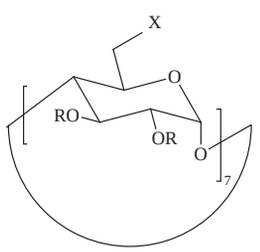
Amphiphilic 6-*S*-alkyl-6-thiocyclodextrins: unimolecular micellar and reverse micellar behaviour

Joykrishna Dey, Pascale Schwinté, Raphael Darcy,* Chang-Chun Ling, François Sicoli and Cormac Ahern

Laboratory for Carbohydrate and Molecular Recognition Chemistry, Department of Chemistry, National University of Ireland, University College, Dublin 4, Ireland

Micellar behaviour of the amphiphilic 6-*S*-alkyl-6-thiocyclomaltooligosaccharides (2) in chloroform has been measured by use of the fluorescent probe 8-anilinonaphthalene-1-sulfonate (ANS) as guest molecule, as well as by water–chloroform interfacial tension measurements. At low concentrations the amphiphiles show behaviour consistent with their being oligomers of monosaccharide amphiphiles, or 'unimolecular micelles'. Thus, with ANS, 1 : 1 complexes are formed for which the association constants are in proportion to the length of the chains. At higher concentrations, the long-chained (C_{16}, C_{18}) alkylthiocyclodextrins form inverted micellar aggregates. The NMR shift changes associated with inclusion of 1-naphthol indicate that even in these aggregates, the carbohydrate cavity plays a part in complexation, while the hydrophobic chains act as extensions of the more hydrophobic primary-hydroxy side of the cavity.

Amphiphilic 6-*S*-alkyl-6-thiocyclomaltooligosaccharides (**2**) are derivatives of cyclodextrins (**1**)[1] which have been shown to



	R	X
1	H	H
2	H	$S(CH_2)_nCH_3$

form thermotropic liquid crystalline phases, as well as monolayers at the air–water interface.[2] Together with aza-crown ethers,[3] these amphiphilic macrocycles are rare examples of molecules which not only behave as host-molecules, but which also show the full range of lyotropic and thermotropic liquid-crystalline properties. In view of their low solubility in water we have examined these macro-amphiphiles for possible reverse micelle formation in chloroform, where such aggregates have been observed before for smaller amphiphiles.[4] Cyclodextrin amphiphiles have also been shown to form supramolecular aggregates such as nanoparticles[5] by precipitation from non-aqueous solvents. Ability to form prior molecular assemblies in non-aqueous solvents would be significant for the structures of these nanoparticles, and for their possible functions as artificial cells[4] and in drug delivery.[6] The stoichiometry of binding of a fluorescent guest-molecule, anilinonaphthalenesulfonate, as well as water–chloroform interfacial tension measurements, was used by us to examine the reverse-micellar behaviour of the amphiphiles **2**.

Supramolecular aggregates of host molecules present the special problem of distinguishing the relative importance of the two microenvironments, that of the individual molecular cavities, and that of the region enclosed by the aggregate, for inclusion of the guest. NMR chemical shift changes induced in the protons of the host molecules by 1-naphthol as guest, were used in an attempt to locate the preferred binding region.

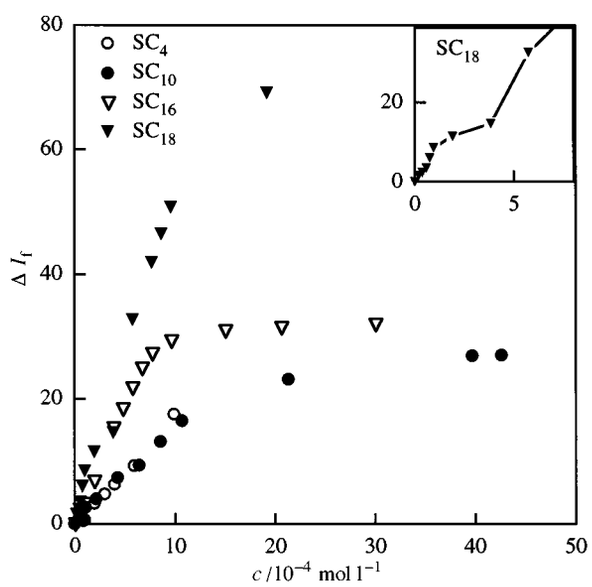


Fig. 1 Fluorescence changes for ANS *versus* concentration of alkylthiocyclodextrin in chloroform [c (ANS) = 1.82×10^{-5} M, 25 °C]

Results and discussion

Inclusion of a fluorescent guest

8-Anilinonaphthalene-1-sulfonate (ANS) is a well-known probe which has mostly been used in aqueous solvents,[7,8] for the determination of the micropolarity of its binding environment. ANS shows a large increase in fluorescence on hydrophobic inclusion which removes it from water. In chloroform, heavy-atom quenching by solvent means that this probe will also signal its sequestration from solvent by an increase in fluorescence. As expected, in the presence of increasing concentrations of CD amphiphiles (**2**) (SC_4, SC_{10}, SC_{16} and SC_{18}), fluorescence of ANS increased (Fig. 1). Limiting values were observed. Below the limiting values, the longer the amphiphile hydrocarbon tails, the greater the fluorescence at a given CD concentration. The increase in fluorescence intensity is accompanied by a blue shift in the emission spectrum (Table 1).

The microenvironment created by the cyclodextrin molecules

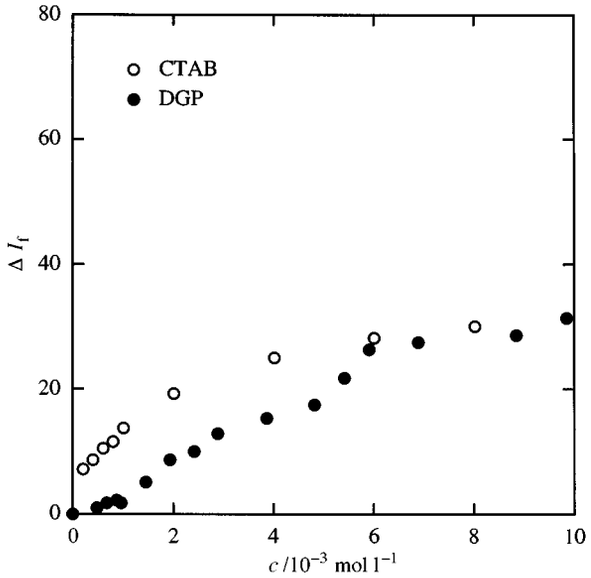


Fig. 2 Fluorescence changes for ANS *versus* concentration of CTAB and DGP surfactants in chloroform [c (ANS) = 10^{-5} M, 25 °C]

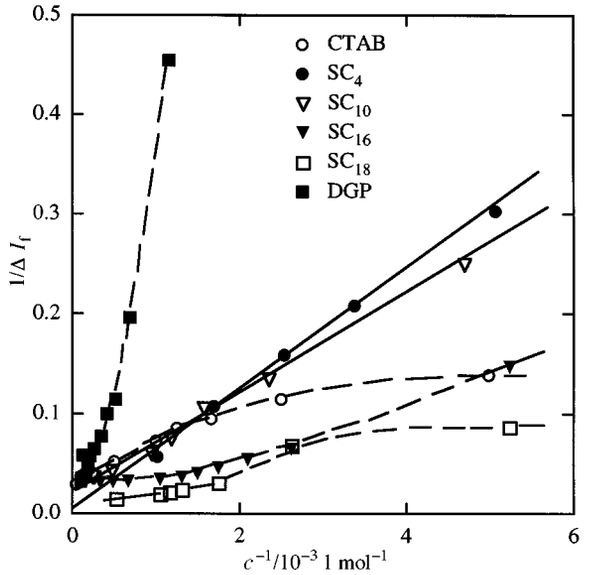


Fig. 3 Reciprocal of fluorescence increase for ANS *versus* reciprocal of amphiphile concentration in chloroform

Table 1 Association constants for 1:1 complexes of alkylthiocyclodextrins with 8-anilinonaphthalene-1-sulfonate fluorescent probe, and blue shifts in the emission maximum wavelength in chloroform

CD[a]	K_a/M^{-1}	Δλ/nm
SC_4–CD	44	18
SC_{10}–CD	460	19
SC_{16}–CD	469[b]	17
SC_{18}–CD	510[b]	16
DiMe–CD	13	14

[a] 5×10^{-4} M. [b] Not only 1:1 complexes are formed.[8]

at higher concentrations is expected to be reverse-micellar in character. There is clear evidence for this in the case of SC_{18}, where the fluorescence variation curve shows two distinct regions. The first region (at $<4 \times 10^{-4}$ M amphiphile) can be attributed to the formation of a 1:1 complex; this is then followed by a sharp increase in fluorescence above 5×10^{-4} M, a concentration which is therefore probably the critical micellar concentration,[9] although cmc values for reverse micelles are not expected to be as well defined as for ordinary micelles.[4]

The surfactant cetyltrimethylammonium bromide (CTAB) is known to form reversed micelles in organic solvents.[10,11] Of the non-ionic sugar-based surfactants,[12] dodecyl glucopyranoside (DGP) may be regarded as a monomer analogue of a cyclodextrin amphiphile oligomer, although it has not previously been studied in chloroform as solvent. We therefore also measured ANS fluorescence as a function of CTAB and DGP concentrations. The fluorescence changes with both surfactants are plotted in Fig. 2. In the case of CTAB the fluorescence reaches a limiting value, and the corresponding concentration (2.5×10^{-3} M) approximates to the cmc. A cmc value of 5.5×10^{-3} M is estimated here for DGP, from the inflection observed in the fluorescence curve at this concentration.

The fluorescence increase observed with CTAB or DGP is due to inclusion of the probe within reverse micelles, equivalent to an n:1 complex where n is the average number of surfactant molecules in a micelle. The indefinite increase with DGP beyond the apparent cmc is probably due to gradual continued aggregation, which can be a feature of reverse micelle formation. The formation equilibrium of the CTAB–ANS and DGP–ANS complexes was compared with that of the CD–ANS complexes by double reciprocal (Lineweaver–Burk) plots.[13] For CTAB and DGP, the plots of the reciprocal of ΔI_f, the increase in fluorescence, against the reciprocal of CTAB molar concentration were hyperbolic curves with inflections in the regions corresponding to the cmc. The plots for SC_4 and SC_{10} were straight lines (Fig. 3), indicating that only 1:1 complexes were formed. However the plots for longer-chain derivatives SC_{16} and SC_{18}, taken over the full concentration range, compare better with the surfactant plots and show the ability to aggregate, while forming 1:1 complexes at low concentrations. The estimated K_a values for 1:1 complexes of the cyclodextrin amphiphiles (Table 1) range from 44 to 510, and show dependence on chain length. For comparison, the reported value[8] for complexation of ANS with β-cyclodextrin in water is 100.

A comparison was also made with 2,6-methylated cyclodextrin, since this highly alkylated derivative is soluble in chloroform. Comparable intensities of ANS fluorescence were obtained with this cyclodextrin only at much higher concentrations (above 10^{-3} M), and the calculated K_a of 13 was insignificant. We conclude from this that inclusion in a cyclodextrin cavity which is merely solubilised in chloroform by alkylation[14] is not very effective. The long alkyl chains of the alkylthiocyclodextrins might be considered as increasing the hydrophobicity of the carbohydrate cavity by altering its solvation. However, when results from NMR measurements (below) are taken into account, a more accurate description is that the chains take part directly in complexation.

Certain sulfonated calixarenes have been shown to behave as 'unimolecular micelles' in water.[15] The hexasulfonated calix[6]arenes showed contrasting properties depending on whether they were C_6(alkyl)-sulfonated or C_{12}-sulfonated. The C_6-sulfonates were reported to form small aggregates of about six molecules, and have other properties similar to conventional surfactants, including a cmc of 5×10^{-4} M, while the C_{12}-sulfonates do not aggregate beyond about three molecules. The effect of chain length on the ability of the calixarene amphiphiles to form micelles is opposite therefore to its influence on the cyclodextrins **2** in the formation of reverse micelles. With the cyclodextrins, the longer-chained compounds SC_{16} and SC_{18} show more tendency to aggregate. These results are significant for the design of amphiphilic macrocycles, since they indicate that the packing of large chains in micelle formation is unfavourable, in contrast to the assembly of the macrocyclic headgroups at the centre of reverse micelles.

Water–chloroform interfacial tension measurements

Pendant drop tensiometry enhanced by video image digitisation is known to be an accurate method for the experimental measurement of the interfacial tension at a fluid–fluid interface,

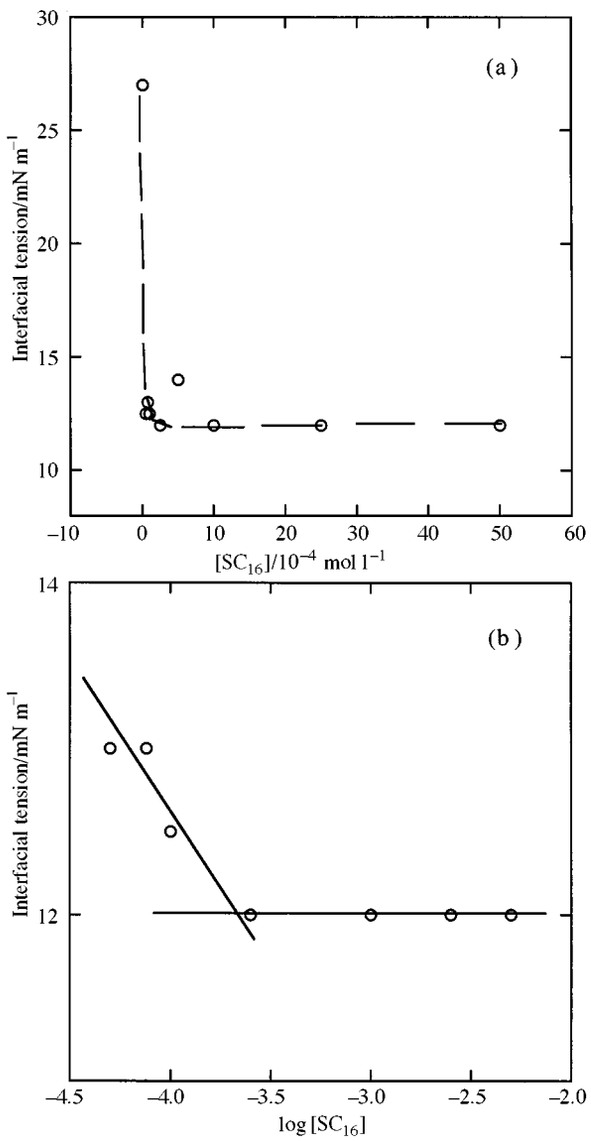


Fig. 4 Water–chloroform interfacial tension of SC_{16} solutions in chloroform: (a) *versus* molar concentration of SC_{16}, (b) *versus* logarithm of the molar concentration of SC_{16}

and for the measurement of the relaxation in interfacial tension due to the adsorption of surfactant molecules at a fluid interface.[16] The water–chloroform interfacial tension was measured for a series of SC_{16} solutions of increasing concentrations in chloroform (0 to 5×10^{-3} M), by creating drops suspended in water (pendant drop method). The dynamic interfacial tension measured in water showed exponential decay with time. The equilibrium value was obtained from the plateau region: a steady value was obtained after 15–30 min. Interfacial tension for pure chloroform was 27 mN m^{-1} after 30 min.[17] As the concentration of CD in chloroform increased, the interfacial tension decreased, as expected when there is aggregation, and fell to 12 mN m^{-1} [Fig. 4(a)]. A value of 3×10^{-4} M for the cmc could be estimated from the plot of the interfacial tension *versus* the logarithm of CD molar concentration [Fig. 4(b)], and this agrees with the result obtained by fluorescence measurements.

NMR measurements

Formation of supramolecular aggregates of a host molecule raises the question of which microenvironment, the host-molecule interior or the aggregate interior, is the more important for guest inclusion.

NMR titration of these hosts with ANS was not possible due to the low solubility of the polar probe in chloroform.

Table 2 Chemical shift changes $\Delta\delta$ induced in NMR spectra of CDs (10^{-3} M) by 1-naphthol (10^{-4} M for SC_{16} and 10^{-3} M for SC_{18}) in $CHCl_3$ at 25 °C

CD	H-3	H-5	H-6,6′	SCH_2
SC_{16}–CD	+0.04	−0.09	−0.08, −0.10	−0.05
SC_{18}–CD	+0.07	−0.08	−0.09, −0.08	−0.06

1-Naphthol was therefore used instead as guest in an effort to discern the mode of complexation of a hydrophobic aromatic group under these conditions from NMR shift changes, $\Delta\delta$. The chemical shift changes which are usually most diagnostic of inclusion of an aromatic guest molecule are those for the intracavity H-3 and H-5 protons.[18] Such shift changes were observed for SC_{16} and SC_{18} in the presence of 1-naphthol. The concentration of CD amphiphiles was above their critical micelle concentrations, and concentrations of naphthol were varied to give guest : host ratios from 0.1 to 1. There was no discontinuity in the chemical shift changes which would have indicated disruption of the cyclodextrin aggregates. The changes for H-3 and H-5 were however accompanied by similar or larger changes for the H-6 protons (Table 2). This phenomenon has been observed before by us for complexation of anthraquinone-2-sulfonate in water by octakis(6-*S*-hydroxyethyl-6-thio)-γ-cyclodextrin.[19] We showed then that the guest molecule is included not only in the cavity, but also within the hydrophobic volume contributed by the side chains. Calculations by Lichtenthaler and Immel have demonstrated the greater hydrophobicity of the primary-hydroxy side of the cyclodextrin cavity,[20] and in these amphiphilic derivatives, the hydrophobic chains create an extension of this effect.

Conclusion

Fluorescence measurements, using a fluorescent probe, have shown that alkylthiocyclodextrins (**2**) at low concentrations behave in effect as fused monosaccharide amphiphiles, functioning as 'unimolecular micelles' in chloroform. With hydrophobic guest molecules, 1 : 1 complexes are formed in which the association constants are in proportion to the length of the chains. At higher concentrations, fluorescence and water–chloroform interfacial tension measurements have shown that the long-chained alkylthiocyclodextrins (SC_{16}, SC_{18}) form aggregates which approximate to inverted micelles. The NMR shifts associated with inclusion of an aromatic guest indicate that even in these aggregates, the carbohydrate cavity plays a part in complexation, while the hydrophobic chains act as extensions of the more hydrophobic primary-hydroxy side of the cavity.

Experimental

Materials

Alkylthiocyclodextrins were synthesised as previously described by us.[2] Complete substitution at position 6 was confirmed by microanalysis, ^{13}C NMR spectroscopy, and FABMS (molecular ion). The CDs were dried at 100 °C and 1.3 Pa for 24 h, after which microanalysis confirmed the absence of water of hydration. 8-Anilinonaphthalene-1-sulfonate (ANS) was purchased from Molecular Probes Europe. Cetyltrimethylammonium bromide was obtained from Aldrich Chemicals, *n*-dodecyl β-D-glucopyranoside from Sigma Chemicals, 2,6-*O*,*O*-methylated β-cyclodextrin (degree of substitution per anhydroglucose, 1.8) from Wacker Chemicals, and Uvasol-grade chloroform from Merck. Uvasol-grade chloroform (500 ml) was thoroughly washed with distilled water, dried over calcium chloride, and passed through a column (1 m × 1.5 cm diam.) of Al_2O_3. It was then refluxed with P_2O_5 for 1 h and distilled, before storage in a dark-glass bottle containing silver foil. This chloroform contained less than 0.1% water, and was used for all measurements.

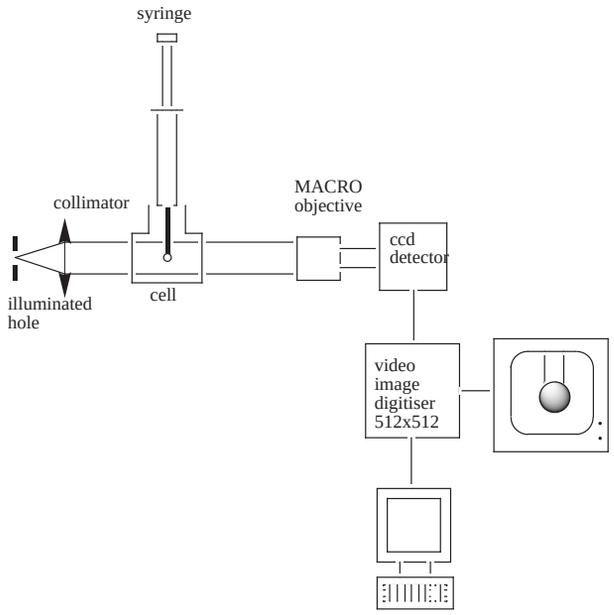


Fig. 5 Pendant drop apparatus

Fluorescence measurements

Fluorescence titration experiments were conducted using a Perkin-Elmer 204 recording fluorescence spectrometer with samples thermostatted at 25 °C. A series of solutions of alkylthiocyclodextrin of increasing concentrations (from 2×10^{-5} to 3×10^{-3} M) was prepared in chloroform. A concentrated solution of ANS (0.2 ml, 5×10^{-4} M) in chloroform was added to 5 ml of chloroform (reference solution) or to 5 ml of the CD solutions. An alternative method was to add 100 μl of the stock solution of probe to 2 ml of CD solution directly into the cuvette. The fluorescence spectra of ANS were recorded between 400 and 600 nm (excitation wavelength 380 nm), after each sample had been thermostatted for 10 min. Relative fluorescence intensities were measured at a constant wavelength (480 nm) near the emission maximum. Binding constants were calculated from double reciprocal plots of change in fluorescence intensity *versus* host concentration.

Interfacial tension measurements

Dynamic measurement of water–chloroform interfacial tension for a series of solutions of SC_{16} ranging from 0 to 5×10^{-3} M in chloroform[9] (negligible partitioning into water) was carried out by creating droplets of chloroform solution suspended in chloroform-saturated water, at 20 °C, using the pendant drop method. This method is a drop shape analysis: a video camera interfaced to a computer is used to record the images of a pendant drop of fluid 1, here chloroform, produced at the tip of a needle into fluid 2, here water. The shape of the drop is analysed and compared to theoretical interfacial models generated by the solution of the Young–Laplace equation[21] and the interfacial tension is then varied until an optimum is obtained between the theoretical and the experimental shape.

The pendant drop apparatus was built by F. Sicoli, in the Complex Fluids Laboratory, Centre for Soft Condensed Matter, in this Department. Water was contained in a spectrophotometric cell (Hellma 1 × 1 × 5 cm). A drop of SC_{16} chloroform solution was formed at the tip of a metallic needle. The diameter of the needle (0.5 mm) was chosen in order to obtain an image of the largest drop which fills the video screen. The needle was attached to a syringe, with a piston which can be displaced with a micrometric screw. The drop was illuminated with a plane parallel beam, obtained with a white light source (filament lamp, 10 V, 100 W), a pinhole and a collimator. The image was formed on a CCD camera with an objective (Macro Zoom 18-200) offering a small distortion. This is particularly important here because the interfacial tension is deduced from the shape and size of the drop. The image was digitised with a video image digitiser (Matrox Magic, 512 × 512 pixels and 256 grey levels) and stored on a PC 486 66 MHz computer (Fig. 5).

In order to analyse the image, the drop boundary was first located, by linear interpolation using the grey levels. The interpolation was done along a horizontal line between the two pixels where the grey level was changing. Close to the bottom, or the top, of the drop, the interpolation was done along vertical lines. The exact size was calculated after calibration with the image of a ruler. Care was taken to calibrate both in the horizontal and vertical directions, the aspect ratio of the pixels being approximately 0.7 in this instrument. The vertical alignment of the detector was made with the help of a plumb line. The shape of the drop is determined by the balance between gravitational energy which tends to elongate the drop and capillary energy which tends to make the drop more spherical. The interfacial tension was then calculated by solving the Laplace equation with a computer program. The precision of the measurements is 0.2 mN m^{-1}.

Acknowledgements

We gratefully acknowledge the hospitality extended to C. Ahern by H.-J. Schneider and his research group for NMR measurements. This work was supported by grants (SC/93/226 and SC/95/237) from Forbairt, the Irish Science and Technology Agency.

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Paper 7/06378D
Received 1*st September* 1997
Accepted 3*rd March* 1998