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CONSIDERATION OF THE NATURE OF THE LECTIN-CARBOHYDRATE INTERACTION

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SUMMARY

Intermolecular forces in lectin-carbohydrate interaction are analyzed on the basis of their structure and chemical features. The role of water, as well as that of other physico-chemical parameters influencing the formation of such complexes in aqueous media, is also discussed in detail. A model depicting the importance of hydrogen-bonding and charge-transfer interactions as the main sources of complex stability in the association between lectins and carbohydrates is proposed.

INTRODUCTION

My first meeting with Professor Porath took place in Mexico City in 1974 when, invited by our National Academy of Sciences, he came to lecture to us on the principles of affinity chromatography. It was by then that in Dr. B. Arreguin's group we had attempted to purify seed glycosidases using electrostatically immobilized oligosaccharides on DEAE-Sephadex[®]¹. Simultaneously, in Dr. F. Cordoba's group, still another type of sugar-binding proteins, namely lectins, was being intensively studied. There is no doubt now about the nature of the topic that aroused Professor Porath's greatest interest. All of a sudden, I found myself studying lectins in Uppsala, instead of glycolytic enzymes at Caltech. Nevertheless, the experience gained at a leading institution on protein fractionation techniques has been extremely rewarding ever since.

In Uppsala I enjoyed the opportunity of meeting a number of personalities and experts, which in recent years has helped me to increase greatly my comprehension of the various physico-chemical parameters that influence the separation of biomolecules and to understand the fundamental principles of biomolecular interactions. Therefore, I considered it appropriate that in the celebration of Professor Porath's 60th anniversary I should present some of the ideas that originated from the discussions with Professor Porath and his group in the form of a review, oriented towards a research field in which I am at present involved, *viz.*, lectin-carbohydrate interactions.

THE BIG QUESTION AND THE BIG PROBLEM

A number of attempts have been made to determine how lectins and oligosaccharides form complexes. Several models for lectin-induced cell agglutination have been proposed but no generalization is considered valid, as lectins differ not only in specificity, but also in size, shape, subunit structure, chemical composition, source and possible functions²⁻⁵. Thus, if we wish to understand the mechanism by which such lectins may interact with sugars, we can only refer to those cases in which full characterization of the lectin has been achieved. I shall therefore describe first the characteristics of the known lectin sugar-binding sites and the structural features of the carbohydrates so far employed for this kind of study. Subsequently I shall refer to the chemical nature of the hydrogen bonds and of the "hydrophobic interactions" that have been regarded as the main sources of stabilization in lectin-sugar complexes.

THE SUGAR-BINDING SITE OF LECTINS

First, it should be mentioned that all of the sugar-binding sites of a particular lectin (they are multivalent) are considered homogeneous, that is, all of them appear identical in chemical composition and hence in specificity. Variations in subunit structures are related to differences that do not seriously affect the mode of binding of the sugar to the lectin. As lectins from different sources showing similar specificities are not identical, the above hypothesis lacks experimental support and it seems likely that all the affinity constant values of lectin-carbohydrate complexes thus estimated reflect the average values of the affinity between the various sugar-binding sites of the lectin and the corresponding carbohydrate inhibitor. Moreover, the features of the sugar-binding sites of two lectins from different sources with specificity for a common sugar are hardly the same. As a consequence, we must disregard the "lock" and "key" classical model proposed for enzyme-substrate interactions and search for alternative models.

The aminoacyl residues involved in the sugar-binding site of lectins have been identified in part by introducing specific chemical modifications on the protein components. Acetylation of the free amino groups and available phenolic groups in concanavalin A, for example, has little effect on its interaction with a variety of substrates⁶. However, with wheat germ agglutinin, the tyrosine residues cannot be acetylated without a marked decrease in agglutinating ability⁷. The lectin from the seeds of the Lentil *Lens esculenta* Moench., on the other hand, is drastically affected with respect to its haemagglutinating properties if acetylation of all free amino groups is carried out, thus indicating in this instance the participation of such groups in sugar binding⁸.

Involvement of tryptophanyl residues in the sugar-binding mechanism of wheat germ agglutinin, in both the native and succinylated states, has been inferred from the shifts observed in their fluorescence spectra before and after saccharide binding occurs⁹⁻¹¹. Carboxyl groups have been also found to participate actively in the sugar-binding process in several instances and their modification reduces significantly the affinity of the lectin molecule for its corresponding substrate¹².

A partial picture of the sugar-binding site in concanavalin A has been obtained

from X-ray diffraction studies^{13,14}. At low resolution, the surface of concanavalin A appears relatively smooth and uninterrupted, except for one large depression, or cavity, extending deep in each promoter. These cavities contain two subsites with different characteristics. The first is large, predominantly hydrophobic and is surrounded by the side chains of Tyr 54, Leu 81, Leu 85, Val 89, Val 91, Phe 111, Ser 113, Val 179, Ile 181, Phe 191, Phe 212 and Ile 214. The second subsite is a more constricted region between the first subsite and the molecule surface and contains predominantly hydrophilic groups, such as the side-chains of Tyr 54, Ser 56, Asn 86, Ser 113 and Ser 189, as well as the main-chain oxygen atoms associated with Lys 114 and Ile 181. Competition studies have led to the conclusion that the specific saccharide-binding site must be outside the cavity, near the intermolecular contacts that stabilize the crystal lattice, as crystal cracking occurs when moderate concentrations of the sugar inhibitor are added to the crystal¹⁴. The cavity, however, is the site of binding of many iodophenylglycosides, probably through hydrophobic interactions.

The carbohydrate binding site structure has been inferred on the basis of experiments on crystals of native concanavalin A cross-linked with glutaraldehyde so that the subsequent binding of sugars would not destroy the crystal lattice. Further studies on the crystalized concanavalin A- α -methyl mannopyranoside complex led to the conclusion that Asp 16, Asp 208, Tyr 12 and Tyr 100 are responsible for the sugar-binding capacity of the lectin. The position of the carboxyl side-chain of Asp-208 is indirectly linked to Ca^{2+} by an H_2O bridge and is suitably placed for hydrogen bonding to the sugar. Asp 16 is also nearby and would interact directly with the sugar. The two tyrosyl residues in the carbohydrate-binding region, Tyr 12 and Tyr 100, can easily account for the increased affinity of concanavalin A for the aryl pyranosides participating in aromatic interactions with the Aryl groups¹⁴.

Further support for the participation of aromatic structures in sugar binding has been gained by means of circular dichroism studies^{15,38-40}. In several instances it has been demonstrated that lectins from various sources and of different specificities suffer conformational changes upon binding of the corresponding sugar inhibitor of substrate. With soy bean agglutinin, for example, the major alterations on the circular dichroism spectrum of the protein occur in the 265-290 nm region, in which aromatic chromophores display Cotton effects. Therefore, it has been concluded that tyrosyl and tryptophanyl residues may be involved in the interaction of this lectin with the carbohydrate substrates¹⁵.

THE SUGARS

The inhibition of lectins by simple sugars was first reported by Morgan and Watkins¹⁶. Later, Krupe¹⁷ investigated the effects of 23 low-molecular-weight carbohydrates on the action of lectins and concluded that specific lectins exhibited a group variation on their behaviour towards these compounds, whereas non-specific lectins showed a completely random variation amongst themselves in this respect. On the basis of further investigations on the inhibition of the reaction of 52 lectins with both human and animal erythrocytes, Mäkelä¹⁸ classified aldopyranoses into four inhibitory classes according to the configuration at C-3 and C-4. Some limitations to this classification, however, have been discussed in several reviews^{2,3}.

Sugar-lectin complementarity is established by comparing the efficiency of

carbohydrates in inhibiting the precipitation reaction between the lectin and a reactive macromolecule, or the haemagglutination reaction¹⁹⁻²¹. The drawbacks to this kind of approach in elucidating the characteristics of the sugar-binding site are obviously determined by the limitations on the availability of different oligosaccharide structures¹⁹⁻²¹. The use of α - and β -glycosides as hapten inhibitors, in addition to free sugars, yields important information about the anomeric specificity of the lectin¹⁹⁻²¹. Glycosides of aromatic aglycones, on the other hand, provide useful information about the nature of the protein site adjacent to the place where carbohydrate binding occurs. However, these later experiments should be interpreted with caution, as the non-specific interaction of the protein with the aromatic moiety may preclude the specific carbohydrate-binding properties of the lectin in question.

The higher affinity constants observed in the binding of lectin to glycoproteins and cell surface carbohydrates in comparison with simple sugars has been rationalized on the basis of multivalent interactions, not necessarily carbohydrate-mediated ones, between the lectin and the complex macromolecule. Further, in the interaction between lectins and macromolecules the influence of steric factors, in addition to the specific interaction between the carbohydrate and the lectin, must be considered.

Most lectins accommodate a single glycosyl residue^{2,3}. In a few instances, however, the nature of the glycoside linkage plays a role in determining the binding specificity^{2,3}. The fact that several lectins bind di- and trisaccharides more strongly than the corresponding alkyl glycosides suggests that the reducing sugar may contribute to the binding energy of the lectin-carbohydrate complex. The precise position of a determinant sugar in an oligo- or polysaccharide is also relevant to lectin reactivity^{2,3} because, with few exceptions, lectins interact with the non-reducing terminal glycosyl groups of polysaccharides and glycoprotein chain-ends.

Finally, many other techniques have been introduced for the study of lectin-carbohydrate interactions and for exploiting this property for purification purposes²²⁻²⁶ or for quantitative assays of carbohydrate solutions²²⁻²⁶. Such methods also permit the study of the lectin-carbohydrate complex formation under conditions otherwise restricted by the fragility of the cells employed during the agglutination assay, but present drawbacks related to the nature of the method itself; in affinity chromatography, for instance, the matrix, the coupling procedure and the length of the spacer arm might interfere with the specificity of the reaction²²⁻²⁶. In affinity electrophoresis²⁷, on the other hand, the experimental conditions are so different from those employed in the haemagglutination assay that comparisons are impossible.

THE MEDIA

Water plays an important role in protein structure and function. Studies of water-protein interactions can be carried out in solution, in protein crystals or with protein powders of variable water content²⁸. In aqueous solutions, or in protein crystals in contact with the mother liquor, the water activity is relatively constant and no important conformational changes have been observed upon protein drying. However, nothing definite can be said about what happens to protein structure and function as water is removed. Removal of water molecules from protein surface groups could give rise to unsatisfied sites for hydrogen bonds or electrostatic interac-

tions; if these sites were to be satisfied by gross refolding it would be necessary to split important hydrogen bonds in the native secondary structure of the proteins. Thus, the fact that the number of strongly held water molecules in the almost completely dry protein corresponds to the number of water molecules observed in the crystal state supports the idea that the conformation of the macromolecule is about the same at different degrees of hydration, at least with the few proteins studied.

Water is the medium in which most biological reactions occur, and therefore a brief discussion on the participation of water in lectin-carbohydrate complex formation appears pertinent.

Liquid water is considered as a system with a certain structural order due to the presence of hydrogen-bonded clusters²⁹. The free energy of the hydrogen bond is assumed to be a continuous and smooth function of the bonding angle, and is highly influenced by small amounts of solutes. The solutes are usually classified with respect to their charges, as cations, anions or neutral species. However, as we are concerned with the structure of liquid water, we shall refer to the hydrophilic and hydrophobic concepts proposed by Bene and Pople³⁰. Accordingly, hydrophilic solutes are "structure breakers" and interact more strongly with water than the water molecules with each other. They are readily soluble and give rise to hydration layers that show structural features different from those of the bulk water. All the "structure breakers" have in common the ability to be hydrated by water molecules by exercising either an electron donor or acceptor (charge-transfer?) function.

In contrast, hydrophobic or "structure-making" solutes are sparingly soluble in water, as the intermolecular forces between water and solute molecules are weaker than the hydrogen-bond interactions between the water molecules. The dissolution of such molecules causes a positive change (loss) in entropy. The solute molecules of ions are placed in holes of the water structure, which are adaptable to the requirement of the solute molecule for free rotation.

In summary, each "structure maker" and each "structure breaker", including each vacant hole, should be considered as a "structure-regulating centre". Their specific nature, their number and their relative positions to each other will be decisive for the range of bond-length variations within the liquid.

The type of salt and its concentration, pH, temperature and other agents present in the media may influence the carbohydrate-lectin association either directly, by complexing with the sugar or the protein, or indirectly, by modifying the water structure in the bulk and/or in the surface of the protein, thus introducing the possibility of changes in the participating molecules³¹⁻³⁴.

The participation of hydrophobic bonding in the binding of lectins to various carbohydrate structures has been acknowledged in several instances^{35,36}. Therefore, polarity-reducing agents such as ethylene glycol and mild denaturants, *e.g.*, urea and detergents, added to the media have resulted in enhancement of lectin specificity³⁴⁻³⁶. Some metal ions also exert a non-specific effect in lectin-carbohydrate interaction by facilitating complexation between entities with a similar charge sign and/or by favouring their hydrophobic association³². On the other hand, relatively high salt concentrations may preclude the lectin-sugar interactions in which electrostatic forces might be critical³⁷.

NATURE OF THE BINDING FORCES INVOLVED IN THE LECTIN-CARBOHYDRATE COMPLEX

Based on the structure of the carbohydrate substrate and of the sugar-binding site, it has been postulated that hydrogen bonds play the main role in complex stabilization. Hydrogen bonds can be described as the result of several different concepts^{41,42}: (a) electrostatic energy; (b) polarization energy; (c) charge-transfer energy; (d) exchange energy; and (e) dispersion energy. Thus, the sum of these five terms should represent the total intermolecular interaction energy, E_{H-B} , *i.e.*, the difference between the energy of the final hydrogen-bonded system at equilibrium and the total energy of the original isolated molecules.

(a) The electrostatic contribution corresponds to the energy change that would result if the free constituent molecules A and B, in some hypothetical way, were brought together into the position corresponding to the hydrogen-bonded complex without any deformation of the original monomer charge distributions and without any electron exchange.

(b) The polarization contribution corresponds to the energy further gained on deforming the monomer charge distributions in the previously hypothetical state to approach more closely the final hydrogen-bond situation but without any transfer of electrons between the original constituents.

(c) The charge-transfer contributions represent the energy improvement by also allowing electron transfer between the systems.

(d) The dispersion contribution corresponds to the attraction between the systems due to the coordinated motion, or correlation of the electrons in the two halves involved (London dispersion forces).

(e) All previous forces are attractive and the two interacting systems are prevented from collapsing owing to the repulsion taking place by exchange energy contribution. This represents the effects of electron exchange between A and B and corresponds more physically to the repulsion of the two electron systems when too many electrons are put in the same volume.

In general, with weak and moderately strong hydrogen bonds, it is found that the polarization, exchange, charge transfer and dispersion contributions approximately cancel each other. Further, the variation in electrostatic energy follows the same trend as the total hydrogen-bond energy. As each of the other binding forces is less sensitive to the relative orientation of the monomers, this finding roughly explains the empirical rule that most geometrical features of hydrogen bonding can be originated by electrostatic behaviour.

Using a very elementary model, the receiver of the hydrogen bond is considered to be a lone pair on the acceptor atom. However, one should take into consideration that if a molecule contains more than one "active" hydrogen atom, as occurs in carbohydrates, then all such hydrogen atoms will tend to participate in hydrogen bonding. For instance, it is very seldom found that the hydrogen atoms of a water molecule are not engaged in hydrogen bonding. If the number of lone pairs available is less than the number of active hydrogen atoms, then the hydrogen atoms tend to arrange themselves around the lone pairs in such a way that all of them participate in hydrogen bonding to the same extent. This can be illustrated by the three hydrogen atoms of solid ammonia forming hydrogen bonds simultaneously.

hydrogen bonds, or the donor hydrogen atom is directed towards some point between several lone pairs. It is clear, therefore, that the hydrogen-bond arrangement is often determined by single geometrical requirements and/or steric hindrances.

On the other hand, it is important to take into account the whole electron and nuclear distribution when discussing the relative arrangements of interacting molecules. Several studies have provided evidence for the directional influence of the lone-

There is also the case in which available lone pairs are in excess, as occurs in many organic compounds. Here, either some of the lone pairs are not involved in pair electrons of the carbonyl group when acting as a hydrogen-bond acceptor. A statistical study⁴³ of the $H \cdots O-C$ angle with certain compounds showed an average value of about 120° , but many deviations from this value were also noted. In the absence of specific interactions between functional groups, the relative arrangement of the molecules is mainly determined by the overall shape and size of the molecules involved. The overall distribution is directly related to the outer contours of the total electron density of the molecule. Specific interactions, such as hydrogen bonding, in contrast, generally result in a crystalline arrangement that is very different from that expected from simple close packing. Statistical analysis of the geometry of 100 $H \cdots O-H$ hydrogen bonds, for example, showed that about 25% of the hydrogen bonds can be described as bifurcated, indicating that this form of association is more common than previously supposed⁴³. However, the study also suggests that there is a preferred direction of hydrogen bonding with respect to the acceptor oxygen atom which is in, or close to, the plane containing the oxygen lone-pair orbitals, but there is no evidence of a preferred direction within the plane. In addition, the so-called bifurcated hydrogen bonds involving the ethereal atoms⁴³ appear to be a common phenomenon in carbohydrate crystal structures. The anomeric oxygen atom, on the other hand, seems to be an exceptionally weak hydrogen-bond acceptor, but an unusually strong hydrogen-bond donor, which may explain its role in determining the α/β -oligosaccharide specificity of lectins.

As mentioned above, it is difficult to assume that all the interactions in which aromatic amino acids participate are necessarily hydrophobic in nature. In fact, the hydrophobic concept as applied to lectin-carbohydrate interactions is misleading. The hydrophobic character of amino acids cannot be given an absolute value. A scale of amino acid hydrophobicity exists, based on the solubilities of the amino acids in water and also in progressively increasing concentrations of some organic solvents, such as ethanol in water⁴⁴. Thus, the solubilities of the amino acids can be extrapolated to pure organic solvents and then the free energy of the transfer of the amino acid from the pure organic solvent to water can be calculated. Such a scale has been constructed using glycine as the reference, and subtracting its free energy of transfer from that of all the other amino acids. It is clear, therefore, that this estimation cannot be correlated with other compounds unless similar criteria, and approaches in the determination of their hydrophobicities have been employed.

In general, the term hydrophobic interaction is used when referring to the association of apolar molecules in aqueous systems⁴⁵. Nevertheless, the mechanism of the association of alkyl groups is different from that involving aromatic structures. In the latter instance, one must also consider the possibility of other kinds of interactions.

It should be noted that all of the studies concerning the aminoacyl structures of

the sugar-binding sites of lectins discussed in the previous sections do not emphasize the participation of side-chain alkyl residues, such as those of Leu, Ile, and Val. Hence, the model for a pure hydrophobic interaction⁴⁵ in this process is not applicable. Only two kinds of functional groups, free-amino and carboxyl groups, and two aromatic structures, tyrosine and tryptophan, have been clearly identified as being involved in the recognition of carbohydrate structures by lectin molecules. Their arrangement and number in the sugar-binding site determine the carbohydrate specificity of the lectin. The free-amino and carboxyl groups probably participate in the formation of hydrogen bonds with the sugar, as pointed out before. Aromatic amino acids, in addition, may form charge-transfer complexes with carbohydrates⁴⁶. This suggestion is supported by the fact that several aromatic structures, including the amino acids, are retained in chromatographic systems using polysaccharide matrices. The influence of temperature, salt concentration and polarity-reducing agents has indicated the charge-transfer interaction mechanism as the reason for adsorption^{46,47}.

It has also been argued that with dextrans the anhydroglucose residues of the polysaccharide matrices furnish a sufficient number of hydrophobic sites by adopting special conformations in a stable and regular array of the fibres^{48,48}. However, this does not seem sufficient to explain the adsorption behaviour of hydrophobic solutes on tightly cross-linked unsubstituted dextran gels. A thermodynamic study pertaining to the transfer of hydrophobic solutes from external water to internal water in Sephadex G-10 has recently indicated that the water structure, and thereby the solubility of the hydrophobic solutes, in such locations is different⁴⁹; the solubility of hydrophobic solutes is higher in the internal phase.

Taking into account these data, Professor Porath and his colleagues have proposed a model that explains the adsorption of aromatic compounds on poly-

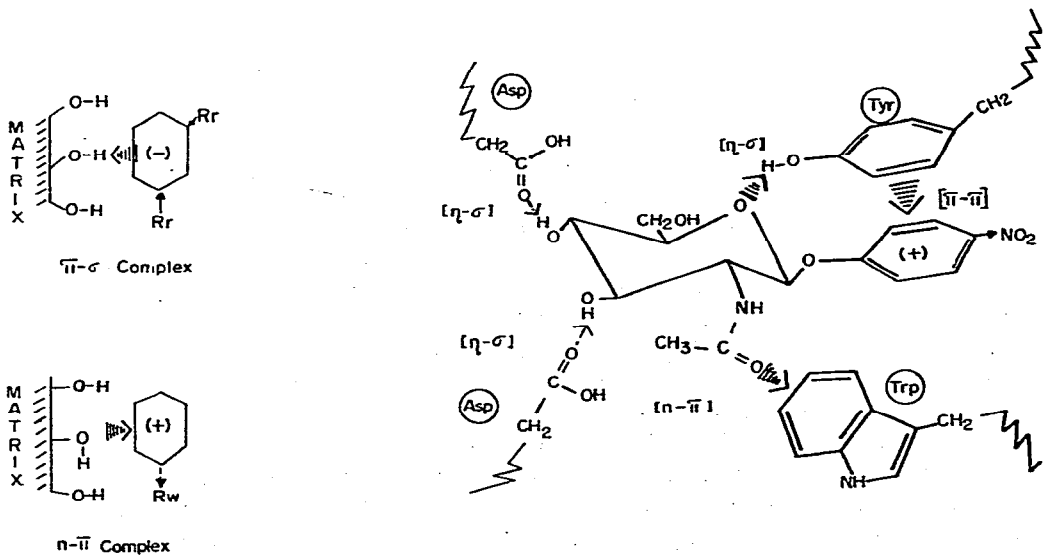


Fig. 1. Mechanism of adsorption of aromatic compounds on polysaccharide matrices and modes of interaction of an oligosaccharide compound with the amino acids of a hypothetical sugar-binding site in a lectin molecule, showing a close resemblance one to another.

saccharide matrices involving predominantly a charge-transfer mechanism⁴⁷. The reverse of this model may be applicable to lectin-carbohydrate interactions if we consider the sugar-binding site as a microenvironment resembling the situation depicted in the chromatographic model (Fig. 1).

CONCLUSIONS

Various factors are considered to be involved in biomolecular interactions. In addition to those determined by the nature of the participating entities, the reaction medium and the experimental conditions play an important role in making the interaction feasible. Concerning the lectin-carbohydrate interaction in particular, we depart from the assumption that it is the lectin that binds the sugar; probably because we follow the reasoning that as for many other macromolecules, *e.g.*, enzymes and antibodies, the binding site in lectins is often more complex than the substrate or the hapten molecules with which they interact.

The sugar binding-sites of lectins vary not only by virtue of their specificity but also owing to the large differences in physico-chemical properties found between lectin molecules from different sources. It is now accepted that the only common feature of lectins is that they all are proteins.

The sugars, on the other hand, may also vary in structure and conformation, as well as in arrangement and composition, between cell receptors. It is therefore extremely difficult to assess the nature of the interaction between lectins and cells. In this paper, an attempt has been made to describe the simplest cases and to discuss the different kinds of forces that participate in the process of the binding of sugars to lectin molecules.

The model proposed is far from being general or complete. Much more work must be carried out if we wish to understand two phenomena that previously have been considered in too simple a manner: the induced cell agglutination by lectin molecules and the mechanisms of lectin-carbohydrate interactions.

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