  Bull. Chem. Soc. Jpn., 61, 3999—4003 (1988)

Footprint Catalysis. II. Molecular Recognition of Footprint Catalytic Sites

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(Received March 22, 1988)

In order to investigate the molecular recognition capability of "footprint" catalytic sites, the affinities of nine "footprint" catalysts for eleven competitive inhibitors, closely related to template molecules, were estimated through the competitive inhibition constants, K_i. An examination of the affinity revealed that the recognition by "footprint" catalytic sites was so highly specific that it could recognize not only the whole, but also the partial, structures of the bound molecules.

Our preceding paper of this series[1] reported that a new imprinting procedure for aluminium ion-doped silica gel by the action of a transition state analog (i.e., dibenzamide or *N*-benzoylbenzenesulfonamide) as a template formed unprecedented "imprinted" Lewis acid sites ("footprints") with a structure complementary to the template used, and that the "footprints" showed enzyme-like specific catalytic behavior regarding the butanolysis of benzoic anhydride.

The unique specific catalysis by the footprints has been assumed to arise from the molecular recognition capability of the "imprinted" catalytic sites through their "complementarity"[1] (or "electronic strain"[2]) to the molecule of transition state analog used.

The present paper describes a further investigation regarding the molecular recognition capability of the "imprinted" catalytic sites in order to prove the assumptive "complementarity"[1] and to obtain fundamental data necessary for designing tailor-made catalysts with substrate specificity by this imprinting method.

Since both substrate specificity in catalysis and susceptibility to competitive inhibitors originate from the same "complementarity" of the footprints, an examination of the suceptibility ($=K_i$) was carried out, before any investigation of the substrate specificity.

Nine "imprinted" catalysts were prepared by the imprinting method using nine different templates which were closely related to dibenzamide or *N*-benzoylbenzenesulfonamide, respectively. They were subjected to a "cross" inhibition study, in which all catalytic sites were crosswise affected by several competitive inhibitors, including the original template, in order to estimate the binding affinity ($1/K_i$) between the catalytic sites and the inhibitors.

The magnitude of the recognition capability of the catalytic sites was defined as $-dG\{=RT\ln(1/K_i)\}$. An examination of the observed $-dG$ values proved that the recognition by the footprints were so highly specific and exact that the catalytic sites could recognize not only whole bound molecules but also even partial structures of the molecules.

Experimental

Materials. Template molecules and related compounds (**1**—**15**) were prepared by the usual pyridine-assisted acylation of benzamide, benzenesulfonamide, and their para-substituted derivatives with benzoyl chloride, *p*-toluoyl chloride, and acetic anhydride, and characterized by mp, IR (JASCO IRA-1), NMR (HITACHI R-600, 60 MHz), and elemental analysis, if necessary. Other materials were the commercial products of guaranteed grade from Nacalai Tesque Co. Ltd., which were used without further purification, if not specified.

N-Benzoylbenzenesulfonamide (**1**); mp 148 °C (lit, 147 °C).

Dibenzamide (**2**); mp 145.5—148 °C (lit, 148 °C); (Found; C, 74.81; H, 4.88; N, 6.14%).

N-Acetylbenzamide (**3**); mp 116—117 °C (lit, 115 °C); (Found; C, 66.25; H, 5.52; N, 8.58%).

N-Benzoyl-*p*-toluenensulfonamide (**4**); mp 145 °C (lit, 147—150 °C).

N-(*p*-Toluoyl)-*p*-toluenesulfonamide (**5**); mp 138—139 °C; IR(Nujol) 3300 (N-H), 1700 (C=O), 1345 and 1175 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$) δ=8.18—6.87 (m, 8H, ArH), 2.36 (s, 3H, CH_3-), and 2.29 (s, 3H, CH_3-); (Found; C, 61.98; H, 5.20; N, 4.78%).

N-Acetylbenzenesulfonamide (**6**); mp 125—126 °C; IR (Nujol) 1690 (C=O), 1360 and 1165 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$) δ=9.47 (s, 1H, NH), 8.40—7.35 (m, 5H, ArH), and 2.05 (s, 3H, CH_3-); (Found; C, 48.38; H, 4.57; N, 7.00%).

N-Benzoyl-4-(decanoylamino)benzenesulfonamide (**7**); mp 211 °C; IR (Nujol) 3285, 3200 (N-H), 1720, 1695 (C=O), 1340 and 1180 cm^{-1} (SO_2); ^1H NMR($CDCl_3$) δ=7.26 (m, 9H, ArH), 1.90 (s, 2H, -CH_2-CO-) and 0.98 (s, 3H, CH_3-); (Found; C, 63.89; H, 7.01; N, 6.66%).

N-Benzoyl-4-(acetamido)benzenesulfonamide (**8**); mp 257—260 °C; IR (Nujol) 3300 (N-H), 1655 (C=O), 1535 (amide II), 1320 and 1170 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$) δ=7.22 (m, 9H, ArH) and 1.47 (s, 3H, CH_3-).

4-(Decanoylamino)benzenesulfonamide (**9**); prepared by chlorosulfonation and succesive amination of decananilide; mp 207—209 °C; IR (Nujol) 3265 (N-H), 1685 (C=O), 1535 (amide II), 1320 and 1155 cm^{-1} (SO_2); ^1H NMR($CDCl_3$) δ=7.24 (m, 4H, ArH), 1.95 (s, 2H, -CH_2-CO-), 1.51 (m, 14H, -CH_2-), and 0.96 (s, 3H, CH_3-); (Found; C, 59.11; H; 8.00; N, 8.62%).

4-(Acetamido)benzenesulfonamide (**10**); mp 219.5 °C (lit, 219 °C).

N-Acetyl-4-(decanoylamino)benzenesulfonamide (**11**); mp

165 °C; IR(Nujol) 3340, 3290 (N-H), 1730, 1690 (C=O), 1520 (amide II), 1320, 1160 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$) δ=8.1—7.1 (m, 6H, ArH), 2.01 (s, 2H, $-CH_2-CO-$), 1.49 (s, 14H, $-CH_2-$) and 1.21 (S, 6H, CH_3-); (Found; C, 58.77; H, 7.72; N, 7.71%).

N-Acetyl-4-(acetamido)benzenesulfonamide (**12**); mp 251°C; IR(Nujol) 3330(N-H), 1705, 1680(C=O), 1550 (amide II), 1320 and 1170 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$) δ=7.24 (m, 4H, ArH), and 1.52 (s, 6H, CH_3-); (Found; 46.79; H, 4.64; N, 11.00%).

N-Benzoyl-4-(methanesulfonamido)benzenesulfonamide (**13**); mp 264—267 °C; IR (Nujol) 3190 (N-H), 1690 (C=O), 1350, 1330, 1170, and 1140 cm^{-1} (SO_2); ^1H NMR (DMSO-d_6) δ=7.50 (m, 9H, ArH) and 3.22 (s, 3H, CH_3-SO_2-); (Found; C, 47.49; H, 3.83; N, 8.04%).

4-(Methanesulfonamido)benzenesulfonamide (**14**); prepared by methanesulfonylation of sulfanilamide; mp 184—187 °C; IR (Nujol) 3400, 3310, 3260 (N-H), 1625, 1350 and 1170 cm^{-1} (SO_2); ^1H NMR (DMSO-d_6) δ=7.6 (m, 4H, ArH) and 3.19 (s, 3H, CH_3-SO_2-); (Found; C, 33.73; H, 3.93; N, 11.10%).

Diacetamide **15**; prepared from acetamide according to a method described in the literature.[2] Mp 75—76 °C (lit, 77.5—78 °C).

Dibenzoylmethane **16**; recrystallized from ethanol. mp 74.5 °C (lit, 77.5—78 °C).

Catalysts. The preparation procedures and acid sites characterizations of catalysts were the same as those described in the preceding paper, except for the use of different compounds as templates.

Kinetic Measurement. The reaction conditions and procedures were the same as those reported in the preceding paper, i.e., 1-butanolysis of benzoic anhydride at 55 °C in 1-butanol-benzene mixture (30:70 v/v), and the kinetic parameters, V_{max}, K_m, and K_i were obtained as usual from Lineweaver-Burk plots.

Reslts and Discussion

All the butanolyses obeyed Michaelis-Menten kinetics with respect to the concentration of benzoic anhydride; the kinetic parameters for catalized reactions are shown in Table 1. Some typical competitive inhibition plots are shown in Figs. 1—5. A coincidence of the intercepts on the $1/V$ axis were observed in all Lineweaver-Burk plots in the presence of different inhibitors (Figs. 2—5), which confirmed that all the different inhibitors were bound onto the same catalytic sites. Therefore, the affinities of a catalytic site for different inhibitors can be estimated and significantly compared with each other. The affinity, molecular recognition, by the catalytic sites is

Table 1. Kinetic Parameters of Footprint Catalysts

Footprint catalyst {N}[a]	K_m	$10^5 \times V_{max}$[b]
	M[c]	M min^{-1}
{**1**}	0.54	40.9
{**1**}[d]	0.57	31.7
{**2**}	0.19	19.0
{**2**}[d]	0.50	35.7
{**3**}	0.54	3.6
{**3**}[d]	1.05	6.5
{**4**}	0.17	19.0
{**5**}	0.29	21.1
{**7**}	0.13	8.1
{**8**}	0.06	4.7
{**8**}	0.23	10.2
{**9**}	0.35	11.2
{**13**}	0.08	1.3

a) Footprint catalyst {N} represents the catalyst imprinted by template N. b) Per catalyst 1.0 g. c) 1 M=1 mol dm^{-3}. d) Other catalyst preparation used mainly in preliminary experiments.

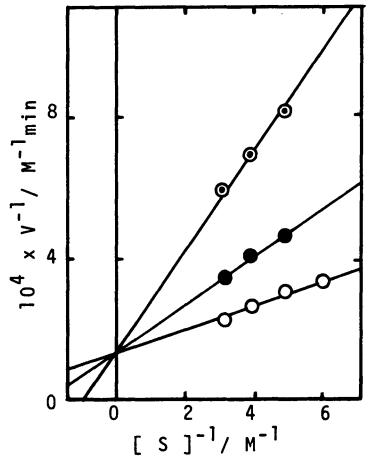

Fig. 1. A criterion for footprint catalytic sites; competitive inhibition of the catalyst {**8**} by the original template **8**. Catalyst {**8**} (K_m 0.23 M), 700 mg; $V=V_{obs}-V_{uncat}$; [S]=benzoic anhydride concentration; [I]=inhibitor (=template **8**) concentration. ○: [I]=0, ●: [I]=3.0×10^{-4} M, ⊙: [I]=5.0×10^{-4} M, K_i=2.79×10^{-4} M, $-dG=RT\ln(1/K_i)$=22.3 kJ.

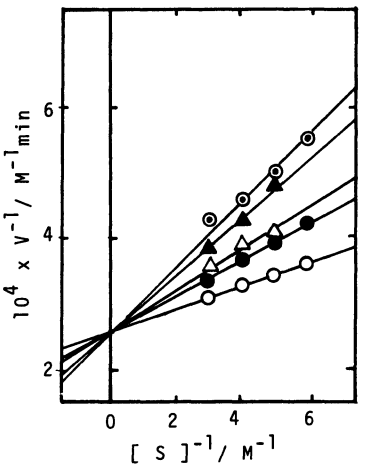

Fig. 2. Competitive inhibition of footprint catalyst {**8**} by **1**, **8**, **10**, and **12**.
Catalyst {**8**} (K_m 0.06 M), 700 mg; [I]=5.0×10^{-4} M; ⊙: by **8**, K_i=2.62×10^{-4} M ($-dG$=22.6 kJ), ▲: by **10**, K_i=3.29×10^{-4} M (21.8 kJ), △: by **12**, K_i=5.46×10^{-4} M (20.5 kJ), ●: by **1**, K_i=8.22×10^{-4} M (20.1 kJ), ○: no inhibitor.

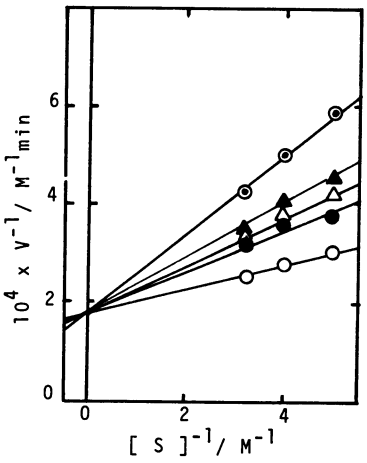

Fig. 3. Competitive inhibition of footprint catalyst {7} by **1**, **7**, **9**, and **11**.
Catalyst {7} (K_m 0.13 M), 700 mg; [I]=5.0×10^{-4} M; ⊙: by **7**, K_i=2.05×10^{-4} M (−dG=23.0 kJ), ▲: by **9**, K_i=3.56×10^{-4} M (21.8 kJ), △: by **11**, K_i=4.53×10^{-4} M (20.9 kJ), ●: by **1**, K_i=5.59×10^{-4} M (20.5 kJ), ○: no inhibitor.

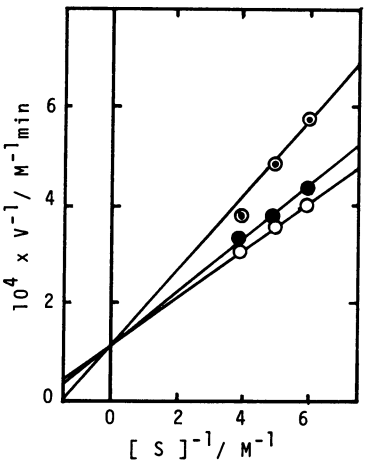

Fig. 4. Competitive inhibition of footprint catalyst {9} by **1** and **9**.
Catalyst {9} (K_m 0.35 M), 700 mg; [I]=3.0×10^{-4} M; ⊙: by **9**, K_i=4.32×10^{-4} M (−dG=21.3 kJ), ●: by **1**, K_i=2.77×10^{-3} M (15.9 kJ), ○: no inhibitor.

expressed by the negative value of the free-energy change on binding; $-dG=RT\ln(1/K_i)$, where K_i is a competitive inhibition constant.

As can be seen in Table 2, each catalytic site reveals the maximum affinity(specified by *) for its original template (the template used in imprinting process.) as an inhibitor without exception. This fact strongly suggests that the molecular recognition capability of the footprints must be closely related to its formation process, and that the it must arise from a "complementarity" to its original template. Since molecular recognition is based on the "complementarity",[1] ("electronic strain"),[2] the magnitude of the affinity for a bound molecule greatly depends upon the resemblance in shapes and structures between the original template of the footprint and the inhibitors, e.g., footprint {1}* clearly differentiates **1** from **3**, **4**, **5**, **6**, and **15** by more than 14.7 kJ (=23.9—9.2 kJ; dG for **3**), whereas it hardly differentiates **1** from the structurally resembling **2** by only 1.3 kJ (=23.9—22.6 kJ), and similar feature is also seen on footprint {2} and {3}. Footprints show less affinity for molecules and/or groups that are too small to fit into the "complementary" structures, e.g., footprint {2} prefers **2** to **3** by 14 kJ, and exclude molecules and/or group that are too large to fit the structures, e.g., footprint {1} excludes **4** and **5** by about 20 kJ.

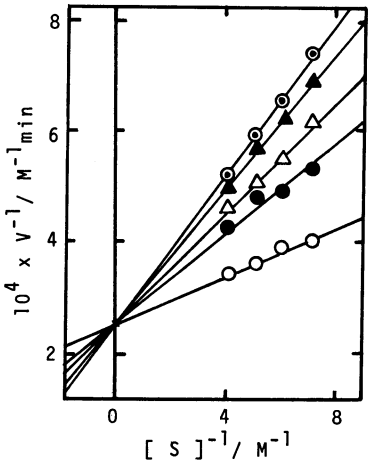

Fig. 5. Competitive inhibition of footprint catalyst {13} by **1**, **8**, **13**, and **14**.
Catalyst {13} (K_m 0.08 M), 300 mg; [I]=5.0×10^{-4} M; ⊙: by **13**, K_i=2.29×10^{-4}M (−dG=23.0 kJ), ▲: by **14**, K_i=2.68×10^{-4} M (22.6 kJ), △: by **8**, K_i=3.68×10^{-4} M (21.8 kJ), ●: by **1**, K_i=5.59×10^{-4} M (20.5 kJ), ○: no inhibitor.

It should be noted that the magnitude of differentiation by the "imprinted" catalytic sites mentioned above is sufficiently large to be comparable to those by the "built-in" receptor sites of macrocycles, considering that 18-crown-6 differentiates K^+ from Na^+ by 10.15 kJ,† and dibenzo-18-crown-6 does so by 3.66 kJ† in methanol at 25 °C.

Furthermore, an examination of the data in Table 2 proves that the affinity for whole molecules(dG_w) can be expressed as the sum of those for partial structures(dG_p s) of the binders(inhibitors and templates); $dG_w=\sum dG_p$, as shown in Scheme 1. Assuming that the footprint {7} consists of three subsites (**X**, **Y**, **Z**) (**X** is situated for the *p*-substituent moiety, **Y** for the benzenesulfonamide moiety, and **Z** for the *N*-acyl

* Footprint{N} represents the footprint imprinted by template **N**.

† Calculated from data in Ref. 4.

Table 2. Molecular Recognition by Footprints; Specific Affinities

Inhibitor	Molecular recognition by footprint {N}; $-dG\{=RT\ln(1/K_i)\}$/kJ								
	{1}	{2}	{3}	{4}	{5}	{7}	{8}	{9}	{13}
1: Ph-SO_2-NH-Bz	23.9*	20.5	8.0			20.5	20.1	15.9	20.5
2: Bz-NH-Bz	22.6	22.6*	8.4						
3: Ac-NH-Bz	9.2	8.4	13.0*						
4: Tosyl-NH-Bz	2.9	2.9		21.3*	4.6				
5: Tosyl-NH-Toluoyl	4.2	0.0	4.2		18.8*				
6: Ac-NH-Tosyl	5.0	0.8	2.9						
	7.1**	3.3**	10.9**						
7: *p*-(C_9H_{19}-CO-NH-)C_6H_4-SO_2-NH-Bz						23.0*			
8: *p*-(Ac-NH-)C_6H_4-SO_2-NH-Bz							22.6*		21.8
9: *p*-(C_9H_{19}-CO-NH-)C_6H_4-SO_2-NH_2						21.8		21.3*	
								21.8**	
10: *p*-(Ac-NH-)C_6H_4-SO_2-NH_2							21.8		
11: *p*-(C_9H_{19}-CO-NH-)C_6H_4-SO_2-NH-Ac						20.9			
12: *p*-(Ac-NH-)C_6H_4-SO_2-NH-Ac							20.5		
13: *p*-(Mesyl-NH-)C_6H_4-SO_2-NH-Bz									23.0*
14: *p*-(Mesyl-NH-)C_6H_4-SO_2-NH_2									22.6
15: Ac-NH-Ac	6.7	2.5	10.9						
	7.3**	6.3**	11.1**						
16: Bz-CH_2-Bz		15.5							

* Maximum values of molecular recognition of footprints to the original template molecules. ** Values calculated from partial affinities shown in Table 4.

Binding of inhibitor **7** onto footprint {7}[a)]
C_9H_{19}-CO-NH-/-C_6H_4-SO_2-NH-/-CO-C_6H_5 $dG=dG_x+dG_y+dG_z=23.0$ kJ
/----**X**-------//-------**Y**--------//------**Z**---/
subsite **X** subsite **Y** subsite **Z**
Binding of **9** onto fotprint {7}
C_9H_{19}-CO-NH-/-C_6H_4-SO_2-NH_2 $dG=dG_x+dG_y=21.8$ kJ
/---**X**------//------**Y**------//---**Z**---/

Binding of **1** onto footprint {7}
C_6H_5-SO_2-NH-/-CO-C_6H_5 $dG=dG_y+dG_z=20.5$ kJ
/----**X**-----//-----**Y**--------//-----**Z**---/
Binding of **11** onto footprint {7}
C_9H_{19}-CO-NH-/-C_6H_4-SO_2-NH-/-CO-CH_3 $dG=dG_x+dG_y+dG_z=20.9$ kJ
/----**X**--------//-----**Y**-------//---**Z**---/

a) Footprint {7} represents the footprint imprinted by template **7**.

Scheme 1. Estimation of partial affinities of footprints.

moiety), then the difference in affinity (1.2 kJ=dG_z) between dG_w (23.0 kJ) of the footprint {7} for inhibitor-(=template) **7** and dG_w (21.8 kJ) of the footprint {7} for inhibitor **9** can be attributable to the partial affinity of the subsite *Z* for the benzoyl moiety of the inhibitor **7**. Similarly, the difference (2.5 kJ=dG_x) between dG_w (20.5 kJ) of the footprint {7} for the inhibitor **1** and dG_w (23.0 kJ) of the footprint {7} for the inhibitor **7** can also be attributed to a partial affinity of subsite **X** for decanoylamino group of inhibitor **7**. The residual affinity (19.3 kJ) should be ascribed to the partial affinity of subsite **Y** for benzenesulfonamide moiety of the inhibitor **7**.

Table 3. Estimated Partial Footprint Affinities

Inhibitors bound	Footprint {N}*	dG	$=dG_x+$	dG_y+	dG_z
		(kJ)			
7	{7}	23.0	= 2.5 +	19.3 +	1.2
8	{8}	22.6	= 2.5 +	19.3 +	0.8
				av.	1.0
13	{13}	23.0	= 2.5 +	20.1 +	0.4
11	{7}	20.9	= 2.5 +	19.3 −	0.9
12	{8}	20.5	= 2.5 +	19.3 −	1.3
				av. 19.5	av. −1.1

* Footprint {N} represents the footprint imprinted by template **N**.

Similar substructive treatments are applicable to footprints {8} and {13}, as shown in Table 3. A closely similar feature is observed in the dG_y and dG_z values, which are identical within experimental errors. The

Table 4. Molecular Recognition of Subsites of Footprints toward Partial Structures of Bound Molecules

Partial structures (**A**–**H**) of bound molecules onto subsite **Y**;	Partial affinity ($-dG_p$/kJ) of subsite **Y**{**N**}* toward **A**–**E**; {**N**} = {**A**},	{**B**},	{**C**},	{**D**},	{**E**}
A; Ph-SO_2-NH-	22.9	19.5		19.5	7.0
B; Ph-CO-NH-	21.6	21.6			7.4
C; *p*-CH_3-C_6H_4-SO_2-NH-			21.3		
D; *p*-NH-C_6H_4-SO_2-NH-				19.3	
E; CH_3-CO-NH-	8.2	7.4			12.0

subsite **Z**;	$-dG_p$ of subsite **Z**{**N**}* toward **F**–**G**; {**N**} = {**G**}	{no footprint}
F; -SO_2-Ph	−1.1	
G; -CO-Ph	1.0	−12.1
H; -CO-CH_3	−1.1	

subsite **X**;	$-dG_p$ of subsite **X**{**N**}* toward **I**–**K**; {**N**} = {**I**}	{**J**}	{**K**}
I; *p*-(CH_3-CO-NH-)	2.5		1.2
J; *p*-(C_9H_{19}-CO-NH-)		2.5	
K; *p*-(CH_3-SO_2-NH-)			2.5

* Subsite **Y**{**N**} represents the subsite **Y** of the footprint imprinted by template **N**.

dG_y values (19.3—20.1 kJ) are much higher than the dG_z values (0.4–1.2 kJ) and the dG_x value (2.5 kJ), which indicates that only one Lewis acid site has been formed and located at subsite **Y** (in each case).

Footprints {**7**}, and {**8**} differentiate acetyl groups of inhibitors **11** and **12** from th benzoyl groups of inhibitors **7** and **8**, respectively, with a negative partial affinity (−0.9, −1.3 kJ). This reveals that an enforced binding occurs to give an intramolecular strain to the bound molecule since the catalytic sites are rigid.

The partial affinities of footprints can be estimated from the data given in Table 2 and shown in Table 4. In the estimation process, the average value of dG_z (1.0 kJ) of footprints {**7**} and {**8**} was used as a starting standard value under the assumption of a priority order for subsite **Y**, *N*-acetyl > -C_6H_4-SO_2-NH- > *N*-benzoyl, because all the inhibitors in Table 2 are ambiguous in action mode during the imprintig and the binding(inhibition) processes. Such a priority order is therefore necessary to define their action mode in the inhibition. Only the above order has offered reasonable estimates agreeable to the observed values shown in the Table 2. The true calculated values (** in Table 2) obtained from the partial footprint affinity (Table 4) agree reasonably well with the practically reliable observed values (> ca. 5 kJ). This finding confirms that the assumption that $dG_w = \sum dG_p$ is reasonable, i.e., the molecular recognition of the footprints can govern even the partial structures of the bound molecules. It also shows that the data given in Table 3 are reliable as a whole.

Though the values given in Tables 2 and 4 have been obtained only from the present catalyst preparations and the reaction system employed in the present work, the whole feature might not change so very much in other reaction systems.

Since the specific affinities and intramolecular strain effects would be essential in enzymatic catalysis,[5] the data for the partial affinities and strains shown in Tables 2 and 3 might be applicable to the design of tailor-made enzyme-simulated catalysts with any substrate specificities. Various attempts are now in progress.

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