Carbohydrate-Aromatic Interactions

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Biographies

Juan Luis Asensio (Barcelona, Spain) received his PhD degree (Chemistry) in 1995. After a postdoctoral period at the National Institute for Medical Research at Mill Hill, he moved back to the IQOG-CSIC in 1998, becoming a tenured scientist in 2000. He was promoted to senior research scientist in 2008, and has focused his research on molecular recognition studies of sugars, nucleic acids and proteins.

Ana Ardá (Coruña, Spain) received her PhD degree (Chemistry) in 2006. After a postdoctoral period at the Bijvoet centre in Utrecht working with NMR, in 2008, she moved to Jiménez-Barbero's group at CIB-CSIC, as Juan de la Cierva scientist, focusing her research on NMR and molecular recognition.

Francisco Javier Cañada (Bilbao, Spain) received his PhD degree (Chemistry) in 1985. He spent a postdoctoral period with Prof Vazquez at the Molecular Biology Centre in Madrid. Since then, his research interest has been at the interface between Chemistry and Biology. In 1988, he moved to Prof. Rando's group at Harvard Medical School. In 1991, he came back to Madrid (IQOG-CSIC) to work with Prof. Martín-Lomas. In 1992 he got a tenured scientist position, focusing his research on molecular recognition processes between carbohydrates and proteins. In 2002, he moved to CIB-CSIC (Madrid), to build up the NMR group in the Chemical & Physical Biology Department. In 2009 he was promoted to Full Professor.

Jesús Jiménez-Barbero (Madrid), got his PhD (Chemistry) in 1987. He did postdoctoral studies at Zürich, at the National Institute for Medical Research at Mill Hill, and at Carnegie Mellon University (1988-1992). His main topic of work focuses on molecular recognition, especially on protein-ligand interactions, with particular emphasis on the application of NMR methods. He was promoted to CSIC Research Professor in 2002 and moved to CIB-CSIC (Madrid), where he is heading the Chemical & Physical Biology Department. Since 2012, he is also the President of the Royal Society of Chemistry of Spain.

CONSPECTUS

Understanding how proteins recognize saccharides represents a fundamental issue in science with far reaching implications in biology, technology or drug design. In the past two decades, considerable efforts have been directed toward this particular objective. Not surprisingly, early crystallographic studies revealed that hydrogen-bonding interactions are usually involved in carbohydrate recognition. Somewhat less expected was the observation that, despite the high hydrophilic character of most sugars, aromatic rings of the receptor often play an important role in this process.

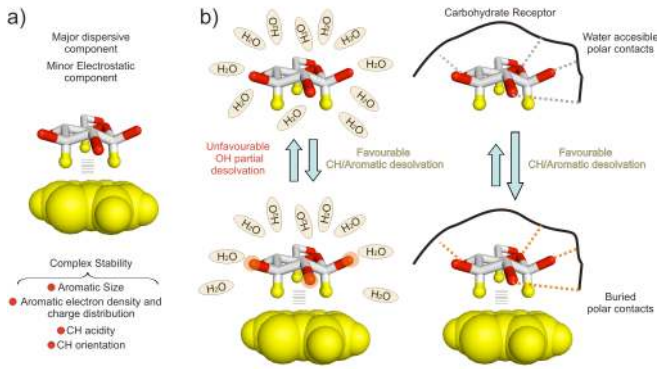
Nevertheless, it is now widely accepted that non-covalent interactions mediated by aromatic rings are pivotal to sugar binding. Indeed, the stacking of aromatic residues against the faces of sugar pyranose rings constitutes a recurring feature of protein-carbohydrate complexes. Such contacts typically involve 2-3 CH groups of the pyranoses and the π electron density of the aromatic ring (the so-called CH/π bonds), and can exhibit a variety of geometries, with either parallel or non-parallel arrangements of the aromatic and sugar units.

The interaction energy between different aromatic rings and simple monosaccharides, in gas phase, has been shown to be in the 3-6 kcal/mol range by quantum mechanics calculations, implying that the stabilization produced by each CH/π bond amounts to 1-2 kcal/mol. These values are somehow larger than those experimentally measured in water, estimated in ca. 1.5 kcal/mol per each carbohydrate-aromatic stacking. The observed variation illustrates the context-dependent character of intermolecular interactions and shows that this stacking is, to some extent, modulated by entropic and solvent effects. Despite their relatively modest influence on the stability of carbohydrate/protein complexes, it is well established that the aromatic platforms play a major role in determining the specificity of the molecular recognition process.

The analysis of carbohydrate/aromatic interactions has become an active field of research. Significant efforts have been devoted to the quantification of carbohydrate/aromatic stacking and to the identification of the different

contributions that stabilize these complexes. Both objectives have been approached employing a variety of experimental and theoretical strategies that, overall, can be grouped in three main approaches. First, the structural and thermodynamic features of carbohydrate recognition by protein receptors have been quantitatively analyzed. This work frequently included the used of site-directed mutagenesis and/or organic synthesis in order to incorporate modifications in the receptor and/or ligand. Second, sugar/aromatic complexes have been analyzed employing a reductionist chemistry-based approach based on the synthesis and characterization of artificial receptors and simple model systems. Finally, the magnitude of the different contributions to the interaction energy has been addressed employing quantum mechanics calculations.

Herein we provide an overview of the current scientific knowledge on this topic. In the first section, we will describe experimental evidences for the relevance of carbohydrate/aromatic interactions, together with experimental approaches employed to dissect their structural and thermodynamic features. Second a summary of the main chemistry-based efforts oriented to analyze stacking complexes will be presented. In third place, we will focus on the theoretical aspects of the stacking. Finally the impact of this fundamental knowledge on our understanding of carbohydrate recognition processes will be briefly outlined.



a) Carbohydrate-aromatic interactions have a major dispersive component and the complex stability depends on several factors. The orientation of the hydroxyl groups of the sugar has a tremendous impact in the interaction. b) The solvation/desolvation process plays a major role in complex formation. The architecture and chemical nature of the binding site is also very important.

Introduction

The essential processes of life largely occur by specific interactions between biomolecules. Among them, carbohydrates are fundamental for cell-cell communications.[1] Carbohydrate-protein interactions are central to a variety of fundamental biological phenomena, including protein trafficking, cell adhesion, fertilization, infection, tumour metastasis, and different aspects of the immune response.[2]

Not surprisingly, early crystallographic studies revealed that, hydrogen-bonding interactions are usually involved in carbohydrate recognition.[3] Somewhat less expected was the observation that, despite the high hydrophilic nature of most sugars, aromatic rings of the receptor often play an important role in this process. The presence of aromatic amino acids in the carbohydrate binding sites of proteins was already observed in lysozyme-chitooligosaccharide complexes,[4] the first enzyme whose 3D structure was determined by X-ray crystallography. The importance of tryptophan residues for carbohydrate binding was further highlighted by NMR strategies available at those times.[5,6] Later in 1986, Quiocho, after connecting the L-arabinose and D-galactose binding bacterial chemotactic proteins to other reported carbohydrate-protein complexes, proposed carbohydrate-aromatic stacking as a common feature for carbohydrate recognition.[6]

Indeed, data-mining tools[7-8] have highlighted the extraordinary high frequency of aromatic amino acids, especially tryptophan, in the carbohydrate binding sites of proteins.

Interestingly, recent studies have revealed that carbohydrate/aromatic interactions are not restricted to protein complexes, but also present in carbohydrate-binding RNAs,[9] highlighting the relevance of the aromatic rings as key elements for carbohydrate recognition. Combined efforts of structural biologists, as well as biological and theoretical chemists have provided insights into the different contributions that stabilize carbohydrate/aromatic complexes.[10]

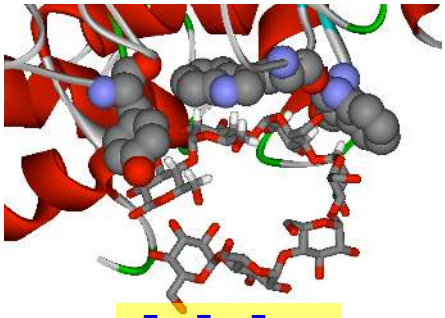
Experimental evidences. The different architectures

Carbohydrate-aromatic stacking has been observed in most carbohydrate-protein complexes, with either enzymes or receptors, for a large variety of protein folds and functions. As examples, it is possible to mention many lectins, including hevein domains,[11] plant toxins or animal galectins.[12] This structural feature is very frequent among those carbohydrate-binding modules (CBM) associated to glycosidases for polysaccharide metabolism.[13] Since there are significantly fewer glycosyl tranferase structures experimentally available, not many examples have been still reported.[8,14] Carbohydrate-aromatic stacking is also frequently found in sugar-sensor/transport proteins, as the chemotactic receptors,[6] and others.[15] In antibodies, the aromatic rings either interact with their own glycans in an intramolecular fashion[16] or do it, intermolecularly, with their polysaccharide antigens.[17]

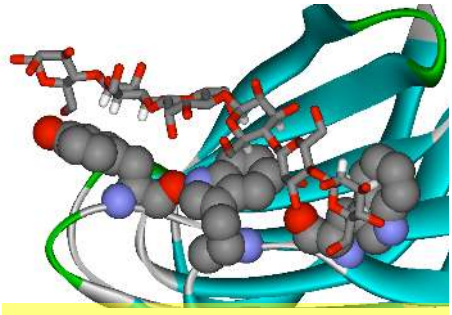
The geometrical features of the interaction are not strictly unique. From the point of view of the protein, different architectures of the binding sites can be delineated (*Figure 1*), depending on the number and relative location of aromatic residues. In many cases, as galectins,[11-12] there is only one aromatic ring providing stacking with the sugar, defining one monosaccharide binding sub-site. In other examples, spatially contiguous aromatic rings are grouped, forming an extended binding site with two (or more) aromatics, which define sequential subsites (n, n+1, etc). Indeed, they are pre-organized to stack with consecutive monomers in oligosaccharides, as in hevein domains.[18] There are also extended binding sites with even 6 sub-sites with aromatic residues located at every other sub-site (n, n+2, n+4, etc). This presentation is observed in polysaccharide degrading enzymes and their associated CBMs.[19] Nevertheless, this organization may adopt different shapes, forming extended surfaces, grooves, or even tunnel-like motifs. Evidences of the importance of the presence of aromatic residues at the entrance of an active site tunnel to provide glycosidase activity have been presented by AFM techniques with native and mutant processive enzymes lacking one specific Trp residue.[20] Two aromatic residues may provide a double aromatic stacking over a monosaccharide, forming a sandwich-type arrangement, which can even give a

more complex architecture, as in *Urtica Dioica* lectin, in which two protein chains wrap around one oligosaccharide chain.[21] *(Figure 1)*

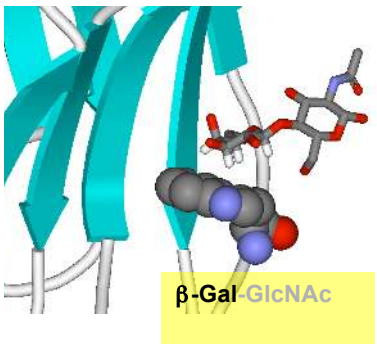
***Figure 1a**.- Selected examples of carbohydrate binding sites, showing the presence of the four aromatic amino acids in homologous hevein domains when complexed with the chitin dimer.*



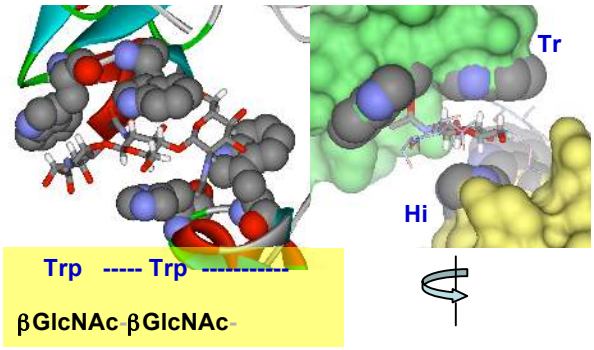
Sequential aromatics. PDB code 3CK9



Alternate aromatics. PDB code 1GWL



Single aromatic. PDB code:1KJL



Sequential aromatics. "Sandwich-like". PDB code: 1EHH

***Figure 1b**.- Examples of the diversity of carbohydrate binding-site topologies and of the structures of carbohydrate ligands. The key hydrogens facing the aromatic rings are shown. In the case of the Urtica dioica agglutinin dimer (bottom right), the chitotriose entity is sandwiched between two different binding sites, each belonging to a different protein monomer.*

The available structural information, with more than 90 non-redundant CBD 3D-structures showing carbohydrate-aromatic stacking, has allowed improving protein-modeling strategies by introducing a "hydrophilic aromatic residue"

parameter as restriction for structural modeling. This approach has been successfully employed to unravel cases where sequence homology was low.[22]

From the carbohydrate perspective, the stacking can take place in different manners. In principle, a pyranose presents two well-defined (α and β) faces (Figure 2), which could interact with the aromatic moieties. Experimental and theoretical evidences have shown that the interaction is favored for that face presenting several axially oriented C-H bonds, and largely disfavored for those faces decorated with axial hydroxyl groups:[23] The interaction is strictly dependent on the sugar configuration. Pyranose-aromatic ring stacking has been documented for galacto- (or fuco)-type configurations, either for α or β anomers, but exclusively through its α-face (Figure 2c). This is also the case for β-mannoses, with exclusive stacking through the α-face (Figure 2b), and no stacking for the α-analogues. For gluco-type sugars, including xylose- or GlcNAc-containing oligosaccharides, the stacking can take place from both faces for β-glycosides (Figure 2d), even simultaneously, while for α-anomers, the aromatic moiety only sits on top of the β-face (Figure 2a).[23-24]

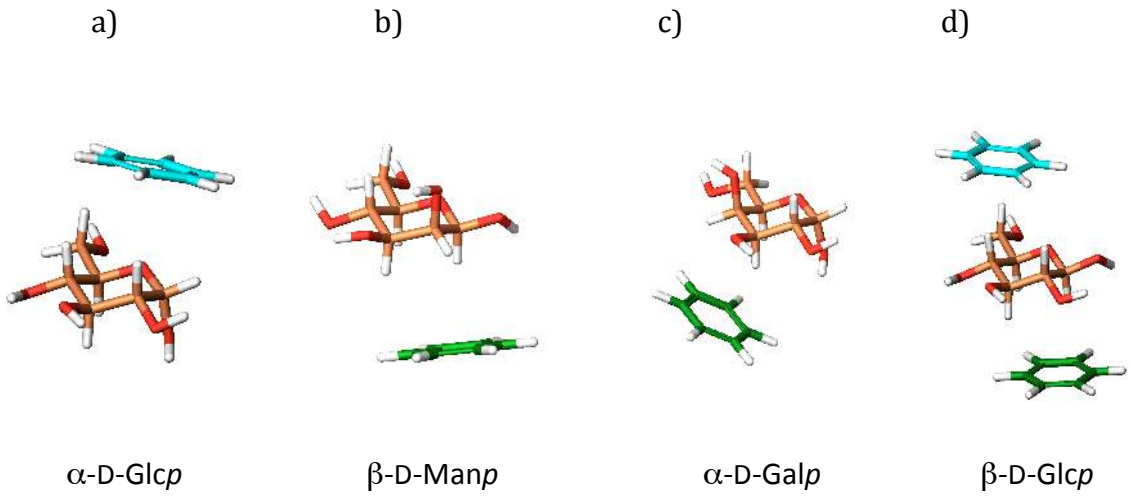


Figure 2.- E*xamples of the different topologies of carbohydrate/aromatic stacking from the sugar point of view. Glcp stands for glucopyranose, Manp for mannopyranose, and Galp for galactopyranose. The α- and β- refer to the anomeric configuration. In the corresponding text, α- and β-faces refer to the spatial location where the corresponding α- and β-substituent is placed.*

However, additional geometrical orientations should also be considered. Sometimes, stacking interactions are not expressed through the exact parallel orientation of the pyranose chair with the aromatic ring. For galacto-type configurations, the sugar chair slides over the aromatic moiety and presents H3, H4, H5 to the amino acid side chain (Figure 2c).[23-25] Indeed, a geometry analysis performed over an extended set of experimental sugar-protein complexes showed that the position of the center of the pyranose ring can take a large set of spatial orientations relative to the aromatic residue.[25]

There are very few reported cases for furanosides, although stacking interactions have been observed when the five-membered ring adopts the proper geometry for the favorable orientation of its CH bonds, as in the complex of an antibody with arabino-containing polysaccharides.[26] In any case, the thermodynamics of furanose-aromatic binding motif deserves further studies.

Aromatic stacking has been scarcely observed for protein complexes with charged saccharides. Indeed, for negatively charged sugars, as heparin glycosaminoglycans, the binding site is composed of complementary cationic amino acids, which establish electrostatic interactions and do not facilitate neighboring of aromatic chains.[27] For positively charged carbohydrates, there is not still enough structural data available to generalize these interactions. Nevertheless, it has been shown in model systems that the interaction with the protein is very dependent on the protonation state of the interacting amino sugars.[28]

Affinity and Selectivity

From the protein perspective, the affinity and selectivity of the interaction depends on the nature of the aromatic residue. Using mutagenesis-based experiments, it has been shown that elimination of aromatic moieties drastically reduces the affinity,[29] while the exchange among aromatics permits the modulation of the receptor properties.

Theoretical calculations have highlighted the importance of stacking interactions, also in the context of enzymatic polysaccharide hydrolysis. It has been hypothesized that the efficiency of processive glycosidases is directly

related to the existence of strategically positioned aromatic residues, since their removal in enzyme tunnels reduced the ligand binding free energy, and switched the enzyme function from processive to nonprocessive.[19]

In a parallel manner, the study of different GH10[8] xylanases with five conserved aromatic residues allowed estimating a favorable 0.5-1 kcal/mol contribution to the ΔG of binding for each subsite, by ITC.[30] However, the geometrical positioning of the residues did not allow the simultaneous establishment of all possible carbohydrate-aromatic stacking interactions. The analysis of the thermodynamic parameters permitted guessing the potential of stacking interactions at the different subsites, relating the aromatic-carbohydrate contact surface area at each subsite to the corresponding changes in ΔCp. In general, the exact contribution of stacking interactions in glycosidases can not be generalized. The aromatic residues conform a binding platform where stacking, solvation-desolvation of the exposed surfaces, conformational perturbations, and other interactions are differently balanced.

Residue exchange among the different aromatic amino acids has indicated that the affinity increases with the size of the aromatic ring. Nature has provided evidences for that: the four aromatic amino acids participate in carbohydrate-aromatic stacking in any of the four hevein domains of WGA.[31] Using X-ray, fluorescence, NMR, and ITC experiments,[11] systematic studies of the importance of the type of the aromatic ring has been performed.[32-33] For the hevein fold, chemically synthesized mutants of the antimicrobial AcAMP peptide, with either Phe, Trp, or unnatural naphtylalanine and 4-fluorophenylalanine amino acids have been studied. The thermodynamic binding parameters were interpreted with the NMR-based 3D structures of the complexes. It was shown that increasing the size of the aromatic ring strongly favored binding, while electron-withdrawal by fluorine significantly reduced the affinity.[33]

The knowledge of the key forces involved in sugar recognition has been also employed for protein engineering. For instance, specifically-designed mutagenesis experiments have been elegantly employed for achieving galactose recognition, starting from a mannose-binding protein[34].

The substrate point of view: Selectivity and Specificity

So far, few studies have quantitatively analyzed the influence that modifications in the sugar length and/or configuration have on the stability of the carbohydrate/aromatic complexes. Using hevein domains,[11] a systematic analysis of the structural and energy features of the interaction of N-acetylglucosamine (GlcNAc) moieties with lectins has been presented (Fig. 3).

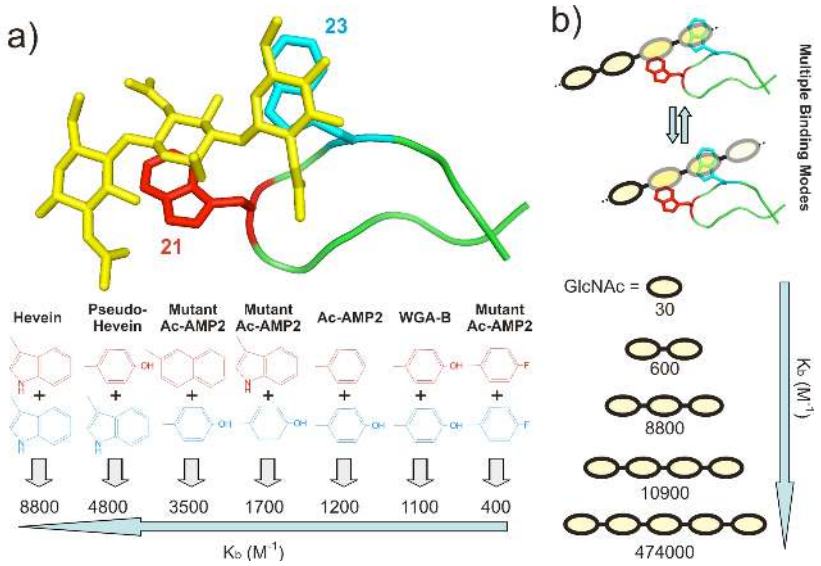


Figure 3.- Hevein/chitooligosaccharide interactions. a) Top: hevein domains display a double stacking of two aromatic residues with two contiguous GlcNAc moieties of the chitin chain. Hevein presents two Trp residues in the binding site. Bottom: changes in binding affinity depending on the chemical nature of the two aromatic residues.[11,18,35-40] b) Top: the interaction of trisaccharide or larger GlcNAc oligosaccharides is dynamic, with exchange among distinct interaction modes. Bottom: the binding affinity increases with the oligosaccharide length, reflecting the importance of the existence of multiple binding modes along with the presence of multivalency. Above the pentasaccharide, two or more hevein domains interact with the same oligosaccharide chain.[35-40]

The protocol implied the use of differently substituted mono- to hexa-saccharides, with different sugar stereochemistry, using NMR methods assisted by molecular modelling, ITC, and fluorescence techniques.[18,35-39] The obtained results were compared to those already published by other authors by employing X-ray crystallography and other procedures.[11] Combining the 3D structural perspective with affinity measurements, the enthalpy change associated to a single carbohydrate-aromatic interaction was estimated

between 1.5-2 kcal/mol. All the observed variations in affinities were explained in structural terms, and key features of the molecular recognition process, including dynamic aspects were unravelled. Thus, restriction to the motion of aromatic rings when passing from the free to bound states was detected, as well as the existence of complexes of different topology in chemical exchange.[35-39] For long oligosaccharides, the existence of multivalent processes in which a single oligosaccharide chain was bound to two protein domains was deduced.[35,40] The employment of sugar ligands with different stereochemistry at specific positions also permitted to deduce that the stacking interactions were extremely sensitive to the glycoside shape. The receptor aromatic rings were major contributors to the selectivity and specificity of the molecular recognition process, and disallowed binding of particular sugar epimers, through steric hindrance and/or by creating unfavourable non-polar environments for axial OH groups.[18,39]

From Nature to the bench: carbohydrate-aromatic interactions in simple models, chemical systems, and artificial lectins

As frequently employed in chemistry-based approaches, reductionism has been used to study carbohydrate-aromatic stacking interactions. Using simple models, composed by just one monosaccharide and one aromatic ring, this interaction has been characterized using different methodologies.[41-44] The recognition process strongly depends on the nature of the sugar, and three CH groups must be on top of an aromatic ring to be an NMR-detectable interaction.[23] Calorimetric studies[45] established its enthalpic nature, while IR[46] has been essential to confirm their major dispersive character, and also to detect OH-π hydrogen bonding in the absence of water.[46] However, the existence of certain hydrophobic component was deduced, since the interaction was not detected by NMR in other polar solvents, such as acetonitrile.[23,24] Interestingly, although the stacking interaction has been demonstrated in the gas phase by IR,[46] using more complex glycopeptide models, this weak interaction in the gas phase is not able to compete with classical hydrogen bonds, even intramolecularly.[47]

Stacking between aromatics and sugars have been observed and used in complex structures, further employed as platforms to design artificial systems,[48-51] to control the conformational behavior of glycomimetics[52-53], or the formation of hydrogel-like supramolecular structures.[54]

The strength of a single carbohydrate-aromatic interaction has been studied in the context of the formation of β-hairpin in aqueous solution, employing model glycopeptides with diverse sugars and aromatics. It was shown that, in the absence of other noncovalent contacts, a single sugar-aromatic interaction may modulate protein folding with a magnitude of ca. −0.8 kcal/mol. Fittingly, replacement of the aromatic ring with an aliphatic group resulted in a decrease in the energy to −0.1 kcal/mol.[55-56] The importance of solvation/desolvation of the interacting groups was also highlighted.[55-56]

The growing knowledge on these interactions has been applied to design artificial carbohydrate receptors,[47,57] which employ a wise combination of hydrogen bonds and stacking interactions (Figure 4), as elegantly illustrated.[57-59] The use of differently substituted synthetic receptors has also highlighted the importance of hydration for effective sugar binding and proper stacking.[60]

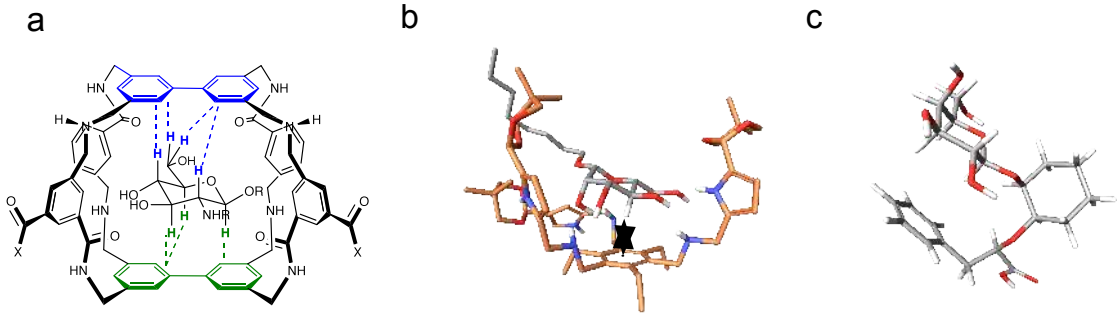


Figure 4.- Different designs of artificial sugar receptors a) The design by Davis[57,58] are able to effectively recognize sugar molecules in water solution by hydrogen bonds and stacking interactions. b) The open receptors developed by Roelens[59] recognize Man moieties in organic polar solvents. Again, stacking interactions and hydrogen bonds provide the driving force for recognition. c) A glycomimetic of GM1 adopts the proper geometry to interact with cholera toxin thanks to intramolecular carbohydrate/aromatic stacking.[52,53]

Theoretical evidences

From a theoretical perspective, initially it could be considered as analogous to a H-bond, although different in its physical origin.[61] It is well known that, for interactions between aromatic surfaces with alkanes, experimental data in the gas phase compared well with those obtained from high-level *ab initio* calculations (CCSD(T)).[61-62] Following this reasoning, a first conclusion, derived from calculations on sugar-aromatic models is that the dispersive component is dominant while the electrostatic contribution is minor.[61-62] Indeed, theoretical[23] and experimental studies[63] have confirmed the presence of electronic density between the sugar hydrogen atoms and the aromatic ring.[23] Also, the dominance of the dispersive contribution implies that the orientation dependence of the carbohydrate-aromatic interactions is weak, conferring to these complexes a dynamic character.

A systematic scan of the potential energy surface of carbohydrate-aromatic complexes, carried out using simple models,[64] showed that the dispersion interactions are highly distance-dependent, and not equally distributed around each carbohydrate atom. The energy for the dispersion interaction was beyond -5.0 kcal/mol, but only in small localized areas, for optimum interatomic distances. In principle, this number could suggest that the energies experimentally found correspond to dynamic systems in solution that could rise up to the theoretical value for rigid complexes, although other factors, as solvation effects, should be contemplated (see below).

The cooperativity between multiple CH/π bonds has been investigated theoretically.[65] The structural information available showed that in most cases, 2-3 CH groups of the pyranose unit participate in CH/π contacts with the same aromatic system. When the additivity of these interactions was explored,[65] the calculations showed that bidentate complexes are weaker than the sum of two monodentate ones, this difference being larger for interactions with naphthalene than with benzene.

Consequences for molecular recognition

The interaction energy theoretically estimated for each sugar-aromatic stacking amounts to 3-6 kcal/mol.[23,64] These values are larger than the experimental ones in water: the contribution of every sugar-aromatic stacking in

hevein complexes has been estimated in ca. 1.5-2.0 kcal/mol.[11] Also, the interaction energy between a single sugar and an aromatic amounted to 0.8 kcal/mol, as revealed by studies on glycopeptides.[55-56] The stacking of glycosides with DNA base-pairs contributes less than 0.5 kcal/mol to duplex stability.[66] These examples illustrate the context-dependent character of carbohydrate/aromatic stacking, modulated by entropic and solvent factors.

A soft nature and a low directionality seem to be essential features of the sugar/aromatic interaction. According to this view, the main role of the aromatic platforms in protein or nucleic acid receptors would be to provide a plastic contribution to the association energy that can be modulated by the local environment of the receptors to achieve both affinity and selectivity.

Theoretical analyses of stacking complexes have shown that dispersive forces play a dominant role. As a consequence, the interaction critically depends on the size and shape complementarity of the interacting surfaces. Regarding the electrostatic component, while relatively minor, it offers interesting opportunities to modulate the attractive forces between pyranoses and aromatic rings. In principle, the stability of the carbohydrate/aromatic complexes could be enhanced by incorporation of electron donating substituents on the aromatic ring. Alternatively, the polar character of the interacting CH groups could be increased by substitutions on the pyranose. The potential of these simple strategies are yet to be explored. In addition, current research support the notion that water plays an essential role in carbohydrate recognition.[25,58,59,63,67]

It should be noted that, despite their overall polar nature, saccharides include hydrophobic patches whose particular topology depends on the axial/equatorial orientation of the OH moieties. These acknowledgements led to the proposal that solvophobic effects represent a key stabilizing influence for the interaction. Studies on simple models have shown that replacement of the aromatic by simple aliphatic chains renders the interaction almost undetectable, suggesting that the hydrophobic component might be lower than originally suspected, even with no key role in the carbohydrate/aromatic interaction.[10] In our opinion, the safest assumption is that desolvation of the aromatic system and the pyranose CH groups constitute a relevant driving force for complex

formation. However, the precise contribution of this hydrophobic component to the net interaction energy remains an open question.

Alternatively, it has also been shown that solvent-dependent contributions to the interaction energy could also contribute to destabilize the stacked complexes.[55,56,60] Thus, depending on the topology of the complex, desolvation of the pyranose CH groups might be accompanied by partial desolvation of the polar substituents. This unfavourable effect seems to be especially relevant in charged glycosides and would also oppose molecular recognition of neutral ligands in a configuration-dependent manner. In agreement with this view, chemical modifications of the sugar, like *O*- or N-methylation, lead to an enhancement of the stacking.[29]

Despite this energy cost, proteins manage to recognize carbohydrates with moderate affinities and exquisite specificities. However, ligand desolvation does not rely exclusively on carbohydrate/aromatic contacts. In most cases, the hydroxyl moieties of the bound oligosaccharides are not free, but involved in extensive intermolecular hydrogen bonding. This observation suggests that Nature uses of cooperativity to achieve affinity, with aromatics and hydrogen-bonding groups synergistically operating to desolvate and bind carbohydrates. By promoting desolvation of the hydroxyl groups, the receptor polar groups would favour the stacking of the pyranoses with aromatics. In turn, the hydrophobic environment provided by these aromatics might cooperatively enhance the strength of the receptor/ligand polar interactions.[55,56] These conclusions have important implications for the design of artificial carbohydrate receptors.

Future will bring further studies and applications of carbohydrate/aromatic stacking in different fields. Understanding the functional role of aromatic residues in glycosidases may be used for rational design of novel carbohydrate-active enzymes.[19,20] In particular cases, optimization of carbohydrate/aromatic contacts might lead to improved protein binders or enzyme inhibitors. This task would greatly benefit from a quantitative understanding of how dispersion, electrostatics and solvent-dependence contribute to the interaction energy. Additionally, stacking can be employed as a portable structural module. This concept has permitted to stabilize native states of glycoproteins up to –2.0

kcal/mol, thanks to the stacking provided by placing a phenylalanine residue two or three positions before a glycosylated asparagine in distinct reverse turns.[68] Additionally, technological applications of these interactions, including the solubilization of carbon nanotubes[69,70] could also be envisaged. We are looking forward to future developments in this field.

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