Asymmetric Transformation of Phenylglyoxal into Mandelic Acid Catalyzed by Cyclodextrin-Based Glyoxalase Models

Seizo Tamagaki,* Jun Narikawa, and Atsushi Katayama

Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558

(Received February 19, 1996)

(2-Mercaptoethyl)amino-attached cyclodextrin provides a highly enantioselective environment during the transformation of phenylglyoxal into (*S*)-mandelic acid, although simple inclusion into native α-,β-, and γ-cyclodextrins and their 2-hydroxypropyl and methyl variants is not sufficient to cause appreciable asymmetric induction.

Enzymes are a class of chiral reagents which generally provide a chiral environment capable of far greater discrimination between enantiotopic or diastereotopic groups than do ordinary chiral chemical reagents.[1] Meanwhile, cyclodextrins (CyDs), composed of glucopyranose units which are linked with each other by 1—4 α-glucoside bonds, have been utilized as small molecule mimics of enzymes and a drug delivery system, since they have an optically active cavity capable of inducing an asymmetric center into the substrate via formation of an inclusion complex;[2,3] for example, 2-naphtyl trifluoromethyl ketone was reported to be reduced with a simple NADH model compound and $NaBH_4$ in aqueous CyD solution, giving the corresponding alcohol with yields as low as only 10 and 36% at the best, respectively.[4,5] Meanwhile, high stereoselectivities by natural CyDs are achieved only in the solid-liquid phase rather than in the homogeneous liquid phase; for example, menthone held in CyDs is reduced with $NaBH_4$ to menthol (65—69%ee).[6] Meanwhile, much attention is currently focused on CyD-based catalysts; their abilities to recognize guest molecules and to catalyze chemical transformations have been assessed. Breslow and coworkers have synthesized keto acid-to-amino acid transamylase models containing one pyridoxamine unit on the primary or secondary hydroxy face, which shows selectivities of 12 and 39%ee, respectively.[7] Particularly high stereoselectivity has been observed in the transamylase model involving one pyridoxamine unit and one ethylenediamine unit on the primary hydroxy side of β-CyD.[8] The NADH model compounds linked with changing spacers to β-CyD exhibited 30 to 50%ee toward the asymmetric reduction of benzoylformate to mandelic acid.[9] As such, there are only a few reports on CyD-linked enzyme models or the combined use of CyDs and simple enzyme model catalysts.

In order to devise effective CyD-based biomimetic catalysts, it is necessary to introduce the catalytic sites to the suitable positions of the CyD. In our recent article we have described the synthesis of a glyoxalase enzyme model involving the catalytic 2-aminoethanethiol unit at the 6-position of β-CyD, i.e., mono[6-deoxy-6-(2-mercaptoethylamino)]-β-CyD (hereforth referred to as MACD) and have kinetically examined its catalytic efficiency using 2-naphthylglyoxal as a substrate, which was transformed into 1-(2-naphthyl)-1-hydroxyacetic acid.[10] In this work we have examined the enantioselectivity in the transformation of phenylglyoxal (hereforth PGO) into mandelic acid catalyzed by MACD, since all the previous investigations were limited to catalysis systems without binding sites.[11] The enzyme-like environments around the catalytic site will create a chiral center by introducing specificity to the reacting substrate.

Experimental

Materials. PGO and hydrochloride of 2-(dimethylamino)ethanethiol (DAET) were purchased from Tokyo Kasei Co., Japan and used without further purification. α-, β-, and γ-CyDs, hexakis-, heptakis-, and octakis[2,6-di-*O*-(2-hydroxypropyl)]-α-, -β-, and -γ-CyDs (2,6-Hp-α-, -β-, and -γ-CyD), and heptakis[2,6-di-*O*-methyl- and 2,3,6-tri-*O*-methyl]-β-CyDs (2,6-DM-β-CyD and 2,3,6-TM-β-CyD) were obtained from the same source and used without further purification. Mono(6-deoxy-6-tolylsulfonyl)-β-CyD was prepared according to the literature procedure.[12] MACD was prepared according to our previously reported method.[10]

Measurements. Absorption spectra were obtained with a Hitachi 220A recording spectrophotometer. Circular dichroism (CD) spectra were recorded on a JASCO J-720 circular dichrometer.

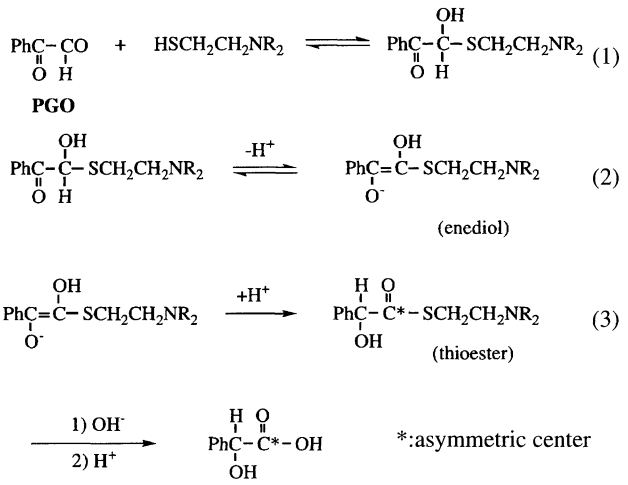
Product Analysis. The aqueous solution of MACD (13×10^{-3} M) or CyD/DAET (13×10^{-3} M each) was pH adjusted with NaOH; then PGO (1.3×10^{-3} M) was syringed into it at 0 °C under nitrogen. (1 M = 1 mol dm^{-3}). Doubly-distilled and deionized water was used as the solvent. The reaction was monitored by following the decreasing absorbance at 280 nm. After 24 h of the reaction, the solution was made alkaline to ca. pH 11 to hydrolyze the thioester product. Then the reaction mixture was acidified to pH 4 with dilute aqueous HCl solution and subjected to analysis for optical resolution on a chiral HPLC column (CHIRALPAK WH purchased from Daicel Chemical Ind., Ltd., Japan; eluant, aq 0.25 mM $CuSO_4$; detection, 230 nm; 50 °C).

Computer-Aided Molecular Design Analysis (CAMD). Computational mechanics calculations for the host–guest com-

plexes were performed with the aid of a CAMD system carrying a software package of CHARMm Ver22/QUANTA Ver 3.3 (Molecular Simulating Inc., USA) on a hardware of INDIGO R-4000 (Silicon Graphics Corporation, USA). The total energies at the global minima attained are listed in Table 2.

Results and Discussion

For transformation of phenylglyoxal (PGO) into mandelic acid, the most reasonable mechanism (Eqs. 1, 2, and 3 in Scheme 1) is shown below. The effects of the medium pH and CyD derivatives on the enantio excess % of the product have been studied. The results are collected in Table 1, which reveals that asymmetric induction leading to formation of an excess of (*S*)- over (*R*)-mandelic acid uniformly took place both with a catalytic system containing a joint use of parent CyDs and 2-(dimethylamino)ethanethiol (DAET) and with MACD having both the binding site and the catalytic site in the same molecule, demonstrating that participation of the chiral CyD cavity which accommodates the PGO phenyl residue is essential in inducing the enantioselectivity. Its diminishing trend: α-CyD > β-CyD > γ-CyD is thus due to



Scheme 1. Overall reaction scheme.

the decreasing rigidity of the inclusion of PGO into the CyD cavities; the largest selectivity observed with α-CyD among all CyDs examined in this study seems to reflect the greatest van der Waals contacts between the binding site of the guest and the inner wall of the α-CyD, because γ-CyD with a bigger cavity exhibits practically no enantioselectivity. Generally, replacement of hydroxylic hydrogens of CyDs by 2-hydroxypropyl or methyl groups had little or no valuable influence on enantioselectivity. In order to provide a theoretical insight into differentiation between the inclusion complexes of enantiomers, molecular mechanics calculations (CAMD analysis) were carried out on the four possible situations of interactions of the methyl esters of (*S*)- and (*R*)-thiomandelic acid with β-CyD as the simple transition state models of the asymmetric protonation to the enediol (Eq. 3). The calculated results are given in Table 2; the total CAMD energies of the inclusion complexes at the global energy minima decrease in the following order: the (*S*)-enantiomer included from the primary hydroxy side > the (*S*)-enantiomer from the secondary > the (*R*)-enantiomer from the secondary > the (*R*)-enantiomer from the primary. Thus, the relative stability of the (*S*)-thiomandelate to the (*R*)-thiomandelate would have to reflect preferential production of (*S*)-over (*R*)-mandelic acid in the actual CyDs-catalyzed reactions. Figure 1 shows a sketch of the optimized geometry for the inclusion complex of β-CyD with the (*S*)-enantiomer.

Meanwhile, the enantioselectivity was significantly higher with MACD than with the system that lacks the linking of the CyD unit and the DAET unit; obviously, the freezing of the kinetic and rotational freedom of the substrate causes the enhancement of the enantioselectivity. However, such a comparatively moderate enantioselectivity as ca. 50%ee, although the present model has not yet been designed to attain optimization, appears to indicate that more effective molecular recognition of the substrate and/or the transition state structure is required to achieve much higher enantioselectivity. To well understand the association mode, circular dichroism (CD) spectrometry has very often been used, be-

Table 1. Enantiomeric Excess Obtained with Phenylglyoxal Transformation

	Enantiomeric excess % (±0.5%)					
	pH					
Catalyst	8.0	9.5	9.75	11.0	11.5	12.5
MACD [a]	45.8	44.2	44.0	36.6	30.9	14.5
α-CyD/DAET [b,c]	17.3		16.2	9.1		2.1
α-CyD/DAET [c]	25.5					
β-CyD/DAET [c]	11.1		10.2	7.4		
γ-CyD/DAET [c]	2.4		2.2	1.7		
2,6-Hp-α-CyD/DAET [c]	4.8					
2,6-Hp-β-CyD/DAET(MS [d] 0.8) [c]	12.6					
2,6-Hp-β-CyD/DAET(MS0.6) [c]	12.5					
2,6-Hp-γ-CyD/DAET [c]	2.1					
2,6-DM-β-CyD/DAET [c]	9.1					
2,3,6-TM-β-CyD/DAET [c]	9.6					

Reaction conditions: 10 °C; N_2; [PGO] = 1.3×10^{-3} M. a) [catalyst]/[PGO] = 10; chemical yield: 98%. b) Chemical yield: 95%. c) [CyD]/[DAET]/[PGO] = 10 : 10 : 1. d) MS denotes average molar substitution.

Table 2. CAMD Calculation Energies for Four Possible Enantio-Recognition Models of Binding of Methyl Esters of (*S*)- and (*R*)-Thiomandelic Acid with β-CyD

Direction of Ph insertion	Energy component	CAMD energy/kcal mol^{-1} (*S*)-PhCH(OH)COSMe	(*R*)-PhCH(OH)COSMe
Primary OH side	Lennard–Jones[a)]	−53.9	−41.9
	Electrostatic	−80.5	−73.5
	Others[b)]	169.0	173.4
	Total	34.6	58.0
Secondary OH side	Lennard–Jones[a)]	−50.5	−42.5
	Electrostatic	−80.0	−75.5
	Others[b)]	172.9	169.3
	Total	42.4	51.3

a) van der Waals interaction + hydrogen bonding interaction. b) Bond energy + dihedral angle energy + improper energy + constrains.

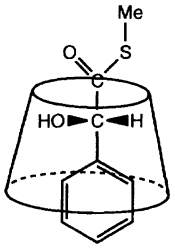


Fig. 1. Schematic illustration of the energetically optimized geometry for the (*S*)-thiomandelate ester immersed in the β-CyD cavity.

cause of its predictability.[13,14] There is also the possibility of gaining information on the precise geometry or mode of the first-formed hemithioacetal intermediate in the MACD reaction. Figures 2a and 2b illustrate the conceivable structures of the intermediate. If the structure shown in Fig. 2a occurs where the phenyl ring is deeply included, the Cotton effect sign of the L_b band (280 nm) should be negative and that of the L_a band (257 nm) should be positive, because the direction of the electronic transition dipole moments is perpendicular and parallel, respectively, to the molecular rotation axis of the cyclodextrin cavity.[15] However, this is not the case, as shown in Fig. 3, which displays CD spectra during the course of the reaction; the observed positive and negative

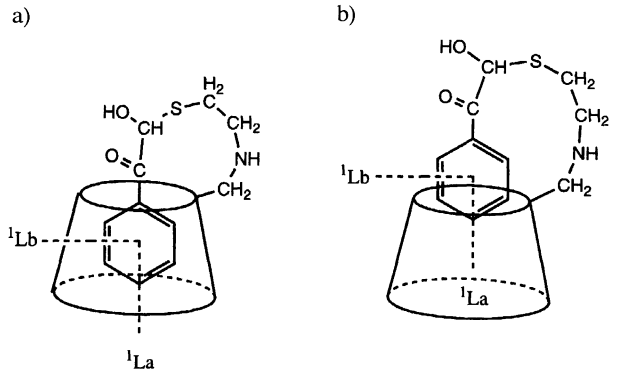


Fig. 2. Possible structures of the inclusion complex between MACD and PGO.

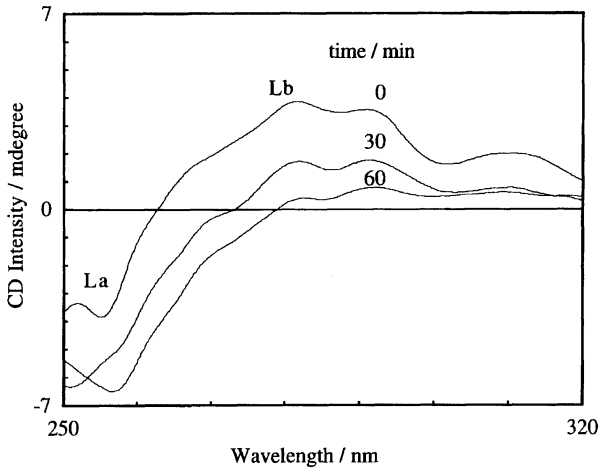


Fig. 3. Circular dichroism spectra of PGO in the presence of MACD. pH = 10.0, [PGO] = 0.7×10^{-4} M, [MACD] = 14×10^{-4} M.

Cotton effects for the L_b and L_a bands, respectively, suggest that, as illustrated in Fig. 1b, the phenyl moiety should protrude appreciably from the CyD cavity with the phenyl π-plane perpendicular to its face, since the short spacer would restrict the deep immersion of the phenyl ring into the cavity owing to the distortion of the CyD skeleton; it is concluded, therefore, that the electronic transition moment due to the L_b band points to the CD-positive direction and that due to the L_a band to the negative direction, as observed.[14,15] On the other hand, no induced CD spectral band appeared with the combination systems of native CyDs and DAET.

Finally, it is important to note that the effect of varying the medium pH on the enantioselectivity was substantial; the increasing of the pH resulted in decreasing enantioselectivity; the decrease was particularly marked above pH 11. It is apparent that this pH effect is not attributable to the hydroxide-catalyzed racemization of the chiral thioester product (Eq. 3)[10] which takes place probably outside the cavity, because even strongly alkaline conditions employed for thioester hydrolysis (pH 12) did not affect the outcome of the enantioselectivity at all. Rather, the decreased value

is reasonably explainable if we make an assumption that the use of such a higher pH medium would change the rate-determining step from the deprotonation (Eq. 2) to the protonation (Eq. 3) and would give rise to the anionic counterpart of the achiral enediol intermediate with its hydrophilicity enhanced. That is to say, the more hydrophilic nature of the enediol counterpart itself would force it to pick up a proton in a rather dissymmetric environment, therefore, disfavoring a high asymmetric induction. The similarity of the pH dependencies on the enantio-differentiation implies, however, that all the CyDs-catalyzed reactions adopt the same mechanism, the one described in the reaction scheme.

We would like to thank Misao Koide of Lion Corporation for his help in the CAMD calculations.

References

1) H. Hirschman, "Comprehensive Biochemistry," ed by M. Florkin and E. H. Stotz, Elsevier, Amsterdam (1964), Vol. 12.

2) Y. Kuroda, T. Hiroshige, and H. Ogoshi, *J. Chem. Soc., Chem. Commun.*, **1990**, 1594; J. Szejtli, "Cyclodextrin Technology," Kluwer-Academy, Dordrecht (1988).

3) Y. Inoue, F. Dong, K. Yamamoto, L.-H. Tong, H. Tsuneishi, T. Hakushi, and A. Tai, *J. Am. Chem. Soc.*, **117**, 11033 (1995).

4) N. Baba, Y. Matsumura, and T. Sugimoto, *Tetrahedron Lett.*, **1978**, 4281.

5) R. Fornasier, F. Reniero, P. Scrimin, and U. Tonellato, *J. Org. Chem.*, **50**, 3209 (1985).

6) H. Sakuraba, N. Inomata, and N. Tanaka, *J. Org. Chem.*, **54**, 3482 (1989); L. Szente, J. Szejtli, and L. T. Chan, *J. Inclusion Phenom.*, **5**, 3 (1987).

7) R. Breslow, A. W. Czarnik, M. Lauer, R. Leppkes, J. Winkler, and S. Zimmerman, *J. Am. Chem. Soc.*, **117**, 11033 (1995).

8) I. Tabushi, Y. Kuroda, M. Yamada, H. Higashimura, and R. Breslow, *J. Am. Chem. Soc.*, **107**, 5546 (1985).

9) H. Ikeda, H.-T. Moon, T. Ikeda, C.-J. Yoon, A. Ueno, and T. Toda, "7th International Cyclodextrin Symposium," Preprint in Tokyo (1994), p. 102.

10) S. Tamagaki, A. Katayama, M. Maeda, N. Yamamoto, and W. Tagaki, *J. Chem. Soc., Perkin Trans. 2*, **1994**, 507.

11) V. Franzen, *Chem. Ber.*, **90**, 623 (1957); S. S. Hall, A. M. Doweyko, and F. Jordan, *J. Am. Chem. Soc.*, **98**, 7460 (1976); T. Okuyama, S. Komoguchi, and T. Fueno, *J. Am. Chem. Soc.*, **104**, 2582 (1982); T. Okuyama, S. Komoguchi, and T. Fueno, *Bull. Chem. Soc. Jpn.*, **55**, 1493 (1982).

12) L. D. Melton and K. N. Slessor, *Carbohydro. Res.*, **18**, 29 (1971); *J. Chem. Soc., Perkin Trans. 2*, **1994**, 2435; "Cyclodextrin," ed by A. Ueno, Sangyo Tosyo, Tokyo (1995).

13) S. Tamagaki, K. Fukuda, H. Maeda, N. Mimura, and W. Tagaki, *J. Chem. Soc., Perkin Trans. 2*, **1995**, 389.

14) L.-H. Tong, Z.-J. Hou, Y. Inoue, and A. Tai, *J. Chem. Soc., Perkin Trans. 2*, **1992**, 1253.

15) K. Harata and H. Uedaira, *Bull. Chem. Soc. Jpn.*, **48**, 375 (1975); P. E. Schipper and A. Rodger, *J. Am. Chem. Soc.*, **105**, 4541 (1983); M. Kodaka, *J. Phys. Chem.*, **95**, 2110 (1991); M. Kodaka, *J. Am. Chem. Soc.*, **115**, 3702 (1993).