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Definitive Evidence for Enantioselective Catalysis over "Molecular Footprint" Catalytic Cavities Chirally Imprinted on a Silica(Alumina) Gel Surface

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"Molecular footprint"-like catalytic cavities were marked on a silica(alumina) gel surface by the authors' molecular imprinting method using a chiral mandelic acid derivative ((-)-(5*R*)-5-phenyl-2,4-diketo-tetrahydro-oxazole); these chirally imprinted cavities displayed distinct enantioselective catalysis on the transacylation of the corresponding substrates, *N*-carboxy-(*R*)-phenylglycine anhydrides and the (*S*)-isomer .

We have investigated the catalyzed reactions over "molecular footprint"-like catalytic cavities imprinted using a template on a surface of silica (alumina) gel.[1-6] Their substrate specificities depend on the templates used in the imprinting, which provides a method for design of tailor- made catalysts. In the previous communications,[5,6] we reported that enzyme-like enantioselective catalyses occured over the catalytic cavities imprinted with chiral amino acid derivatives, Z-(*S*)-Ala-NH-Bz (**4**), and (Z-(*S*)-Ala)$_2$NH (**5**), respectively.

In the present paper, we describe that the similar enantioselective catalysis is operating over the cavities imprinted with a chiral hydroxylic acid derivative, (-)-(5*R*)-5-phenyl-2,4-diketo-tetrahydro-oxazole (**1**).[7] The reason to choose **1** as a template was as follows. i) Template **1** has large value of specific molecular rotation due to a phenyl group attached to the chiral center. ii) **1** of a rigid 5-membered cyclic structure requires 5-membered cyclic substrates, *N*-carboxy-(*R*)-phenylglycine anhydrides (**2**) and the (*S*)-isomer (**3**) ; they might have an advantage of favored entropy loss on binding to the catalytic cavities. iii) Acyclic reaction products of **1** in different molecular shape can avoid the product inhibition caused by rebinding of the products to the cavities; such an inhibition was previously observed in the catalyzed reaction of (Z-(*S*)-Ala-)$_2$ O over the cavities imprinted with **5**.[6]

The catalyst with "molecular footprint" cavities was prepared from Merck Kieselgel 60 by the usual imprinting procedure[3] with a template **1**, as follows. The silica gel was hydrolyzed with 3 mol dm^{-3} HCl for 5 h to yield free silanol groups on the surface. Then the gel was subjected to doping with aluminium ions. Isomorphic substitution of surface silicate by aluminate proceeded to give Lewis acid sites, which were subsequently modified by doping with **1** to form surface acid-base complexes. The gel was aged at pH 4.0 for a week and subsequently dryed for two weeks. The dried gel was subjected to continual methanol extraction, which removed the tempelate molecules off from the surface complexes. Thus the catalytic cavities with complementary structure were left on the silica gel surface. The catalytic activities were determined photometrically as usual[3] using catalyzed transacylation of the substrate **2** and **3**[8] to 2,4-dinitrophenol in

acetonitrile at 30 °C. Pseudo-first-order rate constants, k_{obsd} were obtained from decrease in O.D. at 430 nm. Doublereciprocal plots (Lineweaver- Burk plots), k_{obsd}^{-1} vs. [S], where [S] was the concentration of the substrates, displayed linear relationship. The catalyzed reaction obeyed Michaelis-Menten kinetics,[9] and the kinetic parameters are shown in Table 1.

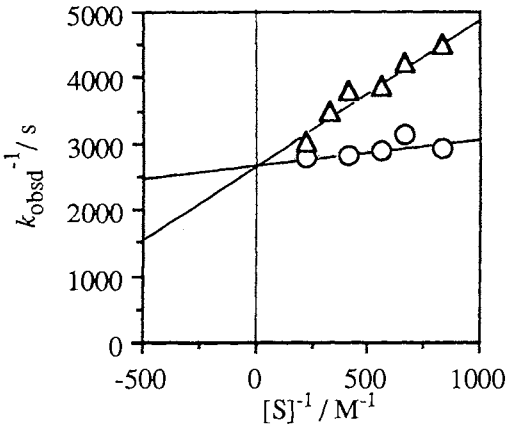


Fig.1. Lineweaver - Burk plots over { **1** }.
[S]: *R* - substrate concentration.
[I]: inhibitor concentration.
○ : [I] = 0. △ : [I] = 2.0 x 10^{-4}M.

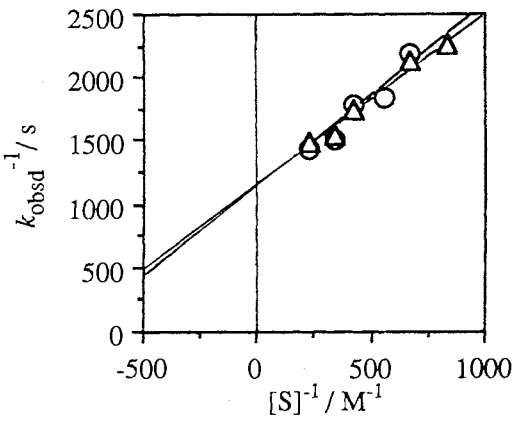


Fig.2. Lineweaver - Burk plots over control gel.
[S]: *R* - substrate concentration.
○ : *R* - substrate. △ : *S* - substrate.

As shown in Fig. 1, the strong competitive inhibition caused by rebinding of **1** evidently proved the formation of the "molecular footprint" catalytic cavities on the surface of the gel.

The Lineweaver-Burk plots in Fig. 2 are for the catalyzed reactions of **2** and **3** over the native Lewis acid sites of the control catalyst. They showed identical catalytic behavior as expected, because the catalytic sites of the control catalyst prepared without the template doping lacked chiral factor at all. Whereas, the distinctly different plots in Fig. 3 are for the catalyzed reactions of **2** and **3** over the chiral cavities imprinted with **1** (The cavities are hereafter referred to as { **1** })

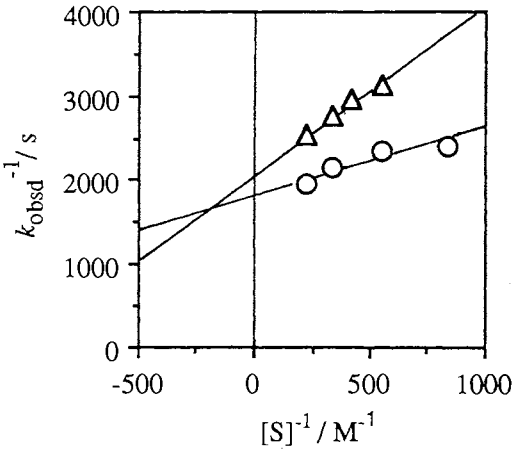


Fig.3. Lineweaver - Burk plots over { **1** }.
[S]: *R* - substrate concentration.
○ : *R* - substrate. △ : *S* - substrate.

It clearly proves the occurence of an enantioselective catalysis based on the chiral recognition of the cavities. This recognition capability cannot come from unextracted template molecules because such remaining template molecules should be a strong competitive inhibitor as mentioned above. Therefore it must originate in the complementary structures of the chiral cavities as expected.

An examination of K_m values suggests that "lock-and-key" mechanism based on simple exclusion effect of the cavities cannot explain the enantioselective catalysis observed. The affinity of **2** for { **1** }, (K_m^{-1}), is naturally the largest, and that of **3** for { **1** } is unexpectedly still larger than those of **2** and **3** for the control catalytic sites. This finding is against the "lock-and-key" mechanism.

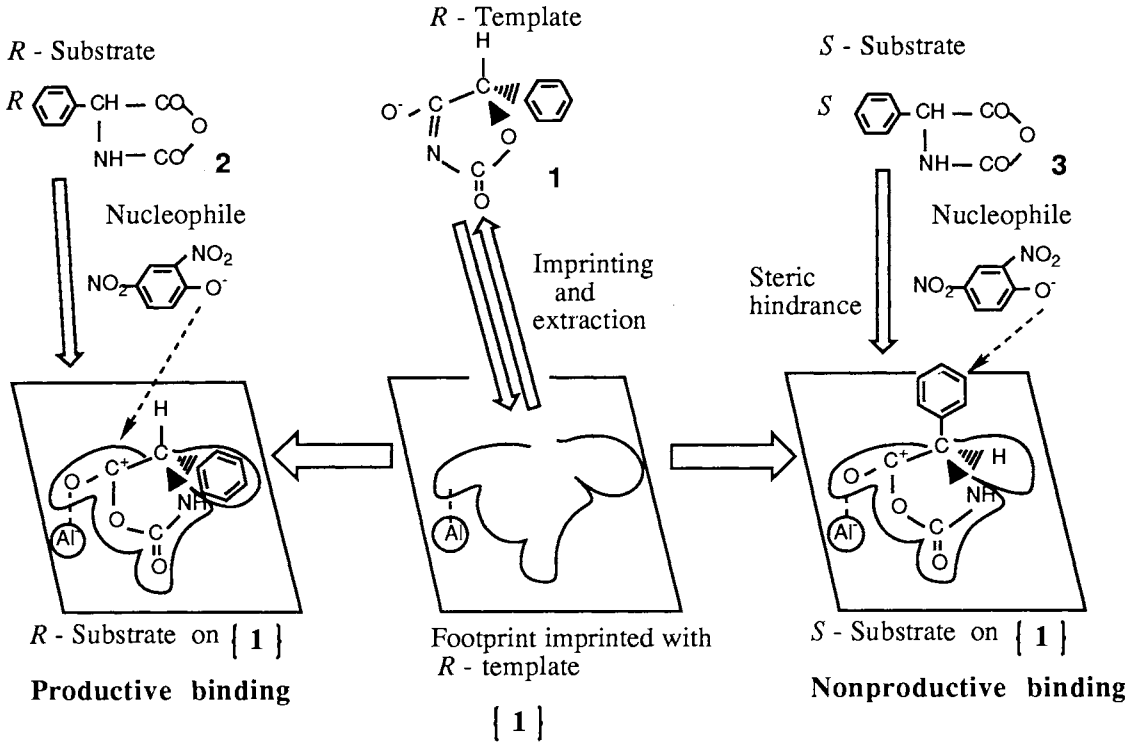
Table 1. Kinetic Parameters of the Catalyzed Reactions by Footprint {**1**}

Catalysts	Substrates	$10^4 \times K_m$ / $dm^3 mol^{-1}$	$10^4 \times k_{obsd\ max}$ / s^{-1}	$10^{-2} \times k_{cat}$ [b] / $dm^3 mol^{-1} s^{-1}$	$10^{-5} \times k_{cat}/K_m$ [c] / $dm^6 mol^{-2} s^{-1}$	$10^5 \times K_i$ [d] / $dm^3 mol^{-1}$
{**1**}[a]	**2**	1.42	3.69	2.80	19.7	3.29
	3	3.33	2.96	2.24	6.73	
Control gel [a]	**2**	11.5	8.77	5.48	4.77	no inhibition
	3	11.9	8.67	5.42	4.55	

a) Amount of catalytic sites, 2.64×10^{-6} mol per gram for {**1**} and 3.20×10^{-5} mol per gram for control.

b) $k_{obsd\ max}$ / molarity of catalytic sites per gram. c)Substrate specificities of catalytic sites.

d) K_i, competitive inhibition constants by the antipode of the substrate.



Scheme 1.

Instead, "productive binding and nonproductive binding" mechanism[10] can explain this enantioselective catalysis. We have been introduced this mechanism to expain similar enantioselective catalyses observed over the catalytic cavities imprinted with chiral alanine derivatives, **4**, and **5**.[5,6] As shown in Scheme 1, **2** can bind onto { **1** } in the same adsorption mode with **1** to make the maximum interaction with the cavities. This binding mode places the phenyl groups of the substrate molecules within the cavities. (Productive binding in Scheme 1). Thus the nucleophile can approach downward to the carbonyl group on a Lewis acid site. Whereas, **3** must bind onto { **1** } in another adsorption mode which forces the phenyl group to stand perpendicularly (Non productive binding in Scheme 1). Such standing phenyl groups might retard the approach of the nucleophiles through their steric hindrance, that causes the enantioselective activity of { **1** } for **2** higher than **3**. The selectivity is manifest in all term, K_m, k_{cat} and k_{cat}/K_m. The ratio of *R*/*S* of the k_{cat}/K_m is 2.5.

The enantioselective catalyses so far observed over imprinted cavities are those on the cavities imprinted with **4**, and **5**. In the former case, where the (*S*)-substrate with sufficient optical purity could not be prepared, only indirect kinetic means examined the selectivity. In the latter case, occurrence of the product inhibition complicated the results; apparent reverse of the enantioselectivity was observed in low substrate concentration. Therefore, the results reported here definitely prove the enantioselective catalysis over the chirally imprinted cavities for the first time.

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7) Template **1** was prepared according to the literature (W. Taube and R. Asher, *Ber.*, **46**, 2082(1913)); Ethyl (R)-(-)-mandelate was allowed to react with cyanamide hydrochloride, and the resulting isohydantoin was hydrolyzed with ethanolic hydrochloric acid to yield **1**, mp 125-127 °C. IR (KBr) 3191 (NH), 1827, 1735 (CONHCOO), 769 and 697 cm^{-1} (CH, phenyl); ^{1}NMR ($CDCl_3$) δ = 5.82 (CH), 7.41 (ArH), 7.91 (NH); (Found; C, 61.42; H, 4.28; N, 7.71%); $[\alpha]_D$ -23.7 °(*c* 2, EtOH).

8) **2** and **3** were prepared according to the usual procedures(J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Jon Wiley & Sons, Inc., New York-London(1961), p. 860). **2**: Mp 115-117 °C; IR (KBr) 3265 (NH), 1847, 1783 (anhydride), 762 and 703 cm^{-1} (CH, phenyl); ^{1}NMR ($CDCl_3$) δ = 5.37 (CH), 6.29 (NH), 7.45 (ArH); (Found; C, 61.04; H, 4.08; N, 7.97%); $[\alpha]_D$ -125° (*c* 2, EtOAc).
3: Mp 115-117 °C; IR and ^{1}NMR were identical with those of **2**; $[\alpha]_D$ +137°(*c* 2, EtOAc).

9) rate = k_{obsd}[2,4-DNP$^-$], k_{obsd} = [Catalyst] [**2**, or **3**] / (K_m + [**2**, or **3**]).

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