**CONTENTS**

I. INTRODUCTION 1-27

I.1. Naturally Occurring Catalytic RNA Molecules 1

I.1.1. Self Splicing of Group I and Group II Introns 1

I.1.2. Ribonuclease P 4

I.1.3. Small Catalytic RNAs 6

I.1.3.1. Hammerhead Ribozyme 7

I.1.3.2. Hairpin Ribozyme 8

I.1.3.3. Hepatitis Delta Virus Ribozyme 9

I.1.3.4. Novel RNA in *Neurospora* Mitochondria 10

I.1.4. Other Self Cleaving RNAs 11

I.1.4.1. Lead-Catalyzed Cleavage of Yeast phe-tRNA 11

I.1.4.2. Manganese-Dependent Cleavage of RNA 11

I.2. *In Vitro* Selection of New Ribozymes 12

I.3. A Comparison of RNA and Protein as Biological Catalysts 13


I.5. Design of New Hammerhead Ribozyme against Chosen Target Molecules 15

I.6. The Structure of Hammerhead Ribozyme 16

I.6.1. The Mechanism and Role of Mg²⁺ 17

I.6.2. Importance of Helix II loop for Activity 17

I.6.3. Importance of 2'-Hydroxyl Group for Activity 18

I.6.4. Tertiary Structure of the Hammerhead Domain 18

I.7. Application of Hammerhead Ribozyme *In Vivo* 19

I.8. Examples of Hammerhead Ribozyme Applications *In Vivo* 24

I.9. SOS Response in *E. coli* and *lexA* Gene as Model Target 25

I.10. Aim of the Present Project 26

II. MATERIALS AND METHODS 28-29

II.1. Materials 28

II.1.1. Materials Used for Vector Construction 28

II.1.2. Materials Used for Sequencing of Plasmid DNA 30

II.1.3. Materials Used for RNA Work 31

II.2. Methods 32

II.2.1. General Procedures 32

II.2.2. Preparation of Competent Bacteria and Their Transformation 32

II.2.3. The LiCl Procedure for Rapid Plasmid Minipreps 33
11.2.4. The Cleared Lysate Method of Plasmid Isolation

11.2.5.1. Isolation of DNA from LMA (Low Melting Agarose)

11.2.5.2. Purification of DNA from Agarose Gel: The Phenol-Freeze-Fraction Method

11.2.5.3. Recovery of DNA Fragment from Native Poly Acrylamide Gels

11.2.6. Synthesis and Purification of Oligonucleotides

11.2.7. Visualization of Secondary Structure

11.2.8. Construction of Cloning and Expression Vectors

11.2.8.1. Cloning of Oligonucleotides Corresponding to Ribozyme Immediately Downstream of the T7 Promoter of pBLoT7

II.3. RESULTS

III.1. Selection of Target Sites in lexA RNA

III.2. Design of Ribozyme

III.3. Construction of Ribozymes in pBLoT7 Vector

III.4. Subcloning of Target Gene (lexA) in pBLoT7 Vector

III.5. Limitation of pBLoT7 Vector

III.6. Transcription Efficiency of T7 Promoter having Different Nucleotides at +1 Position

III.7. Construction of In Vitro Transcription Vector Allowing Precise Initiation of Transcript

III.8. Primer Extension Analysis of New Vectors
III.9. Construction of Ribozyme Clones in pBII Vector

III.10. Construction of Ribozyme for In Vivo Studies

III.11. Subcloning of lexA Gene in pTZ18U Vector

III.12. Subcloning of recA Gene in pBgl II Vector

III.13. Ribozyme-mediated Cleavage of lexA RNA

III.14. Specificity of Cleavage of lexA RNA by Wild Type and Mutant Ribozyme

III.15. Cleavage of Full- and Partial-length lexA RNA by Ribozyme

III.16. Ribozyme-substrate Binding Studies

III.17. Kinetic Characterization of Ribozymes 3.1 and 4.11

III.18. Effect of Factors Interacting with Nucleic Acids on Ribozyme Activity

III.19. In Vivo Studies

III.19.1. Expression of Ribozyme In Vivo

III.19.2. Cellular Ribozyme Activity

III.19.3. Effect of Ribozyme on Expression Pattern of lexA and recA

III.19.4. UV-Sensitivity of Cells after IPTG Induction of Ribozyme

IV. DISCUSSION

IV.1. Selection of Target Sites and Design of Ribozyme

IV.2. Construction of In Vitro Transcription Vectors

IV.3. In Vitro Characterization of Ribozymes

IV.4. Derepression of SOS Regulon without DNA Damage

IV.5. Is Self-cleavage a more Widely Distributed Activity of RNA Molecules?

IV.6. Future Prospects

V. SUMMARY AND CONCLUSION

VI. REFERENCES

APPENDIX

I. Bacterial Strains Used in this Study

II. Plasmids used in this Study