. Place vial onto a magnet (e.g., DynaMag™-2 Magnet, Life Technologies) and allow beads to collect to the side of tube (about 1 minute). Then aspirate supernatant.

d. Wash beads by adding 500 µl of buffer C1 (buffers C1, C2, HB, LB, and SB are components of the Dynabeads antibody coupling kit; Life Technologies). Mix by pipetting.

e. Place vial onto a magnet and allow beads to collect to the side of tube (about 1 minute). Then aspirate supernatant.

f. To the beads, 120 µl of buffer C1 and mix.

g. Add 30 µl of purified 5’ modified aptamer.

h. Add 150 µl of buffer C2 and mix.

i. Incubate with end-over-end rotation overnight at 37 °C.

j. The next day, spin the vials and then place vial onto a magnet and allow beads to collect to the side of tube (about 1 minute). Then aspirate supernatant.

k. Wash the beads with the following buffers:

   i. 800 µl of buffer HB

   ii. 800 µl of buffer LB

   iii. 800 µl of buffer SB

   iv. 800 µl of buffer SB

   v. 800 µl of buffer SB. Incubate for 15 minutes.

   vi. Note: After each wash, place vial onto a magnet and allow beads to collect to the side of tube (about 1 minute). Then aspirate supernatant.

l. Resuspend the beads in 100 µl of PBS-T (10 mM phosphate buffer, 2.7 mM KCl, 140 mM NaCl, 0.05% Tween, pH 7.4, Sigma, St. Louis, MO).

m. Heat the DNA coupled beads to 95 °C for 2 minutes. Then rapidly collect the beads and remove the supernatant. Suspend the beads in 100 µl of fresh PBS-T then place on ice.

4. Resuspend the aptamer-coated beads in 1 ml of an appropriate sample matrix (e.g., 1 µg/µl target protein in PBS-T).

5. Incubate the solutions for 10 minutes at room temperature.

6. Afterwards, wash the beads 3 times with 500 µl of PBS-T.

7. After the final wash is removed, the eluate can be analyzed by a number of readouts. In this work, polyacrylamide gel electrophoresis was employed.

   a. To the beads, add 50 µl of 1X lithium dodecyl sulfate (LDS) sample buffer (Life Technologies) on top of the coated beads, and incubate the mixture at 70 °C with agitation for 10
minutes. For a positive control, add standards of BSA and SEB (200 ng, each) to 50 µl of 1x LDS loading buffer (Life Technologies).

b. Load 25 µl each of the samples and standards, as well as 5 µl of the molecular weight ladder (SeeBlue Plus2 Pre-Stained Standard, Life Technologies), onto a NuPAGE® 4-12% Bis-Tris pre-cast polyacrylamide gel (Life Technologies) with 3-(N-morpholino)propanesulfonic acid (MOPS) as the running buffer. Conduct electrophoresis at 125 V for the initial 5 minutes and then at 200 V for approximately 30 minutes. Visualize the proteins with silver stain according to manufacturer’s instructions (Thermo Fisher Scientific).

3 Results

The above protocol was applied to the discovery of an ssDNA aptamer with affinity and specificity to Staphylococcal enterotoxin B. That aptamer, APT<sup>SEB1</sup>, was then used to purify SEB from a mixture of closely related enterotoxins in non-fat dry milk.

3.1 Bead Coupling Validation via Mass Spectrometry

Thirty micrograms of highly purified SEB (Toxin Technology, Sarasota, FL; 1 µg/µl) were coupled to Dynal M-270 epoxy coated paramagnetic beads. The success of the SEB-bead coupling reaction was determined by LC-MS (see Section 2.1.3). Ten microliters of the tryptic peptide mixture was injected onto a 0.15 x 100 mm C18 column (nanoAcquity HPLC, Waters, Billerica, MA). Mobile phase A (0.1 M acetic acid) was washed over the column for 5 minutes at a flow rate of 1 µl/min. The peptides were eluted over a 10-minute linear gradient from 0% to 70% of mobile phase B (0.1 M acetic acid in acetonitrile). The eluate was analyzed by an LTQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA). After conducting an initial MS survey scan, the top 9 parent ions (by relative intensity) were further analyzed by collision induced dissociation (CID) fragmentation (MS/MS).

Figure 2A shows a characteristic standard SEB MS peak (m/z 655.8 Da) which is consistent with the known tryptic peptide VTAQELDYLTR. The retention time window is from 5 to 10 minutes, and the mass range is filtered to show those ions with a mass-to-charge ratio between 655 and 656 Da. This signature MS peak is present in panel B (SEB-coupled beads) but not in panel C (beads alone). To confirm the presence of SEB, the peptide that comprises the peak in Figure 2A was further fragmented via CID MS/MS analysis; that fragmentation pattern is shown in Figure 2D. The MS/MS spectrum is consistent with the known sequence of the peptide (VTAQELDYLTR). This fragmentation pattern is also present in the analysis of the SEB-coupled beads (Figure 2E). This observation confirms the presence of SEB coupled to the paramagnetic beads.

3.2 SELEX

The SELEX method outlined in section 2.2 was used to discover an aptamer to SEB, which we designated as APT<sup>SEB1</sup>. At the conclusion of the method, the vast majority (98%) of the sequences returned from the sequence reaction were identical. The sequence of APT<sup>SEB1</sup> was identified as 5’-GGT ATT GAG GGT CGC ATC CAC TGG TCG TTG TTG TCT GTT GTC TGT TAT GTT GTT TCG TGA TGG CTC TAA CTC TCC TCT.
Figure 2: LC-MS confirmation of the paramagnetic bead-SEB coupling reaction. Panels A-C are the extracted ion chromatograms (655-656 Da) within a retention time window from 5 to 10 minutes. Panel A shows the signature MS peak (m/z 655.8 Da) from standard SEB. That peak is present in the SEB-bead coupling reaction (Panel B) but not with the beads alone (Panel C). The peptide detected in Panel A was fragmented by CID (MS/MS), and the resultant fragmentation pattern is shown in Panel D. That same characteristic pattern is present when the SEB-coupled beads are similarly analyzed (Panel E).

3.3 Aptamer Isolation Assay

The method described in section 2.3 was applied to the isolation of SEB from skimmed milk using the aptamer APT<sup>SEB1</sup> (Figure 3). We incubated the SEB-coupled beads with two different sample matrices. The first sample matrix consisted of 1 ml of 50% w/v non-fat dry milk (Safeway, Inc. Pleasanton, CA) in PBS-T incurred with 1 µg each of the following proteins: Bovine Serum Albumin (Sigma), SEA, SEB, SEC<sub>1</sub>, SEC<sub>2</sub>, SEC<sub>3</sub>, SED, SEE (Toxin Technologies, Figure 3, Lane 4). The second sample matrix was similar to the first sample, but without the addition of 1 µg of SEB (Figure 3, Lane 5).

The presence of a band in lane 4 (Figure 3), demonstrates the ability of APT<sup>SEB1</sup> to bind to SEB in a complex protein matrix (e.g., milk proteins, bovine serum albumin, SEA, SEC<sub>1</sub>, SEC<sub>2</sub>, SEC<sub>3</sub>, SED, and SEE). The presence of a very faint band in lane 5 (Figure 3) shows the selectivity of the aptamer to predominantly bind to SEB in the presence of other enterotoxins, BSA and milk proteins (compare lane 4 to...
lane 5). The cross-reaction is minor, despite the high similarity shared amongst the enterotoxins (e.g., the primary structure of SEC₁ is ~70% identical to that of SEB).

![Figure 3: Aptamer isolation assay. Lanes 1-3 are standards. Fifty percent non-fat dry milk was incurred with bovine serum albumin, SEA, SEC₁, SEC₂, SEC₃, SED, and SEE (lanes 4 & 5) and also SEB (lane 4 only). Apt⁴¹^SEB₁ successfully isolated SEB from the complex mixture (lane 4), but due to its high specificity, Apt³¹^SEB₁ did not isolate other proteins (lane 5).]

4 Conclusion

The work outlined in this chapter demonstrates that aptamers can be efficiently developed to proteins. Moreover, aptamers possess binding properties similar to receptors or antibodies, without the ethical concerns of animal use. The ability of aptamers to bind and isolate their targets from a complex mixture suggests that aptamers could provide alternatives to commercial antibodies for the generation of aptamer-affinity assays. These assays would be lower in cost and could be widely distributed.

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References


