

Contents

Chapter 1	Atomic Basis of Protein–Carbohydrate Interactions: An Overview	1
	<i>Nathan Sharon</i>	
1	Introduction	1
2	Combining Sites	2
3	Lectin–Carbohydrate Bonds	3
3.1	Hydrogen Bonds	3
3.2	Hydrophobic Interactions	4
3.3	Other Interactions	4
4	Role of Water	4
	References	5
Chapter 2	Mycobacterial Glycolipids and the Host: Role of Phenolic Glycolipid and Lipoarabinomannan	6
	<i>G.J. Blaauw and B.J. Appelmek</i>	
1	Introduction	6
2	Phenolic Glycolipids	7
2.1	Phenolic Glycolipids History	7
2.2	Structure and Biosynthesis of Phenolic Glycolipids	10
2.3	Role of PGL in Pathogenesis	14
2.3.1	Leprosy	14
2.3.2	Tuberculosis	15
2.4	Evaluation	17
2.5	Future Prospects	19
3	Lipoarabinomannan	20
3.1	Introduction	20
3.2	Synthetic LAM Oligosaccharides	21
3.2.1	Synthetic LAM Oligosaccharides: ManLAM–Lectin Interactions	22
3.2.2	Synthetic LAM Oligosaccharides: ManLAM–Antibody Interactions	24

3.3 Future Prospects	24
Acknowledgments	25
References	26

Chapter 3 Structures and Roles of *Pseudomonas aeruginosa* Lectins 30

Anne Imberty, Michaela Wimmerová, Charles Sabin, and Edward P. Mitchell

1 PA-IL: A Calcium-Mediated Galactose-Binding Lectin	31
1.1 Characteristics and Specificity	31
1.2 Structure of PA-IL	33
1.3 Comparison with other Proteins	34
1.4 Roles of PA-IL in Bacterial Infection	36
2 PA-III: A High-Affinity L-Fucose-Binding Protein	36
2.1 Characteristics and Specificity	36
2.2 Affinity and Thermodynamics	37
2.3 Crystal Structures of PA-III	39
2.4 Comparison with other Proteins	41
2.5 Biological Roles	42
3 Perspectives: Targeting <i>Pseudomonas</i> Lectins for Antibacterial Therapy?	43
Acknowledgements	45
References	45

Chapter 4 Protein–Carbohydrate Interactions in Enterobacterial Infections 49

Nathan Sharon and Itzhak Ofek

1 Introduction	49
2 Enterobacterial Surface Lectins	50
2.1 Specificity and Structure	50
2.1.1 Type 1 fimbriae	52
2.1.2 P fimbriae	59
2.1.3 F17 fimbriae	61
2.2 Genetics and Biosynthesis	61
2.3 Functions	65
3 Anti-Adhesion Therapy	68
References	69

Chapter 5	GM1 Glycomimetics and Bacterial Enterotoxins	73
	<i>Anna Bernardi, Ćrtomir Podlipnik, and Jesús Jiménez-Barbero</i>	
1	Introduction	73
2	Structure and Interaction Features of AB5 Toxins: An Overview	74
3	The Interaction with GM1	74
4	The Use of GM1-Glycomimetics	76
5	Multivalency	85
	References	88
Chapter 6	Retrocyclins: Miniature Lectins with Potent Antiviral Activity	92
	<i>Robert I. Lehrer</i>	
1	Introduction	92
2	Antimicrobial Peptides	92
3	Defensins	93
4	Theta (θ)-Defensins	94
4.1	Presence in Nonhuman Primates	94
4.2	Why Humans Lack Them	95
4.3	Resemblance to Protegrins	96
4.4	<i>In Vivo</i> Expression and Chemical Synthesis	98
4.5	Antimicrobial Properties	99
4.6	Activity Against HIV-1 and Herpes Simplex	99
4.7	Mechanism of Activity Against Influenza A	102
5	Concluding Remarks	103
	References	104
Chapter 7	C-type Lectin Receptors that Regulate Pathogen Recognition Through the Recognition of Carbohydrates	106
	<i>Sandra J. Van Vliet, Christian H. Grün and Yvette Van Kooyk</i>	
1	Introduction	106
2	Expression Patterns of C-type Lectins in the Human Body	106
2.1	DC-SIGN (CD209)	107
2.2	L-SIGN (DC-SIGNR, CD299)	108
2.3	ASGP-R	108
2.4	MGL (CD301)	108

3	Binding of C-type Lectins to Carbohydrates	108
3.1	Interactions	109
3.2	DC-SIGN (CD209)	109
3.3	L-SIGN (DC-SIGNR, CD299)	112
3.4	ASGP-R	113
3.5	MGL (CD301)	114
4	C-type Lectin–Pathogen Interactions	114
4.1	C-type Lectins in Viral Infections	114
4.2	C-type Lectins in Parasitic Infections	117
4.3	C-type Lectins and <i>Helicobacter pylori</i>	118
4.4	C-type Lectins and <i>Candida albicans</i>	118
5	Conclusion	119
	Acknowledgement	120
	References	120

Chapter 8	Targeting Microbial Sialic Acid Metabolism for New Drug Development	125
	<i>Eric R. Vimr and Susan M. Steenbergen</i>	
1	Introduction	125
2	What is Virulence?	127
3	Rational Drug Design	128
4	Irrational [<i>sic</i>] Drug Design	129
5	The Sialic Acids	129
5.1	Monosaccharides	130
5.2	Structural Partners, Synthesis, and Catabolism	131
5.3	Supermolecular Structures	134
6	Functions of Sialic Acid Uptake During the Host–Microbe Interaction	135
7	Sialic Acid Uptake	136
7.1	The <i>E. coli</i> Paradigm	138
7.2	The Carboxylic Acid or TRAP Paradigm	140
7.3	ABC Systems	141
8	Polysialic Acid Acetylation	142
8.1	Contingency Loci	143
8.2	<i>E. coli</i> K1 Acetylation	144
8.3	Hypervariation of <i>neuO</i>	145
8.4	Function of <i>neuO</i>	146
9	Conclusion	147
	Acknowledgment	147
	References	147

Chapter 9 Synthetic Carbohydrate-Based Antimalarial Vaccines and Glycobiology	151
<i>Alexandra Hölemann and Peter H. Seeberger</i>	
1 Introduction	151
2 Immunization	152
3 Carbohydrate-Based Conjugate Vaccines	153
4 Development of a Synthetic Carbohydrate-Based Vaccine Against Malaria	155
4.1 Introduction	155
4.1.1 Disease Burden	155
4.1.2 Malaria Infection	155
4.1.3 Drug Treatment of Malaria	156
4.2 Anti-Malarial Carbohydrate-Based Vaccines	157
4.2.1 Identification of the Toxin	158
4.2.2 Synthesis and Testing of Carbohydrate-Based Vaccine Constructs	159
5 Development of a Carbohydrate-Based Vaccine Against Leishmaniasis	166
5.1 Disease Burden	166
5.2 Carbohydrate-Based Vaccine Constructs Against Leishmaniasis	166
6 Conclusions and Outlook	170
Acknowledgments	171
References	171
Chapter 10 Studies Toward a Rationally Designed Conjugate Vaccine for Cholera Using Synthetic Carbohydrate Antigens	175
<i>Pavol Kováč</i>	
1 Introduction	175
2 Synthesis of Carbohydrate Antigens in the Form of Methyl α -Glycosides	179
2.1 Synthesis of Methyl α -D-Perosaminide and its 2-O-Methyl Analog	180
2.1.1 The Kenne Approach	181
2.1.2 The Bundle and the Ganem Approaches	181
2.1.3 Our Approach	181

2.2	Synthesis of Acylating Reagents	
	Derived from 3-Deoxy-L-glycero-	
	Tetronic Acid	183
2.2.1	The Kenne Approach	183
2.2.2	The Verez-Bencomo Approach	184
2.2.3	Our Approach	185
2.3	Synthesis of Methyl α -Glycosides of	
	the Terminal, Upstream Monosaccharide	
	Determinants of <i>Vibrio cholerae</i> O1,	
	Serotypes Inaba and Ogawa	187
2.4	Synthesis of the Terminal, Upstream	
	Oligosaccharide Determinants of <i>Vibrio</i>	
	<i>cholerae</i> O1, Serotypes Inaba and Ogawa	190
3	The Mode of Binding of <i>Vibrio cholerae</i> O1	
	Antigens to Anti-LPS-Specific Antibodies	195
4	Conjugation Chemistry and Optimization of	
	Reaction Conditions	199
4.1	Synthesis of Mimics of the Upstream	
	Terminus of the O-SP of <i>Vibrio</i>	
	<i>cholerae</i> O1 in the Form Suitable for	
	Conjugation	199
4.2	Conjugation by Reductive Amination	207
4.3	Conjugation by Squaric Acid Chemistry	208
5	Determination of the Carbohydrate-Protein	
	Ratio in the Neoglycoconjugates	210
6	Development of Protocols for Efficient and	
	Controlled Preparation of a Series of	
	Neoglycoconjugates with Predetermined	
	Carbohydrate-Protein Ratio	211
7	Serological Evaluation of Responses in	
	Mice Following Immunization with	
	Synthetic Immunogens	214
8	Concluding Remarks	215
	Abbreviations	215
	References	216

Chapter 11 Carbohydrate Microarrays for High-Throughput Analysis of Carbohydrate-Protein Interactions 221

Injae Shin

1	Introduction	221
2	Strategy for Immobilization of Carbohydrate	
	Probes on the Solid Surface	222

3	Synthesis of Maleimide-Linked Carbohydrates	223
4	Fabrication of Carbohydrate Microarrays	226
5	Applications	228
5.1	High-Throughput Analysis of Carbohydrate-Protein Interactions	228
5.2	Quantitative Analysis of Protein-Binding Affinities	233
5.3	Characterization of Carbohydrate-Processing Enzymes	234
6	Other Examples of Carbohydrate Chips and Their Applications	235
6.1	Other Immobilization Methods	235
6.2	Other Applications	240
7	Detection Methods	242
8	Conclusion	242
	References	243

Subject Index

247

1 Introduction

Carbohydrate-protein interactions are the basis of numerous biological processes, both normal and pathological ones. They include the biosynthetic synthesis and degradation of oligo- and polysaccharides, intracellular sorting of glycoproteins, transport of carbohydrates into living cells and of their derivatives into extracellular spaces, the immunological response to carbohydrate antigens, and responses of leukocytes to sites of inflammation. These interactions also play a key role in a variety of cell adhesion phenomena, among them the attachment of parasites, fungi, bacteria, and viruses to host cells, the first step in the initiation of infection.¹⁻³ The high selectivity required for this attachment, as well as the binding of a variety of cellular and matrix to cells, is provided by a specific carbohydrate- α 1-6 hexosamine (amplimannose) molecule, one a carrier of biological information (such as complex carbohydrates) and the other capable of decoding such information (such as lectin-binding proteins, belonging to the class of lectins).⁴ This concept has its origin in the lock-and-key hypothesis formulated by Emil Fischer at the end of the 19th century to explain the specificity of interactions between enzymes and their substrates, i.e., between molecules in solution. It was subsequently extended to describe the interaction of cells with soluble molecules and with other cells.

Complex carbohydrates are commonly found at the cell surface, where they are positioned to interact with soluble proteinaceous molecules, primarily lectins, in solution or on the surface of other cells. These proteins, originally identified as sugar-specific hemagglutinins, are currently known as cell-recognition molecules.⁵ They are geared to distinguish between different oligosaccharides, whether in solu-