

# Contents

<b>Introduction</b> . . . . .	1
<b>DNA Hydrolysis: Mechanism and Reactivity</b> . . . . .	3
N.H. WILLIAMS	
1      Introduction . . . . .	3
2      The Importance of the Background Reaction . . . . .	4
3      Mechanism . . . . .	7
4      Spontaneous Hydrolysis . . . . .	8
5      C-O Cleavage . . . . .	9
6      P-O Cleavage . . . . .	10
7      Base Catalysis . . . . .	12
8      Acid Catalysis . . . . .	13
9      Conclusion . . . . .	14
References . . . . .	14
<b>Active Site of Ribonuclease A</b> . . . . .	19
R.T. RAINES	
1      Introduction . . . . .	19
2      Mechanism of Catalysis . . . . .	20
3      Active-Site Residues . . . . .	21
3.1    Histidine 12 and Histidine 119 . . . . .	21
3.2    Lysine 41 . . . . .	24
3.3    Phenylalanine 120 . . . . .	24
3.4    Aspartic Acid 121 . . . . .	25
3.5    Glutamine 11 . . . . .	27
4      Catalytic Rate Enhancement . . . . .	28
5      Envoi . . . . .	29
References . . . . .	29

<b>Structural Considerations Concerning Cleavage of RNA . . . . .</b>	<b>33</b>	
<b>R. KIERZEK</b>		
1	Introduction . . . . .	33
2	Facts About the Influence of Oligoribonucleotide Structure on Cleavage of Phosphodiester Bonds . . . . .	34
2.1	How to Cleave Oligoribonucleotides . . . . .	34
2.2	Cleavage Requires a Single-Stranded Character of the Oligoribonucleotide . . . . .	34
2.3	Sequence and Position of the Scissile Phosphodiester Bond Within the Oligomer is Important for Cleavage . . . . .	35
2.4	Oligoribonucleotide Length Affects Cleavage of Diester Bond . . . . .	36
2.5	The Functional Groups of the Pyrimidine Nucleobases Flanking the Cleaved Phosphodiester Bond Affect Cleavage . . . . .	36
2.6	The Functional Groups of the Purine Nucleobases Flanking the Cleaved Phosphodiester Bond Influence Cleavage . . . . .	38
2.7	The C5 Substituents of Uridine Affect the Hydrolysis Rate of the UA Phosphodiester Bond . . . . .	39
2.8	Chimeric DNA/RNA Oligomers Affect the Cleavage of Phosphodiester Bonds . . . . .	40
2.9	The Motives of RNA Structure are Affected by Spontaneous Cleavage . . . . .	41
2.10	The Effects of Polyamines on the Cleavage of Oligoribonucleotides . . . . .	43
3	Mechanism of Phosphodiester Bond Cleavage . . . . .	44
4	Conclusions . . . . .	46
References . . . . .		46
 <b>Cleavage of RNA by Imidazole . . . . .</b>		 <b>49</b>
<b>V.V. VLASSOV and A.V. VLASSOV</b>		
1	Introduction . . . . .	49
2	Imidazole as the Simplest Mimic of the Ribonuclease Catalytic Structure . . . . .	50
3	Mechanism of RNA Cleavage by Imidazole . . . . .	51
4	Imidazole as a Reagent for Probing RNA Structure . . . . .	54
5	Conclusions . . . . .	59
References . . . . .		59

Contents	VII
<b>Principles of Nucleic Acid Cleavage by Metal Ions . . . . .</b>	61
A. DALLAS, A.V. VLASSOV, and S.A. KAZAKOV	
1      Introduction . . . . .	61
2      General Mechanisms for the Cleavage of Nucleic Acids . . . . .	62
3      Basic Properties of Metal Ions in Solution . . . . .	65
4      Metal Ion Binding to Nucleic Acids . . . . .	69
5      Transesterification and Hydrolytic Cleavage of Nucleic Acids Catalyzed by Metal Ions . . . . .	72
5.1     Efficacy of Cleavage of Nucleic Acids by Metal Ions . . . . .	72
5.2     Possible Mechanism of Metal Ion Catalysis . . . . .	72
5.3     Nucleic Acid Structure and Specificity of Metal-Catalyzed Cleavage Reactions . . . . .	75
6      Oxidative Cleavage of Nucleic Acids Induced by Metal Ions . . . . .	77
6.2     Cleavage Reactions Promoted by Metal Ions in High Oxidation States . . . . .	77
6.3     Cleavage Reactions Involving Metal-Induced Oxygen Radicals . . . . .	78
7      Probing Metal Binding Sites in RNA by Metal-Induced Cleavage . . . . .	81
References . . . . .	83
 <b>Allosterically Controlled Ribozymes as Artificial Ribonucleases . . .</b>	89
M. IYO, H. KAWASAKI, M. MIYAGISHI, and K. TAIRA	
1      Introduction . . . . .	89
2      Maxizymes are Allosterically Controllable Ribozymes . . . . .	90
2.1     Expression of Ribozymes in Cells . . . . .	90
2.2     The Design of Allosterically Controllable Maxizymes . . . . .	91
2.3     Allosteric Control of Ribozyme Activity . . . . .	94
2.4     Selection of Allosterically Controllable Ribozymes in Vitro .	95
3      Maxizymes . . . . .	96
3.1     Truncated Hammerhead Ribozymes that Function as Dimers . . . . .	96
3.2     General Design of an Allosterically Controllable Maxizyme .	97
3.3     The Antitumor Effects of an Allosterically Controllable Maxizyme . . . . .	101
3.4     General Applications of Maxizyme Technology . . . . .	101
4      Conclusion . . . . .	104
References . . . . .	104

**Small Ribonuclease Mimics . . . . .** 111  
I.L. KUZZNETSOVA and V.N. SIL'NIKOV

1	Introduction . . . . .	111
2	RNA-Cleaving Compounds . . . . .	112
2.1	RNA-Cleaving Compounds Mimicking Ribonucleases A and T1 . . . . .	112
2.2	Mimics of the Active Center of Nuclease S . . . . .	113
3	Design of Active Centers of Natural Enzymes . . . . .	113
3.1	Spatial Organization of RNA-Cleaving Groups in Active Centers of RNases and Nucleases S and Sm . . . . .	114
3.2	RNA-Binding Groups . . . . .	115
4	Design of Artificial Ribonucleases Mimicking RNase A . . . . .	115
4.1	Artificial Ribonucleases with Polycyclic RNA-Binding Domains . . . . .	116
4.2	Polycationic RNA-Binding Groups . . . . .	117
5	Specificity of RNA Cleavage . . . . .	121
6	Effects of Buffer on the Cleavage . . . . .	122
7	Mechanism of RNA Cleavage with the Artificial Ribonucleases ABLkCm and nLm . . . . .	123
8	Potential Applications of Small Ribonuclease Mimics . . . . .	125
9	Conclusions . . . . .	125
	References . . . . .	126

**Copper-Containing Nuclease Mimics: Synthetic Models  
and Biochemical Applications . . . . .** 129  
S. VERMA, S.G. SRIVATSAN, and C. MADHAVAIAH

1	Introduction . . . . .	129
2	Model Systems . . . . .	130
3	Mechanistic Pathways . . . . .	130
4	Copper Complexes with Synthetic Ligands . . . . .	131
4.1	Hydrolysis of Phosphate Diesters . . . . .	131
4.2	Cleavage of Nucleic Acids . . . . .	138
4.3	Copper Complexes of Natural Ligands . . . . .	143
5	Outlook . . . . .	146
	References . . . . .	146

Contents	IX
----------	----

<b>RNA-Cleaving Oligonucleotide-Peptide Conjugates . . . . .</b>	151
N.L. MIRONOVA, D.V. PYSHNYI, and E.M. IVANOVA	

1      Introduction . . . . .	151
2      Proteins Displaying Ribonuclease Activity . . . . .	151
2.1    Natural Ribonuclease . . . . .	151
2.2    Small Natural Peptides . . . . .	154
2.3    Synthetic Polypeptides . . . . .	155
3      Conjugates of RNA-Cleaving Peptides with Constructs Capable of Binding to RNA . . . . .	156
3.1    Conjugates of Enzymes and Oligonucleotides . . . . .	156
3.2    Conjugates of Short Peptides and Intercalators . . . . .	157
3.3    Oligonucleotide-Peptide Conjugates Displaying Ribonuclease Activity . . . . .	158
3.3.1   Influence of Peptide Structure on the Efficacy of RNA Cleavage . . . . .	159
3.3.2   Site-Directed RNA Hydrolysis by Peptidyloligonucleotides .	161
3.3.3   Hydrolysis of RNA by the Conjugates of Random Oligonucleotides . . . . .	163
3.3.4   Influence of Oligonucleotide Sequence on the Specificity of RNA Cleavage . . . . .	164
3.3.5   Cleavage of Short RNA . . . . .	167
4      Discussion . . . . .	168
References . . . . .	170

<b>Sequence Selective Artificial Ribonucleases Employing Metal Ions as Scissors . . . . .</b>	173
A. KUZUYA, R. MIZOGUCHI, and M. KOMIYAMA	

1      Introduction . . . . .	173
1.1    Significance of Artificial Ribonucleases . . . . .	173
1.2    Molecular Design of Artificial Ribonucleases . . . . .	174
2      Metal Ion Catalysts for RNA Cleavage . . . . .	175
2.1    Divalent Metal Ions and Their Complexes . . . . .	175
2.2    Trivalent Lanthanide Ions and Their Complexes . . . . .	177
3      Sequence-Selective Artificial Ribonucleases with Covalently Attached Catalysts . . . . .	178
3.1    Attaching the Catalysts to the End of DNA Oligomers . . . .	178
3.1.1   [Lanthanide Complex]/DNA Hybrids . . . . .	178
3.1.2   [Dinuclear Metal Complex]/DNA Hybrids . . . . .	179

3.2	Attaching the Catalysts at an Internal Position	
	Within DNA Oligomers . . . . .	180
4	Noncovalent Systems for Sequence-Selective RNA Scission .	180
4.1	Molecular Design . . . . .	181
4.2	Site-Selective RNA Scission by Lanthanide Ions . . . . .	182
4.3	Requirements for the Sequence-Selective RNA Activation .	184
4.4	Site-Selective RNA Scission by Non-Lanthanide Ions . . . .	185
4.5	Mechanism of the Site-Selective Scission . . . . .	185
5	Prospect . . . . .	186
	References . . . . .	186

**Site-Specific Artificial Ribonucleases: Conjugates  
of Oligonucleotides with Catalytic Groups . . . . .** 189

M.A. Zenkova and N.G. Beloglazova

1	Introduction . . . . .	189
2	Oligonucleotide Conjugate-Based Artificial Ribonucleases .	190
2.1	Conjugates of Oligonucleotides and Metal Complexes . . .	190
2.2	Conjugates of Oligonucleotides and RNA-Cleaving Molecules . . . . .	197
2.3	Design of Artificial Ribonucleases for Site-Specific RNA Cleavage . . . . .	202
2.3.1	Synthetic Approaches . . . . .	202
2.3.2	Effect of the Catalytic Structure Location in the Conjugate Structure . . . . .	203
2.3.3	RNA Sequences Optimal for Site-Specific Cleavage . . . .	206
2.4	Site-Specific Cleavage of Cellular RNA . . . . .	206
3	Site-Specific RNA Cleavage by Conjugates of Oligonucleotides with Imidazole-Based Catalytic Groups	207
3.1	Mono- and Bis-Imidazole-Containing Conjugates . . . . .	207
3.2	Binary Systems of Oligonucleotide Conjugates . . . . .	209
3.3	Conjugates with Anthracene-Based Linkers . . . . .	210
3.4	Conjugates with Multiple Imidazole Residues in the Catalytic Domain . . . . .	212
4	Conclusions . . . . .	216
	References . . . . .	216

<b>DNA and RNA Cleavage Mediated by Phenanthroline-Cuprous Oligonucleotides: From Properties to Applications . . . . .</b>		223
J.C. FRANCOIS, M. FARIA, D. PERRIN, and C. GIOVANNANGELI		
1	Introduction . . . . .	223
2	The 1,10-Phenanthroline-Cuprous Complex . . . . .	224
2.1	Background . . . . .	224
2.2	Structural Probes for Nucleic Acid-Containing Complexes .	226
3	Phenanthroline Conjugates . . . . .	228
3.1	Conjugation of Biomolecules to Phenanthroline . . . . .	228
3.2	Neighbourhood Sensors . . . . .	230
3.3	Oligonucleotide-Phenanthroline as Site-Specific Ribonuclease . . . . .	232
3.4	Oligonucleotide-Phenanthroline Targeted to Double-Stranded DNA . . . . .	233
3.4.1	Artificial Endonucleases for in Vitro Applications . . . . .	233
3.4.2	Biological Activities of Oligonucleotide-Phenanthroline .	235
3.4.2.1	Phenanthroline Cleavage Activity Inside Cells . . . . .	236
3.4.2.2	Modulation of DNA Metabolism Induced by Oligonucleotide-Phenanthroline . . . . .	236
4	Conclusion . . . . .	237
	References . . . . .	238
 <b>Sequence-Specific Cleavage of Double-Stranded DNA . . . . .</b>		243
A.S. BOUTORINE and P.B. ARIMONDO		
1	Introduction . . . . .	243
2	DNA Cleavage by Natural Enzymes and Their Conjugates .	246
2.1	Restriction Endonucleases . . . . .	246
2.2	Conjugates of Nucleases with Oligonucleotides . . . . .	246
2.3	The Achilles Heel Cleavage Method . . . . .	247
3	Sequence-Specific Cleavage Using Conjugated Chemical Reagents . . . . .	247
3.1	Direct Cleavage by Sequence-Specific Conjugates of Metal Complexes . . . . .	247
3.2	Alkylating Reagents Conjugated to Sequence-Specific Molecules . . . . .	250
4	DNA Photocleavage . . . . .	251
4.1	Photocleavage by Blue Light-Absorbing Reagents . . . . .	251
4.2	Long-Distance Electron Transfer in Photocleavage Reactions	253
4.3	Photocleavage by Red Light-Absorbing Reagents . . . . .	254
5	Recruitment of Intracellular Enzymes for Sequence-Specific DNA Cleavage . . . . .	255

XII		Contents
5.1	RecA Protein . . . . .	255
5.2	Recruitment of Topoisomerases by Conjugated Topoisomerase Inhibitors . . . . .	255
6	Application of the Non-Specific Single-Stranded DNA Cleaving Agents for Site-Specific Scission of Double-Stranded DNA . . . . .	259
7	Conclusions . . . . .	260
	References . . . . .	261
 <b>Bleomycin–Oligonucleotide Conjugates as Site-Specific Nucleases . . .</b>		<b>269</b>
P.E. VOROBJEV and V.F. ZARYTOVA		
1	Introduction . . . . .	269
2	Structure and Properties of Bleomycins . . . . .	270
3	Conjugates of Bleomycin with Oligonucleotides: Synthesis and Structure . . . . .	273
4	Cleavage of Nucleic Acids by Bleomycin–Oligonucleotide Conjugates . . . . .	275
4.1	Site-Specific Cleavage of Single-Stranded DNA Fragments by Bleomycin–Oligonucleotide Conjugates . . . . .	275
4.2	Cleavage of RNA by Bleomycin–Oligonucleotide Conjugates	276
4.3	Cleavage of Double-Stranded DNA by Triplex-Forming Bleomycin–oligonucleotide Conjugates . . . . .	278
4.4	Catalytic Degradation of DNA Target by Oligonucleotide–Bleomycin Conjugate . . . . .	278
5	Bleomycin–Oligonucleotide Conjugates in Tandem Sets with Effectors as Efficient Nucleases . . . . .	282
6	Conclusion . . . . .	284
	References . . . . .	284
 <b>Subject Index . . . . .</b>		<b>289</b>