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New Aspects of Cyclodextrin Chemistry Induced by Outside Type Complex Formation; Asymmetric Reduction of Indol-3-Pyruvic Acid with $NaBH_4$ in Aqueous Solution

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Abstract

Asymmetric reduction of indol-3-pyruvic acid (IPA) with $NaBH_4$ in aqueous solution in the presence of various cyclodextrins (α-, β-, γ-, mono-6-amino-6-deoxy-β- and di-6^{AB}amino-6^{AB}-deoxy-β-cyclodextrin) was investigated. From the NMR and circular dichroism spectral studies, the conformation of the CyD–substrate complexes is suggested; the part of carboxylic group stay in the cavity of α-CyD, whole of IPA in β-CyD, two molecules in a γ-CyD cavity, and IPA(s) is/are on the rim of the cavity of mono-6-amino-6-deoxy-β- and di-6^{AB}amino-6^{AB}-deoxy-β-CyD (AβCyD, DAβCyD) with electrostatic interaction between amino group and carboxylic group. This conformational difference provides in the difference in the optical selectivity of reduction.

Introduction

Outside complex with cyclodextrin (CyD) has reported to indicate unique functions as it as usual inside complexes formation [1]. The advantages of outside complex type are the effect of hydrogen bonding at the rims of the cavity and the micro surface effect. In other hand, many organic reactions mediated by CyDs without covalent bond have been known. The hydrophobic cavity of the CyD gives the reactant access to a new reaction environment, an 'extra reaction field' in which the reactivity, such as rate or selectivity, changes. In these cases, the role of the CyD is not always defined as catalyst. More correctly, the CyD mediates the reactions. Many reports have been published on CyDs and the reactions they catalyze. $NaBH_4$ is the commonly used reduction reagent in aqueous solutions; however, it possesses poor selectivity. The reduction of ketones using CyD crystalline complex is a useful and simple route to chiral alcohols. Using pre-crystalline methods, over 90% ee has been reported [2]. In aqueous or organic solvent, the reagent or substrate still can form a host–guest complex through specific interaction with CyD. Modification of the CyD by attachment to a new interaction point or a change in the hydrophobic cavity can result in significant ee in an asymmetric reaction. Greater enantioselectivity in the reduction of benzoylformic acid (BFA) by $NaBH_4$ has been reported using 6-amino-6-deoxy-β-CyD (AβCyD) as the host instead of β-CyD in neutral aqueous buffer solution (Scheme 1) [3]. The phenyl group is used to control cavity size and the sp [3] carbons between the parent CyD cavity and the phenyl group act as a flexible arm. Asymmetric reactions take upon approaching from only one side face. The rim of CyD cavity has much more differences than inside cavity. We wish to indicate here, high enantioselective redox reaction from indol-3-pyruvic acid (IPA) to D or L-indole-3-lactic acid (ILA) with $NaBH_4$ in aqueous solution mediated modified CyD through outside complex formation. This phenomenon is good example for outside advantage of CyD chemistry.

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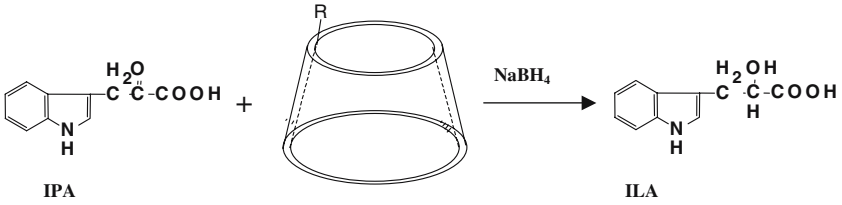
Experimental

General

Materials

α-, β-, and γ-CyD were purchased from Nihon Shokuhin Kako Co. The commercial samples of CyDs were purified by recrystallization from water. Indole-3-pyruvic acid (IPA) (ALDRICH), DL-indole-3-lactic acid (ILA) (ALDRICH) and $NaBH_4$ (Wako Co.) were used without purification.



Scheme 1. Redution of IPA with $NaBH_4$ mediated cyclodextrin.

Preparation of modified CyDs

6-Amino-6-deoxy-β-CyD (AβCyD) was prepared from 6-tosyl-β-CyD according to the previous report. Di-6^{AB}amino-6^{AB}-deoxy-β-CyD (ABAβCyD); Dry β-CyD and 1,3-benzene-disulfonyl chloride were dissolved in dry pyridine and stirred 1.5 h at 60 °C. After the usual treatment, the crude product was purified by hydrophobic chromatography attached with HP-20. 6^{AB}-1,3-benzene-di-sulfonyl-β-CyD (AB capped-CyD) was obtained. AB capped-CyD was dissolved in water and reacted with NaN_3 and then treated H_2 with Pd/C. The reaction mixture was treated with usual process, white powder was obtained (yield: 6.3%). ^{1}H-NMR (500 MHz, D_2O, ppm): δ1.40 (s, 9H, *tert*-butyl), 3.25–4.09 (br, 44H, H2, H3, H4, H5 and H6 of β-CyD and -CH_2– of glycine), 4.91–5.08 (br, 7H, O–1H of β-CyD), MS (FAB^-) *m*/*z*, 1134. TLC; Rf = 0 (eluent; BuOH:EtOH:H_2O:NH_3 = 5:4:3:1).

Measurements

NMR spectra were measured on a JEOL Lambda 500 spectrometer (500 MHz) in D_2O using acetone as internal reference (2.100 ppm) at room temperature NOESY and ROESY spectra were measured with mixing times of 500 ms. Fluorescence spectra were taken on a JASCO FP-770. UV spectra were taken on a JASCO UVDEC-660 or V-570. The circular dichroism spectra were measured on a Jasco J-820 spectropolarimeter at room temperature.

pH Titration of IPA

pKa Of IPA was determined from pH titration measuring UV adsorption. IPA was dissolved in buffer solution (2×10^{-4} mol/L). From the titration curve of absorbance at 330 nm against pH from 2 to 8, pKa of IPA was calculated as 2.87 (Figure 1).

Asymmetric reduction

A CyD derivative (2.5×10^{-5} mol) and the guest (6.25×10^{-6} mol) were dissolved in Tris–HCl buffer (pH 8.13), and stirred for 1 h in an ice bath (below 3 °C). Measuring the absorbance at 305 nm followed the reduction. The reaction was initiated by addition of excess molar of $NaBH_4$. After the kinetic measurement, it was confirmed by analytical HPLC using an Enantio L1 optical resolving column (0.14×150 mm: TOSO) eluted with aqueous 20 mM KH_2PO_4 solution (flow rate 0.7 ml/min) and detected with UV (280 nm). The chemical yield of each reduction was almost 100%.

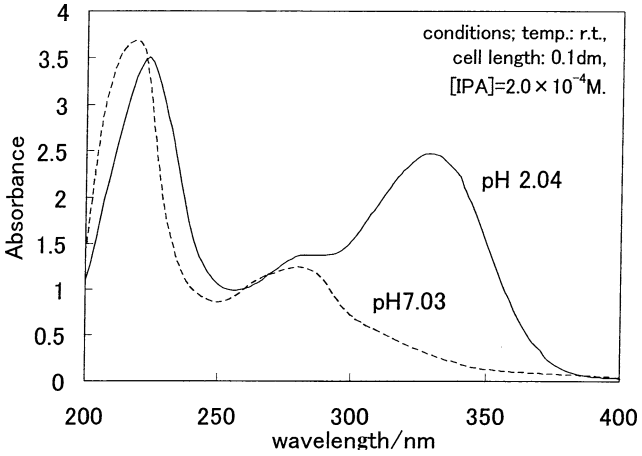


Figure 1. UV spectra of IPA (2×10^{-4} M). Temperature: r.t., cell length: 0.1 dm.

Results and discussion

Asymmetric reactions with various CyDs

The reduction reaction rate from IPA to ILA was accelerated by addition of β-CyD, AβCyD and DAβϖ CyD, however, decelerated by adding of α-CD. Adding of γ-CyD gave no effect on the reaction rate. Table 1 shows the enantio excess of the reaction. Higher enantioselectivity are observed with AβCyD and DAβϖ CyD. With native CyDs, α-, β- or γ-CyD, gives low enantioselectivity.

Circular dichroism spectra

Whenever a chromophore is not optically active, induced circular dichroism (ICD) is observed in a chiral CyD cavity. Figure 2 shows the CD spectra of IPA in neutral aqueous solutions with and without various CyDs. Without CyD, there is no elliplicity both in

Table 1. Asymmetric reduction in the presence of various CyDs with $NaBH_4$

Substrate	CyD	Optical yield%/ee	Configuration
IPA	None	3	S(L)
	α-CyD	1	S(L)
	β-CyD	6	S(L)
	γ-CyD	0	–
	AβCyD	17	R(D)
	DAβCyD	33	R(D)

neutral and acidic conditions. In pH 7 buffer solutions, adding of AβCyD induced positive ellipticity and Cotton effect peaked around 217 and 312 nm, respectively. When DAβCyD was adding, strong Cotton effects were observed peaked at same wavelength, which suggests two IPA molecules associate in the CyD cavity. In acidic solution (pH 2), the maximum point is shifted to a longer wavelength owing to change of emission spectra. The Cotton effect peaked at 330 nm induced by DA$\beta\varpi$ CyD is disappeared. Normal positive ICD was observed. With β-CyD, γ-CyD or AβCyD, positive ICD were observed. α-CyD could not indicate ICD (Figure 3). Applying of the general rule, the indol group of deprotonated IPA is located outside the CyD cavity staying parallel to the rim of the AβCyD and DAβCyD cavity, while the indol group is included in β-CyD and γ-CyD cavity. The cavity size of α-CyD is too narrow to include indol group. The COOH group stays in the cavity. The binding constant between γ-CyD and IPA could not calculate from dependency of concentrations

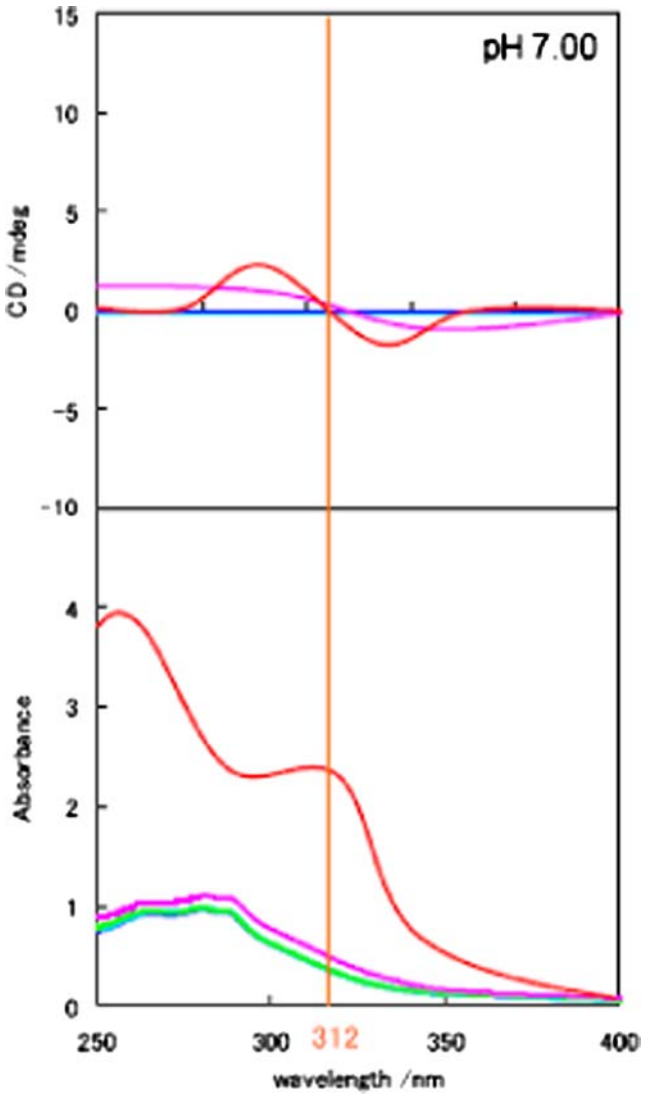


Figure 2. Circular dichroism spectra of IPA at pH 7.00. Conditions: pH 7.00, r.t., cell length: 0.02 dm, [IPA] = 1.0×10^{-4} M, [CyD] = 5.0×10^{-3} M.

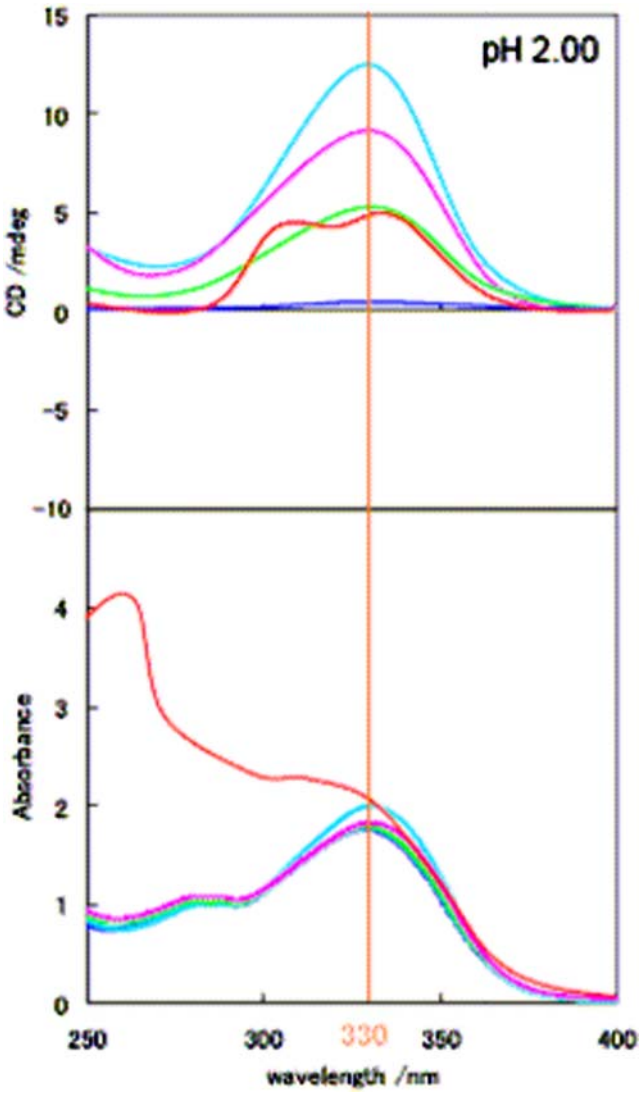


Figure 3. Circular dichroism spectra of IPA at pH 2.00. Conditions: pH 2.00, r.t., cell length: 0.02 dm, [IPA] = 1.0×10^{-4} M, [CyD] = 5.0×10^{-3} M.

as 1:1 host–guest complex formation. Since γ-CyD has wide cavity, it would include two IPA molecules forming opposite conformation each other. This is the reason why weak ICD. This result suggests that the complex formation of IPA is affected by deprotonation of the carboxyl group.

NMR spectra

The NMR studies have provided important information concerning the molecular geometry of the CyD inclusion complexes [4, 5]. Figure 4 shows the NMR spectra of IPA with excess equivalent molar of CyDs in D_2O. Assignments of each proton were carried out by COSY and NOESY methods. The chemical shifts assigned to indol group with α-CyD are almost the same as those without CyD. Interestingly, chemical shift of carboxyl group is observed between 9 and 10 ppm. The result suggests that the carboxyl group stays in a hydrophobic environment and is protected from bark water molecule, whereas indol group is out of cavity. With γ-CyD,

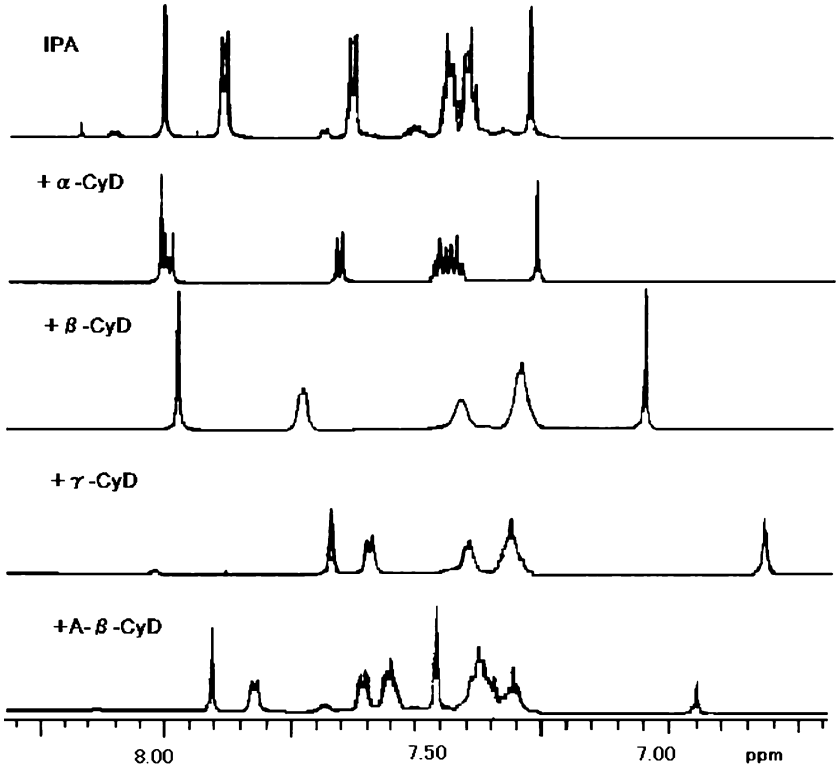


Figure 4. ^{1}H-NMR spectra of IPA in D_2O with and without various CyD. Up to bottom; without CyD, with α-CyD, β-CyD, γ-CyD and AβCyD.

proton resonance of carboxyl group is also observed around 9 ppm. However, improvement low field shift of the protons assigned as indol group is observed. Adding of β-CyD, high field shift of indol group, H3 and H5 protons on the glucose ring of CyD ring are observed.

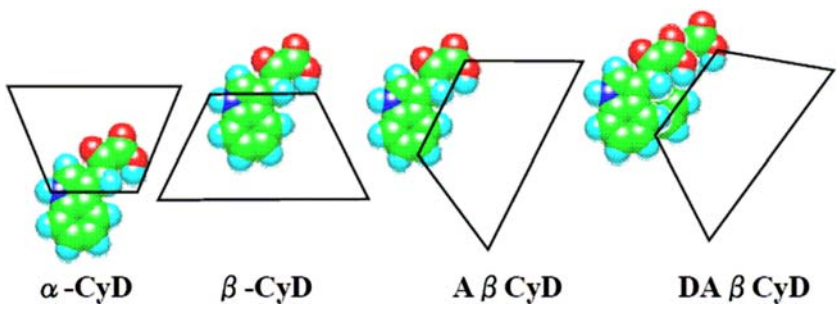
Differences on conformation and the correlation to reduction reaction

Adding with amino group modified CyDs (AβCyD and DAβCyD), the chemical shifts assigned to the indol group shifts to high field and are observed in many well-resolved sharp peaks. The split width and shift with DAβCyD are more than those with AβCyD. The cross peak between protons staying inside cavity (H3 and H5) and indol group were observed clearly only in the case of β-CyD.

Based on the results of the NMR and CD spectra, the conformational difference of each CyD and IPA are suggested as below; under neutral pH conditions, in which the carboxyl groups are protonated, part of the carboxylic groups included into the cavity of α-CyD, the whole IPA is in β-CyD, two IPA molecules in a γ-CyD cavity. On the other hand, IPA stays outside the cavity on the rim of the AβCyD and DAβCyD (2:1 complexation). Under acidic conditions, conformation of the inclusion complex with β-CyD, AβCyD and DAβCyD are almost similar conformation. The advantage of the two amino-CyD derivatives depends upon the ionic interaction between the amino acid and carboxylic acid. This conformational variation provides the difference in the optical selectivities of reduction (Scheme 2).

Conclusion

In the case of an oval-shaped substrate such as IPA, which cannot participate in self-inclusion but forms a complex outside the cavity near the rim of CyD, high enantioselectivities were observed with almost all



Scheme 2. Asymmetric reduction mediated CyD carried out nearby the rims of CyD.

amino-CyD derivatives, irrespective of the substituent position. The greatest enantioselectivity was observed using with DAβCyD. Conformation of the CyD–substrate complexes was determined from NMR and circular dichroism spectral studies. Since the reduction reaction of IPA proceeded in excess molar concentration of CyD, not only 2:1 but also 1:1 complex formation might be existed in DAβCyD system. Higher enantioselectivity should be carried out 2:1 complex system.

The early stages in CyD chemistry suggested us a very impressive concept of host–guest complex, which has been described as a ball in a bottomless pail. We have suggested here, that the ball, or 'guest,' is not always deeply embedded into the pail cavity. Depending on the solvent and the nature of host and guest, the combination of intermolecular interactions, including steric fit, van der Waals forces, dipole–dipole interactions, and hydrogen bond, play significant roles in guest molecular recognition. The 'rims' of the cavity also influence interactions. CyDs contain several hydroxyl groups on the rim of the cavity. CyDs demonstrate the role of hydrogen bonding in aqueous solutions. The results mainly involve no natural systems, however, future investigations of CyDs should provide a new world of CyD as a supramolecule based on hydrogen bonding in the presence of the water molecules, and thus reveal important information on the role of hydrogen bond in mechanisms of natural biosystems.

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