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Aptamers as Biosensors

By

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Abstract

Aptamers are short, single strands of artificial nucleic acids which bind to specific targets. This binding is similar to the binding exhibited by antibodies but aptamers offer improved properties and consequently are replacing antibodies in some applications.

This first part of this project attempted to generate an aptamer which would bind to the molecules amphetamine and methamphetamine, with high selectivity and affinity. The process for generating an aptamer is called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) and is carried out *in vitro*. The strength of an aptamer / target binding interaction can be assessed using a variety of different techniques and in this work an Enzyme Linked Immunosorbent Assay (ELISA) was investigated. Other methods including surface plasmon resonance (SPR) and circular dichroism were attempted.

The phenomenon of naturally occurring RNA molecules with catalytic properties (ribozymes) were the inspiration for the development of catalytic aptamers. Consequently, a novel SELEX system was deigned to select aptamers capable of catalysing a Diels Alder reaction between cyclohexadiene modified DNA and maleimide. A number of potential sequences were isolated and when one such sequence was incorporated into a test reaction a cycloadduct resulting from a successful Diels-Alder reaction was confirmed using MALDI – MS.

In addition to generating novel aptamers, this thesis details work on the application of existing aptamers with the aim of creating novel assays and expanding the potential use of aptamers. Two protein targets, namely Protein kinase C (PKC) and thrombin were chosen and their respective aptamers investigated for use in metallic nanoparticle conjugates. The combination of aptamers and nanoparticles (in particular metallic nanoparticles) has grown in prominence in the literature and is regarded as an exciting development due to their desirable optical properties. Consequently, novel assays based on UV-visible spectroscopy were developed using the two specified DNA aptamers conjugated to gold and / or silver nanoparticles. In addition, the possibility of incorporating Surface enhanced resonance Raman scattering (SERRS) analysis was investigated.

Abbreviations

2^{y} Ab	Secondary antibody
11-MHEG	11-Mercaptoundecyl hexa(ethylene glycol)
11-THEG	Triethylene glycol mono-11-mercaptoundecyl ether
A	Adenine
ADHD	Attention-deficit hyperactivity disorder
AFM	Atomic force microscopy
AGNC	Aptamer gold nanoparticle conjugate
AP	Alkaline phosphatase
API	Active pharmaceutical ingredient
ASNC	Aptamer silver nanoparticle conjugate
BDTDD	(*E*)-4((1*H*-benzo[*d*][1,2,3]triazole-5-yl)diazenyl)-3,5-dimethoxy phenol
BCIP	3-Bromo-4-chloro-5-indolyl phosphate
bp	base pair
BS	Blocking solution
BSA	Bovine serum albumin
C	Cytosine
CCD	Charged coupled device
CD	Circular dichroism
CM	Carboxymethylated dextran
Da	Daltons
DATP	Deoxyadenine triphosphate
DCTP	Deoxycytosine triphosphate
DEPC	Diethylpyrocarbonate
DGTP	Deoxyguanine triphosphate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
dNMP	Deoxynucleotide monophosphate
ds	Double stranded
dTTP	2’ Deoxythymidine 5’-triphosphate
DTT	Dithiothreitol
EDC	Ethylene carbodiimide
EDTA	Ethylene diamine tetraacetic acid
EF	Enhancement factor
ELISA	Enzyme linked immunosorbent assay
ELONA	Enzyme linked oligonucleotide assay
EMSA	Electromobility shift assay
FAM	5-Carboxyfluorescein
FDA	Food and Drug Administration
G	Guanine
GC-MS	Gas chromatography-mass spectrometry

GRO	G-rich oligonucleotide
HBV	Hepatitis B virus
HEG	Hexaethylene gycol
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	Human immunodeficiency virus
HNE	Human neutrophil elastase
HPLC	High performance liquid chromatography
HRP	Horse raddish peroxidase
IgG	Immunoglobulin G
IPTG	Isopropyl-β-D-thiogalactopyranoside
KOD	*Thermococcus kodakaraensis*
LB	Luria broth
LNA	Locked nucleic acid
LPA	Linear polyarcylamide
MALDI	Matrix assisted laser desorption ionisation
MDMA	Methylenedioxymethamphetamine
MWQ	MilliQ water
MS	Mass spectrometry
MWCO	Molecular Weight Cut Off
NECEEM	Non–equlibrium capillary electrophoresis of the equilibrium mixture
NBT	Nitro blue tetrazolium
NHS	*N*-hydroxy succinimide
NP	Nanoparticle
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDGF	Platelet – derived growth factor
PKC (δ)	Protein Kinase C (delta)
pNPP	p-Nitrophenyl phosphate
RNA	Ribonucleic acid
RNases	Ribonucleases
ROX – ITC	X-Rhodamine isothiocyanate
SELEX	Systematic evolution of ligands by exponential enrichment
SER(R)S	Surface enhanced (resonance) raman scattering
SPR	Surface plasmon resonsnce
ss	single stranded
T	Thymine
TBE	Tris borate EDTA
TEAA	Triethyl ammonium acetate
TLC	Thin layer chromatography
TMB	Tetramethylbenzidine
TOF	Time of flight
U Forward (P)	Universal phosphorylated forward primer
U	Uracil
U Reverse	Universal reverse primer
U Reverse (B)	Universal biotinylated reverse primer

UV – vis spectroscopy	Ultra violet - visible spectroscopy
v /v	volume / volume
VEGF	Vascular endothelial growth factor
VSDR2 mid	12 base oligonucleotide containing a cyclohexadiene modified T base located mid sequence
w / v	weight / volume
X – Gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside