Molecular Catalysis: Enhanced Rates of Thiolysis with High Structural and Chiral Recognition in Complexes of a Reactive Macrocyclic Receptor Molecule

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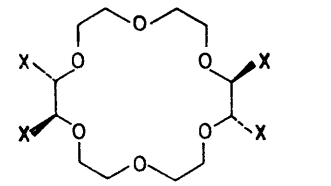
Summary The macrocyclic molecular catalyst (**1**) complexes primary ammonium ester salts and displays enhanced rates of intramolecular thiolysis of the bound substrates with structural selectivity for dipeptide esters and high chiral recognition for the (L) antipode of the enantiomeric glycyl–phenyl–alanine esters.

THE design of highly efficient and selective molecular catalysts may provide enzyme models as well as a new class of 'abiotic' chemical reagents. Rate enhancement by substrate binding has been observed with cyclodextrin derivatives[1] and hydrophobic systems[2,3] bearing reactive groups. Recently, the development of macropolycyclic molecular receptors for polar substrates led to macrocyclic reagents which show enhanced rates of acyl transfer[4,5] and of hydrogen transfer[6,7] with bound substrates.

We now report some of our results on ester thiolysis by a chiral macrocyclic molecular catalyst, (**1**), bearing cysteinyl residues as reactive groups, which displays: (i) complexation of salts of primary aminoesters; (ii) preferential thiolysis of dipeptide esters; (iii) large acceleration of the acyl transfer step; (iv) high chiral discrimination between two enantiomeric dipeptide esters.



(1) X = -CONH-(L)-CH(CH$_2$SH)-CO$_2$Me

(2) X = CO$_2$H

(3) X = COCl

(4) X = -CONH-(L)-CH(CH$_2$-S-CH$_2$Ph)-CO$_2$Me

Condensation of L-cysteine methyl ester with the acid chloride (**3**) of the chiral macrocyclic tetracarboxylic acid (**2**)[8,9] affords the tetra-L-cysteinyl derivative (**1**) (m.p. 204–205 °C; $[\alpha]_D$ +41°, *c* 1, $CHCl_3$; yield 66%). Similarly, compound (**4**) is obtained using *S*-benzyl-L-cysteine methyl ester (m.p. 139—140 °C; $[\alpha]_D$ −44·5°, *c* 1, $CHCl_3$; yield 87%).

TABLE. Kinetic data for release of *p*-nitrophenol from amino acid and dipeptide ester salt substrates in the presence of the macrocyclic reagent (**1**).

Run[a]	Substrate[b] R–OPNP, HBr	Rate constant, 10^5 $k\psi$/s^{-1}[c] solv.	(**1**)	[(**1**) + K$^+$]	Relative rates[d]
1A	Gly	4·8	400	27	15
2A	L or D-Phe	2·9	19	34	0·6
3A	β-Ala	7·8	18	27	0·7
4A	Gaba	75	52	—	—
5A	Gly–Gly	4·8	230	2·9	80
6A	Gly–(L)-Phe	2·4	150	1·5	100
7A	Gly–(D)-Phe	2·4	23	0·7	35
5B	Gly–Gly	390	235,000	1700	140 5B/9B = 1900
8B	Gly–β-Ala	4·3	120	38	3
9B	(L)-Pro–Gly	115	125	590	0·2
10B	CbO–Gly	5·5	14·5	220	0·07
5C	Gly–Gly	59	2600	—	5C/9C = 15,000
6C	Gly–(L)-Phe	74	1600	—	6C/7C = 50
7C	Gly–(D)-Phe	74	30	—	—
9C	(L)-Pro–Gly	77	0·17	—	—
6D	Gly–(L)-Phe	15	1500	—	6D/7D = 90
7D	Gly–(D)-Phe	15	16·5	—	—

[a] The number characterizes the substrate; capital letter indicates the medium. A: MeOH–CH_2Cl_2–H_2O 78·5 : 20 : 1·5, Py–Py.HBr buffer 0·05 M, pH = 6·1; B: MeOH–dimethylformamide–H_2O 78·5 : 20 : 1·5, AcOH–$AcONMe_4$ buffer 0·02 M, pH = 4·8; C: CH_2Cl_2–MeOH–H_2O 97·9 : 2 : 0·1, CF_3CO_2H–*N*-ethylmorpholine buffer 0·3 M, pH = 7·0; D: CH_2Cl_2–EtOH 95 : 5, CF_3CO_2H–*N*-ethylmorpholine buffer 0·03 M, pH = 7·0; the pH indicated corresponds to that of an aqueous solution which would contain the same buffer concentrations as used here. In media A and B, substrate: 10^{-4} M, (**1**): 3·5 × 10^{-3} M, KBr: 1·4 × 10^{-2} M; in media C and D, substrate: 5 × 10^{-5} M, (**1**) and (**4**): 3·5 × 10^{-4} M. [b] All substrates are in the form of ammonium hydrobromides. Gly: glycyl, H_2N–CH_2–CO–; Phe: phenylalanyl, H_2N–CH(CH_2Ph)–CO-; β-Ala: β-alanyl, H_2N–CH_2–CH_2–CO-; Gaba: γ-aminobutyryl, H_2N–$[CH_2]_3$–CO-; Pro: prolyl, HN–$[CH_2]_3$–CH–CO-; CbO: benzyloxycarbonyl $PhCH_2OCO$-. [c] Pseudo-first order rate constants at 20 °C for the release of *p*-nitrophenol determined spectrophotometrically (Cary 118C spectrometer) at 345 nm; (solv.): solvolysis by buffer in the medium indicated without additive; (**1**): with (**1**) added, corrected for buffer solvolysis; [(**1**) + K$^+$]: with (**1**) and excess of KBr added, corrected for buffer solvolysis. Rate 5B (**1**) has been measured by a stopped-flow technique. [d] Relative rates refer to the rate ratio (**1**)/[(**1**) + K$^+$], except when the runs are explicitly indicated.

Compounds (**1**) and (**4**) have spectral (^{1}H, ^{13}C n.m.r., mass spectra) and microanalytical properties in agreement with the assigned structures. The substrates (S) used in the present work were the *p*-nitrophenyl (PNP) esters of amino acids and of dipeptide hydrobromides listed in the Table. They were all prepared and purified by conventional methods.[10] The rates of *p*-nitrophenol release from the PNP–ester substrates have been measured under various experimental conditions (Table).

(i) Compounds (**1**) and (**4**) form complexes [(**1**), S] with primary ammonium salts where the substrate (S) is bound to the macrocycle by its $-NH_3^+$ group (Figure 1).[9,11,12]

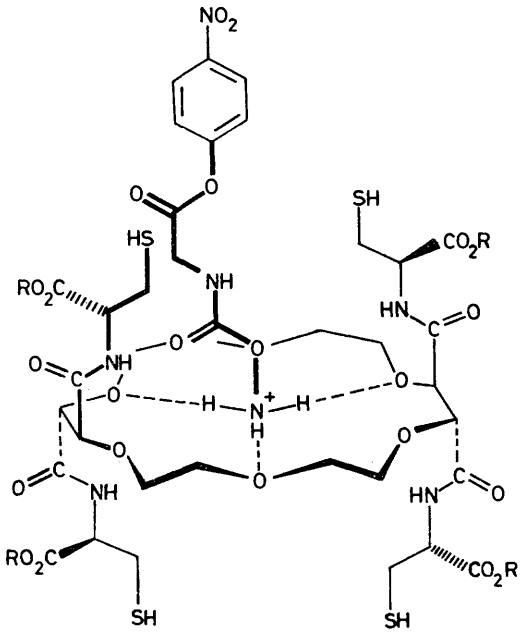


FIGURE 1. Schematic representation of the complex of receptor (**1**) with the dipeptide substrate, glycyl-glycine *p*-nitrophenyl ester salt.

(ii) The rate of release of *p*-nitrophenol from several substrates is much faster in the presence of (**1**) (Table) than when (a) (**1**) is absent (spontaneous solvolysis); (b) excess of KBr is added to inhibit the catalyst by displacing the ammonium salt by K^+;[9] (c) a secondary ammonium substrate like the L-ProGly–OPNP dipeptide ester is used, which is complexed much more weakly by macrocycles like (**1**) than are $R-NH_3^+$ species.[12,13] The rate accelerations observed are therefore due to complexation of the primary ammonium salt by the receptor (**1**). The products expected are the internally complexed *S*-acyl derivatives of (**1**).

(iii) No rate enhancement (even slight inhibition) is observed when the *S*-benzyl derivative (**4**) is used instead of (**1**), showing that the –SH groups of (**1**) are the reactive centres as in the cysteine enzyme papain.

(iv) The reaction of GlyGly–OPNP with (**1**) shows saturation kinetics, which change to second order kinetics when excess of KBr is added ($k = 3{\cdot}2$ l mol^{-1} s^{-1}; medium B). The reaction proceeds intermolecularly in a preformed complex and becomes intramolecular when the substrate is displaced by K^+. A Lineweaver–Burk plot yields a dissociation constant $K_m = 3{\cdot}5$ mM and k (acyl transfer) $= 5{\cdot}4 \times 10^{-3}$ s^{-1} (medium A, Table footnote a; 20 °C).†

(v) The rate acceleration factors show structural selectivity among the substrates used, the best substrates being the dipeptide esters. Large accelerations of acyl transfer

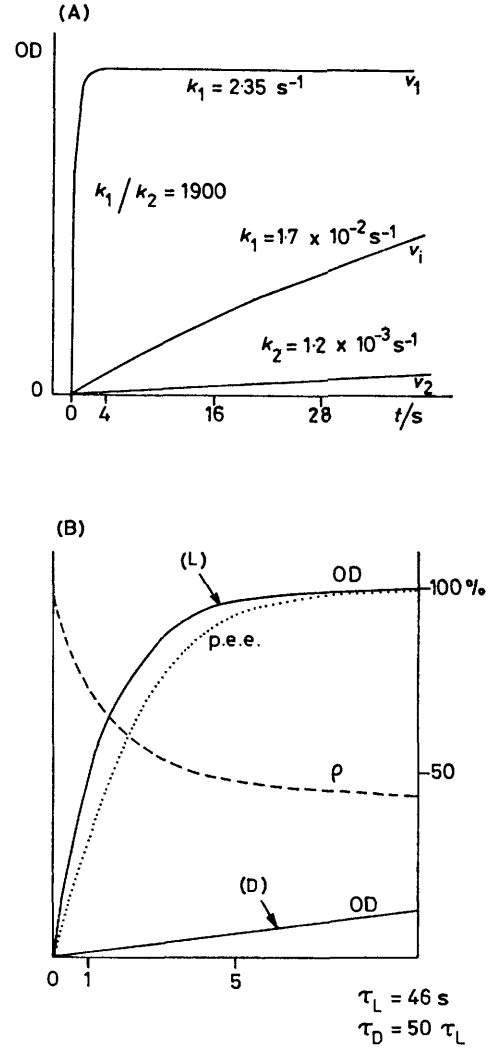


FIGURE 2. (A) Enhanced rates of intramolecular thiolysis; v_1: (**1**) + Gly–Gly–OPNP (run 5B, Table); v_i: same as v_1 with inhibition by KBr (run 5B, Table); v_2: (**1**) + (L)-Pro–Gly–OPNP (run 9B, Table). (B) Chiral recognition in the thiolysis of enantiomeric dipeptide ammonium esters; (—) OD: observed changes in optical density for *p*-nitrophenol release from Gly–(L)-Phe–OPNP (L) and from Gly–(D)-Phe–OPNP (D) [runs 6D and 7D in the presence of (**1**), Table]; (- - -) ρ: percentage of remaining unchanged ester starting from racemic DL ester; (. . . .) p.e.e.: % enantiomeric excess in remaining D ester starting from racemic DL ester, 100[(D) − (L)]/[(D) + (L)] (ρ and p.e.e. are computed from the L and D curves); the abscissa unit is the half-life τ_L (= 46 s) of the reaction L; $\tau_D = 50\ \tau_L$ (not corrected for reaction with buffer).

† In the polar medium A only 50% substrate is complexed with the concentrations used in the Table; the pseudo first order rate constants and the acceleration factors should thus be increased by a factor of *ca.* 2.

by factors of *ca.* 1900 (5B/9B) and *ca.* 15,000 (5C/9B) are found for [(**1**), Gly–Gly–OPNP] with respect to (**1**) + L-Pro–Gly–OPNP (Figure 2A; Table). The latter very weakly complexed substrate may be considered as a more satisfactory reference than runs with the K^+ inhibited catalyst (see below).

(vi) The reactions of (**1**) with the non-complexable CbO–NH–CH_2-COOPNP species is accelerated by a factor of *ca.* 15 when KBr is added (run 10B). Similarly, the the reaction of [(**1**), K^+] with GlyGly–OPNP is *ca.* 15 times faster than that of (**1**) with L-Pro–Gly–OPNP (Figure 1A; runs 5B, 9B). Thus the catalyst becomes more reactive when K^+ is complexed, for instance by a lowering of the pK of the SH group, or by favourable conformational fixation or both. The same is expected to occur when the polar $-NH_3^+$ groups of the substrates are bound to the macrocycle. These observations point to a general phenomenon, which may be of importance in enzyme catalysis itself: activation of the catalyst by the intermolecular interactions introduced by complexation of the substrate.

(vii) Whereas the enantiomeric dipeptide esters Gly–L–Phe–OPNP and Gly–D–Phe–OPNP react with the buffer [alone or in presence of (**4**)] at the same rates, high chiral discrimination is found for their reaction with the chiral reagent (**1**). The L-species reacts *ca.* 50—90 times faster with (**1**) than its D-antipode (runs 6C/7C and 6D/7D, Table) (Figure 2B).‡ Starting from racemic DL-ester this represents a kinetic resolution process in which high optical purity with high chemical yield of the non-consumed D-ester antipode may be achieved (Figure 2B).

Whereas Gly–(L)-Phe–OPNP reacts with (**1**) almost as fast as the achiral Gly–Gly–OPNP, the reaction of the D-ester is much slower. The chiral discrimination may reside in differences in complexation (K_m) or in reactivity [k (acyl transfer)] within the complexes of the two enantiomeric substrates; also both the chiral centres on the macrocycle and in the cysteinyl residues of (**1**) may play a role.

The molecular catalyst (**1**) displays molecular complexation, rate acceleration, and structural and chiral discrimination, *i.e.* properties of an artificial, abiotic, enzyme model. Rapid deacylation of the acyl-catalyst intermediates formed in the reactions described above is required for reagents like (**1**) or analogous systems to be true catalysts. Such processes are being investigated as well as the origins of the rate enhancements and of the high chiral discrimination achieved by system (**1**).

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‡ A rate factor of 8 was observed for thiolysis of enantiomeric substrates by a chiral binaphthyl crown catalyst (ref. 4).

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