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β-Cyclodextrin-Catalyzed Effects on the Hydrolysis of Esters of Aromatic Acids

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Abstract. In order to make the behavior of the hydrophobic cavity of β-cyclodextrin clear, we have studied β-cyclodextrin-catalyzed hydrolysis of a series of nitrophenyl esters of aromatic acids. We defined a new kinetic parameter to determine the structure of the inclusion compounds. The kinetic parameters obtained provide evidence that the aromatic acid moiety rather than the nitrophenyl moiety of the esters mainly enters the hydrophobic cavity of β-cyclodextrin to form the inclusion complex.

Key words. Cyclodextrin-catalyzed, hydrolysis, hydrophobic cavity, inclusion complex, kinetic parameter RMP.

1. Introduction

Cyclodextrin has attracted great attention as an enzyme model for serine acylase enzymes such as chymotrypsin. It has a hydrophobic cavity and shows hydrophilic characteristics by the hydroxyl groups on both sides of the torus. In cyclodextrin-catalyzed reactions, a substrate binds into the cyclodextrin molecular cavity and then undergoes reaction with one of the cyclodextrin hydroxyls. According to Bender's work [1], the cyclodextrins cause a markedly stereoselective acceleration of the release of phenols from substituted phenyl acetates, the rate accelerations with meta-substituted esters being larger than with the corresponding para-substituted esters, i.e., the rate accelerations are independent of the electronic nature of the substituents. In order to make clear the behavior of the hydrophobic cavity of β-cyclodextrin, we have studied the β-cyclodextrin-catalyzed hydrolysis of the esters containing both hydrophobic and hydrophilic groups, such as *m*- and *p*-nitrophenyl nicotinates and 3-β-pyridylacrylates [2], and the other *m*- and *p*-nitrophenyl esters of *p*-*t*-butylbenzoic, *p*-*t*-butylcinnamic, α-naphthoic, acetic and β-anthraquinone carboxylic acids.

2. Experimental

Aqueous buffers were prepared with deionized water. The dimethyl sulfoxide used in the kinetic runs was dried over potassium hydroxide and distilled. β-cyclodextrin was recrystallized from water and dried overnight at 80 °C (0.05 torr) just prior to use.

A Shimadzu UV 260 spectrophotometer was used both for recording UV/visible

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absorption spectra and for the kinetic studies. pH measurements were accomplished with a PHM 84 research pH meter equipped with a GK2401C combined electrode.

2.1. KINETIC MEASUREMENTS

Reaction buffers were prepared by adding 4 volumes of 10 mM aqueous potassium dihydrogen phosphate/sodium hydroxide buffer (pH 6.8–7.6) to 6 volumes of dimethyl sulfoxide. The resulting solutions had pHs ranging from 10.6 to 11.5 as determined with the glass electrode. β-Cyclodextrin solutions (0.9–6.0 mM) were prepared with this buffer and stored under nitrogen. Substrate solutions (3–4 mM in dimethyl sulfoxide) were stored in the dark.

A kinetic run was initiated by equilibrating 3.00 ml of β-cyclodextrin solution to 30.0 ± 0.5 °C in the spectrophotometer chamber. A 30 µl sample of substrate solution was injected (to make the solution 30–40 µM in substrate) and the absorbance at 410 or 415 nm monitored as a function of time. After a suitable interval (10 half-lives), the final absorbance was measured. The pH of the solution throughout the run was found to remain constant to within ± 0.02 unit. In the case of substrates showing monophasic kinetics, data from the first 3–4 half-lives were fitted to a simple exponential by using a standard nonweighted least-squares routine. The resulting rate constants showed standard deviations of less than 1%.

The maximal rate constants (k_c) and binding constants (K_m) were extracted from rates measured at six different concentrations of β-cyclodextrin by using the method of Eadie [3].

2.2. PREPARATION OF MATERIALS

All substrates were prepared by condensation of carboxylic acids using dicyclohexylcarbodiimide or acid chlorides with *p*- and *m*-nitrophenol. The esters were identified

Table I. The melting point of the esters used [a]

Substrates	m.p. (°C)	Ref.[b]
1. *m*-nitrophenyl acetate	55–56	
2. *p*-nitrophenyl acetate	80–81	
3. *m*-nitrophenyl nicotinate	134–135	
4. *p*-nitrophenyl nicotinate	172–173	
5. *m*-nitrophenyl 3-β-pyridylacrylate	163–164	
6. *p*-nitrophenyl 3-β-pyridylacrylate	186–187	
7. *m*-nitrophenyl *p*-*t*-butylbenzoate	85–86	[5]
8. *p*-nitrophenyl *p*-*t*-butylbenzoate	123–124	[6]
9. *m*-nitrophenyl *p*-*t*-butylcinnamate	82–83	[4]
10. *p*-nitrophenyl *p*-*t*-butylcinnamate	147–148	
11. *m*-nitrophenyl α-naphthoate	116–117	[7]
12. *p*-nitrophenyl α-naphthoate	142–143	
13. *m*-nitrophenyl β-anthraquinone carboxylate	212–213	[8]
14. *p*-nitrophenyl β-anthraquinone carboxylate	243–244	

[a] All melting points are uncorrected. Esters, except 1, 2, 3, 4, 8, and 12, are the unknown compounds.

[b] References to the synthetic methods for the esters or for the corresponding acids or the acid chlorides.

by elemental analysis and spectral measurements before use. All reported melting points are listed in Table I.

p-t-Butylcinnamic acid was prepared from *p-t*-butylbenzaldehyde and propanedioic acid [4], m.p. 198–199 °C. Anal. Calcd. (Found) for $C_{13}H_{16}O_2$: C, 76.44(76.22); H, 7.89(8.11).

3. Results and Discussion

According to Bender's work [1], the rate acceleration k_c/k_{un} (k_{un} is the observed first-order rate constant for hydrolysis of the ester in the absence of β-cyclodextrin and k_c is the maximal catalyzed rate due to decomposition of the fully complexed ester) with *m*-nitrophenyl acetate is larger than with *p*-nitrophenyl acetate, since the cyclodextrin forms a complex with the nitrophenyl group of the esters and the *m*-nitrophenyl group is more suitable than the *p*-nitrophenyl group for cyclodextrin catalysis. To express our views clearly, we define a new kinetic parameter, RMP, as the ratio of the rate acceleration for β-cyclodextrin-catalyzed hydrolysis of the *m*-nitrophenyl ester to that of the corresponding *p*-nitrophenyl ester, $(k_c/k_{un})_m/(k_c/k_{un})_p$. So the RMP values of the *m*- and *p*-nitrophenyl acetates is 10.5 (calculated using Bender's data [1]). If the β-cyclodextrin forms a complex with the acyl group of the esters, the complexes of *m*- and *p*-substituted esters are similar from the point of view of catalysis and the rate acceleration will be almost the same and the RMP values will be nearly unity. Otherwise similar results to Bender's work will be observed. Because the methyl group is too small to stay in the cavity of β-cyclodextrin and the anthraquinonyl group is too large to enter the cavity [9], the nitrophenyl groups of these two esters will enter the cavity and we can get a result similar to that from Bender's work.

The maximal rate constants k_c and binding constants K_m of β-cyclodextrin-ester complexes in 6DMSO–4H_2O solvent are shown in Table II. For nitrophenyl acetates the temperature and solvent we employed is different from Bender's [1], so the data obtained are different. From the data in Table II, differences are observed between the substrate pairs (1, 2) and (13, 14) and the others.

The RMP values of the substrate pairs (1, 2) and (13, 14) are 4.5 and 4.8 and are larger than those of the others (less than 1.5). For substrates 1, 2, 13 and 14, β-cyclodextrin forms complexes with the nitrophenyl groups and a markedly stereoselective acceleration is caused. For the other substrates β-cyclodextrin forms the complexes with their acyl groups and a similar complex is formed with the *p*- and *m*-nitrophenyl esters. So it will not cause a markedly stereoselective acceleration and the RMP values will be nearly unity.

On the other hand, from the data in Table II we found that there are differences in the values of K_m only between substrates 1 and 2 or 13 and 14. The values of K_m for the other substrates are almost the same. This can be explained by the fact that if the acyl group enters the β-cyclodextrin cavity, the structures of the inclusion complexes of the corresponding *p*- and *m*-substituted esters with β-cyclodextrin are similar and the values of K_m will be very close. If the *p*- and *m*-nitrophenyl groups enter the β-cyclodextrin cavity, the apparent differences in the values of K_m of the substrates 1 and 2 or 13 and 14 can be expected because of the difference of the steric effect between two nitrophenyl groups.

The k_c values of the *m*-nitrophenyl esters are larger than those of the *p*-nitro-

Table II. Kinetic and Binding Constants at 30.0 ± 0.5 °C

Substrate	k_{un}[b] ($s^{-1} \times 10^5$)	k_c[a] ($s^{-1} \times 10^3$)	K_m[c] ($M \times 10^3$)	pH	k_{un} corrected to pH 10.00 ($s^{-1} \times 10^6$)	k_c corrected to pH 10.00 ($s^{-1} \times 10^4$)	k_c/k_{un}	RMP[d]
1	20.9 ± 0.1	12.6 ± 0.42	20 ± 1	11.45	7.42	4.46	60.1	4.5
2	43.1 ± 0.2	5.78 ± 0.53	8.3 ± 1.1	11.45	15.3	2.05	13.4	
3	15.8 ± 0.1	5.28 ± 0.55	5.3 ± 1.0	11.09	12.8	4.29	33.5	1.2
4	17.6 ± 0.1	5.02 ± 0.75	6.6 ± 2.1	11.05	15.7	4.48	28.5	
5	3.51 ± 0.01	5.10 ± 0.65	6.7 ± 1.4	11.00	3.51	5.10	145	1.5
6	5.74 ± 0.01	5.42 ± 0.82	6.5 ± 1.7	11.00	5.74	5.26	94.4	
7	2.22 ± 0.03	1.81 ± 0.09	0.32 ± 0.03	11.49	0.719	0.587	82	0.66
8	3.80 ± 0.02	4.70 ± 0.12	0.35 ± 0.02	11.49	1.23	1.52	124	
9	2.51 ± 0.01	3.45 ± 0.16	0.39 ± 0.03	11.37	1.07	1.47	137	1.2
10	5.16 ± 0.01	5.70 ± 0.16	0.40 ± 0.02	11.37	2.20	2.43	111	
11	7.40 ± 0.01	1.54 ± 0.16	9.8 ± 1.3	11.50	2.34	0.488	21	0.60
12	5.79 ± 0.01	1.83 ± 0.10	9.5 ± 1.0	11.50	1.83	0.578	35	
13	49.8 ± 0.2	18.5 ± 1.9	11 ± 4	10.66	109	40.5	71.7	4.8
14	62.6 ± 0.3	9.69 ± 2.88	3.6 ± .2.0	10.66	137	21.2	15.5	

[a] Pseudo-first-order rate constants for acylation of β-cyclodextrin by fully bound substrate in 6DMSO–4 H_2O at the 'pH' indicated, as measured with a glass electrode.
[b] Pseudo-first-order rate for hydrolysis of the substrate in the absence of β-cyclodextrin at the 'pH' indicated.
[c] Dissociation constant of the substrate-β-cyclodextrin complex.
[d] $(k_c/k_{un})_m/(k_c/k_{un})_p$.
[e] All errors are standard deviations.

phenyl esters for substrates 1, 2, 13, and 14 while the opposite trend is observed for the other substrates. The nitrophenyl groups do not enter the β-cyclodextrin cavity on complexation and the substrates, apart from 1, 2, 13, and 14 will display the electronic effect of the acylation reaction. The k_c value of the *p*-nitrophenyl ester is larger than that of the corresponding *m*-nitrophenyl ester. For example, the k_c value of substrate 8 is 1.52×10^{-4} and that of substrate 7 is 0.58×10^{-4}.
But for substrates 1, 2, 13, and 14, the nitrophenyl groups enter the β-cyclodextrin cavity and the same result is not observed (for substrate 1, k_c is 4.46×10^{-4} and for substrate 2, $k_c = 2.05 \times 10^{-4}$).

Complexes formed between β-cyclodextrin and the aromatic acid moiety of the esters, apart from 3, 4, 5, and 6 are more suitable for catalysis than those formed with the nitrophenyl group, since the aromatic acid moiety is larger than nitrophenyl. So it is reasonable to draw this conclusion.

The pyridyl group is less hydrophobic than the nitrophenyl group and the volume of the pyridyl group is a little less than that of the nitrophenyl group (from CPK models). Why does the end of the pyridyl group enter the β-cyclodextrin cavity more readily than the end of the nitrophenyl group? We consider that maybe there is a hydrogen bond between the N atom of the pyridyl group and one of the hydroxyl groups of the β-cyclodextrin in the inclusion complex, or that other, more complicated effects occurred. It is necessary to investigate this point further.

From the results and discussion above, we can get the following preliminary conclusion: it is the aromatic acid moiety rather than the nitrophenyl moiety of the

esters that enters the hydrophobic cavity of β-cyclodextrin to form the inclusion complex.

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