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Differential Complexing by Cyclodextrin Twin-cavity Hosts

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Abstract. The twin-cavity cyclodextrin (**1**) in which the link is a dithiotrehalosyl unit, and the flexibly-linked dimer (**2**) were shown to distinguish between the heterocyclic guests **3** and **4** (clofazimine drug) in spite of the guests' small structural difference. Both cyclodextrin dimer hosts form 1:1 complexes with methyl orange and with **3**, as shown by double reciprocal plots of UV-absorbance change and host concentration. However with **4**, both host molecules formed a 2 : 1 (host : guest) complex . Since both dimer cavities are used to create this effect, it is a new type of selectivity for macrocyclic hosts.

Key words: beta-cyclodextrin dimer, clofazimine complex, methyl orange complex, association constant

1. Introduction

Cyclodextrins are well known as macrocyclic water-soluble host molecules [1]. Two potential improvements on their behaviour have been subjects of research in recent years. Extension of the cavity with multiple groups has resulted in interactions with guest molecules which derive from a large effective cavity size [2]. Another approach has been to link two cyclodextrin molecules so that the two cavities can bind to substrates bearing two suitably spaced hydrophobic segments [3, 4]. Ethyl orange for example binds to a disulphide-linked dimeric β-cyclodextrin one hundred times better than to the natural cyclomaltoheptaose [5].

Previous bis-cyclodextrins have had either relatively short aliphatic links, rigid aromatic links, or double links, all of which restrict conformational possibilities. An expectation for a dual cavity is that it might operate as a single extended cavity and show selectivity deriving from different, fairly stable orientations of the two components. This selectivity could be brought about by a link such as a sugar unit which has preferred conformations. Alternatively, a twin-cavity host with a long flexible link might show the same talent if enthalpy of inclusion predominates over entropy. We have synthesised the twin-cavity oligosaccharide (**1**) in which the link is a C_2-symmetric dithiotrehalosyl unit and, for contrast, the flexibly linked

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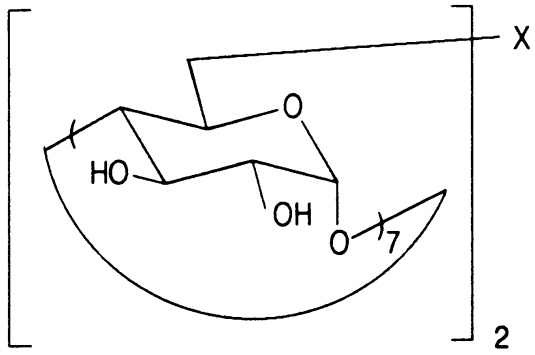
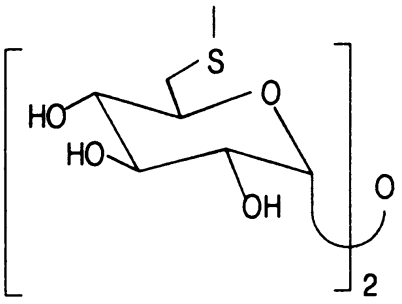
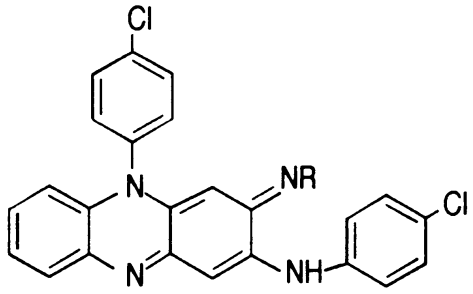


1 X =



2 X = $-S(CH_2)_8S-$



3 R = H

4 R = $CH(CH_3)_2$

Scheme 1.

dimer (**2**). These hosts show the ability to distinguish between the large heterocyclic guests **3** (clofazimine analogue) and **4** (clofazimine drug) in spite of the guests' small structural difference. We have demonstrated that this is a new type of macrocyclic host selectivity derived from the twin-cavity receptor design, since both cavities are used in the formation of a 1 : 1 complex with the NH-compound, but not with its *N*-isopropyl derivative.

2. Experimental

The cyclodextrin dimers were synthesised by methods similar to those already used by us to synthesise *S*-glycosyl-thiocyclodextrins, where the key step is a displacement reaction by thiolate on 6-monotosylated cyclodextrin [6]. In the syntheses used here, α,α-trehalose dithiolate and octane dithiolate, with two equivalents of cyclodextrin tosylate, produced respectively dimers (**1**) and (**2**). The structures of the dimers were confirmed by NMR, FAB-MS and microanalysis [7].

Clofazimine and clofazimine analogue were synthesised by methods already described [8]. Methyl orange (Aldrich) was used as received.

Association constants K_a were measured from the linear regions of "double reciprocal" plots of increase in guest absorbance maxima versus concentration of cyclodextrin dimer. Guest concentrations were 3×10^{-5} M, which were within a range where no self-aggregation was detected by absorption measurements.

The association constants quoted for methyl orange complexation are apparent association constants, equivalent to the sums of the association constants for the protonated and tautomeric forms of the dye [9]. The large blue shift (about 60 nm) on formation of a 1 : 1 complex between a cyclodextrin and a dimethylaminophenylazo dye is attributed to a change in tautomeric equilibrium [10]. Such complexes are in fact mixtures of forms in which similar ends of the molecules are complexed. The assumption is made that the association constants are identical, and this simplification is also made here in discussing both methyl orange and clofazimines.

3. Results and Discussion

In order to confirm that the two cavities of a dimer could be involved in complexation, we compared dimer complexation kinetics with those of β-cyclodextrin. As guest molecules which might fill both cavities in a 1 : 1 complex we used methyl orange, the drug clofazimine (**4**) [8], and a clofazimine analogue (**3**). In tune with a study of hydrophobic binding, the polar sulfonic acid group of methyl orange is absent from the clofazimines. These are however soluble enough in water to show intense UV-absorption.

For complexation of β-cyclodextrin with methyl orange, 1 : 1 kinetics were observed at low host concentrations ($< 4 \times 10^{-3}$ M) and as reported elsewhere [7]. However at higher concentrations there was clear deviation from linearity in the

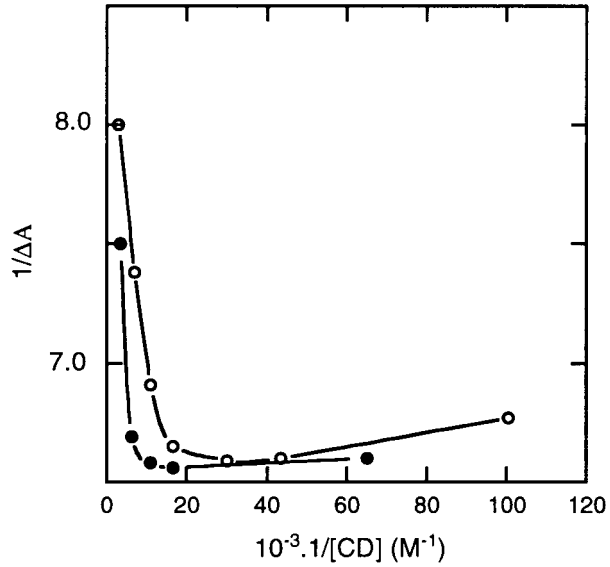


Figure 1. Double reciprocal plots for binding to β-cyclodextrin of: (a) (●) clofazimine **4**, (b) (○) clofazimine analogue **3**.

double reciprocal plot of absorbance change and host concentration, and the data analysed as an equilibrium between 1 : 1 and 2 : 1 (host:guest) complexes [9].

The double reciprocal plots obtained from β-cyclodextrin with clofazimine (Figure 1a) and from β-cyclodextrin with clofazimine analogue (Figure 1b) also show deviation from 1 : 1 kinetics. A better data fit (correlation factor 0.9) was obtained in each case for 2 : 1 complexation [9]. This is most likely to involve complexation of the chlorophenyl and chlorophenylamino groups, since from results with similar complexes formed by substituted naphthalenes [11], the fused aromatic rings are too hindered to complex.

In contrast to cyclodextrin, dimer **1** with methyl orange gave a 1 : 1 complexation profile even at high host-to-guest ratios (Figure 2a). This was expected from previous results with CD dimers. With clofazimine analogue (**3**) as guest 1 : 1 binding was also observed (Figure 2b). The binding constants for dimer **1** (Table) show that use of the neutral guest **3** does improve binding in comparison with methyl orange, however binding constants are still in the lower range for known dimers, since shape and size of the guest are not apparently optimal. The selectivity advantage of the twin cavity becomes apparent instead when these results are compared with results for clofazimine (**4**). With dimer **1** clofazimine produced a double reciprocal plot (Figure 2c) which showed sharp deviation from linearity. Analysis of the data proved it to be consistent with formation of a 2 : 1 complex. There is a significantly lower K_a value (7×10^4) (Table) for the 1 : 1 complex (formed at lower dimer concentrations) than for that formed by clofazimine analogue (2×10^5). In agreement with the concept that this 1 : 1 complex probably involves inclusion

Table I. Association constants in H_2O at 25° of 1 : 1 complexes

Host	Substrate	K_a, M^{-1}
β-CD	Methyl orange	4×10^2
	3	2×10^3
	4	1×10^4
Dimer **1**	Methyl orange	1×10^5
	3	2×10^5
	4	7×10^4
Dimer **2**	Methyl orange	1.5×10^5
	3	1×10^6
	4	2×10^4

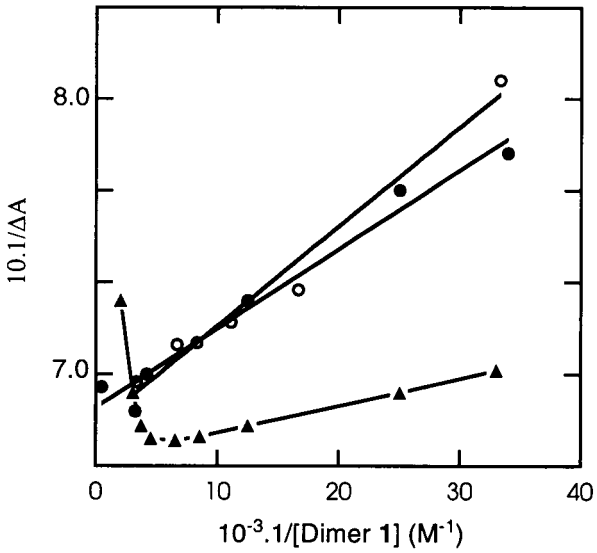


Figure 2. Double reciprocal plots for binding to cyclodextrin dimer **1** of: (a) (○) methyl orange, (b) (●) clofazimine analogue **3**, (c) (▲) clofazimine **4**.

only of one chlorophenyl group by one cyclodextrin cavity, the wavelength shift on inclusion is lower, as with cyclodextrin-clofazimine 1 : 1 complexation. The dual cavity therefore differentiates between two complex heterocycles (**3**, **4**) by virtue of the presence or absence of a single isopropyl group. It forms a strong 1 : 1 complex with the NH-compound by employing both halves of the dual cavity, but cannot do this with the *N*-isopropyl compound, which instead it includes using half the dual cavity.

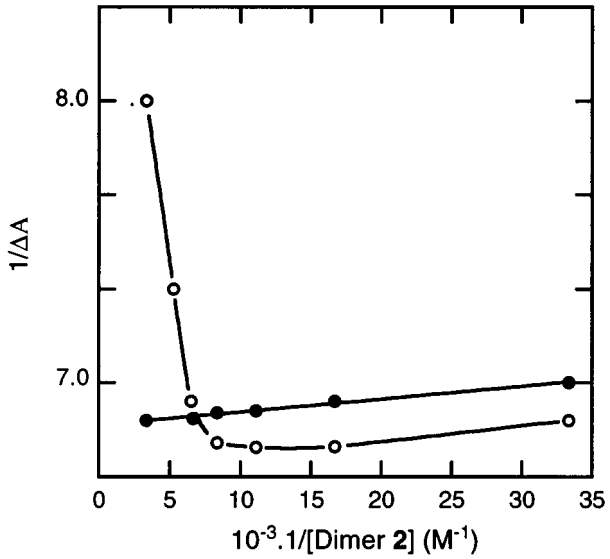


Figure 3. Double reciprocal plots for binding to cyclodextrin dimer **2** of: (a) (●) clofazimine analog **3**, (b) (○) clofazimine **4**.

Inclusion of both chlorophenyl rings can be visualised by taking into account the shape of clofazimine from X-ray crystallographic data [12]. The central chlorophenyl ring is perpendicular to the phenazine ring system, bringing it closer to the chlorophenyl-NH group. This conformation has the result that although clofazimine complexes with polynucleotides, it does not intercalate. The conformation of the chlorophenyl-NH group depends on the identity of the imino-nitrogen substituent, and has been correlated with biological activity [12]. It is possible that a similar effect by the imino substituent operates here; biological activity of **4** is much greater than that of **3**.

Comparison of dimer **1** with the flexibly-linked dimer **2** shows that a conformationally rigid link is less important here than the shape of the guest molecule. The dithio-octyl-linked dimer also differentiates between clofazimine and its analogue as evidenced by the respective double-reciprocal plots (Figure 3). The Table shows the much stronger cooperative binding of **3** ($K_a = 1 \times 10^6$) by the twin cavities than for inclusion of one chlorophenyl ring of **4** by one cavity ($K_a = 2 \times 10^4$). The possibility that this value represents weaker inclusion of both chlorophenyl rings by the cavities of one host molecule cannot be excluded. Breslow has shown the dominance of enthalpy over entropy in complex-formation by the two cavities in a series of cyclodextrin dimers [4]. The results for the flexibly linked dimer here agree with this and show that a design incorporating a rigid link may be more trouble to synthesise than it is worth.

4. Conclusion

Four distinct phenomena are expected to distinguish a dual-cavity host, namely: 1 : 1 semioccupancy by a guest; 1 : 1 binding of a guest which occupies both cavities; 1 : 2 binding of two guest molecules; and 2 : 1 binding. In the last case, the K_{11} values for dual-cavity inclusion must be small, and the guest must be so large that the two dimeric cyclodextrins do not hinder each other. This criterion is apparently fulfilled when either of the cyclodextrin dimers **1** or **2** complexes with clofazimine.

Previous studies on dimeric cyclodextrin complexation have concentrated on ditopic guest molecules where the deciding factor for good binding has been the distance between the two guest segments. Differential complexation of the clofazimines by dimers **1** and **2** is therefore the first demonstration of dimer selectivity as receptors towards guests with minor structural differences, and these dimers operate in a way which is not possible for CD-monomers. Since both dimer cavities are used to create this effect, it is a new type of selectivity for macrocyclic hosts in general.

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