J Incl Phenom Macrocycl Chem (2011) 69:417–423
DOI 10.1007/s10847-010-9774-8

ORIGINAL ARTICLE

Substrate structure governs maximum rate of catalysis exerted by cyclodextrin oxidase chemzymes

Jeannette Bjerre · Mikael Bols

Received: 30 October 2009 / Accepted: 16 March 2010 / Published online: 21 April 2010

Abstract Selectively modified α- and β-cyclodextrin ketones or aldehydes act as artificial oxidases on a variety of small lipophilic substrates. The structure of the substrate is a highly important factor governing how effectively the oxidation reaction can be catalyzed. Amino acid-type substrates were not prone to catalysis, which yields new information about the limits of CD catalysis. Aniline showed some non-quantifiable catalysis, but for quinones and benzyl alcohols no net catalysis was detected. For aminophenol oxidation, *o*-aminophenols are far better substrates than *p*-aminophenols. The CD-catalyzed reaction follows Michaelis–Menten kinetics, involves CD cavity binding of the substrate and substrate recognition, and thus encompasses many of the hallmarks of natural enzymatic catalysis. Strong binding of the cooxidant H_2O_2 to the CD catalytic carbonyl group is a prerequisite for the subsequent oxidation of the substrate and in accordance with this, the binding of H_2O_2 to β-CD dialdehyde was shown to be strong ($K_d = 1.4$ mM). β-CD $6^A,6^D$-diketone which binds H_2O_2 weaker than an aldehyde was accordingly a less efficient oxidase. The wide range of substrates applicable to CD chemzyme catalysis brings about optimism for future scopes of synthetic biology.

Keywords Supramolecular chemistry · Artificial enzyme · Oxidase chemzyme · Cyclodextrin catalysis · Synthetic biology · Substrate specificity

This work was presented at the First European Cyclodextrin Conference, Aalborg, Denmark, 11–13th of October 2009.

J. Bjerre · M. Bols (✉)
Department of Chemistry, University of Copenhagen, Copenhagen, Denmark
e-mail: bols@kemi.ku.dk

Abbreviations

CD	Cyclodextrin
K_m	Michaelis–Menten constant
K_d	Dissociation constant
k_{cat}	Catalyzed reaction rate
k_{uncat}	Uncatalyzed reaction rate

Introduction

Oxidases are an interesting class of enzymes; crucial components in the vast majority of biological processes and natural systems. The specialized natural oxidases respond to the need of oxidation of distinct substrates having widely different chemical design and structure [1, 2]. Mimicking oxidase activity in an artificial enzyme has been the goal of our research in which the cyclodextrin macromolecule is used as a supramolecular host [3]. Modified cyclodextrins functioning as macromolecular ensembles in enzymatic catalysis have been studied before, commonly for esterase activity [4–6], but also for oxidation [7–9]. CDs are cyclic α(1-4)glucopyranosides, i.e., circular structures comprised of 6 (for α-CD) or 7 (β-CD) alphabetically denominated glucose units which form a cone-shaped macrostructure with a primary hydroxyl rim (narrower) and secondary hydroxyl rim (wider). The non-polar cavity can selectively bind small lipophilic compounds whilst the polar CD exterior affords the inclusion complex to be water-soluble, making the CD an attractive structure to base the design of a biomimetic artificial enzyme upon [10]. By selective organic synthetic manipulations, the CD is equipped with catalytic groups which react with the substrates that bind inside the CD cavity,

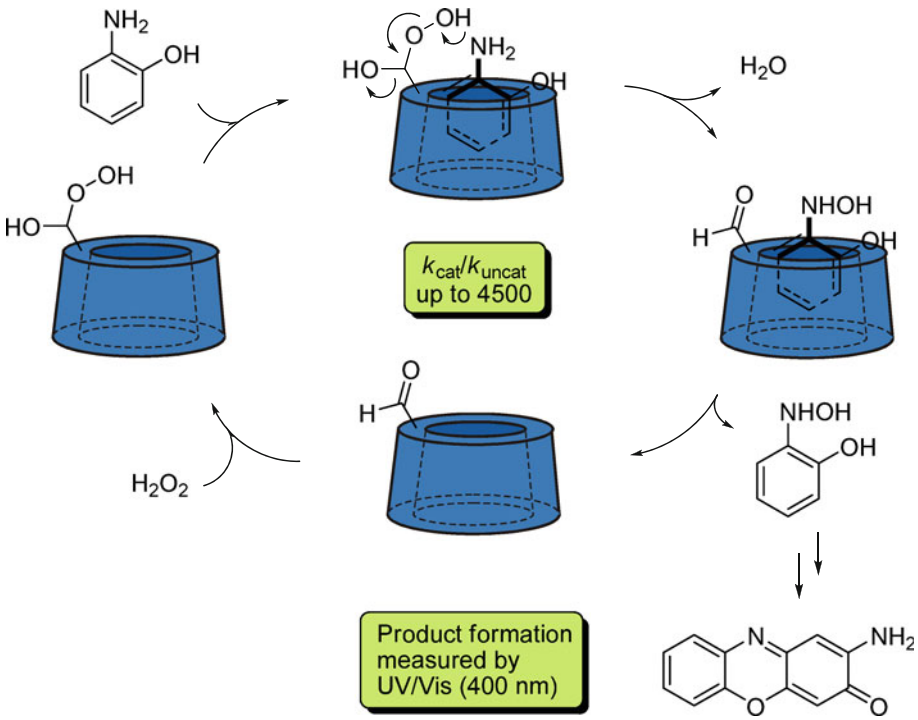


Fig. 1 Proposed catalytic cycle for oxidation of *o*-aminophenol by CD aldehydes

thereby effecting catalysis of the conversion of substrate to product [11, 12]. Most of the CDs previously found to possess oxidase activity contain primary hydroxyl rim ketone bridges as the catalytic entity, but recently it was noticed that CDs equipped with aldehyde groups on the primary hydroxyl rim also catalyze oxidation of amongst others a large range of aromatic amine substrates [13] (Fig. 1).

The catalyzed reaction follows enzyme-characteristic Michaelis–Menten kinetics and employs hydrogen peroxide as a cooxidant. The binding of hydrogen peroxide to the catalytic CD aldehyde groups activates these for subsequent oxidation of the amine group in the bound substrate. A crucial factor in the catalytic context is substrate selectivity, as the structural composition of the substrate sets the boundaries for how effectively the reaction can be catalyzed by the CD chemzyme. A wide range of new substrates tested afford information of the realms of CD catalysis.

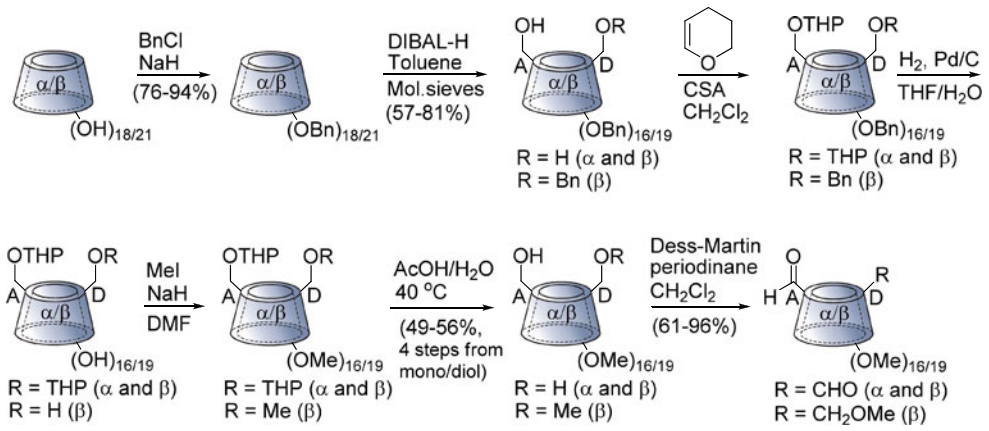
Materials and methods

General

Solvents were distilled under anhydrous conditions. All reagents were used as purchased without further purification. Evaporation was carried out in a rotary evaporator at 40 °C under reduced pressure. ^{1}H NMR experiments were carried out with a Varian Mercury 300 instrument. Catalysis assays were performed using a Spectronic Genesys 5 spectrophotometer.

Determination of catalysis rate by UV

Each assay was performed on 8 samples (1 mL each) of the appropriate substrate at different concentrations in 190 mM phosphate buffer, pH 7.0 containing 72 mM H_2O_2, and either the CD catalyst added (0.04–0.50 mM), or only phosphate buffer and H_2O_2 as control. The reactions were followed at 25 °C (±1 °C) using UV-absorption at 400 nm, and were typically monitored for 30 min for aminophenols and other substrates, and 5 h for benzyl alcohols. Each assay was typically performed three times for validation. At the end of each assay, the phosphate buffered reactions were extracted with CH_2Cl_2, the combined organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residues containing oxidation products from respectively the catalyzed and non-catalyzed reaction fractions were analyzed individually by electrospray mass spectrometry (ES-MS). For calculations of kinetic parameters, velocities were determined as the slope of the progress curve of each reaction. Uncatalyzed velocities were obtained from a series of separate experiments. Catalyzed velocities were calculated by subtracting the uncatalyzed rate from the total rate of the appropriate cyclodextrin containing sample. The catalyzed velocities were used to construct Hanes plots ([S]/V vs. [S]) to ensure that the reaction followed Michaelis–Menten kinetics. In that case K_m and V_{max} were determined using least square non-linear regression fitting to the V_{max} vs. [S] curve. For non-linear regression fitting, the NIST programme Dataplot was employed [14]. k_{cat} was calculated as



Scheme 1 Examples of selective syntheses of some permethylated CD aldehydes

V_{max}/[cyclodextrin]. k_{uncat} was determined as the slope from a plot of V_{uncat} vs. [S]. The following extinction coefficients (25 °C, pH 7.0, $\lambda = 400$ nm) were determined and used: 2-amino-phenoxazin-3-one, 0.42 mM^{-1} cm^{-1}; *N*,*N*′-bis-(*p*-hydroxyphenyl)-2-hydroxy-5-amino-1,4-benzoquinone diimine, 1.59 mM^{-1} cm^{-1}; 4-nitro-3-methylphenol, 5.51 mM^{-1} cm^{-1}; 2-nitro-4-methylphenol, 1.06 mM^{-1} cm^{-1}; 2-nitro-3-methylphenol, 0.68 mM^{-1} cm^{-1}; 2-nitro-5-methylphenol, 2.26 mM^{-1} cm^{-1}; azobenzene, 7.00 mM^{-1} cm^{-1}; azoxybenzene, 7.00 mM^{-1} cm^{-1}.

Investigation of epoxidation of styrene

To a solution of styrene (10.4 mg, 0.10 mmol) and 6^A,6^D-dioxo-$2^{A–G}$,$3^{A–G}$,$6^{B–C,E–G}$-nonadecakis-*O*-methyl-*β*-cyclodextrin (5.6 mg, 0.004 mmol) in H_2O (1.0 mL) at 0 °C were added oxone ($KHSO_5$, 30.6 mg) and $NaHCO_3$ (16.8 mg) every 10 min over an 1 h time period. In total, 3 eq. (183.6 mg) of oxone and 12 eq. (100.8 mg) of $NaHCO_3$ were added. Finally, water was added, the aqueous layer was extracted with CH_2Cl_2, the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. ^1H NMR ($CDCl_3$) of the residue was recorded. Similar control experiments were also performed, with ommitance of either CD or oxone; confirming that no catalysis takes place without the dual presence of both these agents.

Results and discussion

Designing CDs as macromolecular catalysts

The substrates in this paper were all tested with selectively modified aldehyde- and ketone CDs as supramolecular oxidation catalysts. Most of the artificial oxidases studied in the Bols group have originated from selective modifications of the 6^A,6^D-positions, creating catalytic entities on opposite sides of the CD primary hydroxyl rim [15]. Chemical manipulation specifically of these groups is possible by the use of a 6^A,6^D (or just 6^A, depending on reaction conditions) selective de-*O*-benzylation step [16, 17], illustrated in Scheme 1 where it forms part of the synthesis route of some of the permethylated CD aldehydes recently studied [18].

Enzyme kinetics

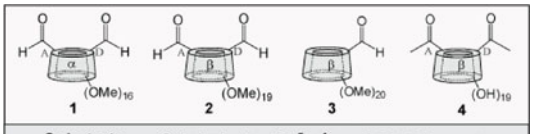
The modified CDs were tested for catalysis of aromatic amine oxidation on a range of structurally different small molecule substrates, in phosphate buffer at pH 7.0 and 25 °C. The catalysis was monitored by measuring the absorbance of the oxidized products by UV/Vis at 400 nm [19, 20]. The assays were performed both with and without CD added, and the net catalysis was obtained by subtracting the non-catalyzed reaction rates from the catalyzed. Via calculations based on the Michaelis–Menten rate law equation, kinetic parameters such as Michaelis-Menten constant (K_m) reflecting the strength of substrate binding to the CD, uncatalyzed (k_{uncat}), and catalyzed (k_{cat}) rate constants were obtained. Thus, the reactions followed the rate Scheme 2.

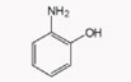
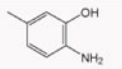
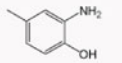
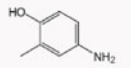
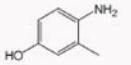
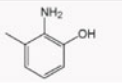
Chemzyme trends

Some general trends for recently tested CD chemzymes **1**–**4** are evident from Table 1. The strength of catalysis is roughly proportional to the number of catalytic groups present in the CD; the dialdehyde **2** is in most cases about

Scheme 2 Michaelis–Menten kinetics; conversion of substrate (S) to product (P)

Table 1 Influence of substrate structure and CD chemzyme identity on catalysis



Substrate	Catalyst	k_{cat} (10^{-5} s^{-1})	K_m (mM)	k_{cat}/k_{uncat}
	1	1881±121	10.8±0.9	4491±311
	2	763±63	8.4±0.9	1821±161
		29±11*	5.1±3.1*	24±9* (MeOH)
	3	306±38	9.6±1.6	730±98
	4	16±1	1.87±0.27	37±2
	2	101±22	11.8±3.3	141±34
	3	17±4	1.9±0.8	24±6
	1	470±45	6.6±0.9	170±18
	2	902±114	14.9±2.3	327±44
	3	564±39	25.6±2.0	204±15
	1	700±59	8.5±1.0	2742±259
	2	63±22	1.34±0.97	247±97
	3	612±134	25.8±6.5	2398±592
	4	59±7	2.5±0.7	231±33
	1	12±4	0.33±0.54	9±3
	2	8.9±0.2	1.40±0.07	6.6±0.2
	3	5.7±1.4	0.09±0.26	4.3±1.2
	1	1.9±0.2	0.20±0.09	9±1
	2	2.0±0.2	0.23±0.11	10±1
	3	2.0±0.6	0.86±0.75	10±3
	4	1.1±0.3	2.4±1.3	5±1
	1	16±6	0.03±0.26	47±25
	2	16.4±5.6	3.2±1.7	47±23
	3	8.4±1.7	0.05±0.23	24±7

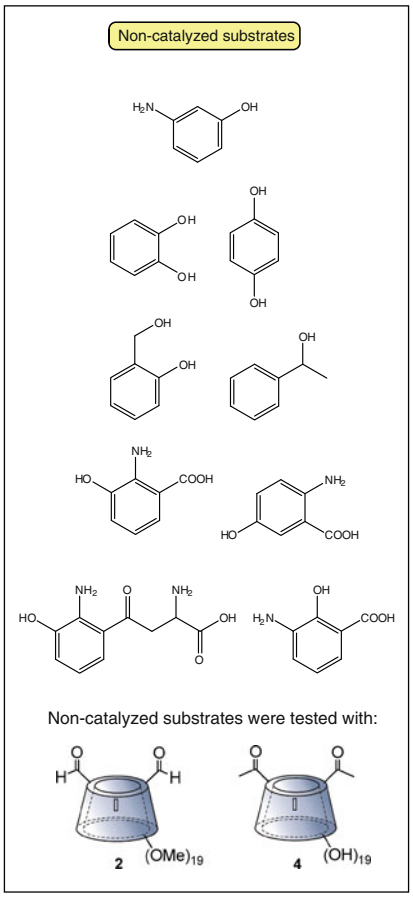


Illustration to the right: substrates that were not subject to CD catalysis

twice as efficient as the monoaldehyde **3**. Even in methanol **2** displays some catalysis, whereas in ethanol catalysis is absent. This is presumably a result of the inclusion of the solvent in the CD cavity, excluding a catalytically productive binding mode of the substrate. The α-CD dialdehyde **1** performs slightly better or comparable to the *β*-CD dialdehyde **2**. In general, good catalysis seems to be associated with weaker binding (high K_m). This may imply that a strong and unproductive substrate binding impedes catalysis.

Aminophenol structure impact on catalysis

The catalysis data in Table 1 reveal that the structural composition of the aminophenol substrate governs how effectively the reaction can be catalyzed, a trend persisting regardless of the nature of the CD catalyst utilized. *O*-aminophenols are far better substrates than *p*-aminophenols. However, if a methyl group is placed *meta* to the hydroxyl group this dramatically lowers catalysis for both *o*- and *p*-aminophenols. If the amino group is placed *meta* to the hydroxyl group, no catalysis can be measured. Catalysis of *p*-aminophenol oxidation is impeded somewhat by methyl substitution in the substrate, whereas for *o*-aminophenol the presence of a methyl group *para* to the hydroxyl group is well-tolerated. These findings are summarized graphically in Fig. 2.

Preparative analysis

The oxidation of *o*-aminophenol catalyzed by *β*-CD $6^A,6^D$-dialdehyde **2** was also examined in a preparative manner in order to attempt to quantify the bulk of the oxidation products. The quantitative assays were made analogous to the standard kinetic assays, but in a larger scale; ending with standard organic synthesis work-up; extraction of the aqueous layer with CH_2Cl_2 and NMR analysis of the organic residue components. The dual solubility of

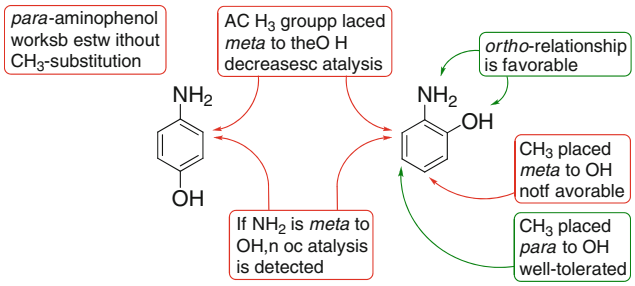


Fig. 2 General structural trends affecting how effectively the aminophenol-type substrate can be catalyzed by the CD chemzyme

permethylated β-CD 6^A,6^D-dialdehyde **2** in both phosphate buffer and organic phase complicated the spectra significantly and made obtainment of unambiguous data difficult. This technique would however be beneficial in case of non-organic soluble catalysts, and provides as such an interesting alternative to analysis of oxidation products.

Rate-determining step

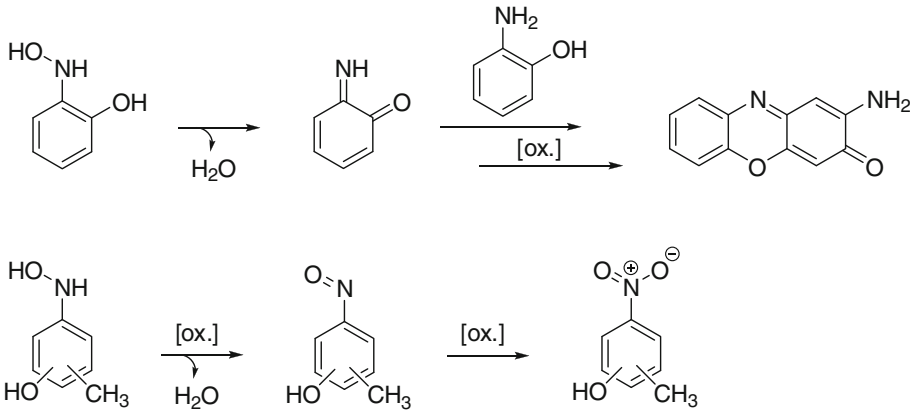
The oxidation of aminophenols is a process involving several steps; it has been confirmed that the biomimetic CDs catalyze the conversion of starting material into product, but it would be of interest to find out exactly which step is the rate-determining and therefore the one prone to CD catalysis. Scheme 3 illustrates the oxidation steps that follow from the initial hydroxylamine oxidation product (see Fig. 1 for its formation), in case of both *o*-aminophenol and the methyl-substituted aminophenols [21].

By measuring the CD catalysis of some of these individual intermediates, it might be possible to pinpoint exactly which step is subject to CD catalysis, which would be of great interest for optimizing the future chemical design of the CD chemzymes. Efforts were however hampered by the lack of commercial availability of most of these compounds and related analogs; probably due to their inherent instability. *p*-nitrosophenol was the only readily available intermediate, in the form of a wetted matrix suspension though, and it was tested for catalysis with β-CD 6^A,6^D-dialdehyde **2**. The matrix seemed to disturb the catalysis and UV-readings excessively, and another obstacle in this sense was that nitrosophenol absorbs at the same wavelength as the oxidation product. Though the hope was to be able to measure a difference in intensity of the measured UV-absorption for the reaction, the instability and formulation of the substrate made the direct obtainment of finite data under catalysis assay conditions non-straightforward. The search for alternative methods to monitor the details of these reaction mechanisms more closely will continue to be of interest.

Probing the limits of CD enzymatic catalysis

Some substrates were not subject to catalysis (depicted right of Table 1). These yield valuable information about the limits of CD catalysis. Several are amino acids and hence catalysis of amino acid oxidation does not seem to be an easy achievement with CDs. In three out of four cases for the carboxylic acid-containing substrates, the carboxylic acid entity or substituent is placed *meta* to the OH which, as illustrated in Fig. 2, is generally not beneficial for catalysis. Having a carboxylic acid group in the substrate has significant steric as well as electronic impacts on the aminophenol substrate, and the catalytically productive binding of substrate to CD could be hampered by this. The mimicking of natural amino acid oxidases might be feasible if based upon another supramolecular structure, however. Benzyl alcohols have previously acted as oxidizable substrates for bridged ketone CD chemzymes [22], but the CDs **2** and **4** show distinct substrate discrimination against these; thus, no catalysis is observed for neither 2-hydroxyphenol nor 1-phenylethanol. The *o*-aminophenoloxidase GriF has been shown to catalyze the oxidation of pyrocatechol with a k_{cat} of 12 s^{-1}, and a K_m of 3.5 mM.

Scheme 3 Intermediates in oxidation of aminophenols with CDs and H_2O_2



GriF also catalyzes the oxidation of *o*-aminophenol with a k_{cat} of 20 s^{-1}, and a K_m of 19 mM, and even seems to be able to combine in the oxidation process, these two substrates, affording a combined dimerization product [23]. Inspired by this, quinone substrates were investigated for CD catalysis. Under the assay conditions employed, no catalysis was to be found for either hydroquinone or pyrocatechol, which illustrates a marked difference in substrate compatibility between GriF and CD chemzymes.

Catalysis of aniline oxidation

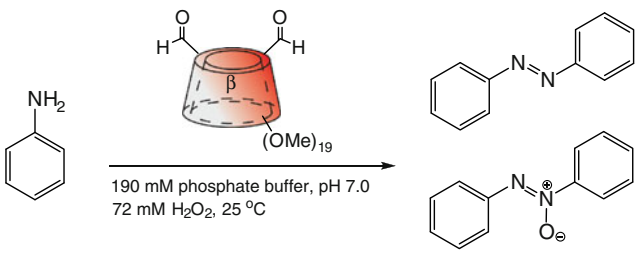
Aniline is an interesting substrate, having an aromatic amine group reminiscent of the majority of substrates tested, but devoid of any hydroxyl groups. In earlier work, a non-quantifiable catalysis of aniline was noted with bridged ketone CDs [24]. With *β*-CD dialdehyde **2**, aniline again showed some non-quantifiable catalysis (Scheme 4).

The collective data suggest, that in order to achieve a significant degree of catalysis, the substrates should preferentially originate from phenol-type structures.

Reactivity differences between CD ketones and aldehydes

Substrate design is obviously very important for catalysis, albeit other factors are of interest as well; Table 1 reveals that diketone **4** is a poor chemzyme compared to the CD aldehydes. This can be attributed to the fact that ketones bind H_2O_2 less efficiently than aldehydes [25]. To measure the binding of H_2O_2 to CD dialdehyde **2**, the catalysis of *o*-aminophenol oxidation was studied at different concentrations of H_2O_2 with **2** (Fig. 3). The hyperbolic curve shows that at $V_{max} = 50\%$, $K_d = 1.4$ mM for binding of H_2O_2. This strong binding of the cooxidant peroxide correlates with the dialdehyde **2** being a potent oxidation catalyst.

In contrast to the previously studied unprotected CD chemzymes tested for styrene epoxidation, the permethylated CD aldehydes have dual solubility, merging both with the aqueous layer and the organic phase upon work-up. Still, it is possible to distinguish starting material clearly in



Scheme 4 *β*-CD $6^A,6^D$-dialdehyde-catalyzed oxidation of aniline

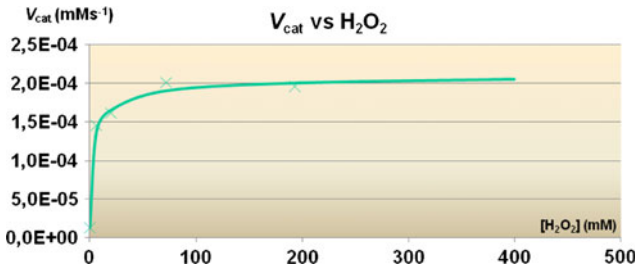
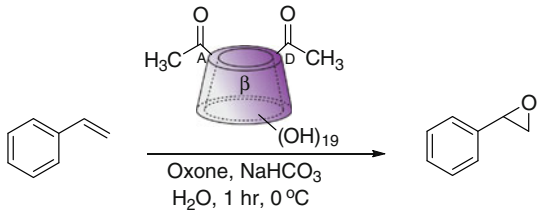


Fig. 3 Catalytic rate vs. $[H_2O_2]$ for **2**-catalyzed oxidation of *o*-aminophenol *β*-CD diketone **4** has previously been shown to catalyze the epoxidation of styrene, with 100% conversion of the substrate within 1 h [26, 27] (Scheme 5)



Scheme 5 *β*-CD $6^A,6^D$-dimethylketone-catalyzed epoxidation of styrene

^{1}H NMR and the conversion of substrate can be effectively monitored. The permethylated *β*-CD dialdehyde **2** was recently tested for catalysis of styrene epoxidation, with oxone as a cooxidant. ^{1}H NMR showed no catalysis, which is possibly due to the reactive aldehyde group in the CD itself being oxidized to acid by oxone and hence not being able to engage in catalysis, like the ketone CD is [28]. This is yet another illustrative example of the chemical differences in CD aldehydes and ketones as oxidation catalysts. With this knowledge at hand, the customized design of CD-based biomimetic catalysts for specialized purposes can be further expanded. In the future, the goal of our artificial enzyme research will be the synthesis of even more potent macromolecular CD chemzymes for various applications in catalysis and green chemistry.

Conclusion

A diverse range of new substrates tested for CD-effected catalysis of oxidation afford new information of the boundaries of CD chemzyme catalysis. Selectively modified *α*- and *β*-CD ketones or aldehydes catalyze the oxidation of different aminophenol substrates, with *o*-aminophenol being the optimal substrate. Interestingly, the oxidation of aniline was also catalyzed. Amino acid-type, benzyl alcohol or quinone substrates were beyond the realms of CD catalysis. The binding of the cooxidant H_2O_2 to CD dialdehyde is strong ($K_d = 1.4$ mM) and the less

reactive CD diketone is therefore a weaker oxidation catalyst. This in turn makes the ketone suited for styrene epoxidation, whereas the aldehyde is uneffective for this purpose. The catalysis is roughly proportional to the number of catalytic groups on the CD, but the structure of the substrate plays an equally important role in determining how effectively the reaction can be catalyzed. These findings on substrate compatibility provide new insight on the potentials of CD catalysis, which fosters optimism for future applications of CD chemzymes in synthetic biology.

Acknowledgments We thank The Lundbeck Foundation for financial support.

References

1. Burton, S.G.: Laccases and phenol oxidases in organic synthesis—a review. Curr. Organ. Chem. **7**, 1317–1331 (2003)
2. Ferguson Miller, S., Babcock, G.T.: Heme/copper terminal oxidases. Chem. Rev. **96**, 2889–2907 (1996)
3. Bjerre, J., Rousseau, C., Marinescu, L., Bols, M.: Artificial enzymes, "chemzymes": current state and perspectives. Appl. Microbiol. Biotechnol. **81**, 1–11 (2008)
4. Zhang, B., Breslow, R.: Ester hydrolysis by a catalytic cyclodextrin dimer enzyme mimic with a metallobipyridyl linking group. J. Am. Chem. Soc. **119**, 1676–1681 (1997)
5. Yan, J., Breslow, R.: An enzyme mimic that hydrolyzes an unactivated ester with catalytic turnover. Tetrahedron Lett. **41**, 2059–2062 (2000)
6. Tsutsumi, H., Ikeda, H., Mihara, H., Ueno, A.: Enantioselective ester hydrolysis catalyzed by β-cyclodextrin conjugated with β-hairpin peptides. Bioorg. Med. Chem. Lett. **14**, 723–726 (2004)
7. Ye, H., Tong, W., D'Souza, V.T.: Efficient catalysis of a redox reaction by an artificial enzyme. J. Am. Chem. Soc. **114**, 5470–5472 (1992)
8. Ye, H., Rong, D., Tong, W., D'Souza, V.T.: Artificial redox enzymes. Part 3: structure and properties. J. Chem. Soc. Perkin Trans. **2**, 2071–2076 (1992)
9. Ye, H., Tong, W., D'Souza, V.T.: Flavocyclodextrins as artificial redox enzymes. Part 4: catalytic reactions of alcohols, aldehydes and thiols. J. Chem. Soc. Perkin Trans. **2**, 2431–2437 (1994)
10. Breslow, R., Dong, S.D.: Biomimetic reactions catalyzed by cyclodextrins and their derivatives. Chem. Rev. **98**, 1997–2011 (1998)
11. Bjerre, J., Nielsen, E.H., Bols, M.: Hydrolysis of toxic natural glucosides catalyzed by cyclodextrin dicyanohydrins. Eur. J. Org. Chem. 745–752 (2008)
12. Ortega-Caballero, F., Bjerre, J., Laustsen, L.S., Bols, M.: Four orders of magnitude rate increase in artificial enzyme-catalyzed aryl glycoside hydrolysis. J. Org. Chem **70**, 7217–7226 (2005)
13. Fenger, T.H., Bjerre, J., Bols, M.: Cyclodextrin aldehydes are oxidase mimics. ChemBioChem **10**, 2494–2503 (2009)
14. Filliben, J.J.: Dataplot—an interactive high-level language for graphics, non-linear fitting, data analysis and mathematics. Comput. Graph. **15**, 199–213 (1981)
15. Bjerre, J., Fenger, T.H., Bols, M.: Synthesis of some trifluoromethylated cyclodextrin derivatives and analysis of their properties as artificial glycosidases and oxidases. Eur. J. Org. Chem. 704–710 (2007)
16. Pearce, A., Sinaÿ, P.: Diisobutylaluminium-promoted regioselective de-*O*-benzylation of perbenzylated cyclodextrins: a powerful new strategy for the preparation of selectively modified cyclodextrins. Angew. Chem. Int. Ed. **39**, 3610–3612 (2000)
17. Lecourt, T., Herault, A.J., Pearce, M., Sollogoub, M., Sinaÿ, P.: Triisobutylaluminium and diisobutylaluminium hydride as molecular scalpels: the regioselective stripping of perbenzylated sugars and cyclodextrins. Chem. Eur. J. **10**, 2960–2971 (2004)
18. Lecourt, T., Mallet, J.-M., Sinaÿ, P.: An efficient preparation of 6I, IV dihydroxy permethylated β-cyclodextrin. Carbohydr. Res. **338**, 2417–2419 (2003)
19. Horvath, T., Kaizer, J., Speier, G.: Functional phenoxazinone synthase models. Kinetic studies on the copper-catalyzed oxygenation of 2-aminophenol. J. Mol. Catal. **215**, 9–15 (2004)
20. Brown, K.C., Corbett, J.F.: Benzoquinone imines. Part 16. Oxidation of *p*-aminophenol in aqueous solution. J. Chem. Soc. Perkin Trans. **2**, 308–311 (1979)
21. Fenger, T.H., Marinescu, L.G., Bols, M.: Cyclodextrin ketones as oxidation catalysts: investigation of bridged derivatives. Org. Biomol. Chem. **7**, 933–943 (2009)
22. Marinescu, L., Bols, M.: Very high rate enhancement of benzyl alcohol oxidation by an artificial enzyme. Angew. Chem. Int. Ed. **45**, 4590–4593 (2006)
23. Suzuki, H., Furusho, Y., Higashi, T., Ohnishi, Y., Horinouchi, S.: A novel *o*-aminophenol oxidase responsible for formation of the phenoxazinone chromophore of grixazone. J. Biol. Chem. **281**, 824–833 (2006)
24. Marinescu, L., Mølbach, M., Rousseau, C., Bols, M.: Supramolecular oxidation of anilines using hydrogen peroxide as stoichiometric oxidant. J. Am. Chem. Soc. **127**, 17578–17579 (2005)
25. Sander, E.G., Jencks, W.P.: Equilibria for additions to the carbonyl group. J. Am. Chem. Soc. **90**, 6154–6162 (1968)
26. Rousseau, C.; Christensen, B.; Bols, M.: Artificial epoxidase II. Synthesis of cyclodextrin ketoesters and epoxidation of alkenes. Eur. J. Org. Chem. 2734–2739 (2005)
27. Rousseau, C., Christensen, B., Petersen, T.E., Bols, M.: Cyclodextrins containing an acetone bridge. Synthesis and study as epoxidation catalysts. Org. Biomol. Chem. **2**, 3476–3482 (2004)
28. Travis, B.R., Sivakumar, M., Hollist, G.O., Borhan, B.: Facile oxidation of aldehydes to acids and esters with oxone. Org. Lett. **5**, 1031–1034 (2003)