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Cyclodextrin-accelerated cleavage of phenyl esters: is it the 2-hydroxy or the 3-hydroxy that promotes the acyl transfer?

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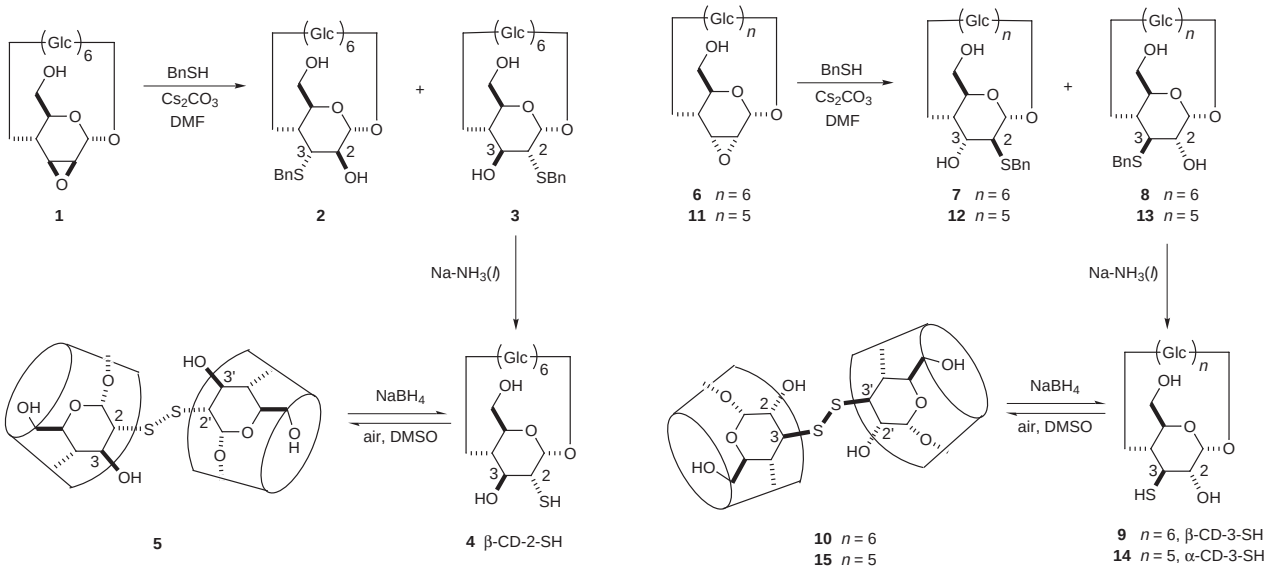
Both 2- and 3-monothiocyclodextrins have been synthesized and used in probing the mechanism of cyclodextrin-mediated cleavage of phenyl esters, showing that the 3-thiols are much more effective than the 2-thiols in promoting the acyl transfer.

Cyclodextrin-accelerated cleavage of phenyl esters is among the earliest examples of enzyme mimetic reactions.[1] Acylated cyclodextrins are usually the products and are difficult to recycle during the reaction. Although this reaction is not a catalytic one, it involves a rapid and reversible pre-binding of the substrate into the cyclodextrin cavity and subsequent acyl transfer to a cyclodextrin hydroxy group in the specifically arranged complex, in the same way that natural serine proteases bind their substrates and promote the transfer of an acyl group to a serine hydroxy group in the first enzymatic step.[2] Therefore, it provides an excellent model for better understanding enzyme action and has attracted a long-standing interest.[3] Some fundamental factors such as the effect of proximity,[4] binding of the substrate in the transition state,[5,6] geometric freedom needed for the entire reaction[7] and so forth have been elucidated, and very large rate-acceleration (up to 6 million-fold[4]) and good structural selectivity or enantioselectivity[8,9] have been obtained. However, the nucleophile in this reaction still remains to be determined. It is certain that the secondary hydroxy groups are engaged in the reaction, but there is no easy way to determine whether the 2-OH or 3-OH is the one to attack the ester first. Bender[10] suggested that the reaction involves the 3-OH group based on the observation that the 3-*O*-methylated derivative causes no rate-acceleration, while Breslow[8,11] believes that the reactive hydroxy group is the 2-OH by analogy with its greater reactivity toward methylation. Computer simulation[12] supports the 2-OH mechanism. Unfortunately, not enough experimental evidence for either mechanism has been obtained. We reason that adjusting the relative reactivity of the two positions can afford some insight into the mechanism. This idea is made practicable by our recent method[13] for selectively functionalizing the secondary face without significantly altering the conformation of cyclodextrin. Replacement of the secondary hydroxy group by a SH group has an interesting influence on the catalytic behaviour of cyclodextrins. Here we describe the preliminary results, which are striking and indicative of the 3-SH being preferred to the 2-SH as the nucleophile.

The syntheses of thiocyclodextrins **4**, **9** and **14** were carried out by nucleophilic ring-opening of cyclodextrin epoxides with BnSH followed by reduction with Na–NH_3 (l) (Scheme 1). The resultant thiols were converted to the corresponding dimers for easy storage. The nucleophilic ring-opening[14] of cyclodextrin mannoepoxide **1** was believed to exclusively result in the formation of a cyclodextrin derivative with a 3-functional altrose sub-unit which slightly distorts the cyclodextrin cavity.[15] We found that, with imidazole as nucleophile, the undistorted product with an imidazolyl group at C-2 of the glucoside unit can be obtained in addition to the distorted one.[13] This abnormal reaction actually occurs with various nucleophiles including BnSH, giving the undistorted cyclodextrin derivative with a functional group at C-2 of the glucoside unit as a minor product.[16] Though in very low yield, this method does afford the undistorted C-2 functional cyclodextrins which are otherwise inaccessible at present. Reaction of **1** with BnSH in DMF gave the undistorted cyclodextrin sulfide **3** in 6% yield in addition to **2** in 77% yield. The replacement of 3-OH was easily realized by the reaction of alloepoxides **6**[13,17,18] and **11** with BnSH which afforded the undistorted cyclodextrin sulfides **8** and **13** as main products. Reduction of the sulfides **3**, **8** and **13**



Scheme 1

Table 1 Kinetic parameters of the cleavage of nitrophenyl acetates by thiocyclodextrins at pH 9.0[a]

		p-Nitrophenyl acetate					*m*-Nitrophenyl acetate				
Catalyst	pK_a[b]	$k_{cat}/10^{-2}$ s^{-1}	$K_d/10^{-3}$ M	$(k_{cat}/k_{un})/10^2$	$(k_{cat}/K_d)/10$ M s^{-1}	$K_{TS}/10^{-4}$ M	$k_{cat}/10^{-2}$ s^{-1}	$K_d/10^{-3}$ M	$(k_{cat}/k_{un})/10^2$	$(k_{cat}K_d)/10$ M^{-1} s^{-1}	$K_{TS}/10^{-4}$ M
α-CD			10[c]	0.028[c]		36[c]	2.4	14	1.5	0.17	0.93
β-CD			7.8[c]	0.081[c]		9.6[c]	1.3	15	0.86	0.086	1.8
α-CD-3-SH	7.7	4.9	18	1.6	0.27	1.1	61	1.9	40	32	0.0049
β-CD-2-SH	7.5	0.80	12	0.26	0.068	4.5	0.4	7.0	0.26	0.058	2.7
β-CD-3-SH	7.7	15	7.6	5.0	2.0	0.15	4.5	3.2	2.9	1.4	0.11

[a] Reactions were carried out in a pH 9.0 standard buffer solution at 25 °C; the release of nitrophenols was monitored at 400 nm. Lineweaver–Burk treatment was applied to derive the k_{cat} and K_d values. [b] Determined by UV titration at 230 nm of the acidic solution of a cyclodextrin thiol with aq. NaOH. [c] Data at pH 11.7 from ref. 19.

with Na–NH_3 (l) gave the cyclodextrin thiols **4**, **9** and **14** which were further converted to the corresponding dimers **5**, **10** and **15** for storage.† The thiols were freshly regenerated with $NaBH_4$ just before use.

The free thiols show no obvious absorption above 215 nm while deprotonation of the thiol group generates a new absorption bond around 230 nm. Titrating the acidic solution of the thiols with aq. NaOH solution and following the absorption changes at 230 nm allows the determination of their pK_a values. As shown in Table 1, the three thiols **4**, **9** and **14** have pK_a values of 7.5, 7.7 and 7.7, respectively, very similar to each other. The behaviour of these thiols on cleavage of phenyl esters was investigated by following the absorption changes of the buffered aqueous solution of *m*-nitrophenyl acetate and its *p*-isomer, which are widely employed as substrates for serine protease mimics. The kinetic parameters of the thiocyclodextrin-mediated decomposition of nitrophenyl acetates at pH 9.0 are summarized in Table 1. β-CD-3-SH **9** significantly facilitates the reactions. It cleaves *p*- and *m*-nitrophenyl acetates 500 and 290 times faster than the buffer solution. Compared with β-cyclodextrin, β-CD-3-SH **9** is 62 times more effective towards *p*-nitrophenyl acetate and 3 times more effective towards the *m*-isomer. The result with β-CD-2-SH **4** is very different. This thiol shows a rate acceleration of 26 fold over the buffer solution in hydrolysing the *p*-nitrophenyl acetate, about 3 times that of β-CD. However, it hydrolyzes the *m*-isomer 3 times less effectively than β-cyclodextrin itself. As a result, β-CD-3-SH **9** is 10–20 times more effective than β-CD-2-SH **4** in promoting the acyl transfer of both substrates. Obviously the thiol group of both β-CD-2-SH **4** and β-CD-3-SH **9** is engaged in the reaction. The kinetic difference between them is most likely a reflection of their geometric specificity rather than their acidity difference since both thiols have similar pK_a values and should deprotonate almost to the same extent at pH 9.0. Only when SH is put in the right position with the correct conformation required to access the transition state can it significantly accelerate the reaction. This position appears to be C-3. Incorporation of SH at C-3 of α-cyclodextrin also gives striking results. α-CD-3-SH **14** accelerates the cleavage of *m*-nitrophenyl acetate by a factor of 4000, 25 times that of its *p*-isomer.

As suggested by the kinetic data in Table 1, the thiocyclodextrins decompose the nitrophenyl acetates in a pre-binding process, just in the same way that the native cyclodextrins do. All the thiocyclodextrins bind the ground state of *p*-nitrophenyl acetate ($1/K_d$) with a comparable strength which is not obviously different from that of native cyclodextrins. However, their transition state (TS) binding ($1/K_{TS}$) is quite different. β-CD-3-SH **9** binds the TS of *p*-nitrophenyl acetate 30 times tighter than its counterpart β-CD-2-SH **4**. Compared with the corresponding native cyclodextrins, β-CD-3-SH **9** and α-CD-3-SH **14** increase the TS binding by 64 and 33 fold, respectively, while β-CD-2-SH **4** does not significantly alter it. A similar result is also obtained in the case of *m*-nitrophenyl acetate. Replacement of one 3-OH of the cyclodextrins by SH greatly increases the TS binding of *m*-nitrophenyl acetate, whereas the replacement of one 2-OH by SH does not significantly change the binding of both ground state and TS. It is apparent that the 3-SH is preferred to the 2-SH as nucleophile in developing the transition state of the acyl transfer reaction of both substrates.

No significant conformational difference should exist between the modified cyclodextrins and the corresponding native ones except that one of the secondary hydroxy groups is replaced by SH. Though the S atom is larger than an O atom and would lead to an earlier TS for the acyl transfer, it appears that this replacement does not reverse the geometric preference of the ester cleavage for the 2- or 3-position. Thus the present research may supply a useful approach to evaluate the reactive group in the cyclodextrin-accelerated cleavage of phenyl esters.

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Notes and references

† All new compounds (**2**, **3**, **5**, **7**, **8**, **10**, **12**, **13** and **15**) were characterized *via* their FAB MS, ^1H and ^{13}C NMR spectra.

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