Photocontrol of Catalytic Activity of Capped Cyclodextrin

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Summary The rate of hydrolysis of *p*-nitrophenyl acetate catalysed by azobenzene-capped β-cyclodextrin (**1**) is accelerated by photoirradiation mainly owing to the increased binding ability of *cis*-(**1**).

LIGHT provides a primary source of information for natural systems,[1] and photosensitive systems are commonplace in nature; light is a trigger which causes direct responses or physiological changes in organisms. Natural systems are very complex, and their mechanisms are still unknown at the molecular level. Using enzymes with a photoresponsive group incorporated near the active site, photocontrol of enzyme activity has been attempted as one approach to elucidate the role of light in biological processes.[1,2] A recent development in the investigation of photocontrol of molecular functions by artificial systems has involved the use of a photochromic capped β-cyclodextrin (**1**) [6,6′,*O*,*O*′-(4,4′-azobenzenedicarbonyl)cyclohepta-amylose],[3] and photoresponsive crown ethers.[2,4] In addition to photocontrolled complex formation by the above systems, photocontrol of catalytic activity has also been accomplished in ester hydrolysis by a β-cyclodextrin–azo inhibitor system.[5] In this case, the photochromic and catalytic components are not covalently bonded. We report here the photocontrol of catalytic activity accomplished by (**1**) where both components are covalently linked.

Compound (**1**) was irradiated with light of 320—390 nm from a 500 W lamp using a Corning 7-37 filter. The percentage of *cis*-(**1**) after photoirradiation was estimated from the decrease in the absorbance at 335 nm assuming that the absorbance of *cis*-(**1**) is negligible in comparison with that of *trans*-(**1**). The following kinetic studies show that a photocontrolled catalytic mechanism does indeed operate in this sort of system. The kinetic experiments were carried out by adding 15 μl of a stock solution of *p*-nitrophenyl acetate in acetonitrile to 3 ml of the dark-adapted or irradiated solutions of (**1**), following the hydrolysis of the ester at 400 nm (25 °C; pH 8·7; Tris buffer). In the irradiated solutions of (**1**), the reversion of *cis*-(**1**) to *trans*-(**1**) during the

TABLE 1. Effect of light on rate constants[a] for reaction of (**1**) with *p*-nitrophenyl acetate.

10^4 [(**1**)]	% *cis*	$10^5 k_{dark}/s^{-1}$ [b]	$10^5 k_{light}/s^{-1}$ [b]	k_{light}/k_{dark}
0·5	50	0·4	1·2	3·0
0·83	38	0·4	2·2	5·5
1·0	45	1·1	2·6	2·4
2·0	32	1·6	2·8	1·8
3·3	32	2·1	4·0	1·9
5·0	26	3·2	5·7	1·8
6·7	16	3·8	5·8	1·5
8·3	16	5·3	7·2	1·4
10·0	7	6·8	7·5	1·1

[a] pH 8·7 solutions (0·05 mol Tris buffer), 25 °C, with $2·58 \times 10^{-5}$ mol l^{-1} of *p*-nitrophenyl acetate. [b] k_{dark} (or k_{light}) = $k_{obs} - k_{un}$ ($k_{un} = 1·17 \times 10^{-4}$ s^{-1}) where k_{obs} and k_{un} denote the rate constants in the presence and absence of the catalyst.

kinetic experiments is negligible since the half life for the reversion is very long (33 h) under the experimental conditions. Good pseudo-first-order rate data were obtained. The resulting rate constants after correcting for buffer catalysis are shown in Table 1. The maximum value of the light-induced rate enhancement, expressed as k_{light}/k_{dark}, was 5·5. The rate enhancement is not significant for solutions containing high concentrations of (**1**) because of the low proportions of the *cis*-isomer attained under our experimental conditions.

The hydrolysis was analysed according to the Michaelis–Menten scheme [equation (1)], where S is the substrate

$$(\mathbf{1}) + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\mathbf{1})\text{–}S \xrightarrow{k_2} (\mathbf{1})' + P \qquad (1)$$

and P is the product. The rate constants for the reaction of the entirely complexed ester, k_2, and the Michaelis constants, K_m, were evaluated from a Lineweaver–Burk plot,[6] and the resultant kinetic parameters are given in Table 2. The kinetic representation for the hydrolysis

TABLE 2. Kinetic parameters.

Catalyst	$10^3 k_2/s^{-1}$	$10^2 K_m$	$10^2(k_2/K_m)$
β-Cyclodextrin[a]	1·09	0·73	15
trans-(**1**)	1·62	2·34	6·9
cis-(**1**)	0·70	0·19	37

[a] Reported values k_2, $1·15 \times 10^{-3}$ s^{-1}; K_m, $0·83 \times 10^{-2}$; k_2/K_m, 14×10^{-2} (A. Harada, M. Furue, and S. Nozakura, *Macromolecules*, 1976, **9**, 705).

after photoirradiation is very complex since both *trans*- and *cis*-isomers of (**1**) are present and compete in substrate binding.[7] The kinetic parameters for *cis*-(**1**) were thus approximately estimated, assuming negligible competition in substrate binding. The maximum rate constant k_2 for *cis*-(**1**) is smaller than that for *trans*-(**1**), indicating its unfavourable geometry for holding a substrate molecule in the correct position; the substrate molecule would be included too deeply in the cavity of *cis*-(**1**) to attain a favourable position between the carbonyl group of the substrate and the alkoxide ion of the cyclodextrin moiety (Figure). The K_m value for *cis*-(**1**) is considerably smaller than that for *trans*-(**1**), indicating that the substrate is included deeply in the expanded hydrophobic cavity of *cis*-(**1**) resulting in enhanced binding, whereas the cavity of *trans*-(**1**) is too shallow to form a stable complex. These situations lead to an increased apparent overall hydrolysis rate k_2/K_m for *cis*-(**1**), which is about five times larger than that for *trans*-(**1**). A comparison of the k_2 value for *trans*-(**1**) with that of β-cyclodextrin indicates that the shallow cavity of *trans*-(**1**) is more favourable for catalytic activity than that of β-cyclodextrin. However, this advantage is cancelled by its inferior binding ability, resulting in poorer catalysis than with β-cyclodextrin. Capped cyclodextrins were reported to be different from the parent cyclodextrin in inclusion-binding ability[8] and stereoselectivity of cyclodextrin-catalysed ester hydrolysis. The behaviour of *cis*-(**1**) in ester hydrolysis is very similar to that of another rigidly capped cyclodextrin reported by Fujita *et al.*,[9] who also demonstrated a change from *meta*-selectivity for the parent cyclodextrin to *para*-selectivity for the capped compound. We also observed a change in selectivity with (**1**); the catalytic activity of (**1**) in the hydrolysis of *m*-nitrophenyl acetate is negligible both before and after photoirradiation.

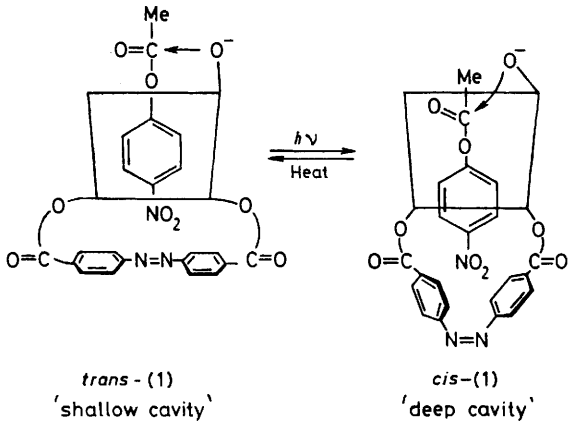

FIGURE. Schematic representation of alkoxide catalysis by (**1**).

This system is reversible, since the *cis*-form of (**1**) is thermally or photochemically† converted into the *trans* form permitting on-off control of the catalytic activity.

† The original *trans*-form was recovered within 10 min by photoirradiation with light of longer wavelength (*ca.* 440 nm).

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