

**Structural Basis for Left-Handed Z-DNA Binding
by Human dsRNA Specific
Adenosine Deaminase ADAR1**

Inaugural-Dissertation
zur Erlangung des akademischen Grades
“Doktor der Naturwissenschaften”
– Dr. rer. nat. –
am Fachbereich Biologie, Chemie, Pharmazie
der Freien Universität Berlin

vorgelegt von Diplom-Biochemiker
Thomas Schwartz

Berlin 1999

Die vorliegende Arbeit wurde im Zeitraum November 1996 bis August 1999 im Labor von Prof. Alexander Rich am Massachusetts Institute of Technology in Cambridge, U.S.A., angefertigt.

1. Gutachter : Prof. Dr. Udo Heinemann
2. Gutachter : Prof. Dr. Volker A. Erdmann

Eingereicht am : 7. September 1999

Tag der mündlichen Prüfung: 18. Januar 2000

I. Index

I. INDEX

II. ABBREVIATIONS

1. INTRODUCTION.....	1
2. SCIENTIFIC BACKGROUND	5
2.1. Z-DNA	5
2.2. DOUBLE-STRANDED RNA ADENOSINE DEAMINASE ADAR1	7
2.3. LIMITED PROTEOLYSIS	10
2.4. PROTEIN-DNA BINDING ASSAYS IN SOLUTION	10
2.4.1. ELECTROPHORETIC MOBILITY SHIFT ASSAY.....	10
2.4.2. CIRCULAR DICHROIC SPECTROSCOPY.....	10
2.5. PRINCIPLES OF MACROMOLECULAR X-RAY CRYSTALLOGRAPHY	11
2.5.1. SCATTERING OF X-RAYS.....	11
2.5.2. CALCULATION OF THE ELECTRON DENSITY.....	12
2.5.3. SOLUTION OF THE PHASE PROBLEM.....	13
3. MATERIALS.....	15
3.1. BACTERIAL STRAINS.....	15
3.2. PLASMIDS.....	15
3.3. <i>E. COLI</i> MEDIA	15
3.4. SOLUTIONS.....	16
3.5. CHEMICALS.....	18
3.6. ENZYMES AND KITS	18
3.7. NUCLEIC ACIDS.....	18
3.8. EQUIPMENT.....	19
3.9. SOFTWARE.....	20
4. BIOCHEMICAL AND MOLECULAR BIOLOGICAL METHODS.....	21
4.1. CLONING OF EXPRESSION CONSTRUCTS	21
4.2. PCR AMPLIFICATION OF DNA FRAGMENTS	22
4.3. DESALTING PCR PRODUCTS.....	22
4.4. PLASMID PREPARATION	23
4.5. RESTRICTION DIGESTION.....	23
4.6. GEL PURIFICATION OF DNA.....	23
4.7. LIGATION	23
4.8. PREPARATION OF COMPETENT <i>E. COLI</i> CELLS.....	24
4.9. TRANSFORMATION.....	24
4.10. COLONY PCR	24
4.11. DNA SEQUENCING.....	25
4.12. DENATURING DOUBLE-STRANDED DNA.....	25
4.13. SEQUENCING REACTION.....	25
4.14. GEL ELECTROPHORESIS	25
4.15. SITE-DIRECTED MUTAGENESIS.....	26
4.16. OVERPRODUCTION OF RECOMBINANT PROTEINS.....	26
4.17. PROTEIN PURIFICATION.....	27
4.18. CONTROLLED PROTEOLYSIS.....	28
4.19. MASS SPECTROMETRIC ANALYSIS OF PROTEOLYTICALLY OBTAINED PROTEIN FRAGMENTS.....	28
4.20. FUNCTIONAL Z-DNA BINDING ASSAYS.....	29
4.20.1. ELECTROPHORETIC MOBILITY SHIFT ASSAY	29
4.20.2. CIRCULAR DICHROIC SPECTROSCOPY	29
4.21. DNA PURIFICATION.....	30
5. CRYSTALLOGRAPHIC METHODS.....	31
5.1. CRYSTALLIZATION	31
5.2. X-RAY DATA COLLECTION	31

5.3. STRUCTURE DETERMINATION USING ISOMORPHOUS REPLACEMENT TECHNIQUES	32
5.4. STRUCTURE REFINEMENT	32
6. BIOCHEMICAL CHARACTERIZATION OF THE Z-DNA BINDING DOMAIN OF ADAR1.....	34
6.1. CLONING OF EXPRESSION CONSTRUCTS	34
6.2. OVERPRODUCTION OF EXPRESSION CONSTRUCTS	34
6.3. DETERMINATION OF THE STRUCTURAL ORGANIZATION OF THE BIPARTITE Z-DNA BINDING DOMAIN ZAB VIA CONTROLLED PROTEOLYSIS.....	35
6.3.1. DEFINING THE BOUNDARIES OF THE MINIMAL Z-DNA BINDING DOMAIN, Z α , OF HUMAN ADAR1	35
6.3.2. INTERACTION BETWEEN THE TWO MOTIFS, Z α AND Z β , TO FORM A SINGLE STRUCTURAL ENTITY.....	38
6.3.3. THE ROLE OF THE LINKER REGION FOR STABILIZING OF THE ZAB DOMAIN.....	43
6.3.4. CONCLUSIONS FROM SECONDARY STRUCTURE EXAMINATIONS OF ZAB	43
6.3.5. PROTEOLYTIC SENSITIVITY OF ZAB IN PRESENCE AND ABSENCE OF LIGAND	45
6.4. Z-DNA BINDING BEHAVIOR OF ZAB AND SUBDOMAINS	47
7. THE COMPLEX OF Zα WITH LEFT-HANDED Z-DNA.....	51
7.1. CRYSTALLIZATION AND CHARACTERIZATION OF THE CRYSTALS.....	51
7.2. DATA COLLECTION AND PHASE DETERMINATION	52
7.3. MODEL BUILDING AND REFINEMENT.....	55
7.4. OVERALL STRUCTURE OF THE Z α -Z-DNA COMPLEX.....	58
7.5. CRYSTAL PACKING INTERACTION	59
7.6. THE Z α DOMAIN.....	63
7.7. THE LEFT-HANDED Z-DNA.....	65
7.8. PROTEIN-DNA CONTACTS.....	66
7.9. COMPARISON OF THE THREE Z α -DNA COMPLEXES IN THE ASYMMETRIC UNIT.....	71
7.10. STRUCTURAL CLASSIFICATION OF THE Z α DOMAIN	73
8. DISCUSSION	75
8.1. THE Z-DNA BINDING MODE OF Z α	75
8.2. Z-DNA <i>VERSUS</i> B-DNA BINDING BY HTH PROTEINS.....	77
8.3. COMPARISON OF THE PROTEOLYTIC EXPERIMENTS WITH THE CRYSTAL STRUCTURE OF Z α	81
8.4. THE <i>IN VIVO</i> FUNCTION OF Z α	82
9. SUMMARY.....	84
10. ZUSAMMENFASSUNG	85
11. REFERENCES	88
11.1. CITED REFERENCES	88
11.2. LIST OF PUBLICATIONS.....	97

ACKNOWLEDGEMENTS

II. Abbreviations

Å	Ångström, $1 \text{ Å} = 10^{-10} \text{ m}$
a.u.	asymmetric unit
aa	amino acid
acc	accession number
ADAR1	dsRNA dependent adenosine deaminase
β-ME	2-mercaptoethanol
bp	base pair
CD	circular dichroic
cDNA	complementary DNA
DNA	deoxyribonucleic acid
ds	double-stranded
DTT	dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylenediaminetetraacetic acid
EMSA	electrophoretic mobility shift assay
FPLC	fast performance liquid chromatography
HEPES	N-[2-Hydroxyethyl]piperazine-N'-[4-butanesulfonic acid]
HTH	helix-turn-helix
kD	kiloDalton, $1 \text{ kD} = 1.66018 \cdot 10^{-21} \text{ g}$
M	molarity, $[\text{mol} \cdot \text{l}^{-1}]$
MALDI-TOF	matrix assisted laser desorption ionization – time of flight
MOPS	3-[N-Morpholino]propanesulfonic acid
mRNA	messenger RNA
NCS	non-crystallographic symmetry
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PDB	Protein Data Bank
PMSF	phenylmethylsulfonyl flouride
rmsd	root mean square deviation
RNA	ribonucleic acid
SDS	sodium dodecyl sulfate
SIRAS	single isomorphous replacement including anomalous scattering
Tris	tris[hydroxymethyl]aminomethane
UV	ultra violet

V8	endoproteinase Glu-C
Z α	Z-DNA binding domain of ADAR1
Z β	Z α -related domain in ADAR1
Z $\alpha\beta$	bipartite domain in ADAR1, consisting of Z α and Z β

Acknowledgements

I would like to especially thank my advisor, Prof. Dr. Udo Heinemann, for his great confidence in my scientific work since I first started my diploma thesis in his lab in 1995. I much appreciate Prof. Dr. Udo Heinemann's support in allowing me to do this thesis work in Prof. Dr. Alexander Rich's lab at the Massachusetts Institute of Technology in Cambridge, U.S.A.

I thank Prof. Dr. Alexander Rich for giving me the opportunity to do this exciting work in his lab. Especially, I remember not only many helpful discussions on the subject of this thesis but most of all about science 'in the big picture'.

This thesis work would not have been possible without the outstanding help and support of Drs. Ky Lowenhaupt and Mark A. Rould. I thank Ky for sharing excitement, and also the opposite of it throughout this entire endeavour. The value of her advice and friendship can hardly be put in words. I am deeply grateful to Mark for teaching me crystallography and for his enthusiasm for this project.

I thank my colleagues Dr. Imre Berger, Dr. Alan Herbert, Dr. Yang-Gyun Kim, Holger Knaut, Dr. Curt Lockshin, Dr. Stefan Maas, Dr. Uwe Müller, Stefan Rothenburg and Dr. Shuguang Zhang for their help and advice on countless occasions. Holly Teichholtz was a great help by editing this manuscript.

The support from the German Academic Exchange Service (DAAD) is greatly appreciated.