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Molecular Recognition by Saccharides. Asymmetric Complexation between Bilirubin and Nucleosides

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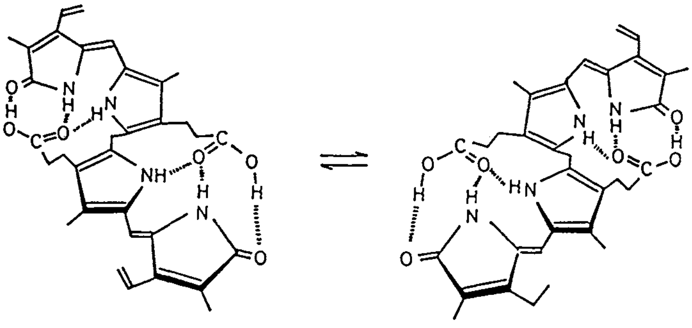
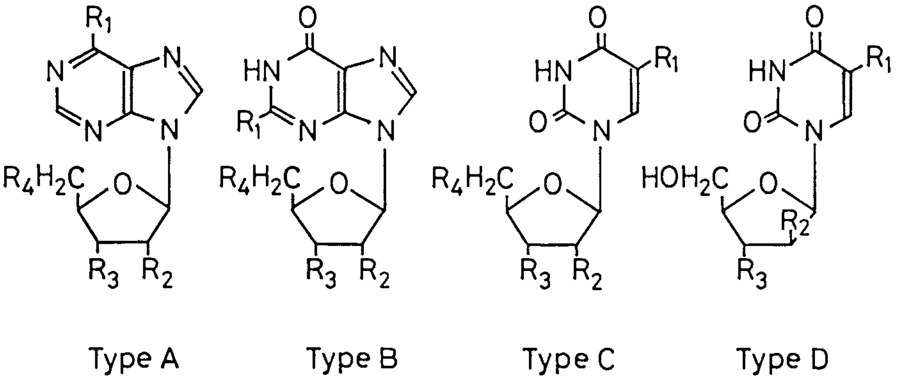
Asymmetric complexation between bilirubin (BR) and nucleosides has been studied in water (pH 10.8) by UV-vis absorption and circular dichroism (CD) spectroscopy. The complexes of BR with ribo- and deoxyribonucleosides show the plus to minus bisignated CD signals, which indicate that BR in these complexes selectively takes the (R)-helix conformation. Thymine and uracil arabinosides exhibit the oppositely signed CD spectra. A multi-point attachment model may explain the asymmetric complexation.

(4Z,15Z)-Bilirubin IX (BR) is a dipyrromethenone compounds generated from a catabolism of heme and two pyrromethenone chromophores are linked each other by a sp^3 carbon at the C-10 position. Although BR does not have any chiral center, intramolecular hydrogen bonds provide two enantiomeric conformations:[1)]



(S)-helix BR (R)-helix BR

Of course, BR is not optically active in homogeneous solution and a fast interconversion is expected between the (R)- and (S)-helix conformers. BR has been known, however, to become optically active when it binds to deoxycholate micelles,[2)] albumins,[3)] cyclodextrins,[4)] and noncyclic oligosaccharides.[5)] In a previous communication, we inferred the intermolecular hydrogen bonding as a main binding force for forming the optically active BR-oligosaccharide complexes.[5)] Although our previous observation is very interesting because one of the enantiomers of BR is selectively bound to the simple saccharides such as maltose and cellobiose through hydrogen bonding in water, we could not specify the binding sites as in other cases.[2-4)] Then we chose nucleosides as the host saccharides in this study. Nucleosides are expected to be suitable hosts for studying the mechanism of enantioselective complexation of BR because (1) their structures are fairly simple, (2) nucleosides have several sites for hydrogen bonding, and (3) various nucleosides are easily available.



Complexation of BR (5×10^{-5} mol dm^{-3}) with nucleoside (5×10^{-3} mol dm^{-3}) was studied by measuring UV-vis absorption and circular dichroism (CD) spectra in water at pH 10.8 (NaOH) at room temperature. Upon complexation, the optical density around at 440 nm, which corresponds to λ_{max} of the BR dianion,[6] decreases. 2-Deoxy-D-ribose did not induce any CD signal. When the ribo- and deoxyribonucleosides were used in place of 2-deoxy-D-ribose, fairly intense bisignated Cotton effects were measured as indicated in Fig. 1 and Table 1. The absolute configuration of BR bound to a nucleoside can be determined easily by using an exciton

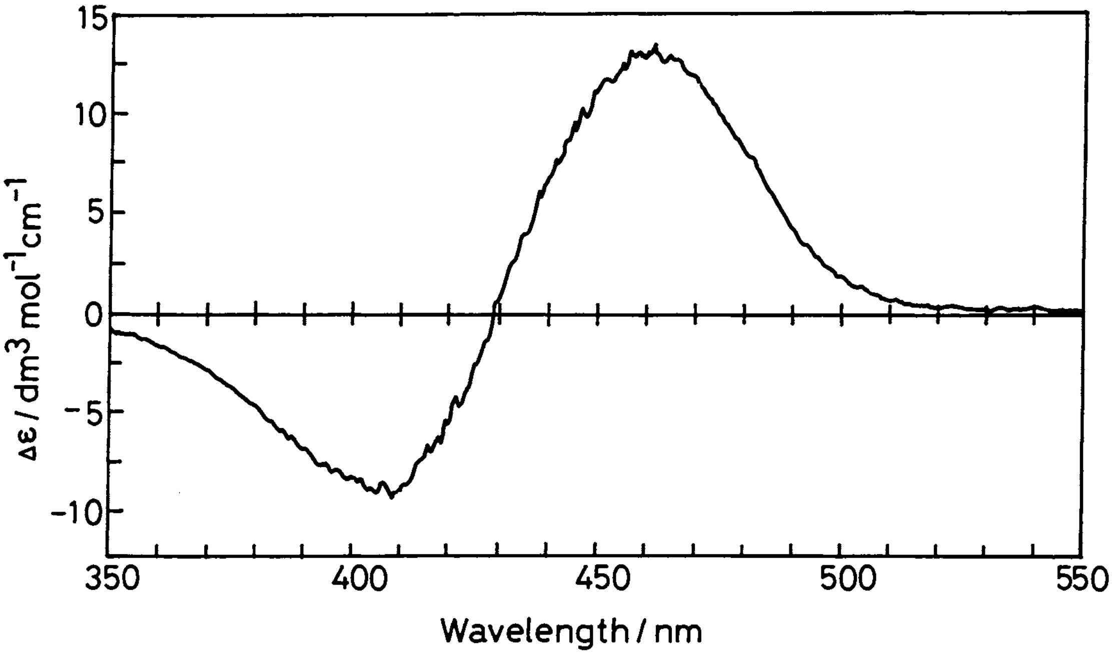


Fig. 1. CD spectrum of BR (5.0×10^{-5} mol dm^{-3}) in water containing 6-chloropurine riboside (5.0×10^{-3} mol dm^{-3}).

Table 1. CD Spectral Data for Complexation of BR with Nucleosides and Nucleotides in Water at pH 10.8

						$\Delta\varepsilon/dm^3mol^{-1}cm^{-1}$	
Type	Abb.	R_1	R_2	R_3	R_4	$\Delta\varepsilon_1$ (nm)	$\Delta\varepsilon_2$ (nm)
A	ClPR	Cl	OH	OH	OH	13.3 (460)	-11.5 (407)
A	PR	H	OH	OH	OH	9.7 (458)	-5.8 (406)
A	MTAdo	NH_2	OH	OH	SCH_3	6.8 (460)	-3.9 (411)
A	Ado	NH_2	OH	OH	OH	5.8 (457)	-3.8 (407)
B	Ins	H	OH	OH	OH	5.5 (458)	-3.3 (405)
A	3'-dAdo	NH_2	OH	H	OH	4.7 (458)	-2.3 (405)
B	dIns	H	H	OH	OH	4.5 (453)	-1.8 (404)
B	dGuo	NH_2	H	OH	OH	4.2 (455)	-2.2 (405)
A	dAdo	NH_2	H	OH	OH	3.1 (457)	-0.8 (407)
C	dThd	CH_3	H	OH	OH	2.5 (450)	-0.8 (400)
C	dUrd	H	H	OH	OH	2.1 (450)	-0.8 (400)
C	TR	CH_3	OH	OH	OH	1.5 (455)	-0.7 (405)
D	UA	H	OH	OH	OH	-1.5 (462)	1.8 (407)
D	TA	CH_3	OH	OH	OH	-1.1 (455)	1.8 (410)
A	5'-AMP	NH_2	OH	OH	OPO_3^{2-}	no bisignated CE[a)]	
A	dAMP	NH_2	H	OH	OPO_3^{2-}	no bisignated CE[a)]	
B	5'-GMP	NH_2	OH	OH	OPO_3^{2-}	no bisignated CE[a)]	
B	dGMP	NH_2	H	OH	OPO_3^{2-}	no bisignated CE[a)]	
C	Urd	H	OH	OH	OH	no bisignated CE[a)]	

a) Abbreviation CE means Cotton effect.

coupling theory.[1,7)] Namely if a BR-nucleoside complex shows a (+)-signed maximum at the longer wavelength transition and a (-)-signed maximum at the shorter wavelength transition,[8)] BR should take an (R)-helix conformation (vice versa). All complexes of BR and ribo- and deoxyribonucleosides revealed the (+) to (-) bisignated Cotton effect, indicating that BR selectively takes the (R)-helix conformation in the complexes of these nucleosides. On the other hand, the complexes of thymine (TA) and uracil arabinosides (UA) show the weak (-) to (+) bisignated CD signals, suggesting that these nucleosides selectively form the complexes with BR having the (S)-helix conformation.

Although we cannot define the binding force for the complexation between BR and nucleosides from the present results, it may be reasonable to consider the hydrogen bonding as the main binding force. It is noteworthy that the CD intensity decreases in the order of 6-chloropurine riboside (ClPR), purine riboside (PR), and adenosine (Ado). This means that the nitrogen atoms at the N-1 and N-3 positions as well as the NH_2 group attached at the C-6 position of Type A nucleosides do not participate in the hydrogen bonding in the asymmetric complexation. The nitrogen atom at the N-7 position, therefore, may accept a hydrogen from BR to form a hydrogen bond. Since BR takes the dianion form under the experimental conditions (pH 10.8), the OH groups in the ribose or deoxyribose residues seem to act as the hydrogen donors to form a hydrogen bond with the propionate anion of BR. The result obtained for 5'-deoxy-5'-methylthioadenosine (MTAdo) indicates that the 5'-OH group of Type A nucleosides does not serve as a hydrogen donor to form a hydrogen bond in the asymmetric complexation. Comparison of the results obtained for Ado, 3'-deoxyadenosine (3'-Ado), and 2'-deoxyadenosine (dAdo) leads to a conclusion that the 2'-OH group participates in the asymmetric complexation with BR more effectively than the 3'-OH group. The significant role of the 2'-OH group is also revealed in the complexation of BR with TA and UA whose 2'-OH groups are in the exo positions relative to the base residues. The chirality of BR bound to these arabinosides is opposite to that of BR bound to the ribo- and deoxyribonucleosides.

The important role of the OH group bound to the asymmetric carbon of the ribose or deoxyribose residues is also suggested from the results of the complex formation between BR and nucleotides. Although the UV-vis absorption spectra indicate the formation of the complexes, no bisignated Cotton effect was observed for the BR-nucleotide systems. The strong ability of the monophosphate dianion, which is attached to the achiral carbon at the C-5' position, to form hydrogen bond may provide the racemates of the BR-nucleotide complexes.

NMR and/or Raman spectroscopic studies should need to clarify the detailed mechanism for the asymmetric complexation between BR and nucleosides. Such studies are now in progress.

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5) K. Kano, K. Yoshiyasu, and S. Hashimoto, J. Chem. Soc., Chem. Commun., 1988, 801.
6) The pK_2 of BR has been estimated to be 9.1-9.8. See: J. D. Ostrow, L. Celic, and P. Mukerjee, J. Lipid Res., 29, 335 (1988).
7) N. Harada and K. Nakanishi, "Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry," University Science Books, Mill Valley, California (1983).
8) Such bisignated Cotton effect will be called as (+) to (-) bisignated Cotton effect.

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