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Peculiarities of β-cyclodextrin acid hydrolysis

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Acidic hydrolysis of β-cyclodextrin in acetic acid solutions was investigated. It was found from kinetic observations and reverse kinetic isotope effect that the reaction proceeded according to the mechanism of specific acid catalysis. Investigation of cyclodextrin hydrolysis in the presence of some aromatic and aliphatic compounds was carried out. The rate of the reaction was found to decrease with an increase in the concentration of a guest.

Key words: acidic hydrolysis, β-cyclodextrin, inclusion complex

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides containing 6, 7 or more units of glucose combined into a closed cycle [1]. Due to the apolar cavity such compounds can form inclusion complexes [2] even with water-insoluble compounds. The stability of such complexes decreases with an increase in temperature [3, 4].

Cyclodextrins undergo hydrolysis in acidic media. The pioneering works on this reaction were done by D. French [5] and K. Freudenberg [6] who examined the structure of cyclodextrins. It is known that the hydrolysis of cyclodextrin proceeds slower than the decomposition of linear oligosaccharides [7]. The data presented in the references on the rate of the hydrolysis of a single cyclic glycosidic bond differ markedly. D. French has found that the acid hydrolysis of β-cyclodextrin proceeds five time slower than that of linear oligosaccharides [5]. K. Myrback has reported that it proceeds only three times slower [8]. The decomposition of cyclodextrins was also investigated by J. Szejtli and co-workers [9, 10]. They indicate that the formation of the inclusion complexes can slow down the rate of hydrolysis of cyclodextrins.

Despite the numerous studies of change in the reactivity of guest molecules in the inclusion complexes, little is known about the changes in hydrolysis of cyclodextrins when the guest molecule is included in the cavity. It has been reported recently [11] that the hydrolysis of β-cyclodextrin is decelerated by some phenols and aromatic amines. Investigation of this reaction is important in order to examine the possibility of using cyclodextrins for the preparation of linear oligosaccharides [12].

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Since data on the acid hydrolysis of β-cyclodextrin in presensce of guest molecules are not numerous, the aim of the present work was to investigate the kinetics of β-cyclodexrtin hydrolysis in presence of acetic acid and its derivates solutions and in the presence of some guest.

EXPERIMENTAL

β-Cyclodextrin (β-CD) containing crystallized water was purchased from "Fluka" (Switzerland). The content of crystallized water was taken into account during the calculations.

Hydrochloric acid (reagent-grade, free from metals and higher oxidized chlorine from "Reakhim", Russia) was used for kinetic investigations. Solutions of both acetic and hydrochloric acids were prepared from ampoules. The concentration of acids was checked titrimetrically. Commercial ("Fluka") 2-substituted acetic acid derivatives were purified by appropriate methods.

d_4-Acetic acid ("Fluka") containing 99.95% of deuterium was used to investigate the kinetic isotope effect. d_{24}-β-Cyclodextrin was prepared from β-CD by drying its solution in D_2O ("Fluka") to complete dryness in vacuum. This procedure was repeated twice. Crystallized water was tested.

Solutions of cyclodextrin containing 2×10^{-3} mol l^{-1} of β-CD were prepared. The concentration of a guest exceeded that of CD up to 50-fold.

The hydrolysis was carried out at different temperatures. The range of temperature variations did not exceed ± 0.2 °C (ultrathermostate UT-10, Poland).

The kinetics of β-cyclodextrin hydrolysis was observed fixing the reducing oligosaccharide groups by the Somogyj–Nelson method [13]. The reducing oligosaccharides were

oxidized with Cu^{2+} to Cu_2O which formed a colour heteropoly-complex with the Nelson reagent $Cu_3[AsMo_{12}O_{40}]$ and after that was determined colorimetrically. It was found by examining the model solutions of maltohexose and glucose ("Serva", Germany) that this method was suitable for the analysis of both oligosaccharides.

Since β-CD has no reducing groups, the rate of the cycle opening (hydrolysis) may be determined by the origin of the reducing groups and may by carried out by the following equation:

$$d[C] / dt = -k[C], \quad (1)$$

where k is the rate constant and C is the concetration of β-cyclodextrin.

The initial rate of reaction v_o shows the rate of cycle opening.

The hydrolysis was carried out to a small extent (5–10% of β-CD was hydrolyzed). The kinetic curve of the reaction was extrapolated to the initial time. The first-order derivative at this point is equal to the initial rate of the reaction [14]. Then the pseudo-first-order rate constant (in the presence of acid excess) was calculated according to the following equation (2):

$$k_1 = v_o / 7\ [\beta\text{–CD}], \quad (2)$$

where [β–CD] is the initial concentration of the host (cyclodextrin). This equation is valid for a single glucosidic bond, because β–CD has seven glucosidic bonds. The pseudo-second-order rate constant was calculated according to the following equation:

$$k_1 = k_2 / [H^+]. \quad (3)$$

Hydrolysis of maltose was examined by using the enzyme methods. The concentration of the glucose formed after hydrolysis of maltose was measured with glucosoxidase.

Hydrogen peroxide produced in the reaction can be estimated amperometrically using an oxygen electrode [15] or peroxydase [16].

RESULTS AND DISCUSSION

1. Acid hydrolysis of β-CD in acetic acid and its derivative solutions

In order to examine the mechanism of the reaction, cyclodextrin hydrolysis in acetic acid was carried out at 120 ± 0.2 °C in sealed vials. The hydrolysis rate of the cyclic glycosidic bond was compared with that of maltose. The data are presented in Table 1.

The pseudo-first-order rate constant increased with an increase in the concentration of acetic acid. We failed to find data on the exact pK_a value at this temperature. To calculate the concentration of H^+ ions at 120 °C, the equations referenced in [17, 18] were used. The second-order-rate constant of the hydrolysis of both the glycosidic bond of maltose and cyclodextrin remained unchanged even when the concentration of acetic acid increased up to 100 times. β-CD hydrolysis in a 90% solution of acetic acid was examined. We detected no maltooligosaccharides in a measurable amount even after 3 hours of experiment run.

Thus, the rate of the reaction was found to be strongly dependent on the concentration of a catalyst (H^+ ion). We may conclude that CD and maltose hydrolysis proceeded according to the mechanism of specific acid catalysis (a possible basic scheme is given in Figure).

Table 1. **Hydrolysis rate constant of β-CD and maltose in acetic acid at 120 °C**

Solution	Concentration of acetic acid, mol l^{-1}	Concentration of H^+ ions, mol l^{-1} × 10^4	Hydrolysis of maltose		Hydrolysis of β-CD	
			Pseudo-first-order rate constant, $k_1 \times 10^{-6}$, s^{-1}	Second-order rate konstant, k_2, x 10^3, l · mol^{-1} · s^{-1}	Pseudo-first-order rate constant, $k_{1CD} \times 10^{-6}$, s^{-1}	Second-order rate konstant, k_{2CD}, × 10^3, l · mol^{-1} · s^{-1}
H_2O	0.01	2.84	0.41 ± 0.06	14.4 ± 2.1	–	–
H_2O	0.1	9.08	1.11 ± 0.04	12.2 ± 0.4	0.191 ± 0.012	2.10 ± 0.14
H_2O	1.0	28.80	3.42 ± 0.24	11.9 ± 2.4	0.456 ± 0.082	1.58 ± 0.28
D_2O	0.11	5.30	1.35 ± 0.18	23.0 ± 2.5	0.200 ± 0.018	3.74 ± 0.34

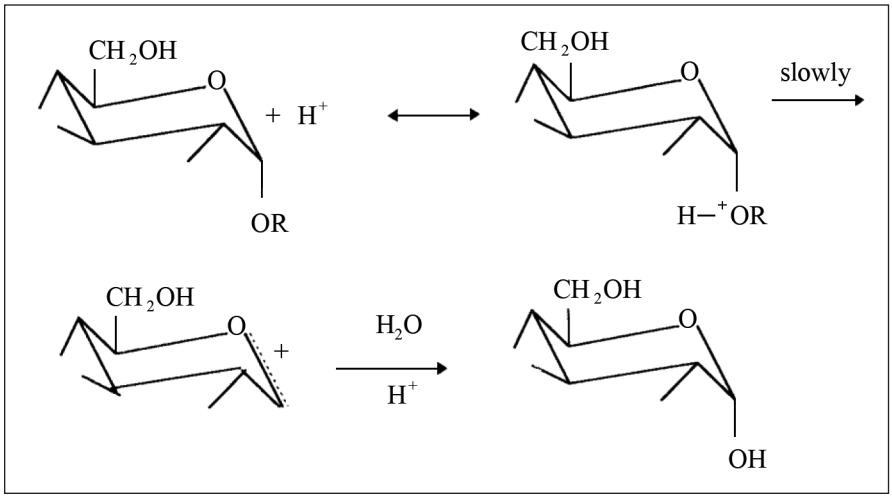

Figure. Scheme of the mechanism of the acidic hydrolysis of cyclodextrin

Table 2. **Characteristics of β-CD hydrolysis in the presence of 0.1 mol l^{-1} 2-substituted acetic acid derivatives at 120 °C**

Acid	pK_a	Pseudo-first-order rate constant, $k_1 \times 10^{-6}$, s^{-1}	Concentration of H^+ ions, mol $l^{-1} \times 10^3$
Acetic acid	4.79	1.91	1.30
3-methoxyphenylacetic acid	4.10	2.34	2.78
Chloroacetic acid	2.82	5.37	12.00
Glycolic acid	3.82	2.78	3.81
Butyric acid	4.82	0.61	1.22

In the CD_3COOD / D_2O mixture, the hydrolysis reaction of oligosaccharides was found to be faster than that in CH_3COOH / H_2O. The kinetic isotope effect was calculated. Comparing the second-order rate constants, we found that it was equal to 23×10^{-3} l mol^{-1} s^{-1} for maltose and 3.7×10^{-3} l mol^{-1} s^{-1} for the glycosidic bond of CD. In both cases, the ratio $k_{D^+} / k_{H^+} = 1.8 \div 1.9$ was found. The D^+ ion is a stronger acid that the H^+; thus, the observed reverse kinetic isotope effect confirmed our conclusions that the reaction proceeded according to the mechanism of specific acid catalysis.

Cyclodextrin hydrolysis with 2-substituted acetic acid derivatives was also carried out. We chose acids of different strength. We could not calculate the concentration of H^+ at 120 °C of these compounds, thus, the first-order rate constant was compared with the pK_a value of an appropriate acid at a standard temperature (25 °C). The data are presented in Table 2.

Interestingly, there was a good correlation between the rate of β-CD hydrolysis and the pK_a value of the acid. Only butyric acid was an exception. We may conclude that cyclodextrin ring hydrolysis was comparatively decelerated by the inclusion of this acid into the cyclodextrin cavity.

2. Acid hydrolysis of β-CD in HCl solutons in the presence of inclusion complexes

Bearing in mind that cyclodextrin can form inclusion complexes with compounds with hydrophobic moieties, we have investigated this reaction in the presence of different ligands as guest compounds at 90 °C in 0.1 mol l^{-1} hydrochloric acid. In this case, only the effect of inclusion on the rate of hydrolysis could be observed. We have chosen p-nitrophenol which is known to form quite a strong inclusion complex with cyclodextrin [19–22]. When increasing the concentration of a guest, the overall rate of the reaction tends to decrease (see Table 3).

According to data presented in Table 3, when the concentration of p-nitrophenol exceeds that of cyclodextrin 10 times, the rate of ring opening reaction decreased about by half. It is obvious that the formation of an inclusion complex inhibits the rate of the reaction. A similar effect was observed by K. Uekama [11]. The reason for this effect is clear from the investigation of the CPK model of cyclodextrin. The oxygen of the glycosidic bond is from the internal rim of the cyclodextrin cavity. So to the catalyst (H^+ ion) it may be difficult to attack the oxygen if an inclusion complex is formed.

Table 3. **Rate constant of β-CD hydrolysis in the presence of p-nitrophenol in 0.1 mol l^{-1} hydrochloric acid at 90 °C**

Ratio of concentration (guest : host)	Pseudo-first-order rate constant, $k_1 \times 10^{-6}$, s^{-1}
0 : 1	5.15 ± 0.52
1 : 10	3.65 ± 0.16
2 : 10	3.15 ± 0.11
1 : 1	2.50 ± 0.52
10 : 1	2.31 ± 0.17

In a number of articles it has been reported that the binding abilities of cyclodextrin depend on the length of the alkyl chain [21]. We have synthesized 2-(4'-sulfo)-phenylnonane in order to compare it with lauryl sulphate and benzene sulfonic acid, and have observed a more than 10-fold deceleration of the reaction. Benzene sulfonic acid practically had no influence on the rate of the reaction. We may conclude that long-chain alkyl groups can decelerate the hydrolysis of β-CD to a greater extent than nitrophenols.

Investigation on the reaction of cyclodextrins hydrolysis is important for a better understanding how cyclodextrins can prolong the action of some medicines. These data could also be used for elaborating the technologies of manufacturing higher oligosaccharides from cyclodextrins.

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β-CIKLODEKSTRINO RŪGŠTINĖS HIDROLIZĖS YPATUMAI

S a n t r a u k a

Buvo tirta β-ciklodekstrino hidrolizė acto rūgšties tirpaluose. Reakcijos kinetikos ir kinetinio izotopinio efekto tyrimai parodė, kad ciklodekstrino hidrolizės, kurios katalizatorius yra hidroksonio jonai, limituojanti stadija yra glikozidinio ryšio nutrūkimas, t. y. kad reakcija vyksta pagal specifinės rūgštinės katalizės mechanizmą. Taip pat buvo tirta β-ciklodekstrino hidrolizė dalyvaujant kai kuriems aromatiniams ir alifatiniams junginiams. Nustatyta, kad susidarę kompleksai keičia β-ciklodekstrino hidrolizės greitį. Į β-ciklodekstrino ertmę įsiterpusi „svečio“ molekulė trukdo katalizatoriui, hidroksonio jonui, prieiti prie reakcijos centro, kartu lėtina hidrolizę.