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Amphiphilic Cyclodextrin Complexation of Clofazimine

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Abstract

The cyclodextrin amphiphiles heptakis[6-(1′-sulfonato-3′-propyl)-6-thio-2,3-di-O-acetyl]-β-cyclodextrin, heptakis[6-(6′ -sulfonato-2′-benzimidazolyl)-6-thio-2,3-di-O-acetyl]-β-cyclodextrin, and heptakis[6-(β-D-glucosyl)-6-thio-2,3-di-O-acetyl]-β-cyclodextrin have been shown to form aggregates in water by fluorescence measurements on the binding of 2-anilinonaphthalene, and by laser light-scattering measurements. Estimates of aggregation number have been obtained. These aggregates successfully incorporate clofazimine, a lipophilic heterocyclic drug, and increase its water solubility by a factor of 30 to 50.

Introduction

Natural cyclodextrins (**1**) are produced enzymatically and have been subjected to many modifications for such objectives as increased solubility, improved binding of guest molecules in their hydrophobic cavities and catalytic behaviour [1].

With amphiphilic cyclodextrins we attempt to exploit the oligosaccharide nature of cyclodextrins by developing their potential for liquid crystalline behaviour [2] and for molecular recognition as epitopes. Our interest is in the development of host-molecular assemblies such as nanospheres or vesicles, which would act as vectors for the trans-membrane transport of drugs.

It has previously been shown that modification of the primary face by attachment of thio- or aminoalkyl groups allows the preparation of Langmuir layers and lyotropic liquid crystals [2]. Hydrophobic esterification at the secondary face allows the formation of mixed vesicles with phospholipids [3].

Here we report the behaviour of water-soluble amphiphilic cyclodextrins (**2–4**) which combine a hydrophilic thio-derivative on the primary face with complete esterification of OH-2 and OH-3.

Clofazimine, 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)-phenazine (**5**) is the prototype anti-mycobacterial riminophenazine [4]. It was originally described in 1957 as an anti-tuberculosis agent, and has been used for the treatment of leprosy since 1962. Riminophenazines have recently been identified as potential anti-cancer drugs [5]. Unlike the existing chemotherapeutic agents which primarily affect cytoplasmic or nuclear targets, the riminophenazines act at the level of the plasma membrane; being highly lipophilic, they distribute into fatty tissues. The primary molecular target of the riminophenazines is Na^+, K^+-adenosine triphosphatase (Na^+, K^+-ATPase), a membrane-associated enzyme, which is essential for tumor cell proliferation. The riminophenazines have been found to be active against all cancer cell lines tested to date, including those which possess intrinsic multidrug resistance. Riminophenazines are therefore ideal candidates for intensive investigation as anti-cancer agents.

The potential therapeutic value of clofazimine is greatly limited due to the fact that it is virtually insoluble in water. To increase absorption, clofazimine is orally administered in the form of a micro-crystalline suspension in an oil-wax base. It is well known that cyclodextrins can improve the solubility of poorly soluble guest molecules upon molecular encapsulation. They are non-toxic and may be administered orally or given by intravenous, intraperitoneal or intramuscular injection. Solubilisation of the drug into water would be further improved by its incorporation into the core of amphiphilic CD aggregates.

Experimental

Syntheses

Amphiphilic cyclodextrins **2–4** were synthesised by methods already used by us to synthesise modified thio-cyclodextrins [6, 7], involving thiolate displacement of a leaving group, in this case bromide [8]. Details will be published elsewhere. Structures were confirmed by NMR (500 MHz) and elemental analysis. Clofazimine was synthesised by methods already described [4].

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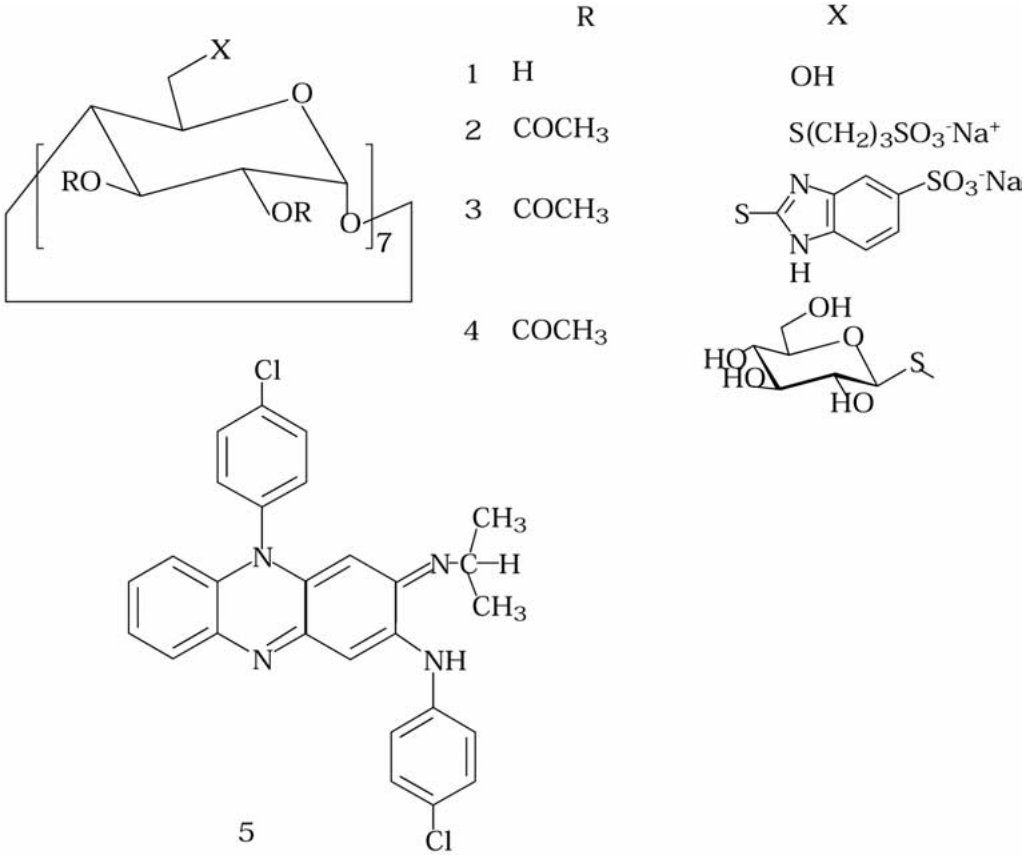

Scheme 1.

Fluorescence measurements

2-Anilinonaphthalene was purchased from Aldrich and used without further purification.

Fluorescence titration experiments were conducted using a Perkin-Elmer 204 recording fluorescence spectrometer in which the temperature was set at 25 °C by means of a thermostated water bath. A typical experiment consisted in preparing a series of solutions characterised by a constant concentration of probe (2-anilinonaphthalene) and an increasing concentration of host. Relative fluorescence intensities were measured at a constant wavelength close to the emission maximum. Binding constants were calculated from double reciprocal plots of variations in fluorescence intensity with host concentration.

Photon correlation spectroscopy (PCS)

Light scattering measurements were conducted with an Autosizer Lo-C (Malvern Instruments) equipped with a series 7032 Multi-8 Correlator (Malvern Instruments) and an Argon Laser 3.5W (Cyonics).The samples were filtered with 0.2 μm or 0.45 μm microfilters before measurement. Data were treated with the programme PCS for windows.

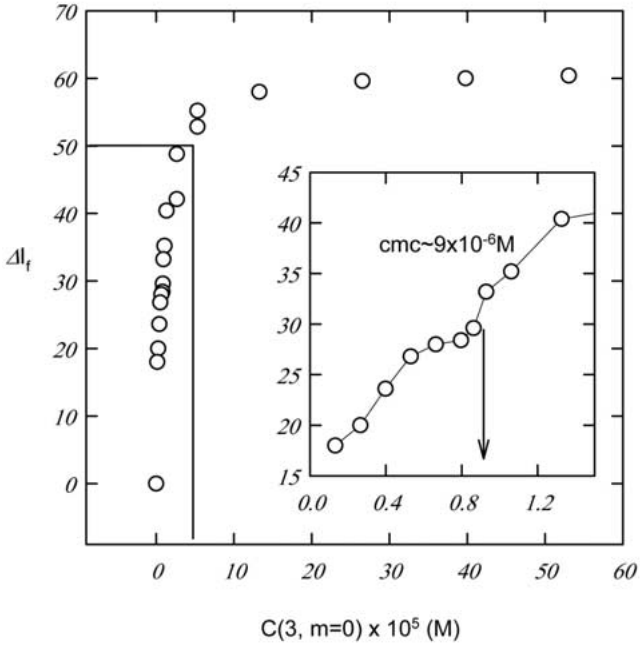

Figure 1. Fluorescence changes for 2-N versus concentration of **3** in water [C(2-AN) = 1.97 × 10^{-6} M, 25 °C].

Solubilisation of clofazimine

An excess of clofazimine (2 mg) was added to increasing concentrations of cyclodextrin host in water (2 × 10^{-6} M–2 × 10^{-4} M). The volumetric flasks (5 ml) were strongly agitated for 15 min at 25 °C. After 15–24 h each sample was filtered through a 0.45 μm microfilter. A sample of filtrate (2.5 ml) was diluted with 2-propanol (2.5 ml). The concentration of clofazimine solubilised in water was estimated spectrophotometrically using a calibration curve at 464 nm.

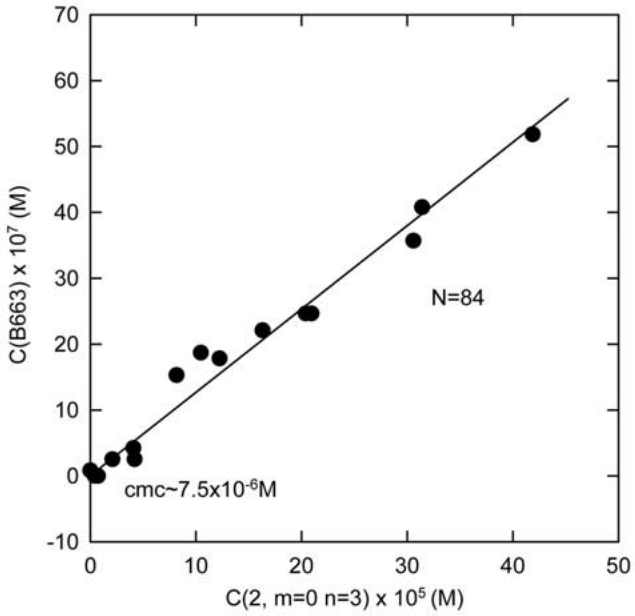

Figure 2a. Concentration of clofazimine solubilised in water (mol l^{-1}) versus concentration of (a) **2**, (b) **3**, (c) **4**, (d) sodium dodecyl sulfate.

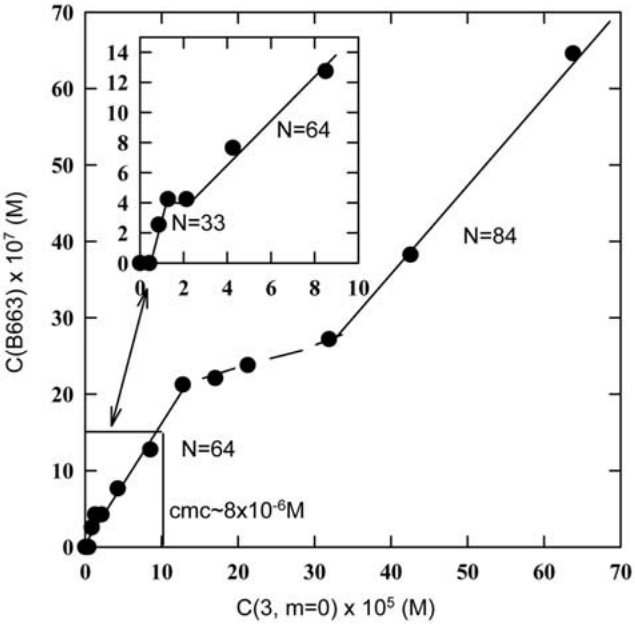

Figure 2b.

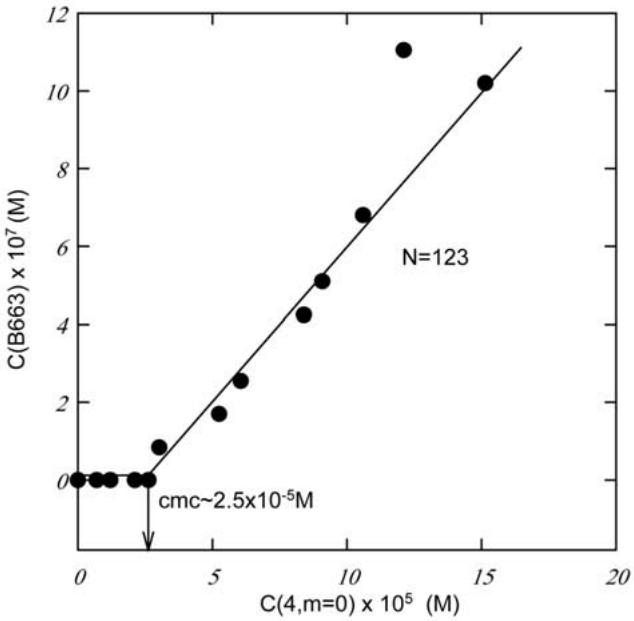

Figure 2c.

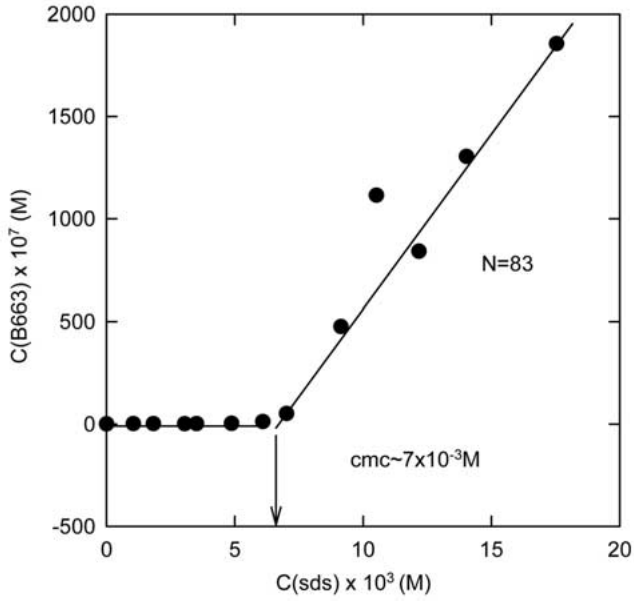

Figure 2d.

A similar test was carried out with sodium dodecyl sulfate for comparison.

Results and discussion

Formation of aggregates in water

In contrast to our alkylthio-CDs [2], these new amphiphilic derivatives **2-4** show water solubility. This means that the reverse micelles observed for SC_{18}-alkylthio-CDs for example in organic solvent [9] are now expected to be paralleled by normal amphiphilic aggregates in aqueous solvents.

This effect was first confirmed by fluorescence measurements on the binding of 2-anilinonaphthalene fluorescent probe in water (Figure 1).

The double reciprocal plot of fluorescence intensity shows that a 1 : 1 complex with 2-AN is formed when conc(host) < apparent c.m.c (M); aggregation occurs above this concentration, as indicated by the presence of an inflection at this point on the I_f/C(host) graph followed by an enhanced fluorescence intensity with increase of host concentration.

Aggregation was confirmed by light scattering measurements, which show a size distribution of the particles ranging from 5 to 20 nm in diameter, giving an average size of 10 nm.

Table 1. Apparent critical micellar concentrations and aggregation numbers obtained from fluorescence and solubilisation measurements. Efficiency factor: concentration of clofazimine solubilised in water by the CD (10^{-4} M) divided by the solubility of clofazimine (1.7×10^{-7} M)

Compound	Fluorescence of 2-AN	Solubilisation of clofazimine		
	c.m.c. (mol l^{-1})	c.m.c. (mol l^{-1}	Aggregation number (N)	Efficiency factor (at 10^{-4} M)
2	8×10^{-6}	7.5×10^{-6}	84	9.5
3	9×10^{-6}	8×10^{-6}	84	9.5
4	3×10^{-5}	2.5×10^{-5}	123	3.5
s.d.s.	7.5×10^{-3}	7×10^{-3}	83	–

Solubilisation of clofazimine

The solubilisation of lipophilic dyes such as 1-(2′-methylaminophenylazo)-2-naphthol (Orange-OT) is frequently used to evaluate the solubilisation power of surfactants [10]. The method has been applied here to the solubilisation of clofazimine (**5**). The concentration of clofazimine (B663) solubilised in water by compounds **2–4** was plotted against the concentration of cyclodextrin host (Figure 2a–d). In each case solubilisation of clofazimine is observed only above the apparent c.m.c. and the concentration increases linearly with increase in surfactant concentration. The slope may be regarded as the molar ratio of the clofazimine-surfactant complex and gives an estimate of the aggregation number N [10].

The apparent c.m.c. values measured are very low (Table 1), which is consistent with the "oligo-surfactant" nature of these compounds. A good accord is found between c.m.c. values obtained by fluorescence and solubilisation measurements. The two sulfonato derivatives **2** and **3**, with a higher water solubility than **4**, have lower apparent c.m.c.'s (by a factor of 3) and appear to be three times more efficient than **4** in solubilising clofazimine.

It appears that the nature of the primary substituents (imidazole, thioalkyl) bearing the sulfonate groups has no influence, neither on the c.m.c. value nor on the aggregation number nor efficiency of solubilisation.

Conclusions

We have synthesised a range of new water-soluble CD amphiphiles and shown the formation of micelle-like aggregates in water by fluorescence and light-scattering measurements. Aggregates of about 100 molecules and 10 nm in diameter solubilise within their core the water-insoluble drug clofazimine, and can increase its solubility by a factor of 30–50, within limits set by the solubility of the CD itself.

Acknowledgements

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