CHAPTER 3

INTERACTION WITH AMPHIPHILES
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3.0. INTRODUCTION

Cyclodextrins are known to interact with fatty acids and related amphiphiles and include them within the apolar cavity (Bender and Komiyama, 1978). In comparison, oligomeric dextrans are curved ribbons, with a relatively apolar interior surface, as section 2.2.5 shows. The free energies of interaction of dextrans are quite low indicating that they perhaps display modest amphiphilic tendencies unlike the cyclodextrins wherein the cavity formation results in a considerable hydrophobicity. Palepu and Reinsborough (1988) have carried out a detailed study of interactions of cyclodextrins with surfactants using conductance measurements. They have found that the cyclodextrins destroy micelles by competing with the surfactant monomers more strongly than they are bound in self-association. Probe experiments suggest that the polarity experienced by the probe molecules in dextrans is roughly comparable to 30% dioxan. Jiang et al. (1984) had shown that amylose, the α-1,4 linked polymer of D-glucose, uncoils self-coiled amphiphiles such as 16-substituted palmitic acid p-nitrophenyl esters. Considering all these points together one can envisage that the maltodextrins would enter into hydrophobic interactions with other amphiphiles. Gratzer and Bevan (1969) had shown earlier that sucrose advances the micelle formation (due to aggregation) in the nonionic detergent Triton X-100. Thus the heteromolecular interactions between the sugar and the target molecules may thereby hinder or alter the characteristic homomolecular interactions (self-association) among the molecules of the target amphiphiles. This property might be
of relevance in studies of membranology and in intermolecular recognition on cell surfaces.

In this chapter, we present further evidence to support the results that have led us to the conclusion that 1a,4e linked α-D glucose chains possess amphiphilic surfaces while the other linkages such as β-1,4 (1e,4e) and α-1,6 linkages do not display such tendencies. We have studied the effect of various sugars on the critical micelle concentration (cmc) values of conventional surfactants by using surface tensiometry and pyrene fluorescence method. The cmc values of the detergents studied were seen to be postponed in the case of dextrins while dextran and other smaller sugars advanced the cmc, or promoted micellization. We have determined the minimal hydrotrope concentration (mhc) of sodium cumene sulfonate, a classical example of a hydrotrope in the presence of linear dextrins and found that the self-association was postponed. We have also looked at the interaction of these on another amphiphilic molecule, namely the 11-bromoundecanoyl tryptophan (BUT) (see Figure 3.1) which tends to self-coil in water (Shoba and Balasubramanian, 1986). The variation in the fluorescence quantum yields of this molecule in water and other sugars has been determined. The α-1,4-linked oligomers of D-glucose seem to straighten out the self-coiled chain of BUT.

3.1. MATERIALS AND METHODS

The detergents, namely cetyltrimethylammonium bromide or CTAB and Triton X-100, and the neutral arene probe, pyrene, were purchased from Sigma Chemical Company. Dextrin 10, Dextrin 20 and Dextran 4 were purchased from Serva Chemical Company. All other reagents used were of analytical grade.
Figure 3.1. Structure of 11-Bromoundecanoyltryptophan (BUT)
3.1.1. Determination of Critical Micelle Concentration (cmc) by Surface Tensiometry

The surface tension of the two surfactants CTAB and Triton X-100 were measured using a White-Fischer tensiometer with a platinum ring. A graph of concentration vs surface tension was plotted. The break point, indicative of a transition (due to aggregation), denotes the cmc of the surfactant. These measurements were carried out in different concentrations of each sugar individually, and a graph of concentration of the sugar vs the cmc of each surfactant was plotted.

3.1.2. Determination of Critical Micelle Concentration (cmc) of Surfactants by Fluorescence Method

Pyrene (10 µl of 100 µM in methanol) was added to 1 ml of each concentration of the surfactant with and without the additive sugar. The λex was set at 335 nm. The relative emission intensity ratio of the third and the first vibronic bands of the emission spectrum, i.e. I3/I1, called the Ham ratio was calculated.

3.1.3. Preparation of 11-Bromoundecanoyltryptophan

11-bromoundecanoyltryptophan was synthesised by the condensation of N-methyl ester of tryptophan to the succinimide ester of 11-bromoundecanoic acid.

N-methyl ester of tryptophan was prepared by the thionyl chloride-methanol procedure (Brenner and Huber, 1953) as follows: To absolute methanol (5 ml) which was cooled in an ice salt bath (-10° C), 0.5 ml of thionyl chloride was added dropwise with shaking. To this mixture 5 mmol of tryptophan was added and the reaction mixture was slowly allowed to thaw to room temperature and kept overnight as such. Then the methanol and the acid were evaporated under reduced pressure and water (10 ml) was added followed by the addition of sodium carbonate.
to make it alkaline and the methyl ester was extracted with chloroform (3 x 10 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to obtain the product as oil, which was used immediately for coupling.

The succinimide ester of 11-bromoundecanoic acid was prepared by the hydroxysuccinimide ester method as follows: The acid (1 equivalent) was dissolved in dichloromethane and cooled in an ice bath. Equivalent amounts of N-hydroxy succinimide and dicyclohexyl carbodiimide were added and kept at room temperature for 72 hrs. The resulting side product dicyclohexyl urea was filtered off and upon evaporation of the organic solvent the succinimide ester of the acid was obtained, which was coupled to the freshly prepared N-methyl ester of tryptophan.

In the coupling reaction, equal amounts of N-methyl ester of tryptophan and succinimide ester of 11-bromoundecanoic acid were dissolved in dimethyl formamide and N-methyl morpholine was added as a catalyst and the reaction mixture was kept stirring overnight at room temperature. The reaction mixture was diluted with ethyl acetate and washed twice with 0.5 M citric acid. The organic layer was then washed with water, sodium carbonate and sodium chloride solutions, dried under anhydrous sodium sulphate and evaporated to obtain the crude product, which was purified by silica gel column chromatography.

Sodium salt of 11-bromoundecanoyltryptophan was prepared by saponification with 0.5 M sodium hydroxide and the product characterized by NMR and mass spectroscopy. For fluorescence experiments the optical density at 280 nm was taken as a measure of the concentration.
3.1.4. Fluorescence of BUT in the presence of sugars

A stock solution of BUT was made in methanol and added to 1ml of the additive. The emission spectrum was recorded by exciting at the characteristic $\lambda_{ex}$ of 280 nm for tryptophan and the changes in the maximum intensity of emission with and without the additive sugar was monitored.

3.2. RESULTS AND DISCUSSION

3.2.1. Effect of Sugars on the Self-aggregation of a Cationic Detergent

The critical micelle concentration of the cationic detergent CTAB is 0.9 mM in water. It was found to be to be advanced to a limiting value of 0.6 mM by D-glucose and trehalose ($\alpha$-1,1-linked dimer of glucose), as shown in Figure 3.2. The dextrins are seen to postpone the cmc to about 4.2 mM in 100 mM Dextrin 20, and almost to 6.2 mM in 50 mM Dextrin 10. The standard free energy of the transfer of the detergent from water to aqueous solutions of the sugars was estimated (Gratzer and Bevan, 1969) by substituting the cmc values in the equation

$$\Delta G^0 = -RT \ln (\text{cmc})_s / (\text{cmc})_w,$$

where, the subscripts s and w refer to the aqueous sugar solution and water respectively. The values of transfer free energies of CTAB and Triton X-100 from water to various sugar solutions are listed in Table 3.1.

An additive that increases the cmc of a surfactant is thought to weaken the hydrophobic self-association between the detergent molecules, by binding to them. This mechanism must be operating in the case of dextrins, wherein they might interact directly with the detergent monomers through their hydrophobic surfaces thereby inhibiting the micellar aggregation. A 10 mM $\alpha$-cyclodextrin delays the micellization from 0.9 mM to 7 mM. This interaction between the dextrins and the
Table 3.1: Standard free energy of transfer $\Delta G_{tr}$ of some detergents from water to aqueous sugar solutions at 298 K

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>$\Delta G_{tr}$ Transfer free energy from water (cal / mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTAB</td>
</tr>
<tr>
<td>10 mM $\alpha$-Cyclodextrin</td>
<td>-1220</td>
</tr>
<tr>
<td>50 mM Dextrin 10</td>
<td>-1040</td>
</tr>
<tr>
<td>50 mM Dextrin 20</td>
<td>-770</td>
</tr>
<tr>
<td>200 mM Maltose</td>
<td>-30</td>
</tr>
<tr>
<td>250 mM D-Glucose</td>
<td>+240</td>
</tr>
<tr>
<td>1 M Sucrose</td>
<td>+410</td>
</tr>
<tr>
<td>200 mM Cellobiose</td>
<td>+240</td>
</tr>
<tr>
<td>10 mM Dextran 4</td>
<td>+120</td>
</tr>
<tr>
<td>0.025% Xylan</td>
<td>+350</td>
</tr>
</tbody>
</table>
Figure 3.2. Effect of various sugars on the cmc of CTAB in water. Curve (1) glucose, (2) trehalose, (3) Dextrin 20, (4) Dextrin 10 and (5) α-cyclodextrin. The limited data points collected for Dextran 4, cellobiose and xylan fell on curves (1) and (2). The cmc values were measured using the pyrene fluorescence probe method.
detergent would necessarily have to be significantly hydrophobic in character, since
the monomeric glucose itself does not interact with CTAB. Therefore it appears that
a hydrophobic surface of appropriate chain length and conformation is a prerequisite
for the binding.

3.2.2. Effect of Sugars on the Self-aggregation of a Nonionic Detergent

We next studied a nonionic detergent, so as to exclude the possibility of any
coulombic factors involved in the process. Here too the linear dextrins, as seen in
Figure 3.3, postpone the cmc substantially. The cmc of Triton X-100 is postponed
from 0.24 mM in water to beyond 1 mM in Dextrin 20 and Dextrin 10. While an
additive like sucrose that is supposed to enhance water structure would promote the
hydrophobic interaction between the nonpolar moieties, linear dextrins should
behave oppositely. As shown in the previous chapter with solubilization of tyrosine,
the values of free energies indicate that α-cyclodextrin and linear dextrins solubilize
the amphiphilic substances and thereby postpone the cmc substantially. Dextran and
xylan are quite different in their behaviour. They advance the cmc of CTAB and
affect that of Triton X-100 very little.

3.2.3. Effect of Sugars on the Self-aggregation of a Hydrotrope

We have also studied the effects of the linear dextrins on hydrotropic self-
association. Hydrotropes are compounds which tend to self-aggregate in water,
beyond a given concentration termed as minimum hydrotropic concentration (mhc),
to form non-covalent stacked molecular assemblies (Balasubramanian et al., 1989,
Balasubramanian and Frieberg, 1993). We have found that the mhc of a hydrotrope,
sodium cumene sulfonate was increased from 0.1 M in water to 0.4 M in 50 mM
Dextrin 10. This result provides further evidence wherein dextrin interacts with
another amphiphile through its apolar surface thereby inhibiting the self-association.
Figure 3.3. Effect of various sugars on the cmc of Triton X-100 in water. Curve (1) glucose, (2) trehalose, (3) Dextrin 20, (4) Dextrin 10 and (5) α-cyclodextrin. The limited data points collected for Dextran 4, cellobiose and xylan fell on curves (1) and (2). The cmc values were measured using the pyrene fluorescence probe method.
3.2.4. Uncoiling of a Hairpin-looped Amphiphile by Linear Dextrins

Another example of the hydrophobic interaction of dextrins was observed when it was found that these linear chains were able to 'uncoil' the self-coiled amphiphiles. Linear amyloses have been reported to be able to uncoil or straighten-out self-coiled amphiphiles such as 16-substituted palmitic acid p-nitrophenyl esters (Jiang et al, 1984). These fatty acid derivatives are terminally functionalized alkanoates, which loop like a hairpin (or self-coils) when placed in an aqueous environment, because of intramolecular hydrophobic interaction. We have synthesized a self-coiling molecule, namely, 11-bromoundecanoyltryptophan or Br-(CH$_2$)$_{10}$-CO-trp, (see structure Figure 3.1). The heavy atom Br present at one terminal quenches the fluorescence of tryptophan, when the molecule is hairpin looped in aqueous solution. Any agent that inhibits or releases this intermolecular self-coiling would tend to remove the Br atom from the proximity of the tryptophan moiety, and thus reduce the heavy atom quenching of the emission of tryptophan and increase the fluorescence intensity. In order to test the effects of the various sugars due to the invocation of the hydrophobic force, on the hairpin looping property of BUT, we have studied the variation in the fluorescence intensity of BUT in the sugar solutions.

The fluorescence spectrum of BUT (which is known to self-coil in water (Shoba and Balasubramanian, 1986),) is found to be relieved of the heavy atom quenching upon the addition of maltodextrins. Upon excitation at 280 nm, BUT shows an emission band around 357 nm in 1 M maltose with a relative emission intensity of 1 in 1 M maltose. This value was found to increase to 1.53 in 300 mM Dextrin 20 with a blue-shift in the emission maximum to 354 nm. Further increase and blue-shifting was observed in the case of Dextrin 10, the higher oligomer of glucose to an emission intensity of 1.7 and a band maximum at 351 nm in 150 mM Dextrin 10. Such intensification can be attributed to the straightening of the hairpin
loop of BUT, which in turn would result in the relief of the tryptophan molecule from the heavy atom quenching by the Br moiety. Also since the tryptophan emission is polarity sensitive (Lackowicz, 1983), the blue-shift observed upon addition of the maltodextrins suggests a decrease in the polarity in the medium/ environment.

In conclusion, linear dextrins interact with other amphiphiles such as detergents and hydrotropes thereby inhibiting their self-aggregation. This ability of dextrins to bind to surfactant monomers may be utilised in their removal from solution in various industrial applications and studies in biotechnology.