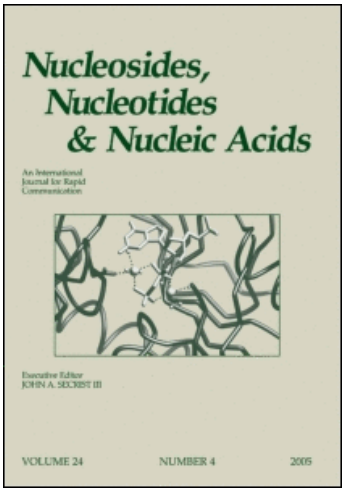

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In Vitro Selection of Single-Stranded DNA Aptamers that Bind Human Pro-Urokinase

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In Vitro Selection of Single-Stranded DNA Aptamers that Bind Human Pro-Urokinase

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ABSTRACT

Single-chain pro-urokinase is an inactive proenzyme form of human urokinase (urinary plasminogen activator) with a Mr of 50,000 which is converted to the active two-chain form by catalytic amounts of plasmin. It is used for thrombolytic therapy of acute myocardial infarction and acute ischemic stroke. We have isolated single-stranded DNA molecules with significantly increased binding affinity for human pro-urokinase by SELEX (systematic evolution of ligands by exponential enrichment) procedure from a pool of 10^{15} molecules containing 24 randomized positions which are flanked by defined regions. ssDNA from this library was hybridized with helper «fixture», thus allowing the central random chain to fold into complex three-dimentional shapes. Sequencing data from pro-urokinase aptamers obtained after 12 selection cycles displayed a highly conserved 12–14 base region.

Key Words: Aptamer; SELEX; Pro-urokinase.

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INTRODUCTION

The systematic evolution of ligands by exponential enrichment (SELEX) process is a combinatorial chemistry method for the isolation of nucleic acid ligands (aptamers) that bind to a desired target molecule with high affinity.[1] Ligand-binding DNAs might be more suitable than RNAs as potential reagents because of the greater stability of DNA. DNA molecules are capable of folding into the necessary complex shapes, and single-stranded DNAs were more likely to fold into complex three-dimentional shapes than double-stranded molecules.[2–4] Here we describe the selection of ssDNA-aptamers that bind to human pro-urokinase.

RESULTS AND DISCUSSION

A 77-mer oligonucleotide library consisting of 24-nucleotide random central sequence, which is flanked by defined regions, was synthesized chemically by Operon Technologies Inc., CA, USA: 5′-GACACCTGCGGATCCAAAGCGATGGC$(N)_{24}$CC-CATCGCAGCCCTGCAGGCTCTTCAG. The two constant regions were designed to contain annealing sites for PCR primers and restriction enzyme sites that allow cloning into pUC19. Other individual oligonucleotides were synthesized by Biosset Ltd., Novosibirsk, Russia. ssDNA from this library was radiolabelled by polynucleotide kinase and hybridized with a 37-nucleotide helper «fixture» (Fig. 1).

Radiolabelled mounted single-stranded DNA was first applied to a Sepharose column to remove DNA with affinity for the sorbent matrix, and the eluent applied to human prourokinase immobilized on Sepharose. Human prourokinase (PLAU) was prepared as recombinant protein from an *Esherichia coli* expression system. PLAU was coupled to CNBr-activated Sepharose (Pharmacia Biotech) according to Ref. [5]. The bound DNA was then eluted with 5 M urea and amplified in 30 cycles of asymmetrical PCR at a 50:1 ratio of primers.[6] Single-strand product was then purified by native electrophoresis in 8% polyacrylamide gel and Nen-Sorb column, radiolabelled by polynucleotide kinase and subjected to further cycles of selection and amplification.

Only 0.5% of the input DNA was eluted from PLAU-Sepharose during the first selection cycle; this percentage increased to 14% by selection cycle 9, 10 and 11.

Figure 1. The structure of 77-mer oligonucleotide aptamers with 24-nucleotide random sequence mounted with helper 37-nucleotide «fixture».

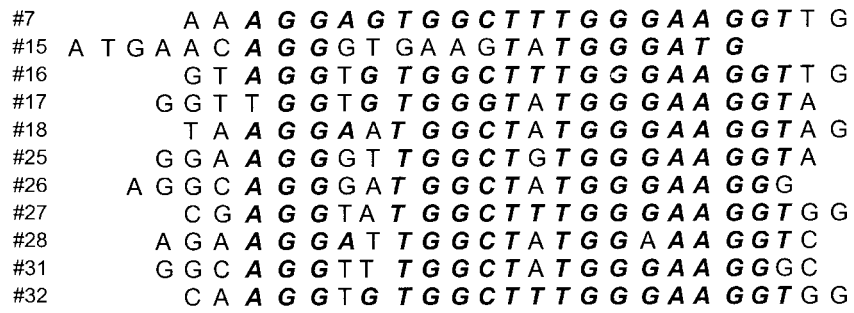

Figure 2. Human pro-urokinase sequence homology. The identified region of homology is shown in bold.

Nitrocellulose filters binding assay has determined increased affinity of ssDNA from selection cycle 12 for PLAU.

DNA from the 12th cycle of PLAU selection was amplified by PCR with the primers 5′-GACACCTGC*GGATCC*AAAGCGATGGC and 5′-CTGAAGAGC*CTG-CAG*GGCTGCGATGGG, digested with *BamHI* and *PstI* (restriction sites are indicated as *italic* letters on the primers sequenses), and subsequently cloned into the appropriate sites in the plasmid pUC19. The sequences of randomly generated 24-mer inserts were determined for 14 clones, and a a highly conserved 12–14 base region was identified for 11 analyzed clones (Fig. 2).

REFERENCES

1. Bock, L.C.; Griffin, L.C.; Latham, J.A.; Vermaas, E.H.; Toole, J.J. Selection of single stranded DNA molecules that bind and inhibit human thrombin. Nature **1992**, *355*, 564–566.
2. Breaker, R.R. DNA aptamers and DNA enzymes. Curr. Opin. Chem. Biol. **1997**, *1*, 26–31.
3. Ellington, A.D.; Szostak, J.W. Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. Nature **1992**, *355*, 850–852.
4. Blank, M.; Weinschenk, T.; Priemer, M.; Schluesener, H. Systematic evolution of a DNA aptamer binding to rat brain tumor microvessels. Selective targeting of endothelial regulatory protein pigpen. J. Biol. Chem. **2001**, *276*, 16464–16468.
5. Blackburn, P.; Wilson, G.; Moore, S. Ribonuclease inhibitor from human placenta. J. Biol. Chem. **1977**, *252*, 5904–5910.
6. Meyerhans, A.; Vartanian, J.P.; Wain-Hobson, S. Strand specific PCR amplification of low copy number DNA. Nucleic Acids Res. **1992**, *20*, 521–523.

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