Substrate control through per-*O*-methylation of cyclodextrin acids†

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Received 24th June 2010, Accepted 2nd September 2010
DOI: 10.1039/c0cc02071k

Per-*O*-methylated cyclodextrins containing a single 2-*O*-(2-acetate), 2-*O*-(3-propanoate) or a 6-carboxylate were investigated for glycosidase activity on *p*-nitrophenyl glycosides. The former two compounds displayed enzyme catalysis giving rate accelerations of 500–1000, while the latter compound gave marginal catalysis. These results show that per-*O*-methylated cyclodextrins direct substrate binding from the secondary face leading to better catalysis.

Enzymes have fascinated chemists since the time of Emil Fischer. This is not surprising since they are crucial for essentially every biological event, and their power and selectivity are of a magnitude beyond normal chemical standards.[1] While a century long major research effort in protein chemistry, biochemistry, molecular biology and crystallography has unraveled much about existing enzymes, actually building new enzymes is still very difficult.[2] Thanks to a monumental effort by Breslow[3] and others,[4] cyclodextrin derivatives have proven to be the most successful artificial enzymes, but the number of compounds that actually display Michaelis–Menten kinetics is still quite small.[5] One of the problems with cyclodextrins is perceived to be a tendency to indiscriminate binding allowing guests to bind from both faces of the cavity and opposite orientations.[3b]

Diacid **1** (Fig. 1) is an example of a cyclodextrin based artificial enzyme.[7] It catalyses hydrolysis[8] of nitrophenyl glycosides at 59 °C, pH 8 in phosphate buffer with a rate increase (k_{cat}/k_{uncat}) of bound substrate (relative to unbound) of 989 (Table 1). The catalysis is presumably a result of electrostatic effects: when the substrate binds to the primary face of **1** (Fig. 2, top left) the anomeric center comes close to the negatively charged carboxylate, which promotes the formation of a positively charged transition state. It is likely that the substrate can bind in several orientations only some of which allow catalysis. Thus a complex between **1** and substrate with the sugar at the secondary face (Fig. 2, top right) will be unproductive and, as it undoubtedly also is formed, leads to a decrease in overall catalysis. If binding orientations could be limited it would obviously pave the way for better artificial enzymes. In this communication we report that per-*O*-methylation can be used to obtain such substrate control.

Per-*O*-methylation of cyclodextrins is potentially attractive since it gives these molecules a wider range of solvent solubilities, while retaining or even increasing water solubility.

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† Electronic supplementary information (ESI) available: Experimental procedures, plot of k_{cat} *vs.* phosphate, kinetic plots and data with errors included and Roesy spectra. See DOI: 10.1039/c0cc02071k

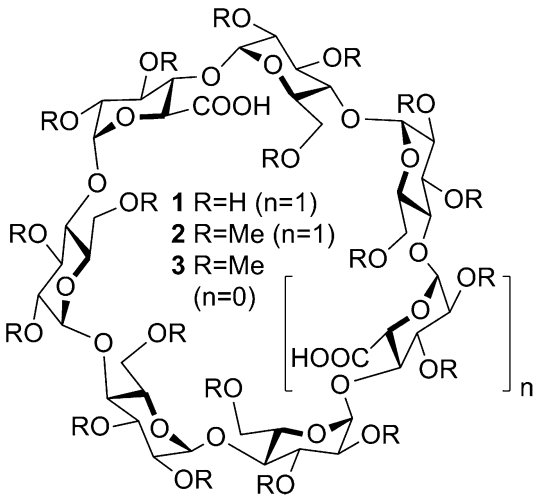

Fig. 1 Cyclodextrin derivatives **1–3** having carboxylic acids at the primary rim.

Table 1 Kinetic parameters for hydrolysis of nitrophenyl glycosides in 0.5 M phosphate buffer, pH 8.0, 59 °C

Catalyst	Substrate[a]	k_{cat} ($\times 10^7$ s^{-1})	K_m/mM	k_{cat}/k_{uncat}
1[7]	PNP β-glc	188	7.90	989
2[6]	PNP β-glc	17	—	122
3	PNP β-glc	1.5	—	14
3	PNP α-glc	—	—	—
3	PNP α-gal	—	—	—
3	ONP β-gal	—	—	—
4	PNP β-glc	269	4.68	975
4	PNP α-glc	217	4.88	538
4	PNP α-gal	289	3.02	763
4	ONP β-gal	55	0.59	56
5	PNP β-glc	173	5.53	517
5	PNP α-glc	273	6.37	646
5	PNP α-gal	131	1.97	326
5	ONP β-gal	31	—	19

[a] PNP = *p*-nitrophenyl, ONP = *o*-nitrophenyl, glc = D-glucopyranoside, gal = D-galactopyranoside.

More important in this context it is known that per-*O*-methylated cyclodextrins have a more narrow primary face opening.[9] Also it has been shown by crystallography that several guests, among them *p*-nitrophenol, bind to cyclodextrin and per-*O*-methylated cyclodextrin in opposite orientations.[10]

The per-*O*-methylated analogues of **1**, **2**[6] and **3**[11] (Fig. 1) were quite disappointing: both **2** and **3** displayed poor or no enzymatic activity (Table 1). However in contrast the *O*-methylated derivatives **4**[11] and **5**[11] that have a single carboxylic acid attached to the secondary face (Fig. 3) were found to be much better. Under similar conditions **4**, which has an acetic acid residue attached to 2-OH, catalysed hydrolysis of *p*-nitrophenyl β-glucoside displaying Michaelis–Menten

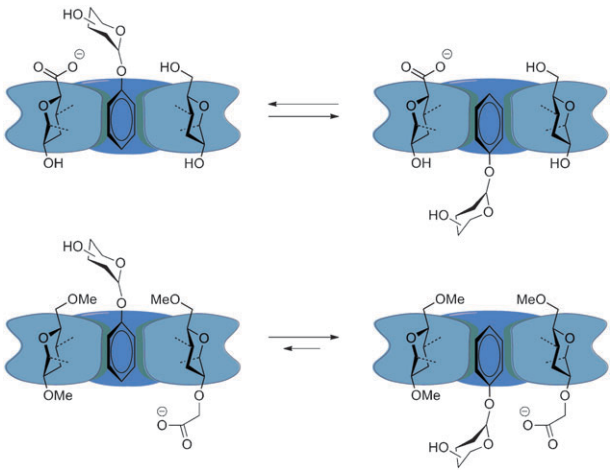

Fig. 2 Binding modes of phenyl glycoside substrates to cyclodextrin acids **1** (top) and **4** (bottom). The glycoside can bind from the primary face (left) or the secondary face (right).

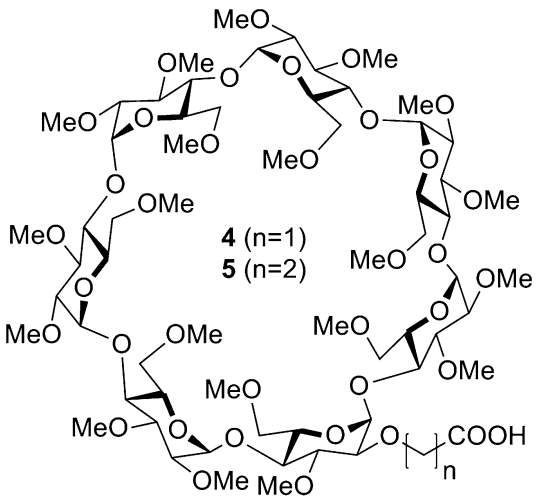

Fig. 3 Cyclodextrin derivatives **4–5** having carboxylic acids at the secondary rim.

kinetics and giving a k_{cat}/k_{uncat} of 975 (Table 1). The corresponding α-glucoside and galactoside substrates were also accepted with k_{cat}/k_{uncat} of 538 and 763, respectively. Only the *o*-nitrophenyl β-galactoside was a poor substrate probably because the change in aromatic substitution leads to unproductive or poor binding.

The analogue **5**, which had a 3-propionate at a 2-OH (Fig. 3), behaved very similar to **4**, the only difference being that the rate accelerations for most substrates were slightly smaller (Table 1).

On first sight the catalysis displayed by **4** and **5** appears very similar to that of unmethylated primary face substituted cyclodextrin acids such as **1**. Thus the catalysis by **4** shows similarly a linear dependence in phosphate (Fig. 4), which indicates that phosphate is involved in the reaction as a nucleophilic catalyst.[12] However, only one acid group is required for the catalysis here while for the primary face substituted acids two carboxylate groups are necessary. Also **4** (unlike **1**) does not need phosphate assistance for catalysis as a component of the catalysis will occur without the presence of

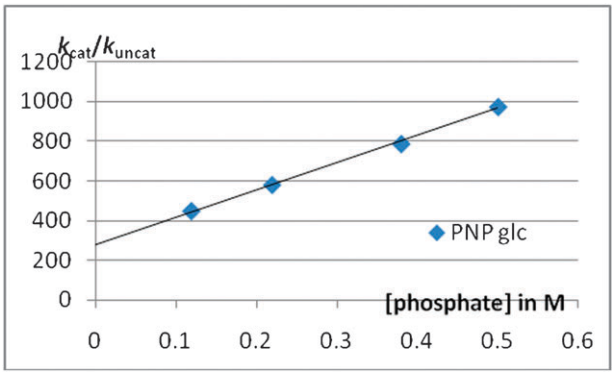

Fig. 4 Dependance of k_{cat}/k_{uncat} for **4** on hydrolysis of PNP α-glc of phosphate concentration at $T = 59$ °C in phosphate buffer.

phosphate (Fig. 4). Therefore these secondary face substituted cyclodextrin acids appear more potent than the primary face substituted cyclodextrin acids.

Overall the data show that while per-*O*-methylation decreases catalysis for cyclodextrin acids with the carboxylate at the primary face, per-*O*-methylated cyclodextrins with a carboxylate at the secondary face works well. This behavior can be explained by a change in binding equilibria as shown in Fig. 2. If unmethylated cyclodextrin derivatives, such as **1**, bind nitrophenyl glycosides both from the primary and the secondary face (Fig. 2, top), while per-*O*-methylated derivatives predominantly bind at the secondary face (Fig. 2, bottom) catalysis as the one observed would be expected. To verify this binding behaviour NMR spectra of the complexes of 4-nitrophenyl β-D-glucoside (PNP glc) with either β-cyclodextrin (CD) or per-*O*-methyl-β-cyclodextrin (MCD) were analyzed. In the complex between CD and PNP glc NOEs were observed between the aromatic protons (in PNP glc) and H-5 and H-3 (in CD), while in the complex with MCD the *o*-nitro protons (in PNP glc) show NOE to both H-3 and H-5 (in MCD), while the *m*-nitro protons only have NOE to H-3 (see ESI†). This clearly shows a change in binding conformations towards more predominant binding from the secondary face when MCD is host.

In summary this work shows that per-*O*-methylation of a cyclodextrin artificial enzyme can direct its substrate binding towards the secondary face. In this particular case the more discriminative binding appears to cause more efficient catalysis. Catalysis is presumably caused by electrostatic interactions (for suggested transition state see ESI†). Limited substrate control is undoubtedly one of the drawbacks when cyclodextrins are used in artificial enzymes and per-*O*-methylation may be one of the solutions to this problem.

FNU and KU are thanked for financial support, and Christian Thorzen for assistance with NOE.

Notes and references

1 R. Wolfenden and M. J. Snider, *Acc. Chem. Res.*, 2001, **34**, 938–945.
2 A. Kirby and F. Hollfelder, *From Enzyme Models to Model Enzymes*, RSC, Cambridge, 2009.
3 (*a*) R. Breslow, *Artificial Enzymes*, Wiley-VCH, Weinheim, 2005; (*b*) R. Breslow and S. Dong, *Chem. Rev.*, 1998, **98**, 1997–2011.
4 (*a*) Y.-H. Zhou, M. Zhao, Z.-W. Mao and L.-N. Ji, *Chem.–Eur. J.*, 2008, **14**, 7193–7201; (*b*) M. Fukudome, K. Shimosaki, K. Koga,

D.-Q. Yuan and K. Fujita, *Tetrahedron Lett*., 2007, **48**, 7493–7497; (*c*) P. G. McCracken, C. G. Ferguson, D. Vizitiu, C. S. Walkinshaw, Y. Wang and G. R. J. Thatcher, *J. Chem. Soc., Perkin Trans. 2*, 1999, 911–912; (*d*) H. Tsutsumi, H. Ikeda, H. Mihara and A. Ueno, *Bioorg. Med. Chem. Lett*., 2004, **14**, 723–726; (*e*) N. Masurier, F. Estour, M.-T. Froment, B. Lefèvre, J.-C. Debouzy, B. Brasme, P. Masson and O. Lafont, *Eur. J. Med. Chem*., 2005, **40**, 615–623; (*f*) A. Fragoso, R. Cao and M. Banõs, *Tetrahedron Lett*., 2004, **45**, 4069–4071.

5 L. Marinescu and M. Bols, *Trends Glycosci. Glycotechnol*., 2010, **21**, 309–323.

6 J. Bjerre, PhD thesis, University of Copenhagen, 2010.

7 C. Rousseau, F. O. Caballero, L. U. Nordstrøm, B. Christensen, T. E. Pedersen and M. Bols, *Chem.–Eur. J*., 2005, **11**, 5094–5101.

8 The reaction is dependent on phosphate which presumably as a phosphorolysis external nucleophilic catalyst, but the final product is the glycose.

9 S. Immel and F. W. Lichtenthaler, *Starch*, 1996, **48**, 225–232.

10 (*a*) K. Harata, *Bull. Chem. Soc. Jpn*., 1977, **50**, 1416–1424; (*b*) K. Harata, K. Uekama, M. Otagiri and F. Hirayama, *Bull. Chem. Soc. Jpn*., 1982, **55**, 3904–3910; (*c*) K. Harata, K. Uekama, M. Otagiri, F. Hirayama and Y. Sugiyama, *Bull. Chem. Soc. Jpn*., 1981, **54**, 1954–1959; (*d*) K. Harata, K. Uekama, M. Otagiri, F. Hirayama and Y. Sugiyama, *Bull. Chem. Soc. Jpn*., 1982, **55**, 3386–3389.

11 Prepared from oxidation of the corresponding aldehyde, see ESI†.

12 We have not been able to show glucose-1-phosphate (Glc-1P) as a product in these reactions only glucose, but Glc-1P is likely to hydrolyse quite readily under the conditions.