  Bull. Chem. Soc. Jpn., 65, 2690—2696 (1992) [Vol. 65, No. 10

Enantioface Differentiating Reduction of Keto Acid in the Presence of 6-Deoxy-6-amino-β-cyclodextrin with $NaBH_4$ in Aqueous Media

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(Received May 30, 1992)

The formation of an inclusion compound and the interaction of both ionic and hydrogen bonding were shown to be indispensable in the asymmetric reduction of keto acid in the presence of 6-deoxy-6-amino-β-cyclodextrin with $NaBH_4$ in an aqueous buffer solution. Furthermore, multiple interactions were elucidated and the enantioface differentiating reduction was induced in the concerted interactions. A mechanistic scheme for the enantioface differentiating attack of a reducing-reagent on the included substrate was suggested.

Many biologically related substances (for example, amino acids, peptides, steroids, alkaloids, and terpenes) are optically active compounds. A useful compound is often either one of the enantiomer pairs. In terms of the enzymatic reaction process, an asymmetric selective reaction has been realized. Such a natural high selectivity[1,2] was obtained through such procedures as an ion interaction, an electronic charge-transfer interaction, a hydrophobic interaction, and a van der Waals interaction. They play concerted roles and a recognition on the molecular level occurs, the molecular geometries in the mutual positional and orientational relationships are determined and, consequently, the product of either enantiomer configuration is favored. Accordingly, the application of a host-guest complex should make it possible to form asymmetric compounds that are analogous to an enzyme reaction in a natural system.

The first attempt to recognize molecules with a host compound was carried out by Cram et al[3,4] using a crown ether that incorporated optically active binaphthyl derivatives. The asymmetric reduction of the aromatic ketone was carried out in the presence of crown ether[5] and an asymmetric reduction in the presence of a certain type of protein.[6,7] There was a report that showed an optical yield of 20—80% e.e. with the reduction of an aromatic ketone using bovine serum albumin (BSA) as a matrix for this kind of attempt. Also, the asymmetric selectivity of the keto acid of optically active cyclophan was demonstrated by Koga et al.[8] For an example using cyclodextrin (CD),[9,10] an asymmetric reaction in a solid crystaline complex by Sakuraba et al. gave 91% e.e. in one case.[11] Further, a system with an aromatic ketone used by Fornasier et al. gave 36% e.e. at maximum.[12] Both short reports showed a high possibility for use as a "Microreactor" reaction vessel at a crystaline complex for asymmetric reduction using a cavity of cyclodextrins.

Generally, the use of $NaBH_4$ in an aqueous solution has the advantage of reducing functional groups only mildly and selectively. However, $NaBH_4$ does not have an asymmetric center in its molecule. The design of an asymmetric field to carry out asymmetric reactions with inclusion using the modified cyclodextrin was the key target of the present research.[13] Thereupon, in this research a host-guest inclusion phenomenon was emphasized for the molecular recognition capability for asymmetric differentiating with multiple interactions between the host and guest in an aqueous media. An examination was carried out for the purpose of controlling the asymmetric reaction, as well as simulating an enzyme reaction and evaluating the function of modified cyclodextrin to realize a "Microreactor" at the molecular level, even in an aqueous media. By using benzoylformic acid (BFA) of an aromatic keto acid included into the cavity of 6-deoxy-6-amino-β-CD (ACD), one amino group at the C-6 pyranose ring of β-CD was obtained. The amino group was expected to increase the interaction due to the electrostatic force.

Experimental

Reagent: 2-naphthoylformic acid (2-NFA) was synthesized according to a method described in a previous report.[14] The yield was 35%. ^{13}C NMR δ=191 (>C=O), 170 (>C=O). *p*-Hydroxybenzoylformic acid (pHBFA) was purchased as sodium salt of 99% purity from Aldrich. Phenylpyruvic acid (PPA) and BFA from Tokyo Kasei Co., and the other reagent from Wako Pure Chemicals Co., were used without any further purification. α-, β-, and γ-cyclodextrins, which were gifts from Japan Meize Product Co., were recrystallized from water. 6-Deocy-6-amino-β-CD (ACD) and 6-deoxy-6-amino-α-CD (A-α-CD) were synthesized according to previous reports.[15] ACD; yield 15%, $[\alpha]_D^{25}$ +117 (c=0.4, H_2O), Anal. Calcd for $C_{36}H_{64}NO_{31}$: C, 42.91; H, 6.42; N, 1.39%. Found: C, 42.89; H, 6.47; N, 1.37%. ^{13}C NMR δ=103 (C1), 82 (C4), 74 (C2), 73 (C3), 72 (C5), 61 (C6).

Reduction Conditions: A typical run for the reduction reaction was as follows. ACD (0.38 g, 0.25 mmol) was completely dissolved in 22 ml distilled water, and 0.038 g (0.25 mmol) BFA was dissolved in 3 ml water. Both of the solutions were then quickly mixed. At an ice-cold temperature, 25 ml of ice-cooled 70 mM (pH 7.0) (1 M=1 mol dm^{-3}) phosphoric acid buffer solution was added and stirred for one hour at 0±2 °C. After adding 0.010 g (5 mM) $NaBH_4$ it was mixed for one hour. After adjusting the pH of the reaction solvent to be below 2.5 with dilute hydrochloric acid, it was extracted 3 times with 50 ml ethyl acetate. After drying over Na_2SO_4, the solvent was evaporated. In the case of *p*-

hydroxyphenylpyruvic acid (pHPPA) and *p*-hydroxybenzoyl formic acid (pHBFA), the following operation was added because of its slight solubility in water. The sodium salt of pHBFA was dissolved in 3 ml of an ice-cold 1% KH_2PO_4 aqueous solution, and then added to the reaction solvent. pHPPA was dissolved in 3 ml ice-cold methanol and then added to the reaction solvent.

Cyclohexanol (0.4—19 g, 2—40 mM) was added to the reaction solvent in order to examine the inhibition effect. As for the urea addition effect, 0.3—15 g (0.1—5 M) urea and 0.4—19 g (0.1—5 M) thiourea were added to the reaction solvent. The ionic strength was adjusted to be 0.5—3.0 by the addition of KCl to a 25 ml phosphoric acid buffer solution (70 mM, pH 7.0). The reaction solvents for the pH-dependency of optical yield were prepared as follows: pH 3—4, 50 mM aqueous succinic acid solution+50 mM borax aqueous solution, pH 5—9, 70 mM phosphoric acid buffer solution; pH 10—11, 50 mM sodium carbonate aqueous solution+100 mM sodium hydrogencarbonate aqueous solution; pH 12—13, 50 mM sodium hydrogencarbonate aqueous solution+100 mM sodium hydroxide aqueous solution. By adding KCl to each 25 ml solution, the ionic strength was adjusted to 0.2.

Identification of the Product: A quantitative analysis of the product was carried out using HPLC. The main product was hydroxy carboxylic acid with trace by-products, such as benzoic acid and phenol from the keto acid. For a product analysis, HPLC equipped with a diethyl-aminoethyl silicagel (DEAE-2SW, TOSOH) column, eluted with a 0.05 M phosphoric acid buffer solution (pH 6.0) with 20 microliter injection, was detected. at UV 216 nm. By preparing a calibration curve, the chemical yield of the product was calculated. The optical yield (% e.e.) was calculated from the rotation angle of the product in ethanol solvent measured at 25 °C with a 50 mm cell using a DIP-360 (JASCO) polarimeter. The specific roiation of (*R*)-mandelic acid was $[\alpha]_D^{25}=-147.25$ (c=0.75, methanol). For benzoylformic ester as substrates the produce was hydrolyzed with stirring for 5 h at 30 °C in 5 ml of a 1% Na_2CO_3 aqueous solution.

Results

1) Examination of the Reaction Conditions of Asymmetric Reduction. In order to examine whether asymmetric selectivity exists various CD's were first added to the aqueous solution. During an examination of the conditions, the optimum conditions for the asymmetrical reduction were also examined. These were the temperatutre dependency as well as the molar ratio of BFA to ACD. Finally, by changing the type of substrate, the adaptability as an asymmetric field by CD was examined.

1-1) Effect of Added Various Cyclodextrins on Asymmetric Selectivity: The results are shown in Table 1. In either case, the chemical yield was 100%. The asymmetric selectivity was not observed for mandelic acid from BFA in neutral aqueous solution. However, asymmetric selectivity appeared when cyclodextrin was added. The results of Run 5 showed a 13% enantiomer excess(e.e.) of the *R*-form in the presence of ACD, which had the most significant effect among the various CDs. β-CD showed a product in the *R* configuration of 4%e.e. The smaller ring α-CD comprising six glucoses showed little selectivity at 2%e.e. However, aminated α-CD showed no asymmetric selectivity.

Table 1. Asymmetric Reduction of BFA in the Presence of Various CDs

Run No.	CD	$[\alpha]_D^{25}$/deg[a)]	Optical yield	Configuration
1	None	0	0	
2	β-CD	0	0	
3	A-α-CD	0	0	
4	β-CD	−6	4	*R*
5	ACD	−20	13	*R*
6	γ-CD	+3	2	*S*

Reaction Conditions: [ACD]=[keto acid]=5.0 mM, [$NaBH_4$]=50 mM, in 70 mM phosphate buffer (pH 7.0, I=0.20) at 0±2 °C for 1 h. a) Maximum rotation value of $[\alpha]_D^{25}$ for the (*R*)-mandelic acid was −174.3 (c=0.75, methanol).

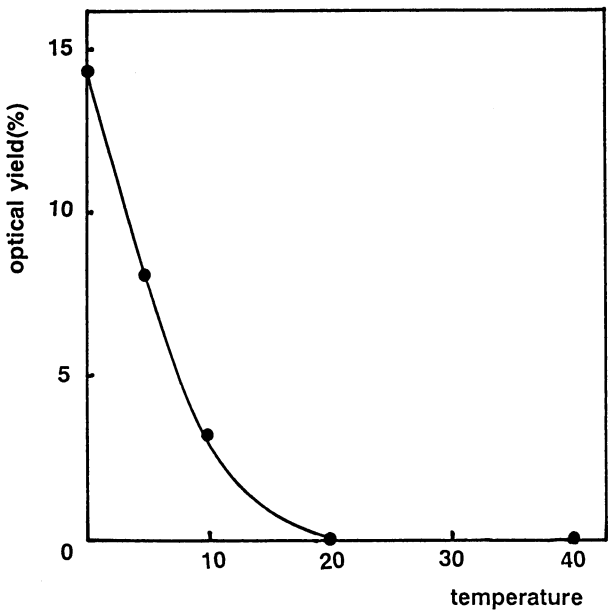


Fig. 1. Dependence of optical yield on temperature. Reaction conditions: [ACD]=5.0 mM, [BFA]=5.0 mM, [$NaBH_4$]=50 mM, in 70 mM phosphate buffer (pH 7.0, I=0.20) at 0—40 °C for 1 h.

1-2) Temperature Dependency on the Asymmetric Selectivity: The temperature dependency on asymmetric selectivity is shown in Fig. 1. The chemical yields were 100% for any experimental run. As the temperature increased, the optical yield decreased. At a temperature greater than 20 °C, the optical yield was 0% e.e. Furthermore, the attempt to improve the asymmetric selectivity at temperatures lower than 0 °C also failed. In these cases, pyridine, or ethylene glycol was mixed with water as an antifreezing liquid. The reaction was carried out for 4 h at either −38 or −10 °C for 48 hours. In either case, in spite of chemical yields of 100%, no asymmetric selectivity was observed. Thereafter, only the experimental results from the reaction conditions at around 0—5 °C are shown.

1-3) Dependency of ACD Concentration on Asymmetric Selectivity: At concentrations of 0.5 to 20 mole ACD to BFA, the reaction was carried out. An *R* selectivity of 13% e.e. was shown for [ACD]/[BFA]>1 in Fig. 2. There was no obvious change in the optical yield for 1—20 equimoles of ACD to substrate BFA.

1-4) Asymmetric Reduction of Various Keto Acids in the Presence of ACD: A reaction was carried out with various keto acids other than BFA. The substrates examined were methyl and etyhl ester of benzoylformic acid (MBFA and EBFA), *p*-hydroxybenzoylformic acid (pHBFA), phenylpyruvic acid (PPA), *p*-hydroxyphenylpyruvic acid (pHPPA), and 2-naphthoylformic acid (2-NFA), as illustrated in Scheme 1. The results are summarized in Table 2. Run 1,4,6,7 substrates showed asymmetrical selectivity. Most of all, pHPPA in Run 6 produced the *S* configuration of 35% e.e. It was of the *R* form of 30% e.e. in the case of 2-NFA, then the *S* form of pHBFA at 3% e.e. There was no selectivity for the methyl and ethyl esters of BFA. The chemical yields were 100%, except for Runs 6 and 7. The PPA of Run 5 had decomposed before the reduction reaction and thus, the pure reactant could not be obained.

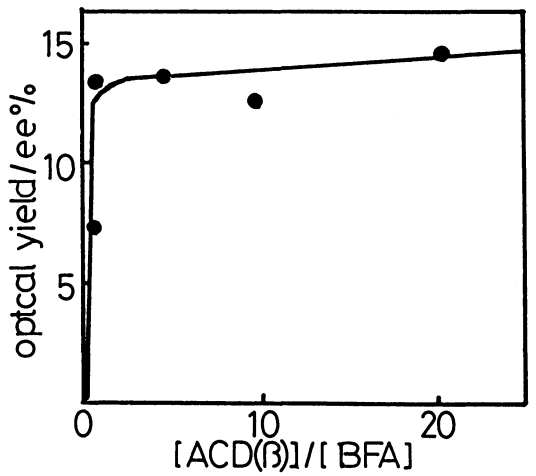
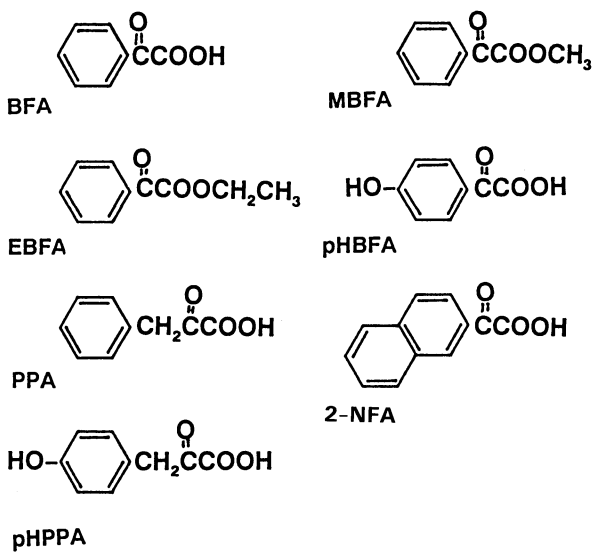


Fig. 2. Dependence of optical yield on molecular ratio of ACD and BFA.
Reaction conditions: [ACD]=0—32.0 mM, [BFA]=5.0 mM, [$NaBH_4$]=50 mM, in 70 mM phosphate buffer (pH 7.0, *I*=0.20) at 0±2 °C for 1 h.



Scheme 1. Structure of guests.

2) Examination of the Conditions for the Asymmetric Selectivity. The mechanism of asymmetric selectivity was examined in the BFA system in the presence of ACD using $NaBH_4$ in aqueous solution. The existence of the following three interactions were proposed as asymmetrical selective factors. First, there should be a hydrophobic inclusion between the CD cavity and the aromatic ring of a substrate. Second, a hydrogen bond between the hydroxyl group and the carbonyl group in BFA can be presumed. And third, there is an ion interaction between an amino group in ACD and a carboxyl group in BFA. In order to prove these assumptions, the addition effect of cyclohexanol, the addition effect of urea and thiourea, and the effect of the ionic strength and pH on the reaction were examined.

2-1) Effect of Added Cyclohexanol. The results concerning the optical yield on the addition of cyclohexanol is shown in Fig. 3. The maximum concentration of added cyclohexanol was 8 mole to the ACD host. The optical yield decreased along with the addition of cyclohexanol and became 5% e.e. at 40 mM cyclohexanol. Above this concentration the reaction solution turned into a heterogeneous system. The chemical yield of mandelic acid was 100%, even if cyclohexanol was

Table 2. Asymmetric Reduction of $NaBH_4$ of Various Keto Acids in the Presence of ACD

Run No.	Keto acid	$[\alpha]_D^{25}$/deg[a]	Optical yield	Configuration
1	BFA	−20	13	*R*
2	MBFA	0	0[b]	
3	EBFA	0	0[c]	
4	pHBFA	−4.1	3[d]	*R*
5	PPA		—[e]	No reaction
6	pHPPA	+3.1	35[f]	*S*
7	2-NFA	−44.9	30[g]	*R*

Reaction Conditions: [ACD]=[keto acid]=5.0 mM, [$NaBH_4$]=50 mM, in 70 mM phosphate buffer (pH 7.0, *I*=0.20) at 0±2 °C for 1 h. a) Conditions for rotation measurement: Run 1; *c*=0.5 in methanol, Runs 2 and 3; *c*=1.8 in methanol and water, Run 4; *c*=1.8 in methanol Run 6; *c*=0.5 in methanol, Run 7; *c*=1.1 in ethanol. b) Reported $[\alpha]_D^{20}$=+141.1 (*c*=1.0, methanol) for the *S*; *Beli.* **10**(2), 114. c) Reported $[\alpha]_D^{20}$=+134 (*c*=3.0 $CHCl_3$) for the *R*. d) Reported $[\alpha]_D^{20}$=+144.4 for the *S*; S. G. Bhat, et al., *Eur. Biochem.*, **68**, 323 (1976). e) Reported $[\alpha]_D^{25}$=+20.0 (H_2O) for the *R*; M. Winitz, et.al., *J. Am. Chem. Soc.*, **78**, 2423 (1956). f) Reported [α]=+8.0 (*c*=0.5, methanol) for the *S*; M. W. A. Ayer, *Can. J. Chem.*, **64**, 904 (1986). g) Reported (95% e.e) $[\alpha]_D^{21}$=+144.2 (*c*=0.98, ethanol) for the *R*; *J. Med. Chem.*, **16**, 1020 (1973).

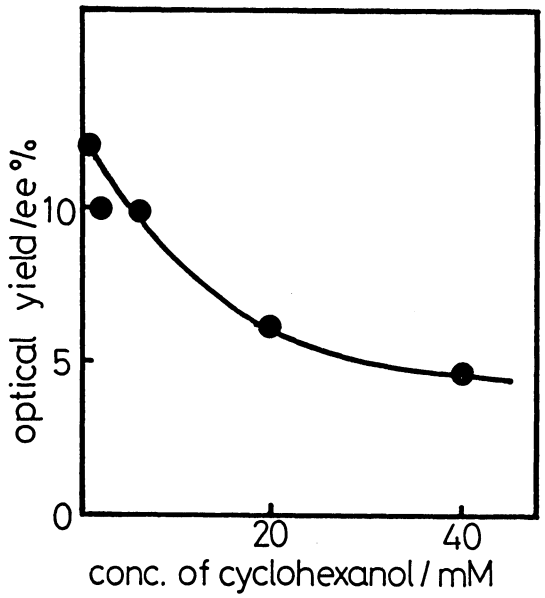


Fig. 3. Dependence of optical yield on the cyclohexanol concentration.
Reaction conditios: [ACD]=5.0 mM, [BFA]=5.0 mM, [$NaBH_4$]=50 mM, in 70 mM phosphate buffer (pH 7.0, I=0.20) at 0±2 °C for 1 h.

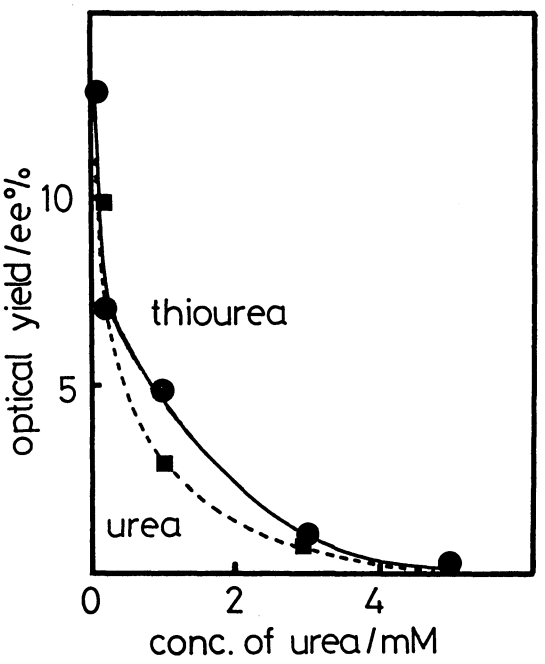


Fig. 4. Dependence of optical yield on urea.
Reaction conditios: [ACD]=5.0 mM, [BFA]=5.0 mM, [$NaBH_4$]=50 mM, in 70 mM phosphate buffer (pH 7.0, I=0.20) at 0±2 °C for 1 h.

added.

2-2) Effect of Added Urea and Thiourea: Figure 4 shows the change in the optical yield in the presence of added urea and thiourea from 0.1 to 5 M for each reaction solution. The optical yield decreased along with the increase in concentration in either case and

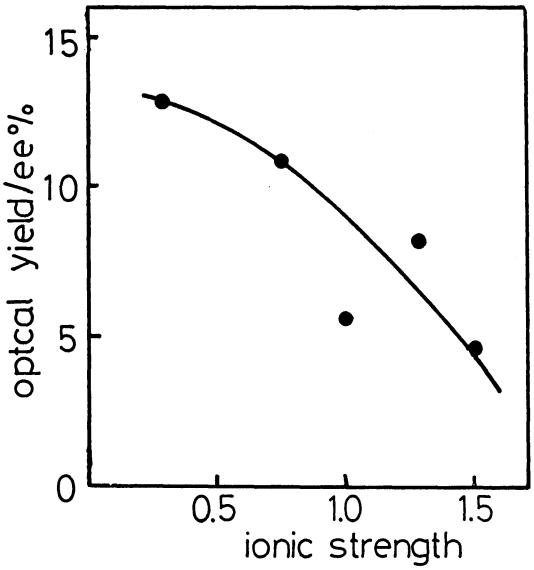


Fig. 5. Dependence of optical yield on ionic strength.
Reaction conditios: [ACD]=17 mM, [BFA]=5.0 mM, [$NaBH_4$]=50 mM, in 70 mM phosphate buffer (pH 7.0, I=0.20) at 0±2 °C for 1 h.

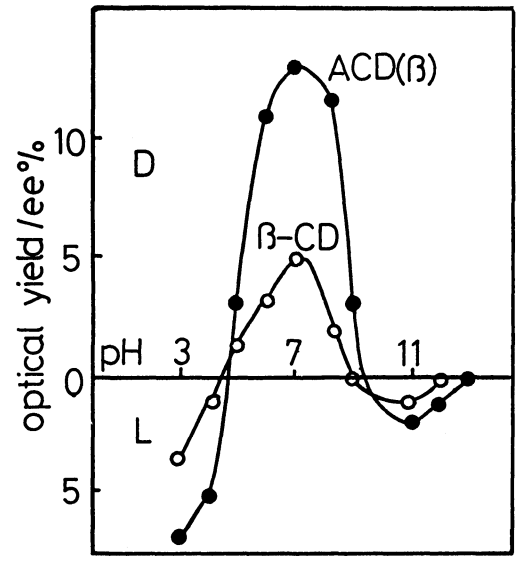


Fig. 6. Dependence of optical yield on pH.
Reaction conditions: [ACD]=17 mM, [BFA]=5.0 mM, [$NaBH_4$]=50 mM, in various buffer referred in the experimantal section (I=0.20) at 0±2 °C for 1 h.

selectlvity became 0% e.e. with the addition of 5 M urea. The chemical yield was 100%, even if ureas were added.

2-3) Effect of Ionic Strength Change: The optical yield of the case in which the ion concentration of the buffer solution was changed from 0.1 to 0.2 is shown in Fig. 5. The buffering ability in the range of these ionic strengths was sufficient; even for a solution with an ionic strength of 0.1, the solution pH showed 7.2, and thus remained unchanged. As the ionic strength increased, the optical yield decreased. It is conceivable that the

change in the optical yield strongly suggests an ionic interaction in the reaction. Even in this case there was no influence on the chemical yield of 100%.

2-4) Effect of a pH Change: The asymmetric selectivity, depending on the pH change with ACD compared with β-CD, is shown in Fig. 6. Without CD, asymmetric selectivity did not exist. A small *S* selectivity appeared at pH 5 and higher *R* selectivity from pH 5 to 11. The optical yield decreased at over, pH 11. Also, with β-CD a similar trend was shown, although the extent of the change was small. The doubtful racemizing of mandelic acid in the procedure was examined at these pH values. There was no racemization until pH 13 and the CDs had no effect on the racemization of mandelic acid. Therefore, the results shown here reflect the asymmetric selectivity, along with the reduction reaction, suggesting that the ionic interaction depended on the pH.

Discussion

1) Effects of Various Added Cyclodextrins on the Asymmetric Selectivity. An examination using the CPK model to explain the results in Table 1 suggests that the size of the α-CD and amino-α-CD cavity is big enough to include BFA. α-CD is better suited to the included BFA. However, the present results concerning asymmetric selectivity with bigger β-CD, ACD, and γ-CD were better than that for α-CD. This fact suggests that the size of the CD requires some degree of "affordness" in order to show asymmetric selectivity. Namely, in α-CD, BFA was included vertically in the cavity, and the plane including the carbonyl carbon of BFA may not be differentiated by the attack of a BH_4^- ion. The asymmetric selectivity should appear when the BFA molecule is included in the "reclined" mode in the afforded CD cavity space. The ionic interaction of the amino group further guides the BFA molecule advantageously. This conjecture does not contradict the later results concerning an examination of the ionic interaction. There might be a possibility that asymmetric selectivity might have occurred during the procedure when the reaction mixture was extracted with organic solvent in the presence of the CDs. However, a blank test using racemic mandelic acid showed no asymmetrical selective results. Also, there is no asymmetrical difference in the association constants of ACD and β-CD with mandelic acid enantiomers, as is summarized in Table 3.

Table 3. Association Constants(K) between CDs and Guests

CD	K/M^{-1}		
	BFA	(*D*)-Mandelic acid	(*L*)-Mandelic acid
A-α-CD	765±7	40±1	45±3
ACD	995±11	56±4	47±1
β-CD	445±4	18±0	16±0

Determined with fluorescence intensity of ANS at 540 nm excited around 350 nm, pH 6.0 solution containing less than 0.6 v/v% acetonitrile at 25.0±0.2 °C. [CD] $=1.0\times10^{-3}$ M, [ANS]$=2.0\times10^{-5}$ M, [guest]$=0.5-6\times10^{-3}$ M. The association constant of CDs and ANS is 96, 60 and 92 for A-α-CD, ACD, and β-CD respectively.

2) Temperature Dependency of Asymmetrical Selectivity. Generally, in a asymmetrically selective reaction the difference of the free energy of the reactions between enantiomers increases at lower temperature.[16] Furthermore, the molecular complex is stable at low temperature. For example, Sakuraba reported on the complex of cinnamic acid and β-CD.[17] With hydrogen bromide, the reaction was carried out at 0 °C, giving 23% e.e. of optical yield whose value was about 2-times the optical yields at 25 °C. Low temperature favors high asymmetric selectivity for two reasons: The formation of inclusion and larger difference in the free energy of the enantioface differentiating reaction. However, it did not show any selectivity at temperatures lower than 0 °C. Generally, CD does not form a host-guest inclusion complex in a nonaqueous solution. It is reported that the limited substrate made inclusion only in ethylene glycol and DMSO, and that the association constant was very small. Furthermore, it is also reported the pyridine and ethylene glycol forms a complex with $NaBH_4$, and promotes the reduction reaction. In these cases, the solvated $NaBH_4$ complex may be formed with pyridine and the mode of attack to BFA should be largely changed. Furthermore, it is proposed that the selectivity did not appear without an inclusion of BFA into ACD in these media.

3) Structural Effect of Various Substrates. The configuration of the reduced product from pHPPA and pHBFA, which has a hydroxyl group at the *p*-position on the phenyl ring, is reversed to *S*-configuration, differed with *R*-configuration in case of BFA. It is thought that the above mentioned substrates should interact with hydroxyl groups at the heme of the CD cavity; this might cause a reversed configuration of the product. This would be especially true for pHPPA, which has a greater motional degree of freedom through part of the methylene group. This should reflect an improvement in the optical yield. It is known that the naphtyl group generally forms a stronger complex than does phenyl in the case of β-CD. This directly reflects the optical yield up to 30% e.e. Esters of BFA have no ionic site for an ionic interaction with host ACD, and vastly changes the orientation between host and guest, as reflected in the optical yield of 0% e.e.

4) Frormation of Inclusion Complex. Cyclohexanol is a well-known compound that forms inclusion compounds and inhibits cyclodextrin catalysis. The decrease in the optical yield appeared because the present reaction was inhibited by cyclohexanol. Also, ACD, and various guests were examined using both fluorescence and circular dichroism spectra. The association constant is shown in Table 3 and the circular dichroism spectrum is shown in Fig. 7. ACD, even β-

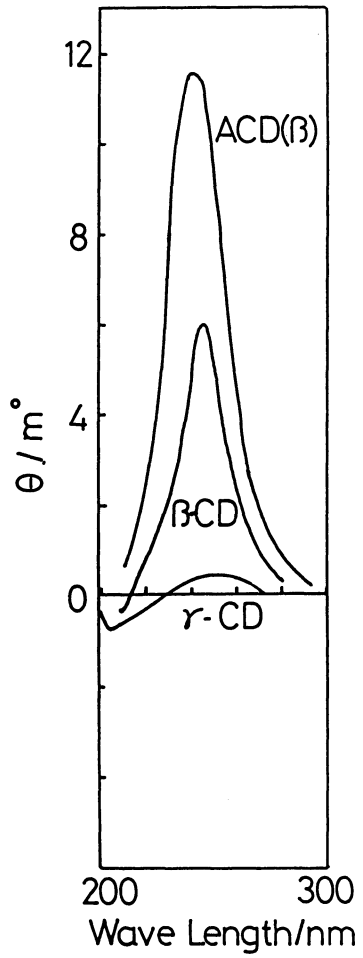


Fig. 7. Induced circular dichroism spectra of BFA in the presence of various CDs Conditions: [CD]=10 mM, [BFA]=0.8 mM, in 33 mM phosphate buffer (pH 7.0, I=0.10) at room temperature.

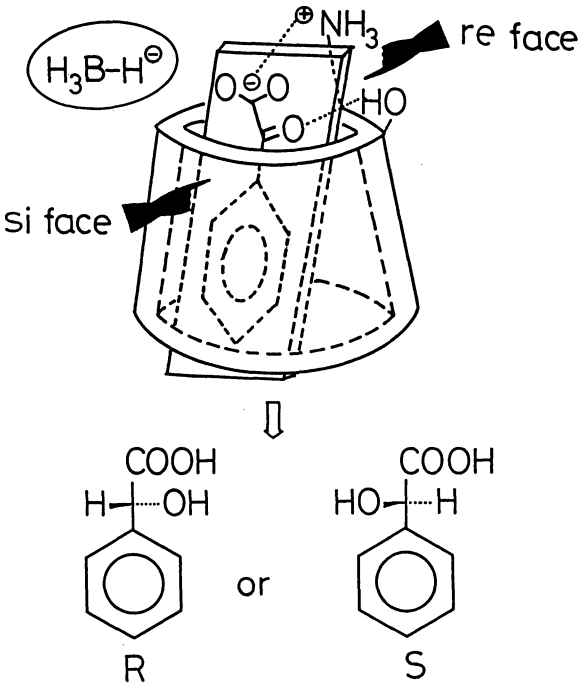
CD, definitely formed inclusion compounds with BFA. This was observed using the circular dichroism that the substrate BFA was assumed to be included in the hydrophobic cavity of CD. The association constant of ACD was more than 2-times that of β-CD. About 70% of BFA is assumed to be included in the ACD cavity, whereas 55% of BFA is included in β-CD. It can be seen that the optical selectivity should be induced by an attack of $NaBH_4$ on the included BFA.

5) Interaction of Hydrogen Bonding. Urea and thiourea are known to form inclusion compounds and act as an inhibitor against hydrogen bonding.[18,19] When 20 equimoles of urea were added to the reaction system, it was observed that the inclusion behavior of CD was affected.[6] It was concluded that urea interacted with the hydroxyl groups at the circular heme of CD and inhibited hydrogen bonding. It was reported that the urea did not inhibit inclusion in the CD cavity. Accordingly, the effect of urea in this reaction system is to inhibit hydrogen bonding between the hydroxyl group and substrate BFA. As shown in the pH-dependency graph (Fig. 7), in the neutral pH range, β-CD also showed asymmetric selective results. This should mean that the hydrogen bond forms the essential geometry to determine the "reclined" position of substrate BFA, in other words, to allow the asymmetrically selective attack of BH_4^- on BFA molecules in inclusion compounds.

6) Ionic Interaction. Esters of BFA as guests that have no ionic site showed 0%e.e. This essentially suggested the existance of an ionic interaction. The optical yield was decreased along with an increase in the ionic strength in the case of ACD. This would favor the presence of an ionic interaction exerting some influence in the asymmetric induction. The proportion of included BFA with ACD in a reaction system is about 70% and about 56% with β-CD at pH 7. Namely, the ionic interaction caused the stronger inclusion of BFA in the reaction process. Furthermore, based on the assumption that the included ACD only caused an asymmetric reduction, the optical yield actually increased to 21%e.e. to the included BFA in ACD, and 9%e.e. to the included BFA in β-CD. This increase, the asymmetrical selectivity of ACD against β-CD by over 2-times. Therefore, regarding the ionic interaction, it can be said that it not only increased the inclusion, but also the asymmetrical selective attack of the reagent was enhanced Also, the reaction showed *R* selectivity when ACD and BFA were dissociated, but *S* selectivity when the ionic group of ACD and BFA was not dissociating. As shown in the result regarding the pH dependence of the asymmetric reduction, the pK_a of BFA and ACD were 4.2 and 8.4, respectively. This fact also showed the same tendency for the case that of β-CD. Therefore, merely an ionic interaction cannot explain the asymmetrical selectivity, though the presence of an ionic interaction significantly amplified the asymmetrical selectivity. Rather, the orientation and geometry in the inclusion complex were determined through hydrogen bonding, depending on the ionic interaction among the host and guest.

Conclusions

First of all, it is indispensable that the substrate be included through the hydrophobic interaction into the CD cavity for an asymmetric reduction in this system. In a larger cavity with some "affordness" may be desirable to have asymmetric selectivity of the attacking reagent, since "reclining" of the BFA molecule may cause asymmetric results. As illustrated in Scheme 2, it can be thought that some ionic interaction amplified the including ability of the complex. It is also necessary that the hydrogen bonding of the hydroxyl group at the hem of the CD cavity determines the orientation of the BFA molecule in the CD cavity. Further, the total concerted effects of these three interactions control the complex conformation, then the *R*-configuration selectivity of the reduction product ((*R*)-mandelic acid) through an attack from the *si* face of the prochiral keto



Scheme 2. Suggested mechanism.

carbonyl carbon in BFA by the BH_4^- ion. The experimental results that examined for the substrates other than BFA showed the carbonyl carbon through the *si* face also in the case of 2-naphthyl-substituted keto acid and *R* selectivity in the product were obtained. However, as for *p*-hydroxylphenyl compounds (pHBFA and pHPPA), a BH_4^- attack occurs through the *re* face on the opposite side of *si*, and the *S* selectivity of the product was preferential. A clear explanation is not yet available concering these results.

As previously mentioned, when ACD is added to the aqueous solution by controlling the attacking direction of the reducing reagent, asymmetric selectivity could be realized. It can be said that this is the first "Microreactor" system which shows the asymmetric reaction function using cyclodextrin derivatives in a sense with molecule recognition. To improve the optical yield, the appropriate orientation of the substrate in the included form should be suggested. For example, an enforcement of the ionic interaction may be effective.

A detailed spectrum as well as kinetic examination are necessary concering the conformational structure and various interactions of the inclusion complex in order to develop and generalize this asymmetrical reduction. Up to now only systems which are limited to the reducing agent $NaBH_4$ and substrate BFA have mainly been examined.

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