Artificial Enzymes

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Very High Rate Enhancement of Benzyl Alcohol Oxidation by an Artificial Enzyme**

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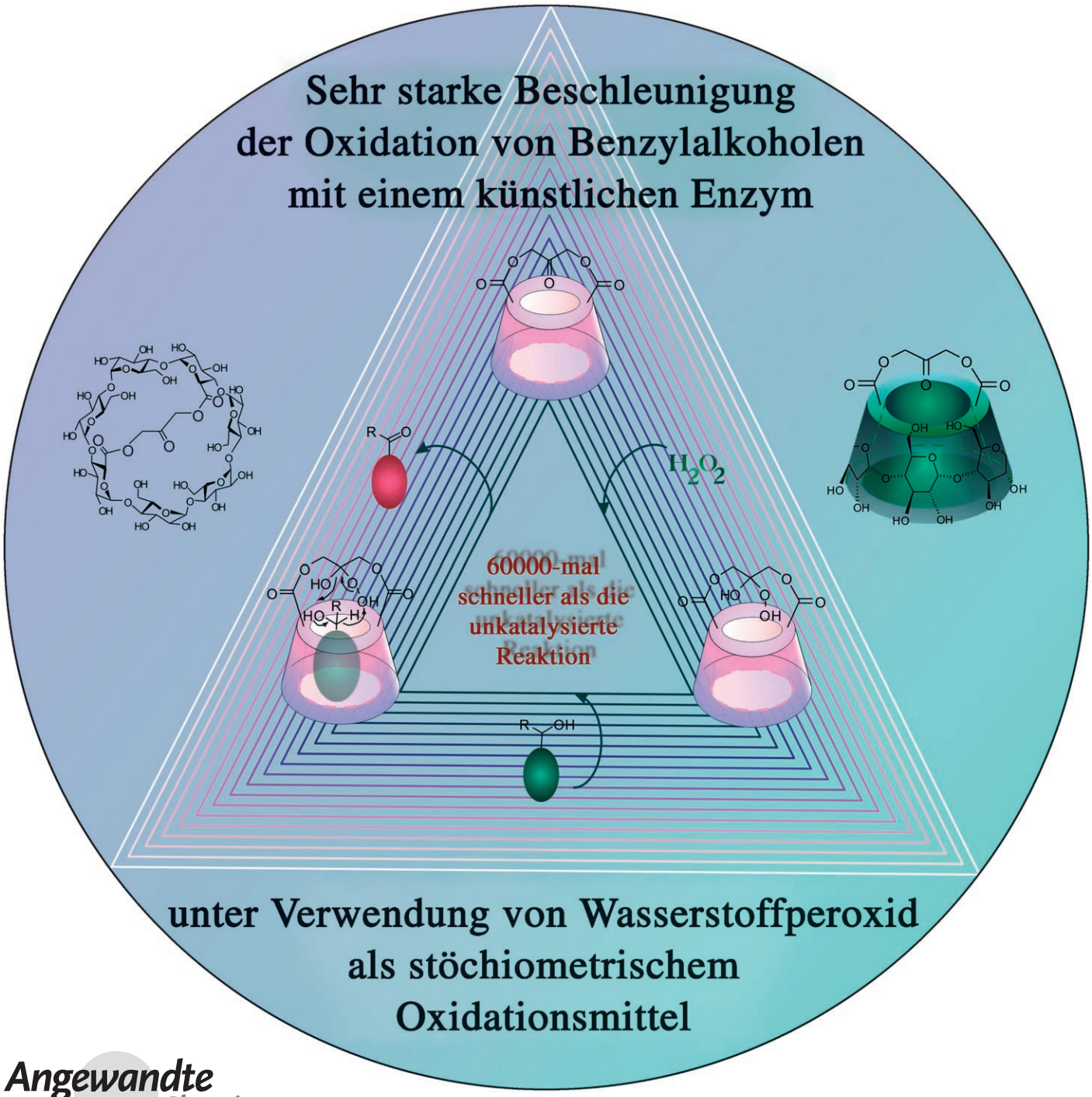
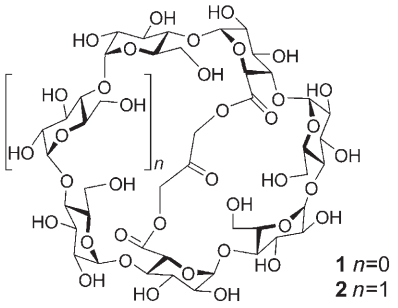


Enzymes have fascinated scientists since their discovery, and, over some decades, one aim in organic chemistry has been to create molecules that mimic the active sites of enzymes and promote catalysis.[1–6] Nevertheless even today there are relatively few examples of enzyme models that actually perform Michaelis–Menten catalysis under enzymatic conditions (i.e., water, pH 7, ambient temperature),[7] and very high rate accelerations under these conditions are rare.[8] On the other hand progress in synthetic chemistry, in this context, in carbohydrate chemistry,[9] now makes it possible to prepare more sophisticated rigid catalysts.

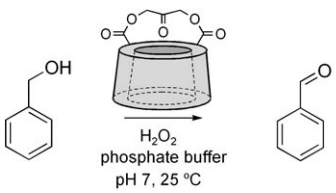
Recently we reported the bridged ketocyclodextrins **1** and **2** (Scheme 1)[10] and that they catalyze the oxidation of



Scheme 1. Catalysts **1** and **2**, which consist of a core of either α- or β-cyclodextrin with dihydroxyacetone attached to the primary rim through ester bonds.

anilines to nitrobenzenes in the presence of hydrogen peroxide (H_2O_2) in a reaction with enzyme kinetics and with a ratio k_{cat}/k_{uncat} up to 1070.[11] The cup-shaped compounds **1** and **2** bind the aromatic amino group in their hydrophobic cavities ($K_m \approx 1$–5 mM), while the ketone functionality is believed to form a hydroperoxide adduct with H_2O_2 that is responsible for the oxidation of the bound amine.[11] We have now found that **1** and **2** also catalyze the oxidation of benzylic alcohols to aldehydes, and that for this reaction the rate acceleration for the catalyzed reaction is very high.

The cyclodextrins **1** and **2** catalyze the transformation of benzylic alcohols into aldehydes (or ketones) in the presence of hydrogen peroxide (72 mM) in aqueous solution, at room temperature, and at pH 7.0 [Eq. (1)]. The reaction rate of the



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catalyzed reaction is observable even at cyclodextrin concentrations that are 10000 times lower than the substrate concentration and as low as 1 μM. GC-MS analysis revealed that complete oxidation of the starting material can be achieved. The reaction follows Michaelis–Menten kinetics (Figure 1), can be inhibited by addition of cyclopentanol, and

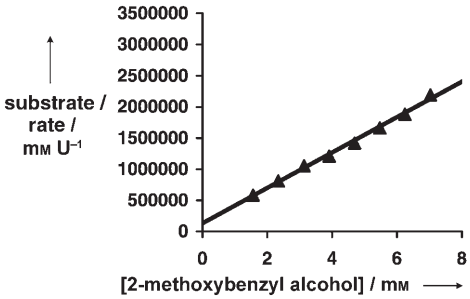


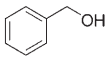
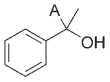
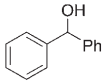
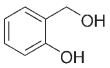
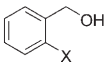
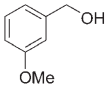
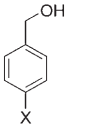
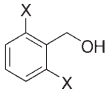
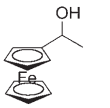
Figure 1. Hanes plot of the **2**-catalyzed ([**2**] = 0.38 mM) oxidation of 2-methoxybenzyl alcohol to 2-methoxybenzaldehyde in the presence of 72 mM H_2O_2, 25 °C.

is catalyzed neither by β-cyclodextrin nor by 1,3-dichloroacetone (DCA, Table 1). 1,3-Diacetoxyacetone (DAA) catalyzes the reaction slightly (Table 1). Under these reaction conditions **1** and **2** are stable and preincubation with hydrogen peroxide for a couple of hours does not decrease the catalytic activity.

The kinetic data (K_m and k_{cat}) for a range of benzyl alcohol substrates were obtained from v_{cat} vs. [S] data (v_{cat}: velocity of the catalyzed reaction, S: substrate) in the usual manner using nonlinear least-squares fitting and are shown in Table 1. For pH 7 and 25 °C, the K_m values range from (0.21 ± 0.07) to (5.0 ± 1.6) mM which are typical values for the binding of small aromatic molecules by cyclodextrins. The k_{cat} constants vary from about 10^{-6} to about $2.5 \times 10^{-4}\ s^{-1}$ and since the first-order rate constant for the background oxidation (k_{uncat}) under these condition is 10^{-8}–$10^{-9}\ s^{-1}$ the ratio between the reaction rates for the reactions inside and outside the cavity (k_{cat}/k_{uncat}) varies from about 400 to about 29000 at 25 °C. The comparison with diacetoxyacetone is also significant: DAA catalysis gives a second-order rate constant $k_{cat,2}$ of $4 \times 10^{-4}\ M^{-1}s^{-1}$, which has to be compared to the k_{cat}/K_m values for **1** and **2** that are 1.35×10^{-2} and $1.75 \times 10^{-2}\ M^{-1}s^{-1}$, respectively; it follows that the supramolecular catalysts are 30 to 45 fold more efficient than DAA. As can be seen from Table 1 secondary alcohols (Me or Ph substituents) are oxidized as well. A range of substituents on the aromatic ring is tolerated though with variations in the catalysis rate.

For three reactions, a study of pH and temperature effects was performed (see Table 1, entries marked with [a]). The reaction rate increases with temperature and pH value, but above pH 9 the catalyst starts to decompose, presumably as a result of the lability of the esters At pH 8.0 and 45 °C the rate increase observed for the oxidation of 2-hydroxybenzyl alcohol is approximately 6.3×10^4. Though this value still is lower than that found for natural enzymes, where rate increases have been calculated[12] to lie between 10^6 and 10^{18}, it is fascinating to consider that since **2** has a 10^2 times

Table 1: Kinetic data for the oxidation of various benzylic alcohols to aldehydes catalyzed by **1** or **2** and compared with those of other potential catalysts. Except otherwise noted the experiments were performed in a 95 mM phosphate buffer at 25 °C and pH 7 with a H_2O_2 concentration of 72 mM and with a concentration of the catalyst of 0.38 mM.

Substrate	A or X	Catalyst	k_{cat} [$\times 10^6$ s^{-1}]	K_m [mM]	k_{cat}/k_{uncat}
		**1**	26.9 ± 3.2	2.0 ± 0.7	1690 ± 230
		2	26.3 ± 2.0	1.5 ± 0.4	1650 ± 150
		2[a]	244 ± 6	–	989 ± 28
		2[c]	no catalysis	2.2 ± 0.2	1
		DCA	no catalysis	–	1
		DAA[d]		–	
	^{1}H	**1**	24.4 ± 1.5	0.72 ± 0.30	5930 ± 360
	^{1}H	**2**	22.6 ± 1.7	5.0 ± 1.6	5490 ± 420
	^{2}H	**2**	15.8 ± 1.4	0.77 ± 0.63	not determined
		**1**	18.6 ± 0.3	1.1 ± 0.1	973 ± 22
		2	18.3 ± 0.4	0.84 ± 0.1	960 ± 34
		β-CD	no catalysis	–	1
		**2**	6.24 ± 0.63	1.3 ± 0.2	28 600 ± 9200
		2[a]	62.1 ± 0.8	1.20 ± 0.15	62 900 ± 5500
		2[b]	no catalysis	–	1
		DCA	no catalysis	–	1
	OMe	**2**	3.41 ± 0.07	0.52 ± 0.08	2740 ± 130
	Cl	**2**	8.90 ± 0.35	0.61 ± 0.17	971 ± 76
	Br	**2**	66.8 ± 4.5	0.71 ± 0.19	366 ± 27
	Br	**2**[a]	591 ± 62	1.2 ± 0.4	82 ± 13
		**1**	5.62 ± 0.44	1.83 ± 0.51	748 ± 106
		2	4.55 ± 0.47	1.09 ± 0.56	887 ± 103
	OMe	**1**	9.63 ± 0.60	2.52 ± 0.43	6760 ± 570
	OMe	**2**	3.85 ± 0.12	0.97 ± 0.16	2700 ± 110
	OMe	**2**[a]	159 ± 12	6.1 ± 0.8	1760 ± 130
	OH	**2**	1.28 ± 0.08	0.09 ± 0.08	not determined
	F	**2**	9.10 ± 0.49	0.66 ± 0.23	402 ± 41
	Cl	**2**	21.5 ± 0.4	0.37 ± 0.05	707 ± 155
	Br	**1**	33.7 ± 0.9	1.15 ± 0.12	1030 ± 30
	Br	**2**	24.2 ± 0.1	0.93 ± 0.02	739 ± 4
	Me	**2**	3.93 ± 0.98	0.78 ± 0.93	945 ± 236
	OMe	**2**	1.57 ± 0.03	0.50 ± 0.06	2120 ± 86
	Cl	**2**	16.0 ± 0.8	0.21 ± 0.07	446 ± 81
		**2**	293 ± 51	11.8 ± 2.3	539 ± 94

[a] $T = 45$ °C, pH 8. [b] Cyclopentanol (109 mM) was added as inhibitor. [c] H_2O_2 was replaced with *tert*-butyl hydroperoxide. [d] $k_{cat,2} = 0.4$ mM^{-1} s^{-1}; $k_{cat}/k_{uncat} = 9.8$ mM^{-1}.

smaller molecular weight than a typical enzyme, the efficiency per mass unit is in the same range.

From the series of *p*-substituted benzyl alcohols (see Table 1) one can construct a Hammett plot (Figure 2), which gives a correlation between k_{cat} and σ of $r^2 = 0.82$, and 1.9 as the reaction constant (ρ).[13] This value suggests that the transition state is ionic and has some negative charge at the reaction center. A comparison of 1-phenylethanol with its 1-deuterated analogue (Table 1) reveals a small isotope effect ($k_H/k_D = 1.4$). Based on this we suggest a mechanism (Scheme 2) related to what has been formulated for the tungsten-catalyzed H_2O_2-oxidation of alcohols, which also gives a relatively low isotope effect ($k_H/k_D = 2$).[14] This mechanism is also similar to what we have proposed for amine oxidation.[11] The somewhat high ρ value[15] could be an indication that the reaction is facilitated by hydrogen bonding between the proton of the benzylic alcohol and a Lewis base, such as the keto hydroxy group, thus invoking the cyclic mechanism outlined. The remarkable supramolecular catalysis displayed by **1** and **2** under mild aqueous conditions is, as discussed above, in the best case as efficient per mass-unit catalyst as that of some natural enzymes. When this level of catalysis can be achieved, practical applications of such molecules are not far away.

Experimental Section

Determining the rate of oxidation: Each reaction was performed on 4–16 samples (2 mL each) of the appropriate substrate at different concentrations in 95 mM phosphate buffer containing 72 mM H_2O_2 and either **1** or **2** (1 mg) or nothing (as control). The reactions were followed at 25 °C using UV absorption at an appropriate wavelength[16] and typically monitored for 5 h. Velocities were determined as the slope of the progress curve of each reaction. The velocities of the

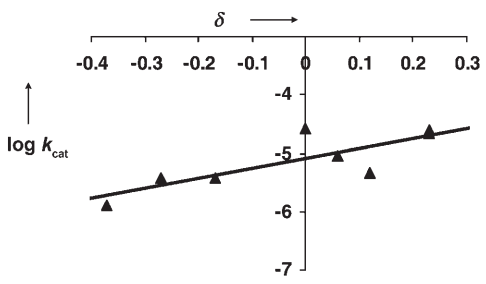
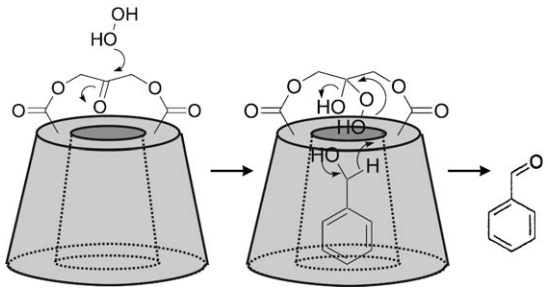


Figure 2. Hammett plot of the **2**-catalyzed oxidation of *p*-substituted benzyl alcohols at pH 7.0 in the presence of 72 mM H_2O_2, 25 °C. The plot has a slope (ρ) of 1.93 and a correlation r^2 of 0.82.



Scheme 2. Proposed mechanism of the catalytic process. First hydrogen peroxide is added to the ketone, and then bound substrate is oxidized. The oxidation step may be assisted by a hydrogen bond.

uncatalyzed reactions were obtained directly from the control samples, those of the catalyzed reactions were calculated by subtracting the uncatalyzed rate from the total rate of the appropriate cyclodextrin-containing sample. The v_{cat} values were used to construct Hanes plots ($[S]/v$ vs. $[S]$) to ensure that the reaction follows Michaelis–Menten kinetics. In that case K_m and v_{max} were determined using least-squares nonlinear regression fitting to the v_{max} vs. [S] curve. k_{cat} was calculated as v_{max}/[cyclodextrin]. k_{uncat} was determined as the slope from a plot of v_{uncat} vs. [S].[16]

GC-MS experiments: A pseudo-preparative experiment was performed by mixing 0.5 mmol substrate with 0.02 mmol catalyst **1** or **2** in 1.5 mL H_2O and slowly adding 1.5 mL H_2O_2, 35%. The mixture was incubated at 25°C from 3 h to several days. After extraction of the mixture with dichloromethane and evaporation the sample was analyzed in a Hewlett-Packard 5890A gas chromatograph equipped with a 5971A MSD mass-selective detector.

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[16] The following extinction coefficients ε [$mM^{-1}cm^{-1}$] (25°C, pH 7) and wavelengths λ [nm] of the products were determined and used: benzaldehyde (1.23, 285), acetophenone (0.32, 300), benzophenone (4.03, 280), 2-hydroxybenzaldehyde (2.92, 325), 2-methoxybenzaldehyde (2.75, 323), 2-chlorobenzaldehyde (1.24, 300), 2-bromobenzaldehyde (0.47, 292), 3-methoxybenzaldehyde (2.48, 313), 4-methoxybenzaldehyde (7.53, 300), 4-hydroxybenzaldehyde (6.1, 329), 4-fluorobenzaldehyde (0.88, 292), 4-chlorobenzaldehyde (1.09, 286), 4-bromobenzaldehyde (1.59, 295), 4-methylbenzaldehyde (3.37, 285), 2,6-dimethoxybenzaldehyde (3.63, 323), 2,6-dichlorobenzaldehyde (1.03, 309), acetylferrocene (1.68, 345 nm).

