CHAPTER I

HYDROPHOBIC INTERACTIONS INVOLVING SUGARS - AN OVERVIEW
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1.0. INTRODUCTION

While two amino acids can build only one (or at best 8 distinct) dipeptide, two monosaccharides can form dozens of different disaccharides depending on the nature of the glycosidic linkage. This brings in the variegation, specificity and selectivity in their interactions with their receptors and other molecules. Molecular recognition in carbohydrates is thus far more varied than amino acids and nucleic acids. Hence it becomes important to investigate the mechanistic variations in their interactions that arise due to the different linkages and also the different types of cohesive forces involved. It becomes even more complicated when one looks at the polysaccharides wherein the possibilities of the different glycosidic linkages, the epimeric structures and the different conformations are multifold.

Polysaccharides are usually considered to be hydrophilic molecules. They are highly soluble in water, rich in hydroxyl groups and contain no obvious apolar groups. All these properties together justify and favour their hydrophilic nature; thus to consider the possibility of any hydrophobic character in these molecules seems counter-intuitive. Yet it is possible to generate amphiphilicity in these saccharide chains based on the conformation of the monomeric sugars, the epimeric structure and the stereochemistry of the glycosidic linkages and the chain conformation. Certain special features in these molecules and the aspects of their interactions with
other molecules reflect the amphiphilicity. Several reports are available to-date wherein the interactions of these sugars have been shown to be apolar in nature.

1.1. EARLY SUGGESTIONS ABOUT HYDROPHOBIC INTERACTIONS INVOLVING SUGARS

Neal and Goring suggested that some sugars may have hydrophobic surfaces (Neal and Goring, 1970). They compared molecular models of maltose and cellobiose (α-1,4 linked and β-1,4 linked dimers of D-glucose respectively) and found that maltose readily folds into a L-shaped conformation with the inner face largely containing the methine hydrogens and the outer face dotted with all the hydroxyl groups. Such a fold appeared less likely or favourable in the case of the β-1,4 linked cellobiose. In the absence of any alkyl or aryl substitution, the hydrophobic character is derived here from the methine CH groups alone and hence negligibly weak in the monomer itself. But in the dimer maltose and higher oligomers, as monomers are linked in a row, an increasing and regular array of these weakly nonpolar faces can develop, thus increasing the hydrophobic character. Regular repetition and consolidation of the hydrophobic faces into a strip or a ribbon-like surface would be dependent on the type of the glycosidic linkage. The α-1,4 linkage in D-glucose reinforces the hydrophobic surface while β-1,4 linkage breaks this order (Figure 1.1). It also became possible to estimate molecular surface areas and define hydrophobic and hydrophilic areas of molecules, using a computer program developed by Hermann (1972) based on atomic coordinates from X-ray crystallography.

Several other groups have reported interactions that were suggestive of forces that are hydrophobic in nature. In 1975, Holmes et al. fractionated tRNAs on
Figure 1.1: The preferred conformations of α-(1,4) and β-(1,4) linked glucosides. The A and B faces are defined as those for which the numbering is clockwise and anti-clockwise, respectively. The CH bonds define the nonpolar faces (hatched).
unsubstituted agarose in a decreasing salt gradient wherein the interaction of the tRNAs to the gel matrix is attributed to be due to the hydrophobicity. Nakatani et al. (1977) were able to detect nonpolar interactions in oligosaccharides. They have used a fluorophore probe method, exploiting the fact that the fluorescence intensity of the probe 2-p-toluidinynaphthalene-6-sulfonate (TNS) is enhanced upon moving from aqueous to a more nonpolar environment. Binding to linear α-amyloses (α-1,4-linked glucose) enhances its emission intensity remarkably and in a manner dependent on the length of the amylose chain. Pullulan (largely α-1,6-linked maltotriose) enhances TNS emission, though rather weakly while dextran (strictly α-1,6-linked glucose, with no α-1,4-linkage) fails to influence the fluorescence of TNS, thus showing the importance of the type of glycosidic linkage in generating apolar surfaces in glucans. From another study using TNS as a probe the binding constant $K_b$ of TNS and Dextrin 10 was estimated to be around $90 \pm 20 \text{ M}^{-1}$ (Das et al., 1995).

During the late 1970s, Masanobu Janado and coworkers showed that in frontal phase chromatographic experiments, the amphiphilic detergent sodium dodecyl sulphate (SDS), at premicellar concentrations, preferentially bound to the matrix material Biogel P-2 which is made of dextran or α-1,6 linked D-glucose polymer. The binding was found to increase when the temperature and the concentration were increased. They also found that aromatic dyes such as azobenzene and dimethylaminobenzene, which are sparingly soluble in water, dissolved in the internal water of the Sephadex gels (Janado et al., 1980a) and in highly concentrated (>20%) dextran solutions in water (Janado et al., 1980b).

Miyajima and coworkers (Miyajima et al., 1985) have defined a parameter called the hydrophobic index, which is the ratio of the hydrophobic and hydrophilic
surface areas of the molecules. The hydrophilic and hydrophobic areas were calculated by a computer program developed by Hermann (1972). The total hydrophobic surface area was defined as the area occupied by the CH and CH$_2$ groups of monosaccharides. The hydrophilic surface area is the area occupied by the -OH and -O- groups and corresponds to the difference between the total surface area and the hydrophobic surface area. The hydrophobic index thus calculated, was seen to correlate well with the partition coefficient of the sugar between polystyrene gel and water, and with the free energy of transfer of the monosaccharide from water to n-butanol, indicating that the fraction of the hydrophobic surface area would be a factor in the interaction of monosaccharides and lipophilic compounds in water. The hydrophobic index was estimated to be in the order

\[
galactose < glucose < arabinose < mannose < xylose < ribose < deoxyribose.
\]

This correlation seems to apply to glucodisaccharides (such as cellobiose, maltose, trehalose, gentobiose and laminarabiose) as well, where the hydrophobic areas are larger, as anticipated, and their affinity to interact with polystyrene gels as well.

Amphiphiles in general enhance the solubility of lipophilic and nonpolar substances in water. Remarkably, some sugars have been shown to display this tendency. Janado and Yano (1985) showed that among the pentoses, arabinose solubilized naphthalene and biphenyl to appreciable extents (twice as good as water), while among the hexoses it was D-mannose.

Last, and most notably, cyclodextrins have been demonstrated to display amphiphilic nature. Cyclodextrins are cyclooligoamyloses, which are like bracelets or bottomless buckets whose inner cavity is hydrophobic while the outer surface is
polar. This amphiphilic feature of cyclodextrin molecules arise from the $^4C_1$ conformation of the monomeric $\alpha$-D glucose units and the 1a,4e-glycosidic linkage, which disposes all the hydroxyl groups on the outer rim of the bucket, with the methine hydrogens protruding inwards making the inner cavity less polar. As a consequence, they form “inclusion complexes” with one or more molecules of nonpolar substances such as aromatic hydrocarbons by solubilising them within the apolar inner cavity (Bender and Komiyama, 1978, Clarke et al., 1988).

1.2. FOUR MAIN CONFORMATIONAL TYPES OF POLYSACCHARIDE CHAINS

The conformational features of polyglucosides have been clarified through X-ray diffraction studies of the polymer fibres and small molecule crystals. Homopolysaccharides are known to take on the extended ribbon (Type A), the flexible helix (Type B), the crumpled ribbon (Type C) and the flexible coil (Type D) conformations, depending on their chain linkage types (Rees 1977; Atkins, 1986; Kennedy, 1988). These four types of structures and the chains that adopt each of these types are shown in Figure 1.2. Of these the Type B helical structure, which $\alpha$-amylose adopts, has an internal surface that is relatively apolar since all the methine groups point inwards. This is the reason why $\alpha$-amylose is able to complex nonpolar substances and include them within - e.g., iodine, fats and polar organic solvents; though amylose adopts multiple conformations in solution, and adopts a flexible coil structure at neutral pH, complexing agents such as the above readily induce the helical fold in amylose, with about six D-glucose residues per helical turn and the complexing agent included within the helix (Kennedy, 1988). The Type B helical conformation comes in three types; cyclodextrin is thought to be one of these, called the V-form (Rees, 1977, Brisson et al., 1991). Table 1.1 lists the sets of homopolysaccharides that adopt each of the four main structural types.
Figure 1.2: The four major conformational types of homopolysaccharides. (Taken from Kennedy, 1988). Only Type B is expected to be amphiphilic.
Table 1.1. *Various conformational types found in homopolysaccharides, and examples of each type*

<table>
<thead>
<tr>
<th>Type A</th>
<th>Type B</th>
<th>Type C</th>
<th>Type D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extended Ribbon</td>
<td>flexible helix</td>
<td>crumpled ribbon</td>
<td>flexible coil</td>
</tr>
<tr>
<td>α-(1,3)-D-galactan</td>
<td>β-(1,3)-D-galactan</td>
<td>α-(1,2)-D-galactan</td>
<td>all 1,6</td>
</tr>
<tr>
<td>α-(1,4)-D-galactan</td>
<td>β-(1,4)-D-galactan</td>
<td>β-(1,2)-D-galactan</td>
<td>disubstituted</td>
</tr>
<tr>
<td>β-(1,3)-D-glucan</td>
<td>β-(1,3)-D-glucan</td>
<td>β-(1,2)-D-glucan</td>
<td>homoglycans</td>
</tr>
<tr>
<td>β-(1,4)-D-glucan</td>
<td>α-(1,4)-D-glucan</td>
<td>β-(1,2)-D-glucan</td>
<td></td>
</tr>
<tr>
<td>α-(1,3)-D-mannan</td>
<td>α-(1,2)-D-mannan</td>
<td>β-(1,2)-D-mannan</td>
<td></td>
</tr>
<tr>
<td>β-(1,4)-D-mannan</td>
<td>β-(1,3)-D-mannan</td>
<td>α-(1,2)-D-mannan</td>
<td></td>
</tr>
<tr>
<td>α-(1,3)-D-xylan</td>
<td>α-(1,4)-D-xylan</td>
<td>β-(1,2)-D-xylan</td>
<td></td>
</tr>
<tr>
<td>β-(1,3)-D-xylan</td>
<td>β-(1,3)-D-xylan</td>
<td>α-(1,4)-D-xylan</td>
<td></td>
</tr>
</tbody>
</table>

* (taken from Kennedy, 1988)
The three dimensional structure of polysaccharides is constrained by the glycosidic link geometry and the spatial disposition (axial or equatorial) of the groups. Figure 1.3 shows the sugars that exist in the usual \textit{4}C\textsubscript{1} chair form, the following possibilities have been listed by Atkins (1986). The 1e, 4e-linked chains exist as two- or three-fold extended chains, with polar groups occupying the surface. This would make the 1e, 4e-linked sugars (cellulose, 1e, 4e- or \beta-D-mannan) simply hydrophilic and not amphiphilic. The 1e, 3e-chains from triple helices with a rather inaccessible interior, while the outer face is solvated. While cellulose, the 1e, 4e-linked ribbon like polymer, is similar to the \beta sheet in proteins, the 1a,4e-linked V-form is a helical tube. Here the conformation is stabilized by intrachain hydrogen bonds and resembles the \alpha-helix in proteins. In curdlan the 1e,3e-linkages forms triple helices, bearing a striking resemblance to collagen (Atkins, 1986).

It appears that, in general, those chains that can adopt the Type B structure in solution may show amphiphilic character, while those than can adopt Type A, C and D may not. However, in interacting with small molecules, some saccharide chains that adopt the flexible coil form (Type D) may be able to present small or limited regions or subregions of nonpolarity with which lipophilic substrates may interact. Among the naturally occurring common polysaccharides, plant pectic substances, carrageenan, some yeast 1,2-mannans, amylose, pullulan, glycogen (in parts) and laminaran are amphiphilic. It would thus appear that oligosaccharides that can adopt incipient helical structures, particularly of Type B, might display amphiphilicity. In biochemistry and cell biology, this property would be relevant to the processes of intermolecular recognition on cell surfaces (between sugars and lipids, glycolipids, proteolipids and proteins at the peripheral and integral regions of membranes), lectin-sugar binding, antigen-antibody interactions and the like, and might be manifested more in a heteromolecular recognition event than as
Figure 1.3 Three structures of polyglucose. (a) Cellulose, 1e-4e linked polyglucose is a gentle undulating ribbon-like polymer. The conformation is similar to β-conformation in proteins. (b) Amylose, 1a-4e linked polyglucose in the V form is a hollow helical tube. The conformation is stabilized by intrachain hydrogen bonds and resembles the α-helix in proteins. (c) Curdlan, 1e-3e linked polyglucose forms a triple-strand rope stabilized by buried hydrogen bonds in the core. The conformation bears a striking resemblance to the protein collagen. (Taken from Atkins, 1986).
homomolecular self-aggregation. (Self-aggregation would at best occur as a face-to-face dimer, with the hydrophobic faces in contact; on the outer face would be the multiple hydroxyl groups which should find hydrogen bonding with the solvent water just as attractive with the hydrophilic outer faces of neighbouring chains).

Extensive reports are available wherein the nonpolar surfaces present in sugars have been demonstrated to enter into non-covalent interactions with a large variety of macromolecules such as nucleic acids and globular proteins, sugar-binding and -transporting proteins, lectins and antibodies and with other carbohydrates too (Sivakama Sundari and Balasubramanian, 1997). The cohesive forces that favour, facilitate and stabilise these interactions seem to be predominantly hydrophobic in nature. The proteins bind to the characteristic-binding sites on the saccharide molecules, by making van der Waals contacts predominantly through their nonpolar amino acid residues. The salient features of these interactions and the molecules involved are briefly discussed below.

1.3. EXAMPLES OF HYDROPHOBIC INTERACTIONS

1.3.1. Carbohydrate-Carbohydrate Interactions

The involvement of nonpolar forces in carbohydrate-carbohydrate interactions is increasingly being studied. Aoyama and coworkers (1992a and 1992b) studied the selective binding of sugars to the inner apolar cavity of the \( \beta \)-cyclodextrin as a prototype for sugar-sugar interactions in water solutions. They monitored the ability of added aldopentoses, aldotetroses and some aliphatic alcohols to displace a fluorescent probe that was housed in the cavity of \( \beta \)-cyclodextrin. The ability to displace the probe was in the order
cyclohexanol > cyclohexane 1,2-diol > t-butanol > aldopentose.

This is essentially in the decreasing order of hydrophobicity. Nonpolar contacts between the ligand and the cyclodextrin appear to be important to the process. Further insight into these lipophilic aspects of carbohydrate-carbohydrate interactions in water comes from the work of Penades and coworkers from Spain (Barbero et al., 1995). This group has used "glycophane," a cyclodextrin-cyclophane hybrid, a cyclic molecule wherein two trehaloses are connected symmetrically on either side by a naphthalene molecule each as cyclic trehalose-naphthalene-trehalose-naphthalene (see Figure 1.4). These results, plus those from molecular dynamics simulation calculations of the global minima structures of some of the complexes, have led Barbero et al. (1995) to conclude that apart from the expected polar interactions, lipophilic forces or hydrophobic contacts between carbohydrate surfaces determine the stability of the association.

All these results are in agreement with the suggestion of Raymond Lemieux (1990), that "the main driving force for complex formation occurs when the adjacent complementary nonpolar surfaces came together", and of Hakomori (1991) that "complementarity of two interacting carbohydrates could be based on hydrophobic interactions between the respective hydrophobic surfaces". It also suggests that the role of the hydroxyl groups in a sugar is not only to form hydrogen bonds but also to modulate the shape of the complementary amphiphilic surfaces and strengthening van der Waals contacts and hydrophobic interactions (Carver, 1993). In this context, it is relevant to note that the critical micelle concentration (cmc) value of a bile acid is lowered when it is glycated (Venkatesan et al., 1994). The lower cmc or easier tendency for aggregation in the case of the glycated detergent may be due to the hydrophobic interaction between the carbohydrate moieties; crystal structural
Figure 1.4. Schematic representation of the complexes of (a) glycophane 1-PNP-α-D-glucopyranoside and (b) α-cyclodextrin-PNP-α-D-glucopyranoside based on CPK models. (Taken from Barbero et al., 1995)
analysis of those glycated bile acids (Cheng et al., 1992) reveals such hydrophobic contacts between the sugar moieties, in addition to the ubiquitous intermolecular hydrogen bonding. Likewise, the dimerization of the chromomycin A₃ - Mg²⁺ complex has been interpreted to be driven by nonpolar interactions between the oligosaccharide moiety and the aromatic (chromomycin) chromophore (Silva and Kahne, 1993).

1.3.2. Interactions with Proteins

Sugars such as sucrose seem to stabilize globular proteins by strengthening the hydrophobic effect, in other words by “sugaring out” the molecules (Lakshmi and Nandi, 1976). Lee and Timasheff (1981) have shown that sucrose stabilizes the proteins by increasing the solvent cohesive forces. Kabat et al. (1981) had found that the monoclonal antibody anti-I Ma recognizes a large lipophilic surface presented by the hapten trisaccharide β-D-Gal-(1,4)-β-D-GlcNAc-(1,6)-β-D-Gal (Lemeuix et al., 1984; see Figure 1.5) and when necessary the carbohydrate will assume intramolecular hydrogen bonds in order to become compatible for hydrophobic bonding with the antibody. Lemeuix and his coworkers (Lemeuix et al., 1988; Delabaere et al., 1990) have suggested a pattern of interactions involving the recognition of the amphiphilic surface of the oligosaccharide that is presented to the protein.

Avadesha Surolia and his group have done extensive work on carbohydrate binding to lectins which offers further information about the nonpolar interactions involved between the sugar and the protein. Their results on the interaction of labelled oligomers of GlcNAc with coccinia indica agglutinin (CIA), a chitooligosaccharide-specific lectin with two binding sites per homodimer of 32 kDa, wherein the umbelliferyl (Umb) group acts as a fluorescent label whose
Figure 1.5: The trisaccharide (compound I) in the conformation expected to be bound by the anti-I-Ma antibody. The binding region is shown in bold face. (Taken from Lemieux et al., 1984).
fluorescence quantum yield decreases as the polarity of the medium drops are suggestive of the nonpolar interactions involved. Sanadi and Surolia (1994) have studied the thermodynamics and kinetics of the binding of the di-, tri- and tetrasaccharides Umb(GlcNAc)$_2$, Umb(GlcNAc)$_3$ and Umb(GlcNAc)$_4$ and have found nonpolar interactions dominating the binding. That the ligands bind to a nonpolar surface was evident from the quenching of the umbelliferyl group emission, which occurred in a manner keeping with the thermodynamic parameters: quenching was 62% when the dimer Umb(GlcNAc)$_2$ bound to lectin, while it was total (100%) when the trimer and tetramer bound to the lectins. They have also compared the amino acid sequences of several lectins (Puri and Surolia, 1994) and found high levels of homology in the sequence of residues constituting the carbohydrate binding site and the hydrophobic cavity. An interesting observation is the complete conservation of the trp (133) residue in all legume lectins, which appears important to the forces involved in the binding of sugars to these proteins. The winged bean acidic agglutinin called WBA II, where again sugar (2"'-fucosyllactose) binding is accompanied by a positive entropy change ($\Delta G = -25.5$ kcal m$^{-1}$, $\Delta H = -23.4$ kcal m$^{-1}$ and $\Delta S = +6.8$ e.u. cf. the values of -13.8, -44.9 and -106.2 respectively for lactose itself), suggesting that nonpolar interactions participate in stabilizing the sugar-lectin complex (Acharya et al., 1990). The binding of WBA II to ($\alpha$-L-Fuc(lc$\rightarrow$2b)- $\beta$-D-Gal-(1b$\rightarrow$4a)- $\beta$-D-GlcNAc-OMe), the H-type human blood group-related trisaccharide is attributed (Lemieux et al., 1994) to changes in hydration that lead to enthalpy-entropy compensation, a phenomenon characteristic of bipolar (hydrophobic and hydrophilic) modes of interactions.

The basic features of the interactions between oligosaccharides and the enzymes lysozyme, phosphorylase and amylases on the one hand (Johnson et al., 1988) and sugar-binding and transporting proteins on the other (Quiocho and Vyas
1984; Quirocho, 1986; 1989; 1990) have been extensively studied. While lysozyme hydrolyzes glucosides in the $\beta$-1,4 conformation, phosphorylase and amylases act on $\alpha$-1,4 linked glucosides. An important conformational consequence of the difference between $\alpha$-1,4 and $\beta$-1,4 linked glucosides (e.g. maltose and cellobiose), shown in Figure 1.1 earlier, is that the $\alpha$-1,4-linked glucosyl oligomers present a continuous modulating nonpolar face or sheet to the protein, with a twist of about $-55^\circ$ between adjacent sugars, while in the case of $\beta$-1,4 linked glucosyl polymers, the oligosaccharide presents an alternating nonpolar and polar face every 0.5 nm, with a twist of about $180^\circ$ between adjacent sugars.

Detailed structural features are available (Johnson et al., 1988) from studies of the crystal structures of the complex between the lysozyme and the inhibitor $\beta$-1,4 linked trisaccharide (GlcNAc)$_3$. This sugar is in a hydrophobic environment, making extensive nonpolar contacts with ile 98, ala 107, trp 108 and val 109. There are several other such nonpolar contacts, at the key catalytic residues in particular. Considerable contacts of this type are seen in the sugar-enzyme complex.

Glycogen phosphorylase is an enzyme that degrades glycogen and generates a D-glucose-1-phosphate per enzymatic cycle, which is used in the glycolytic pathway to generate energy as ATP. The enzyme acts on $\alpha$-1,4 glycosidic links rather than $\beta$-1,4, as lysozyme does. The crystal structures of phosphorylase complexed with glucose, heptenitol and heptulose 2-phosphate have been solved and their binding site features compared (Johnson et al., 1988). The sugar-binding site is a cavity at the centre of the molecule contributing both polar and nonpolar interactions that match the corresponding moieties in the ligand. Thirdly, alpha amylase catalyses the hydrolysis of $\alpha$-D-(1,4) glycosidic bonds of starch components (amylose and amylpectin). In the crystal structural studies of pörcline
pancreatic α-amylase, Buisson et al. (1987) have compared the different Fourier maps between the native and the substrate-analogue-soaked crystals and have observed an extensive amphiphilic contact between the side chains of amino acid residues of the enzyme and the maltotriose analog in this system. In addition to this active site, a second binding site for substrate analogs was identified about 2 nm away. The detergent bile salts bind here, indicating the amphiphilic nature of this protein surface. It is hence to be expected that the ligand sugar that binds this site would engage in hydrophobic interactions.

One of the sugar-binding-proteins, the maltose- or maltodextrin-binding protein, a primary receptor of bacterial active transport and chemotaxis, abbreviated as MBP, has two distinct globular domains divided by a groove that is 1.8 nm deep with a box that is about 0.9 x 1.8 nm in dimension, in which the sugar (maltose or maltodextrin) sits. This is a generalized feature found in other binding proteins/receptors such as those that bind arabinose (ABP), D-galactose/D-glucose (GGBP), the sulphate ion (SBP), the phosphate ion (PBP), leucine/isoleucine/valine (LIVBP) and leucine (LBP) (Quiocio, 1986; 1989; 1990). MBP is a large two-domain protein that undergoes a reversible two-state denaturation, both upon heating (T_m = 336 K at pH 7.41) and upon cooling (T_m ca 278 K, pH 7.4) and binds maltose with a K_a value of 1 mM (Ganesh et al., 1997). The bound maltose in MBP is buried in the groove, inaccessible to the solvent. The groove is populated by polar and aromatic side chains of the protein, which interact extensively through hydrogen bonding and van der Waals contacts (< 0.4 nm) with the sugar. The sugar is wedged between four aromatic residues, with the aromatic rings stacking on the faces of the glucosyl units, providing much of the van der Waals contacts. There are about 60 van der Waals contacts seen between glucose and GGBP in the crystals of the complex, about 47 between arabinose and ABP and 65 between maltose and
MBP (Spurlino et al., 1991).

It is important to point out another general feature of such binding proteins, namely the stacking of the aromatic side chains of the proteins against the pyranose ring. This feature has been observed in ABP and GGBP (Quirocho, 1986, 1989), and in MBP. Such stacking against the apolar face of the sugar ring has since been seen in the crystals of D-xylose isomerase-substrate complex (Lavie et al., 1994), and in maltoporin which facilitates the diffusion of maltodextrins across the outer membrane of Gram-negative bacteria (Schirmer et al., 1995), where the aromatic residues are arranged along a left-handed helical path in the channel lining, providing a "greasy slide" for the sugar to translocate along the channel.

1.3.3. Interaction with Nucleic Acids

Next we turn to hydrophobic aspects of the interaction between sugar chains and nucleic acids. One of the earliest reports has been the use of the saccharide unsubstituted agarose as the stationary phase in chromatographic separation of t-RNA molecules (Holmes et al., 1975). Evidence for hydrophobic interaction has been reported between DNA and the sugar component of some antitumor antibiotics of the ene-diyn type (Ding and Ellestad, 1991). Minor groove binding drugs such as netropsin and distamycin have molecular structures with an inherently bent crescent shape that complements the minor groove of DNA (Berman et al., 1979; Neidle et al., 1987), a shape found in α-1,4- dextrins but not in the α-1,6- dextrans. The minor groove region of the DNA double helix is known to be a relatively hydrophobic low dielectric region in comparison to the major groove (Barawkar and Ganesh, 1995). This has been visualized through the use of a non-intercalating polarity-sensitive fluorophore such as DNS (N,N-dimethylamino-3-naphthalene sulfonate chloride), which binds to DNA (Gao and Patel, 1989) preferentially in the minor groove, just as other
nonpolar dyes (Jin and Breslauer, 1988) and displays a consequent enhancement in its fluorescence intensity but not when placed in the major groove region (Barawkar and Ganesh, 1993). As seen in Figure 1.6, addition of linear dextrin enhances DNS emission, while dextran is ineffective, suggesting that a ternary complex (DNA-DNS-dextrin) is perhaps formed in which the dye DNS is sandwiched between the hydrophobic face of α-1,4-dextrin and DNA in the minor groove (Gopala Krishna et al., 1994). It may also be pointed out that the DNA-binding saccharide units in ene-diyne antibiotics are mostly α-1,4-linked and also comprise 6-deoxy sugars and rhamnose units that are suggested to bind DNA through hydrophobic interactions. Recent reports have shown that aminoglycoside antibiotics of the neomycin family, which contain three or four sugar rings and are characterized by the 2-deoxystreptamine-cyclitol moiety, binds selectively to specific RNA sequences (Fernandez-Saiz et al., 1996).

1.4. APPLICATIONS

From mechanisms of nonpolar interactions of some sugars, we next turn our attention to the application of these interactions. A practical application of the amphiphilic properties of saccharide chains is their use as stationary phase electrolyte supplements and as enantioselective electrolyte supplements in capillary electrophoresis (CE), capillary zone electrophoresis (CZE), and electrokinetic chromatography (EKC), and their particular advantage as chiral selectors therein, for the separation of enantiomers in a racemic mixture (Terabe et al., 1994; Ward, 1994; Fanali, 1996; Nishi, 1996 a and b). D’Hulst and Verbeke (1992) have tried a variety of chiral ‘pseudostationary phases’ i.e. chiral modifiers added to the background electrolyte in CE, for the enantiomer separation of the 2-arylpropionic acid (2APA) class of non-steroidal anti-inflammatory drugs (NSAID such as racemic flurbiprofen, ibuprofen and ketoprofen. The phases tried were β-cyclodextrin and its
Figure 1.6. Fluorescence spectra of DNS in 10 mM Tris buffer, pH 8.0 (—); DNS + 260 nm DNA fragment CGCGAATTCCCG (sequence I) (-----); DNS + sequence I + 30 mM Dextran 4 (.....); DNS + sequence I + 30 mM Dextrin 10 (-o-o-o-). (Taken from Gopalakrishna et al., 1994).
hydropropyl and dimethyl derivatives, maltose oligomers, corn starch hydrolysates, stachyose, raffinose, Dextran 600 and 1500 (α-(1,6)-linked D-glucoses) and linear β-(1,6)-linked D-glucoses. Of all these, only the α-(1,4) linked D-glucoses and amylose showed sufficient enantioselective abilities towards these drugs. Based on these, the authors suggest that the proper hydrophile-lipophile balance (HLB) and the appropriate steric environment necessary for chiral interactions are most optimally displayed by these sugars. With maltodextrins, the efficiency of chiral separation improves with increasing sugar concentration and higher chain length (Figure 1.7). In more recent papers, these authors find that maltodextrin-supplemented electrolytes are able to generate extremely high enantioselectivities for a wide range of acidic (D’Hulst and Verbeke, 1994a and 1994b) and basic (D’Hulst and Verbeke, 1996) racemic drugs. A wide range of basic drugs (antiarrhythmic, anticholinergic, antifungal, antihistaminic, antidepressant and antipsychotic) could be resolved with maltodextrin as the modifier, and with a properly chosen single enantiomer background electrolyte, chiral separation of some drugs (aminopromazine) was complete (Suzuki et al., 1996, see Figure 1.7). The fact that enantioselectivity occurs with the neutral sugars indicates that binding occurs, and considering the lipophilicity and the structures of the drugs, it would be hydrophobic in nature; that dextrin is more efficient than dextran is also in keeping with this idea.

Another practical application would be to use the hydrophobicity for specific removal of other amphiphiles or molecules with hydrophobic pockets by binding specifically to the amphiphilic surface present on the polysaccharide.

In this thesis we have attempted to address the issue of whether linear oligosaccharides namely the linear dextrins, that are analogous to the cyclic
Figure 1.7: (Panel a): Electrophoretogram representing chiral separation of three racemic coumarinic anticoagulant drugs and two chlorinated derivatives. Electrolyte: Glucidex2, 3% Tris phosphate 10mM, pH 7. (Taken from D'Hulst and Verbeke,1994). (Panel b): Influence of chiral electrolyte concentration (mM) on enantioselectivity (%CS) towards aminopromazine. (Taken from Suzuki et al., 1996).
oligosaccharides, the cyclodextrins, would also display amphiphilicity; and whether this amphiphilic property can be exploited for some use in improving the yields of proteins during \textit{in vitro} refolding. We have studied aqueous solutions of smaller sugars such as glucose, sucrose, maltose and of oligosaccharides like the linear dextrins ($\alpha$-1,4 linked glucosics) and compared with those of dextran ($\alpha$-1,6 linked glucosics) and of cellulose ($\beta$-1,4 linked glucosics), as well as of xylan. We have also studied the interactions of these saccharides with other molecules such as detergents, globular proteins, nonpolar substances and other amphiphiles. In many of these instances, we find that linear dextrins, which are more abundant and readily available at far less costs, can effectively replace cyclodextrins in several applications; this has made us dub linear dextrins as 'poor man's cyclodextrins'. We have also used this amphiphilic nature of dextrins in the "artificial chaperoning" of denatured aggregates of globular proteins into refolding back to their native and active states. Various techniques such as absorbance, fluorescence and circular dichroism spectroscopy have been used in this study.