

Binding Mechanisms of RNA Aptamers

Thesis for Obtaining
the Degree of
Doctor of Philosophy
Submitted to the Department of
Biology, Chemistry and Pharmacy
of the Free University of Berlin

by
Stephanie Oestreich
Born May 06, 1973
in Berlin

October 2003

Bindungsmechanismen von RNA-Aptameren

Dissertation
zur Erlangung des Doktorgrades
Eingereicht im Fachbereich
Biologie, Chemie, Pharmazie
der Freien Universität Berlin

Vorgelegt von
Stephanie Oestreich
Geb. am 06.05.1973
in Berlin

Oktober 2003

Declaration of Honesty

(Eidesstattliche Versicherung)

Hereby I declare that I have written this thesis by myself, marked the sources of any quotations or content obtained otherwise and mentioned any personal help by name.

(Ich erkläre hiermit, dass ich die vorliegende Doktorarbeit selbständig verfasst, die wörtlich oder inhaltlich anderen Quellen entnommenen Stellen als solche kenntlich gemacht und die Inanspruchnahme persönlicher Hilfe namentlich aufgeführt habe.)

Boston, October 2003

Stephanie Oestreich

The work for this PhD thesis was performed from April 2000 to October 2003 in the laboratory of Prof. Jack Szostak in the Department of Molecular Biology at Harvard Medical School.

1st supervisor: Prof. Jack W. Szostak

Harvard Medical School
Department of Molecular Biology
Massachusetts General Hospital
50 Blossom Street, Wellman-9
Boston, MA 02114
USA

2nd supervisor: Prof. Volker A. Erdmann

Freie Universität Berlin
Thielallee 63
14195 Berlin
Germany

Date of Defense: March 05, 2004

Table of Contents

	Page
1. Introduction	8
1.1 RNA - Functions and Properties	8
1.2 The RNA World Hypothesis	9
1.3 RNA Structure, Fitness and Information Content	10
1.4 <i>In Vitro</i> Selection	11
1.5 Brief History of <i>In Vitro</i> Selection	13
1.6 Aptamers and Catalytic RNAs Generated by <i>In Vitro</i> Selection	14
2. Task	17
3. Methods	18
3.1 Oligonucleotide Synthesis and Purification	18
3.2 <i>In Vitro</i> Transcription and Removal of DNA	18
3.3 Reverse Transcription	19
3.4 Polymerase Chain Reaction (PCR)	19
3.5 Functional-Domain Mapping by Alkaline Hydrolysis	20
3.6 Library Design and Synthesis	21
3.7 Immobilizing GTP- γ -S on Thiopropyl Sepharose 6B	22
3.8 <i>In Vitro</i> Re-Selection Procedure	23
3.9 TOPO Cloning, Preparation of Plasmid DNA and Sequencing	24
3.10 Determination of Aptamer Secondary Structures and Their Minimum Free Energy	24
3.11 Spinfiltration and Equilibrium Dialysis: Methods for Determining K _d Values and Fraction of Correctly Folded Aptamer	25
3.11.1 Determining the Apparent K _d s of GTP Aptamers	25

Table of Contents

3.11.2	Derivation of the Equation Used for K_d Determination	27
3.11.3	Competitor Experiments	32
3.11.4	Determining the Real K_d and Percentage of Correctly Folded Aptamer	34
3.11.5	Determination of GTP Background Binding	39
3.11.6	Alternative Methods for K_d Determination	39
4.	Results	40
4.1	Testing the Effectiveness of Enriching Functional RNA Sequences to Optimize Selection Conditions	40
4.1.1	Inhibition of Aptamer Column Binding Activity by Pool RNA	40
4.1.2	Pre-Column and Pre-Incubation of the Column Material with tRNA	41
4.1.3	Amount of Washing	41
4.1.4	Examining Aptamer Association and Dissociation	42
4.2	Characterization of GTP Aptamers	44
4.2.1	GTP Selection	44
4.2.2	Aptamer Optimization	44
4.2.2.1	Doped Re-Selections	45
4.2.2.2	Aptamer Sequence Alignments and Comparisons	45
4.2.2.2.1	Optimization of the Class II Aptamer	46
4.2.2.2.2	Optimization of the Class III Aptamer	49
4.2.2.2.3	Results of Aptamer Optimization	51
4.2.2.2.4	Optimized GTP Aptamers	52
4.3	Aptamer Affinity and Stability	57
4.3.1	RNA Secondary Structure Prediction	57
4.3.2	Equilibrium Dissociation Constants and Aptamer Fraction Correctly Folded	58
4.3.3	Relationship of Aptamer Quality and Free Energy of Secondary Structure Formation	59
4.3.4	Investigation of Aptamer Binding Mechanisms with GTP Analogs	62
		5

Table of Contents

4.3.4.1	Overall Interaction Pattern	63
4.3.4.2	Interaction with the Phosphate Region of GTP	68
4.3.4.3	Interaction with the Sugar Region of GTP	68
4.3.4.4	Interaction with the Nucleobase Region of GTP	68
4.3.4.5	Differences in Binding Energy	69
4.3.4.5.1	Comparison of Binding Energy Distributions	71
5.	Discussion	72
5.1	Optimization of <i>In Vitro</i> Selection Conditions	72
5.1.1	Conclusions from Optimizing Selection Conditions	72
5.2	Mechanisms of Aptamer Binding	75
5.2.1	Aptamer Stability	75
5.2.2	Correlation of Aptamer Structure and Quality	75
5.2.3	Aptamer Binding Mechanisms	77
5.2.4	Similar Interaction Patterns of Different Aptamers	77
5.3	Evolutionary Potential of Aptamers	79
5.4	Future Studies	79
6.	Summary	81
7.	Zusammenfassung	82
8.	References	83
9.	Materials	94
9.1	Chemicals	94
9.2	Media	95
9.3	Buffers	95
9.4	Laboratory Equipment	96
		6

Table of Contents

9.5	Other Materials and Equipment	97
9.6	Enzymes	98
9.7	Kits	98
9.8	Oligonucleotides and Randomized Pool Sequences	98
10.	Abbreviations	101
11.	Curriculum Vitae	103
12.	Publications	104
13.	Acknowledgements and Words of Thanks	105