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Tetrahedron Letters
Tetrahedron Letters 45 (2004) 4069–4071

Esterase activity of cyclodextrin dithiocarbamates

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Received 22 March 2004; accepted 25 March 2004

Abstract—The catalytic activity of dithiocarbamate appended α- and β-cyclodextrins in the hydrolysis of *p*-nitrophenyl acetate (PNPA) was studied at alkaline pH. The values of k_{cat}/k_{uncat} for the hydrolysis of PNPA ranged from 8 to 165 in the presence of the catalyst and are pH independent in the range 7.5–9.0. Addition of Cu(II) increases the activity by factors of 75–515 and k_{cat} values increase with increasing pH in the same range. The differences in the obtained kinetic parameters are interpreted on the basis of two different mechanisms.

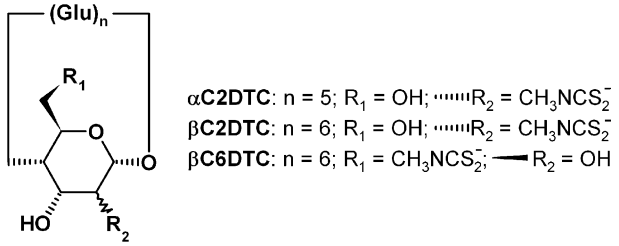
Cyclodextrins (CDs) are a class of cyclic oligosaccharides composed of α-(1→4)-linked D-glucopyranose units featuring an essentially hydrophobic central cavity. Such a structure allows them to form stable inclusion complexes with a wide variety of guests.[1] This remarkable property has been successfully applied in the past to study biomimetic reactions in which CDs behave as artificial enzymes.[2]

Structural and functional models of several enzymes have been prepared so far by attaching a catalytic moiety to one of the rims of CD, which acts as a substrate recognition site.[3] Among them, hydrolase models have attracted the attention of many research groups in the past years and several elegant examples can be found in the literature.[3,4] Research on artificial hydrolases has been mainly focused on CDs attached to nitrogen containing residues such as amines and imidazole but there are only few reports on sulfur-containing CDs.[5] On the other hand, substituents capable to coordinate transition metals have been used to construct metalloenzyme models.[6] The presence of the metal ion usually changes the classical acyl-transfer mechanism of CDs by coordination to the substituent donor atoms. Therefore, these systems are also of theoretical interest to study hydrolysis reactions.

We have been interested in the past on the biological activity of dithiocarbamates and their coordination complexes with Cu(II).[7,8] In the present letter we wish to report on the esterase activity of several monosubstituted CD-dithiocarbamates (Scheme 1) and their Cu(II) complexes. The role of the metallic centre on the hydrolysis mechanism is also discussed.

Table 1 shows the kinetic parameters obtained for the studied systems. As can be seen, αC2DTC, βC2DTC and βC6DTC accelerate the hydrolysis of PNPA with respect to the buffer alone in factors of 165, 88 and 8.2, respectively. The dithiocarbamate moiety is responsible for a 100-, 51- and 4.1-fold activity increase with respect to the corresponding native CD. At the same time, the role of the CD cavity in assisting the catalysis is evident considering that it causes up to a 121-fold increase in the activity. On the other hand, $1/K_m$ also decreases in the order αC2DTC > βC2DTC > βC6DTC indicating that PNPA is bounded more tightly to the smaller cavity of αCD.

Figure 1 shows the UV–vis spectrum of 6.25×10^{-5} M αC2DTC in the presence of 10^{-3} M *p*-nitrophenolate



Scheme 1.

Keywords: Cyclodextrin; Dithiocarbamate; Esterase activity; Copper.
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doi:10.1016/j.tetlet.2004.03.156

Table 1. Kinetic parameters for the hydrolysis of PNPA by cyclodextrin dithiocarbamates (in 10 mM Tris buffer pH = 9)

Catalyst	$k_{cat}\times10^{-3}$ s^{-1}	$K_m\times10^{-3}$ M	k_{cat}/K_m M^{-1} S^{-1}	k_{cat}/k_{uncat}	k_{cat}/k_{MorDTC}
αC2DTC	49.5	1.34	36.9	165	121
βC2DTC	26.3	2.11	12.5	88	64
βC6DTC	2.58	2.44	1.05	8.2	6.3
MorDTC[a]	0.41	—	—	1.3	—
Cu(αC2DTC)$_2$	154	3.21	48.0	515	376
Cu(βC2DTC)$_2$	96.1	5.11	18.8	320	234
Cu(βC6DTC)$_2$	22.5	5.68	3.96	75	55

[a] Morpholyldithiocarbamate.

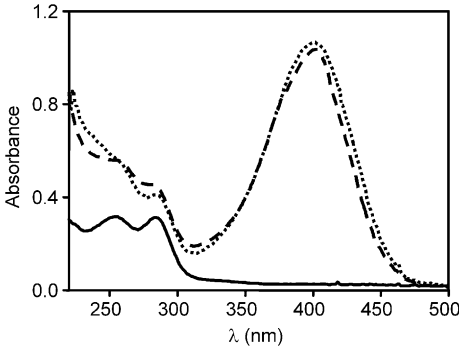

Figure 1. UV–vis spectra at pH 9 of 6.25×10^{-5} M αC2DTC (—), in the presence of 10^{-3} M *p*-nitrophenolate (----) and a mixture of 6.25×10^{-5} M αC2DTC and 10^{-3} M PNPA 12 min after reaction (....).

paired with that obtained 12 min after mixing in the same concentrations αC2DTC and PNPA. As can be seen, both spectra are coincident and the bands of αC2DTC are clearly visible at 253 and 282 nm, demonstrating that αC2DTC acts catalytically in the hydrolysis of PNPA. Figure 2 shows the spectral changes and the time course of PNPA hydrolysis by αC2DTC. Under these conditions, the acyl-CD recycle rate was 78 h^{-1}.

When a Cu(II) salt is added to a solution of αC2DTC, βC2DTC or βC6DTC, a complex of general formula CuL_2 is formed.[8] These complexes show even higher activity in the cleavage of PNPA than the corresponding ligands (Table 1) and also obey LWB behaviour. The highest acceleration is shown by Cu(αC2DTC)$_2$ in a factor of 515 with respect to the background buffer (3.1 with respect to αC2DTC). The values of $1/K_m$ for the complexes are higher that those of the corresponding ligands, suggesting that the substrate interaction is now

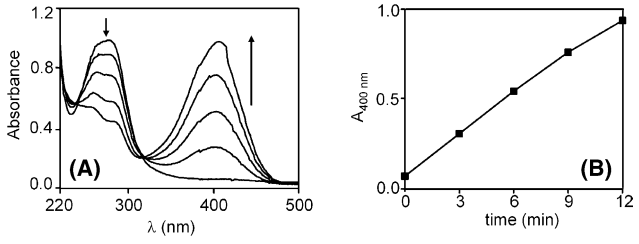

Figure 2. (A) Spectral variations for the hydrolysis at pH 9 of 10^{-3} M PNPA by 6.25×10^{-5} M αC2DTC recorded in 3 min intervals. (B) Time course of the reaction.

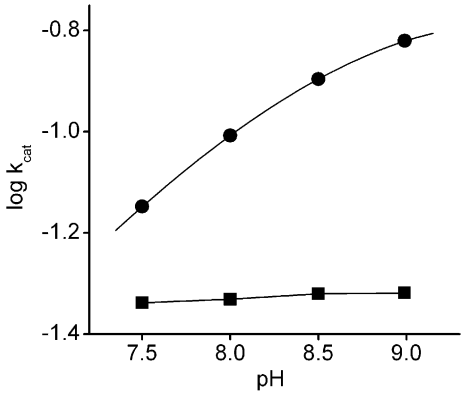

Figure 3. k_{cat} Versus pH profile for the hydrolysis of PNPA by αC2DTC (■) and Cu(αC2DTC)2 (●) in Tris buffer pH 7.5–9.0 at 25 °C. The solid curve for the Cu(II) system was calculated for pK_{ES} = 8.73 (see text for details).

hindered by the presence of the CuS_4 moiety. These systems constitute another example of the effectiveness of metal coordinated CD derivatives as enzyme mimics.[4,6]

The k_{cat} versus pH profiles for αC2DTC and Cu(αC2DTC)$_2$ resulted to be very different, as shown in Figure 3. The activity of αC2DTC is almost pH independent suggesting that the hydrolysis takes place through a nucleophilic attack of one of the sulfur atoms of the dithiocarbamate moiety to the carboxyl atom of PNPA without participation of the buffer. By contrast, k_{cat} increases with pH under the same conditions for Cu(αC2DTC)$_2$, suggesting a basic catalysis mechanism. Since coordination of Cu(II) to the dithiocarbamate moiety suppresses the possibility of a nucleophilic catalysis carried out by the sulfur atoms of the dithiocarbamate group, the catalytic function should be carried out by an activated water molecule axially bounded to the copper atom. In this case, the pH dependence of k_{cat} can be represented by the equation:[9] $k_{cat} = (K_{ES}k_{cat}^{max})(K_{ES} + [H^+])^{-1}$ where K_{ES} is the ionization constant of the bounded water. For Cu(αC2DTC)$_2$, pK_{ES} = 8.73 and $k_{cat}^{max} = 180\times10^{-3}$ s^{-1}. Figure 4 shows a schematic representation of the proposed hydrolysis mechanisms.

In conclusion, we have demonstrated that dithiocarbamates attached to cyclodextrins and their Cu(II) complexes possess hydrolase activity towards activated esters.

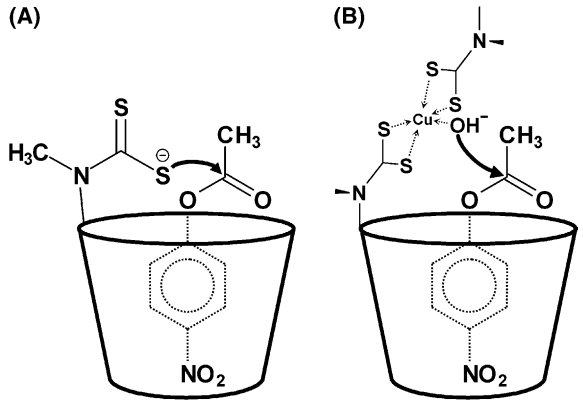

Figure 4. Proposed mechanisms for the hydrolysis of PNPA by αC2DTC (A) and Cu(αC2DTC)$_2$ (B).

Experimental: The CD derivatives αC2DTC, βC2DTC, C6DTC and their Cu(II) complexes were prepared as previously reported.[8] PNPA (Aldrich) and Tris (Merck) were used as received.

The kinetic studies were performed on a Ultrospec 2100 spectrophotometer (Amersham Biosciences) by measuring the release of *p*-nitrophenolate at 400 nm in Tris buffer pH 9.0 (10 mM) at 25 °C. The catalyst concentration was fixed at 6.25×10^{-5} M while PNPA concentration was varied from 10^{-3} to 10^{-2} M. Lineweaver–Burk approach was applied to evaluate the values of the catalytic (k_{cat}) and dissociation (K_m) constants. The influence of pH was studied in the same conditions but using 10 mM Tris buffer of the appropriate pH value.

Acknowledgements

Financial support from the Third World Academy of Sciences (Grant 02-334 RG/CHE/LA to A.F.) is gratefully acknowledged.

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