Enantioselective Complexation of Bilirubin with Cyclodextrins and Non-cyclic Oligosaccharides

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Circular dichroism spectra indicate that intermolecular hydrogen bonding plays an essential role in enantioselective binding of bilirubin to cyclodextrins and non-cyclic oligosaccharides.

Bilirubin (BR) is a cytotoxic pigment observed in jaundice,[1] and can assume enantiomeric conformations with *R*- and *S*-helices *via* intramolecular hydrogen bonding.[2] BR bound to sodium deoxycholate[3] and albumins[4] is known to show bisignate circular dichroism (c.d.) spectra in water. Recently, Lightner and his co-workers found that α-, β-, and γ-cyclodextrin (α-, β-, and γ-CDX) also induce the bisignate c.d. Cotton effect; this is attributed to an exciton coupling interaction of the chiral bichromophoric pigment bound to the CDX.[5] Although these results are of biological importance, the mechanism of chiral recognition has not yet been clarified. We report here new aspects of the mechanism for enantioselective binding of BR to cyclic and non-cyclic oligosaccharides.

C.d. spectral measurements of BR (5×10^{-5} M) in aqueous saccharide solutions [pH 10.8 (NaOH)] are summarized in Table 1. As Lightner *et al.*[5] have revealed, BR in aqueous β- and γ-CDX solutions showed bisignate c.d. spectra: Δε at longer wavelength ($\Delta\varepsilon_1$ at λ^1_{ext}) is negative and that at shorter wavelength ($\Delta\varepsilon_2$ at λ^2_{ext}) is positive. The exciton-coupling theory predicts that an enantiomer of BR having an *S*-helix conformation is bound preferentially to CDX. The c.d. signal markedly diminished when heptakis-(2,6-di-*O*-methyl)-β-CDX (DM-β-CDX) was used in place of β- or γ-CDX. No distinguishable c.d. signal was observed for the BR–heptakis-(2,3,6-tri-*O*-methyl)-β-CDX (TM-β-CDX) system. These results imply intermolecular hydrogen bonding. Maltoheptaose, an open-form β-CDX, also formed a chiral complex with BR; its c.d. behaviour was almost the same as that of the β-CDX complex. It can be concluded, therefore, that the lipophilic cavities of CDX are not essential for preferential binding of *S*-helix BR to CDX.

D-Glucose, a monosaccharide and a unit which can form CDX, did not induce any c.d. signal. All disaccharides used in which the pyranose residues were connected by (1 → 4) linkages recognized the chirality of BR. An enantiomer of BR having the *S*-helix conformation is bound preferentially to maltose, which is an α-(1 → 4)-disaccharide. Interestingly, cellobiose– and cellotetraose–BR complexes exhibited more intense but oppositely signed c.d. signals. Cellobiose and cellotetraose are β-(1 → 4)-linked saccharides and prefer to bind the *R*-helix BR molecule rather than the *S*-helix. The enantioselectivity of disaccharides, however, is confused by the result obtained for lactose, the residues of which are connected by a β-(1 → 4)-galactoside linkage: we found that in

Table 1. C.d. spectral data for bilirubin in various saccharide solutions at pH 10.8.

Saccharide	λ^1_{ext}/nm	$\Delta\varepsilon_1$/l mol^{-1} cm^{-1}	λ^2_{ext}/nm	$\Delta\varepsilon_2$/l mol^{-1} cm^{-1}
β-CDX (0.01 M)	455	−9.0	405	+5.1
γ-CDX (0.01 M)	455	−10.4	405	+7.3
DM-β-CDX (0.01 M)	435	+1.8	378	−2.1
TM-β-CDX (0.01 M)		no Cotton effect		
D-Glucose (2.0 M)		no Cotton effect		
Maltose (0.1 M)	455	−1.2	395	+0.6
Maltose (0.5 M)	455	−2.1	400	+1.8
Maltose (1.0 M)	460	−3.5	397	+2.4
Maltotriose (0.1 M)	455	−3.6	402	+1.8
Maltoheptaose (0.01 M)	458	−5.5	408	+4.1
Cellobiose (0.1 M)	455	+7.6	405	−4.8
Cellotetraose (0.025 M)	455	+1.5	408	−0.9
Lactose (0.1 M)	455	−7.3	405	+5.2
Gentiobiose (0.1 M)	450	+1.5	*ca.* 400	*ca.* 0

this case the *S*-helix conformer of BR was preferred as guest molecule. On the basis of these results, it became obvious that a (1 → 4)-linked disaccharide is the minimum requirement for recognition of the chiral BR conformers, and that the enantioselectivity is very sensitive to the conformation of the disaccharide. A very weak c.d. signal was observed for the BR–gentiobiose (0.1 M) system. The two D-glucopyranose residues of gentiobiose are linked by a β-(1 → 6)-glucoside linkage and the residues are further apart than in the (1 → 4)-linked disaccharides. It seems, therefore, that at least two neighbouring hydroxy groups attached to the separate saccharide residues participate in the enantioselective complexation of BR with saccharides.

The c.d. signal intensity increased in the order maltose < maltotriose < maltoheptaose; this may be ascribed to the difference in the binding constants of these saccharides. The hydrophobic environment provided by saccharides seems to play an important role in the formation of hydrogen bonds.

The present system is one of the simplest models for molecular recognition by saccharides. Chemical modifications of saccharides may make it possible to understand the complete mechanism for enantioselective binding of BR. Such studies are in progress.

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