Enzyme-like Enantioselective Catalysis over Chiral 'Molecular Footprint' Cavities on a Silica (Alumina) Gel Surface

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Chiral catalytic cavities were imprinted on a silica gel surface using a chiral template, *N*-benzoyl-(*N*$^{\alpha}$-benzyloxycarbonyl)-L-alanineamide, by an imprinting procedure; these cavities display enantioselective catalysis in 2,4-dinitrophenolysis of the corresponding substrate, benzoic *N*-benzyloxycarbonyl-L-alanine anhydride.

According to our 'molecular footprint' imprinting method for tailored specific catalysts,[1–3] chiral catalytic cavities capable of chiral recognition have been designed and successfully imprinted on the surface of aluminium ion-doped silica gel using chiral template molecules, *N*-benzoyl-(*N*$^{\alpha}$-benzyloxycarbonyl)-L-alaninamide (Z-L-Ala-NH-Bz).† The templates were referred to as the transition state or reactive intermediate analogues[4] of benzoic *N*-benzyloxycarbonyl-L-alanine anhydride (Z-L-Ala-O-Bz),‡ the corresponding substrate for the catalysed transacylation, 2,4-dinitrophenolysis.[3]

The 'footprints' are specific adsorption sites with complementary structures to the template molecules. They involve Lewis acid sites[5] and can function as specific catalytic sites, because their specific affinity to the templates can also act on the substrate to stabilise their transition state or reactive intermediate in the reaction,[6] as the recently developed catalytic antibodies do.[7,8]

The 'footprint' catalysts were prepared from Merck Kieselgel 60 *via* imprinting procedures using L- and DL-template molecules.§ The catalysed reactions were followed photometrically,[3] and found to obey Michaelis–Menten kinetics,¶ and catalytic activities were estimated from values of k_{cat}/K_m.

As the mixed anhydride substrates were too labile to repeat recrystallization and so the D-substrate with sufficient optical purity had not been prepared as yet from commercially available D-alanine, it was necessary to study enantioselective catalysis by indirect kinetic means: two catalysts imprinted with L- and DL-templates (hereafter referred to as {L} and {DL}) were allowed to react with the L- and DL-substrate, respectively, and their differences in catalytic activities were analysed kinetically.

† Z-L- and -DL-Ala-NH-Bz were prepared from Z-Ala-NH_2 by the action of NaH (2 equiv.) in tetrahydrofuran (THF) and subsequent acylation with benzoic anhydride (1 equiv.), and recrystallised from EtOH–water. IR (KBr): ν/cm^{-1} 3356 and 3303 (N–H), 1692 (CO, amide I), 1677 and 1524 (amide II), 1454 (CH_3) and 1256 (amide III); ^{1}H NMR($CDCl_3$): δ 1.49 (d, CH_3), 5.03 (CH), 5.14 (CH_2), 5.37 (NH), 7.2–7.8 (m, ArH) and 9.16 (NH). L-Form, m.p. 122–123 °C; $[\alpha]_D^{21}$ −12 (*c* 2, EtOH) (Found: C, 65.0; H, 5.6; N, 8.5. $C_{18}H_{18}N_2O_4$ requires C, 66.2; H, 5.6; N, 8.6%); DL-form, m.p. 119–121 °C (Found: C, 64.45; H, 5.6; N, 8.1%).

‡ Z-L- and -DL-Ala-O-Bz were prepared according to the usual procedure for mixed anhydride: J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, New York–London, 1961; pp. 970–978, and recrystallised from ethyl acetate–light peteoleum; IR (KBr): ν/cm^{-1} 3309 (N–H), 1820 and 1754 (CO, anhydride), 1693 (amide I), 1542 (amide II), 1456 (CH_3), 1259 (amide III) and 1096 (C–O–C). L-Form, m.p. 107–111 °C; DL-form, m.p. 60–62 °C, lit. 61 °C: T. Wieland, W. Kern and R. Sehring, *Liebigs Ann. Chem.*, 1950, **569**, 122.

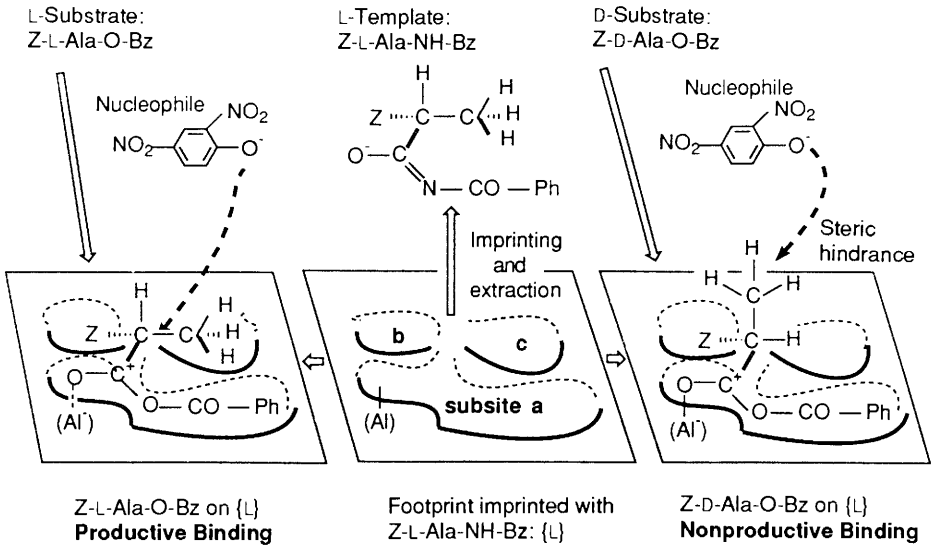
§ The silica gel surface was activated by acid hydrolysis prior to subsequent doping with aluminium ions and template molecules. After ageing and drying, the template molecules were removed by methanol extraction. Details are given in ref. 1.

¶ $v = k_{obs}$ [2,4-DNP$^-$], $k_{obs} = k_{cat}$ [Cat.] [Z-Ala-O-Bz]/(K_m + [Z-Ala-O-Bz]).

Table 1 Kinetic parameters for catalysed reaction (30 °C, in MeCN)

Run	Cat.	Substr.	K_m[a]/10^{-2} dm^3 mol^{-1}[a]	V_{max}[b]/10^{-2} dm^3 mol^{-1} s^{-1}	(K_m/V_{max})[c]/s	k_{cat}[d]/10^3 s^{-1}	(k_{cat}/K_m)/10^6 dm^3 mol^{-1} s^{-1}	K_i[e]/10^{-3} dm^3 mol^{-1}
1	{L}[f]	L	1.32	2.35	0.56	16.38	1.24	(0.0012)[g]
2	{L}	DL	0.75	0.62	1.20	4.30	0.58	
		DL	(0.37)[h]	(0.62)	(0.60)	(4.30)	—	4.7
3	{DL}[f]	L	2.49	1.31	1.90	4.30	1.72	
4	{DL}	DL	0.15	0.16	0.97	0.52	0.34	1.60
5	Control[f]	L	1.72	0.81	2.10	2.83	0.16	
6	Control	DL	1.07	0.53	2.02	1.84	0.17	

[a] Apparent K_m. [b] $k_{obs,max}$ for 50 mg catalyst. [c] Slope of Lineweaver–Burk plots. [d] k_{cat} for 1 g catalyst. [e] K_i, competitive inhibition constants by the antipode of the substrate calculated from eqn. (2) or eqn. (4). [f] Amount of catalytic sites, 28.7 × 10^{-6} mol per gram for {L}, 61.1 × 10^{-6} mol per gram for {DL} and 57.5 × 10^{-6} mol per gram for control. [g] K_i of original template, Z-L-Ala-NH-Bz. [h] (Corrected value) according to eqn. (2), wherein intrinsic substrate is L-form and [S] is half of [S_{DL}].



Scheme 1

As shown in Table 1, the native catalytic sites of the control catalyst naturally showed identical activities (k_{cat}/K_m) towards the L- and DL-substrate, whereas the imprinted catalysts {L} and {DL} exhibited distinctly different catalytic behaviour towards the L- and DL-substrate. This clearly demonstrates that a certain enantioselective mechanism is operating involving the chiral 'footprint' catalytic cavities. A 'lock-and-key' mechanism[9] through simple exclusion effects of the cavities, however, is not sufficient to explain the results because, if such a mechanism were operative, Runs 1 and 3 should show the same K_m as Runs 2 and 4, respectively, and the slope of Run 3 should be half that of Run 4. However, a 'productive binding and nonproductive binding' mechanism[10,11] could explain the results based on the following assumptions (Scheme 1). (*i*) The L-templates mark chiral 'molecular footprints', {L}, which consist of three subsites[2] (a, b and c in Scheme 1) corresponding to three partial structures of the template molecules, *i.e.* -CO-NH-Bz, Z-NH-, and Me-groups (not α-H) of the alanine residue. (*ii*) The L-substrate molecules bind onto {L} through three-point adsorption (a, b and c),[12] keeping the same α-carbon configuration as the templates; this places the carbonyl group of the reaction centre for activation just on the Lewis acid site (Al), thus allowing transformation by nucleophilic attack with 2,4-dinitrophenoxide (DNP^-) (productive binding). (*iii*) The D-substrate molecules bind onto {L} through another three-point adsorption; -CO-O-Bz and Z-NH-groups, which are capable of hydrogen bond formation, preferentially bind onto their corresponding subsites (a and b), while the hydrophobic α-H is forced into subsite c in place of the Me-group. Consequently, the Me-groups of the D-substrate stand perpendicularly, and shield the carbonyl carbon from nucleophilic attack (nonproductive binding), thus showing that the D-antipode is a very poor substrate and acts as a competitive inhibitor.‖ From these assumptions, the Lineweaver-Burk equations (1)–(4) were easily derived.

$$1/v = 1/V_{max} + K_m/(V_{max}[S_L]) \quad (1)$$

$$1/v = (1 + K_m/K_i)/V_{max} + K_m/(V_{max}[S_L]) \quad (2)$$

$$1/v = 2/V_{max} + 2K_m/(V_{max}[S_L]) \quad (3)$$

$$1/v = (1 + K_m/K_i)/V_{max} + K_m/(V_{max}[S_{DL}]) \quad (4)$$

Eqn. (1) was the original Lineweaver–Burk equation for Run 1, and eqn. (2) for Run 2 was derived from the usual Michaelis–Menten equation in the presence of a competitive inhibitor, wherein [S] = [S_L], [I] = [S_D], and [S_L] = [S_D]. Eqn. (3) for Run 3 was derived from eqn. (1) considering that only {L}, a half of the catalytic sites {DL}, participated in the catalysis, and eqn. (4) for Run 4 was derived as eqn. (2) except [S_L] = [S_{DL}].

‖ Preliminary confirmation of this assumption was obtained in a separate competitive inhibition experiment using the dominant L-substrate plus a small amount of the D-substrate; a K_i value of 3.18 × 10^{-4} dm^3 mol^{-1} was obtained.

According to these equations, the slopes of Runs 1 and 2 should be equal (obs. K_m/V_{max}: 0.56 and 0.60, respectively); Run 1 should have a larger intercept ($1/V_{max}$) by a factor of ($1 + K_m/K_i$) than that of Run 2 (obs. intercepts: 162.2, 42.6, respectively); Run 3 should have a slope which is twice that of Run 4 and an intercept smaller by a factor of $(1 + K_m/K_i)/2$ than that of Run 4 (obs. slopes: 1.90, 0.97, respectively; intercepts 76.2, 627.8 respectively). This fair coincidence of experimental data with the theoretical values confirms the operation of an enantioselective mechanism. This enantioselective catalysis should extend the scope of our strategy for tailored specific catalysts by the molecular imprinting method.

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References

1 K. Morihara, S. Kurihara and J. Suzuki, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 3991.
2 K. Morihara, E. Nishihata, M. Kojima and S. Miyake, *Bull. Chem. Soc. Jpn.*, 1988, **61**, 3999.
3 K. Morihara, E. Tanaka, Y. Takeuchi, K. Miyazaki, N. Yamamoto, Y. Sagawa, E. Kawamoto and T. Shimada, *Bull. Chem. Soc. Jpn.*, 1989, **62**, 499.
4 R. Wolfenden, *Acc. Chem. Res.*, 1972, **5**, 10; *Annu. Rev. Biophys. Bioeng.*, 1976, **5**, 271.
5 M. W. Tamele, *Faraday Discuss.*, 1950, **8**, 270.
6 L. Pauling, *Chem. Eng. News*, 1946, **24**, 1375; *Am. Scient.*, 1948, **36**, 51.
7 A. Tramontano, K. D. Janda and R. A. Lerner, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 6736.
8 J. Jacobs, P. G. Schultz, R. Sugasawara and M. Powell, *J. Am. Chem. Soc.*, 1987, **109**, 2174.
9 E. Fischer, *Chem. Ber.*, 1894, **27**, 2985.
10 S. A. Bernhard and H. Gutfreund, *Proc. Intern. Symp. Enzyme Chem.*, Tokyo Kyoto, 1958, p. 124.
11 C. L. Hamilton, C. Niemann and G. S. Hammond, *Proc. Natl. Acad. Sci. USA*, 1966, **55**, 664.
12 A. G. Ogston, *Nature (London)*, 1948, **162**, 963.