Communication

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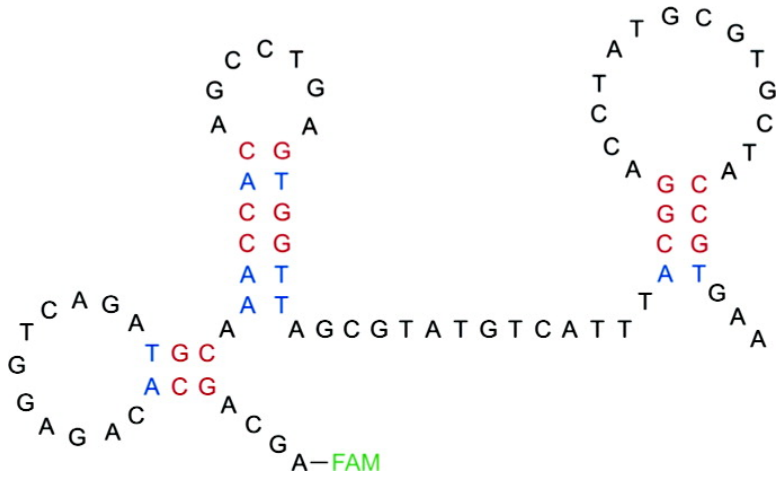

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In Vitro Selection of Aptamers with Affinity for Neuropeptide Y Using Capillary Electrophoresis

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Systematic evolution of ligands by exponential enrichment (SELEX) is a process for isolating high affinity ligands, referred to as aptamers, from random-sequence nucleic acid libraries.[1−3] A number of reviews describing the SELEX process have been published.[4−6] In general, a large pool of random-sequence ssDNA or RNA is generated. Sequences with affinity for the target are enriched through some selection process, usually nitrocellulose filtration or affinity chromatography. Retained sequences are amplified using PCR to generate a new nucleic acid pool for further selection. Aptamers with low nanomolar dissociation constants are typically obtained for large protein targets after 8−12 selection cycles. Aptamers have successfully been isolated for a wide range of targets. These aptamers have demonstrated great potential for use as pharmaceuticals or diagnostic agents.[7−9]

We have recently demonstrated SELEX using capillary electrophoresis selection (CE-SELEX).[10,11] To perform CE-SELEX, the target is incubated with the nucleic acid library in free solution. Less than 50 nL of this solution is then separated using CE. Sequences bound to the target travel through the capillary at a velocity different from those not bound to the target, allowing binding sequences to be isolated from nonbinding sequences. Advantages of CE-SELEX include increased separation power, reduced nonspecific binding, and ability to perform the selection in free solution. As a result of these advantages, high affinity aptamers can be obtained in 2−4 rounds of selection instead of the 8−12 typical of conventional selections.

An obvious limitation to CE-SELEX is that the nucleic acid must exhibit a significant mobility shift when it binds the target if it is to be separated from the nonbinding sequences. To date, CE-SELEX has been used to isolate aptamers with low nanomolar dissociation constants for IgE[10,11] (∼200 kDa) and protein farnesyltransferase[12] (45−48 kDa). These are large protein targets that significantly retard the mobility of ssDNA (∼25 kDa) upon binding.

Small targets would be expected to have only a minimal effect on the mobility of ssDNA upon complexation, making collection of these binding sequences difficult. In the current communication, we have performed selections against neuropeptide Y (NPY) to determine if CE-SELEX can be used to isolate aptamers for targets smaller than the ssDNA sequences used in the initial nucleic acid pool. NPY is a 36-amino acid peptide (MW = 4272 g/mol) found in the central and peripheral nervous system.[13] NPY is significantly smaller than the 80-mer ssDNA (∼25 kDa) used in the selections. To further complicate matters, NPY is negatively charged at neutral pH (pI = 5.52), reducing the drag that the aptamer will experience upon binding. Considering these factors, the mobility of a DNA sequence would only be expected to change slightly, if at all, upon binding NPY.

To perform the selection, 10 nM NPY was incubated with 10 μM of the nucleic acid library (10 μL total volume) for 20 min at room temperature. The library contained 80-mer ssDNA's containing a 40 base random region flanked by two 20 base primer regions (5′-FAM-AGC AGC ACA GAG GTC AGA TG-(40N)-CCT ATG CGT GCT ACC GTG AA-3′). The 5′ end was 6-carboxyfluorescein (FAM) labeled to facilitate fluorescence detection. Approximately 5 nL of this mixture was injected onto an uncoated CE capillary. The injection volume and concentration of the ssDNA library were reduced from those used in earlier CE-SELEX selections[10,11] to improve the peak shape of the nonbinding DNA. Considering the injection volume and library concentration, only 3×10^{10} DNA sequences and 3×10^{7} NPY molecules are injected for each CE selection. Three CE collections were performed for every round of selection to increase these numbers to 10^{11} DNA sequences and 10^{8} NPY molecules. The number of DNA sequences used is low by typical SELEX standards. It is desirable to use as many sequences as is reasonably possible in the first round of selection to increase the abundance of high affinity aptamers. The lower number used here may limit the effectiveness of the selection but was considered necessary to increase resolution of the NPY−aptamer complexes from the nonbinding sequences.

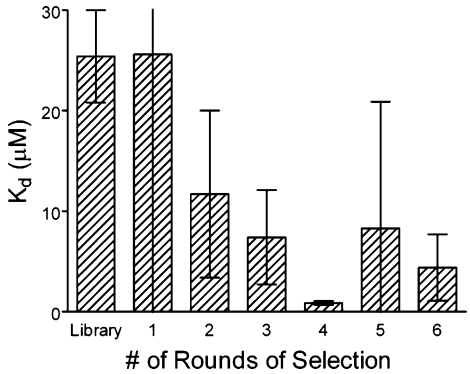

Figure 1. Bulk K_d values for the unselected DNA library and DNA pools after each CE-SELEX round. Error bars represent standard errors.

After injection, 30 kV was applied across the capillary for 1.2 min to migrate binding DNA sequences into a collection vial containing 48 μL of CE separation buffer (see Supporting Information for electropherograms). Nonbinding sequences remain on the capillary and are later washed to a waste container using pressure. In an uncoated capillary, the NPY−DNA complexes are expected to migrate earlier than unbound DNA. The concentration of unbound DNA is relatively high, making detection straightforward. Collecting the bound sequences is a simple matter of collecting anything that migrates before the peak corresponding to the unbound DNA. The collected fraction is PCR amplified, purified, and made single stranded to prepare the pool for further rounds of selection.

The affinity of the ssDNA pool for NPY was assessed after every round using affinity CE to monitor the progress of the selection. Figure 1 shows the "bulk" K_d of the pool after each round of selection. No improvement in affinity was observed after the first

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Table 1. Dissociation Constants of the NPY−Aptamer Complexes

sequence	K_d (μM) NPY	K_d (μM) hPP	selectivity
library	25 ± 5		
	Four Rounds		
4.3	1.0 ± 0.3	19 ± 8	18
4.9	0.5 ± 0.3	14 ± 2	30
4.13	1.0 ± 0.4	12 ± 4	12
4.20	0.8 ± 0.3	14 ± 7	16
4.21	1.0 ± 0.1	6 ± 3	6
4.31	0.3 ± 0.2	15 ± 3	42
average[a]	0.8 ± 0.3	13 ± 4	20 ± 13
	Six Rounds		
6.1	5 ± 2	5 ± 2	1
6.11	9 ± 2	7 ± 2	0.7
6.14	4 ± 1	18 ± 5	4
6.18	11 ± 3	11 ± 4	1
6.21	1.6 ± 0.5	8 ± 2	5
6.26	1.1 ± 0.4	7 ± 3	6
average[a]	5 ± 4	9 ± 5	3 ± 2

[a] Confidence intervals of the averages are the standard deviations of the mean. All other confidence intervals are standard errors.

round. Steady improvement in binding was observed in rounds 2−4. Surprisingly, affinity worsened with further rounds of selection.

Clones were sequenced from the pools obtained after the fourth and sixth rounds of selection. A random number generator was used to choose six sequences from each group for synthesis and affinity measurements. Table 1 lists the K_d for each sequence. Note that, since sequences were chosen at random, this can be considered a true statistical sampling of the pool as a whole. The observed affinities of the selected aptamers were significantly stronger than that of the unselected library. K_d values after four rounds of selection were in the high nanomolar range. This compares well with the nuclease-resistant RNA aptamers for NPY selected by Proske et al., which had K_d values of 370−470 nM after 12 rounds of selection.[14] To the best of our knowledge, there are no pre-existing ssDNA aptamers for NPY. In line with the trend shown in Figure 1, the affinity of the clones from the sixth round of selection was actually worse than that obtained after four rounds.

The specificity of the aptamers for NPY was assessed by measuring their affinity for human pancreatic polypeptide (hPP). hPP is a 36-amino acid peptide that shares ∼50% sequence homology with NPY and is known to act on some of the same physiological receptors.[15] Up to 42-fold selectivity for NPY over hPP was observed in the sequences obtained after four rounds of selection. Selectivity after six rounds of selection dropped significantly. As shown in Figure 2, there was a strong inverse correlation between aptamer K_d and selectivity. This is the trend that would be expected if selection increased the affinity for NPY without generating affinity for hPP.

In summary, we have demonstrated for the first time that it is possible to obtain aptamers for targets that are even smaller than the DNA molecules themselves using CE-SELEX. The aptamers that we obtained for NPY have affinities similar to those for RNA aptamers previously selected using more conventional selection techniques.[14] A major advantage of the CE-SELEX approach was that aptamers with ∼800 nM dissociation constants were obtained

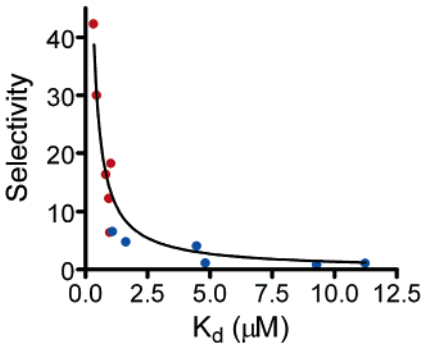

Figure 2. Plot of selectivity vs K_d. Red and blue points correspond to sequences obtained after four and six rounds of selection, respectively. Data were fit to the following equation using the method of least squares: $y = 13.6/x$ ($R^2 = 0.9187$).

in only four rounds, significantly reducing the time required for selection. It should be emphasized that the selection was performed on the native target in free solution. In conventional filter or affinity-based SELEX selections, small molecule targets must be physically linked to a stationary support or large carrier molecule to facilitate selection. This linkage modifies the chemical structure of the target and has the potential for biasing the selection. The low number of sequences used in the initial round most likely limited the effectiveness of the selection. Aptamers with even greater affinity for NPY may be obtained if the number of sequences can be increased. Strategies for increasing the number of sequences introduced in the initial round of selection include using a higher concentration ssDNA library, increasing the inner diameter of the separation capillary, or increasing the number of CE collections performed.

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Supporting Information Available: Experimental procedures, electropherograms, and aptamer sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818−822.
(2) Joyce, G. F. *Gene* **1989**, *82*, 83−87.
(3) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505−510.
(4) Bowser, M. *Analyst* **2005**, *130*, 128−130.
(5) Famulok, M.; Mayer, G.; Blind, M. *Acc. Chem. Res.* **2000**, *33*, 591−599.
(6) Osborne, S. E.; Ellington, A. D. *Chem. Rev.* **1997**, *97*, 349−370.
(7) Clark, S. L.; Remcho, V. T. *Electrophoresis* **2002**, *23*, 1335−1340.
(8) Mayer, G.; Jenne, A. *BioDrugs* **2004**, *18*, 351−359.
(9) Rimmele, M. *ChemBioChem* **2003**, *4*, 963−971.
(10) Mendonsa, S. D.; Bowser, M. T. *J. Am. Chem. Soc.* **2004**, *126*, 20−21.
(11) Mendonsa, S. D.; Bowser, M. T. *Anal. Chem.* **2004**, *76*, 5387−5392.
(12) Berezovski, M.; Drabovich, A.; Krylova, S. M.; Musheev, M.; Okhonin, V.; Petrov, A.; Krylov, S. N. *J. Am. Chem. Soc.* **2005**, *127*, 3165−3171.
(13) Gehlert, D. R. *Neuropeptides* **2004**, *38*, 135−140.
(14) Proske, D.; Hofliger, M.; Soll, R. M.; Beck-Sickinger, A. G.; Famulok, M. *J. Biol. Chem.* **2002**, *277*, 11416−11422.
(15) Michel, M. C.; Beck-Sickinger, A.; Cox, H.; Doods, H. N.; Herzog, H.; Larhammar, D.; Quirion, R.; Schwartz, T.; Westfall, T. *Pharmacol. Rev.* **1998**, *50*, 143−150.

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