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Interaction of Pharmaceuticals with Betacyclodextrin III*

Influence of Betacyclodextrin on Phenobarbital Hydrolysis

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Abstract—The hydrolysis of phenobarbital is decelerated in alkaline solution by betacyclodextrin. The betacyclodextrin inhibits the degradation of phenobarbital up to 1.5 fold in the system containing 1% betacyclodextrin. The degradation mechanism in systems containing betacyclodextrin is the same that in system without complexing agent, although the rate constants are different. The pH dependence of the hydrolysis rate deceleration is compared with the ionization percent of betacyclodextrin. The results indicate that a direct relationship does not exist between the ionization of betacyclodextrin. It seems reasonable therefore that the phenobarbital undergoes a stable complex with betacyclodextrin and complex formation would provide a better shield for the phenobarbital from hydroxyl ion attack.

As the name implies the cycloamyloses are cyclic 1,4- linked d-glucose polymers and have 6, 7 or 8 glucose residues per molecule. X-ray crystallographic studies have firmly established the structure[1] and stereochemistry[2] of the cycloamyloses.

They are doughnut-shaped molecules with the glucose units in the Cl conformation. The primary hydroxyl groups are located on one side of the torus while the secondary hydroxyls (carbons 2 and 3 of the glucose units) are located on the other side of the torus. The interior of the cavity contains a ring of C-H groups, a ring of glycosidic oxygens, and another ring of C-H groups. As a consequence the interior of the cycloamylose torus is relatively hydrophobic when compared to H_2O.

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The previous reports[3-4] in these laboratories indicated that the betacyclodextrin exhibits a marked tendency to solubilize various compounds.

The cyclodextrin has been shown to impose both the acceleration and deceleration on various organic reaction.

Cramer[5-7] studied the cyclodextrin catalyzed decarboxylation of various organic acids and Bender[8-11] *et al.*, reported that the cyclodextrins accelerated the hydrolysis of the various esters.

On the other hands cycloheptaamylose was found to inhibit the basic hydrolysis of ethyl p-aminobenzoate, the complexed ester being completely unreactive in 0.04N $Ba(OH)_2$.[12]

Ethyl esters of aminobenzoic acids, acetylsalicylic acid and atropine in alkaline solution were shown to be either accelerated or decelerated in the presence of cyclodextrin[13].

The unstability of phenobarbital in aqueous solution has been discussed by many investigators[14-16]. According to these studies, the decomposition of phenobarbital depends on the pH value and temperature of the solution. In the previous report[2] it was evident that phenobarbital was shown to interact with betacyclodextrin and formed a relatively stable complex.

The present investigation deals with the effects of betacyclodextrin on the alkaline hydrolysis reaction of phenobarbital from the standpoint of chemical kinetics in an accelerated condition.

EXPERIMENTAL

Materials and Reagents—Betacyclodextrin, $[\alpha]_D^{25}=+162°$ ($c=1$, in H_2O), phenobarbital, m.p. 176° (recrystallized from diluted EtOH).

Buffer Systems—pH range, 8, 9, 10, alkaline borate buffers (U.S.P. XVIII, standard buffer solution).

Apparatus—A constant temperature incubator, set at 30±0.5°, 45±0.5°, 60±0.5°, Freund life tester (model LT-6), Knick pH meter (Type pH 34), Beckman DU-2 spectrophotometer, 2 ml-ampoules.

Procedure—About 100mg of the phenobarbital was dissolved in 200 ml of the buffer solution containing various concentration of betacyclodextrin and 2 ml of the solution were sealed in an ampoule under N_2.

A series of ampoules was kept in a thermostatically controlled life tester or incubator of the desired temperature. When the content of ampoule reached to be examined temperature, three ampoules were removed, chilled, and each 1 ml of the solution was placed in a volumetric flask, and diluted to 50 ml with the buffer solution of pH 9.6. The extinction of the unhydrolyzed phenobarbital was determined at 240 nm.

This value was considered as the initial concentration and after a suitable intervals of time for experimental periods the sample was taken and treated in the same manner. Suitable blanks were prepared to correct for the spectral contributions of betacyclodextrin.

RESULTS AND DISCUSSION

The degradation of phenobarbital in alkaline solution, according to Hasegawa[14], is considered as the first order reaction. The close agreement of the experimental data obtained in this experiment and their results give additional support to this reaction order.

The results of phenobarbital hydrolysis in aqueous alkaline solution with and without betacyclodextrin at 30°, 60° and 45° after a period of a suitable intervals of time are given in Table I, II and shown in Fig. 1.

Table I—Influence of betacyclodextrin on phenobarbital hydrolysis in pH 9 buffer solution at 30°.

Time (hr)	Betacyclodexrtin concentration	
	0.00%	1.00%
0	2.093×10^{-3}M	2.078×10^{-3}M
500	2.048	2.048
1000	1.951	2.022
2000	1.913	1.969
3000	1.831	1.805
5000	1.681	1.520

Table II—Influence of betacyclodextrin on phenobarbital hydrolysis in pH 9 buffer solution at 60°.

Time (hr)	Betacyclodextrin concentration	
	0.00%	1.00%
0	2.074×10^{-3}M	2.059×10^{-3}M
14	1.962	1.980
21	1.928	1.951
38	1.801	1.887
46	1.707	1.835
111	1.333	1.584

From Table I, II and Fig. 1 it is evident that the rate of hydrolysis of phenobarbital in alkaline solution in the presence of cyclodextrin is decelerated. The deceleration tendency is not so markedly within the range of 0 to 1% of betacyclodextrin concentration, but the effect of betacyclodextrin on the phenobarbital hydrolysis is negligible at high pH value.

The influence of betacyclodextrin on half-life and rate constant of phenobarbital is summerized in Table II.

In a system containing 1% betacyclodextrin about a 1.5 fold incrase in half-life of this reaction is observed at pH 8 and 9. It is reasonable to expect that inclusion formation

between phenobarbital and betacyclodextrin would provide a better shield for the phenobarbital from hydroxyl ion attack.

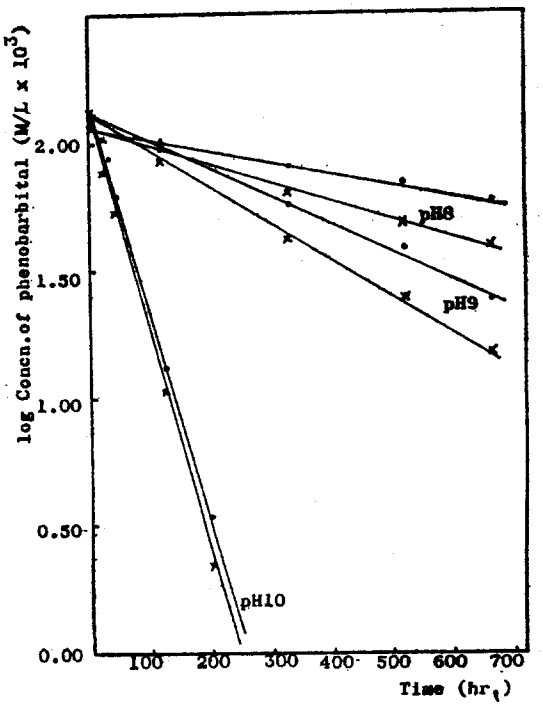

Fig. 1—Betacyclodextrin influence on phenobarbital hydrolysis at 45°.
Key: x, 1.00% betacyclodextrin; •, 0.00% betacyclodextrin

The hydrolysis of acetyl salicylic acid[13] and the decarboxylation of acid phenyl ester[9] in the presence of cyclodextrin was accelerated. This is the result of a nucleophilic reaction of an alkoxide ion derived from either the C-2 or C-3 secondary hydroxyl groups of the cyclodextrin.

The rate effects of cyclodextrins on the hydrolysis of alkylbenzoate esters[12] contrast sharply with their effects on reaction of acid phenyl esters. This effect was explained in two ways by Bender, *et al.*[9]. The unreactivity of the complexed ester can be explained on the basis of nonproductive binding in which the carbonyl carbon atom of the ester molecule is located in the cycloamylose cavity at some distance from the secondary hydroxyl groups. If, for example, the ester was complexed in such a way that the carbonyl group was positioned near the primary hydroxyl groups of the cyclodextrin, it would be well protected from reaction in solution.

Alternatively, the unreactivity of alkyl benzoate esters in the cycloamylose complexes may be due to an unfavorable partitioning of the tetrahedral intermediate[17]

The inhibition of the phenobarbital hydrolysis can be attributed to the total inclusion formation between phenobarbital and cyclodextrin, so phenobarbital is shielded from nucleophilic attack by the alkoxide ion and hydroxyl groups.

The kinetic hydrolysis rate constants of phenobarbital were determined at temperatures between 30° and 60° and the temperature effect on phenobarbital degradation is illustrated by an Arrhenius plot as shown in Fig. 2. From the same slope it is evident that the degradation mechanism in systems containing the betacyclodextrin is the same that in system without complexing agent, although the rate constants are different.

Betacyclodextrin has been reported to exhibit behavior similar to that shown by particular enzyme systems regarding interaction with various molecules. Because of this similarity in activity[5,6,9,18], cyclodextrins have been used as model systems in the study of enzyme mechanisms.

Table II—Influence of betacyclodextrin on rate constant and half-life of phenobarbital hydrolysis.

Temp.	pH	Betacyclodextrin(%)	Rate const. $(hr^{-1}) \times 10^5$	Half-life (hr)
	8	0.00	1.99	34,800
		1.00	1.41	49,500
30	9	0.00	4.47	15,500
		1.00	2.75	25,200
	10	0.00	39.9	1,740
		1.00	34.7	2,000
	8	0.00	36.4	1,900
		1.00	24.5	2,830
45	9	0.00	71.2	970
		1.00	51.3	1,350
	10	0.00	562	123
		1.00	479	144
	8	0.00	200	346
		1.00	135	513
60	9	0.00	398	174
		1.00	251	276
	10	0.00	3,000	31
		1.00	2,400	28

As the degradation of phenobarbital depends on the concentration of hydroxyl ion and hydrolysis in an alkaline region is very much accelerated, the pH dependence of the cyclodextrin effect was also studied.

This is very important problem because the hydroxyl groups of the cyclodextrin must be in the ionized form in order that they act as a nucleophile. A plot of log percent of hydrolytic rate decrease against pH and also a plot of log percent of ionization of

betacyclodextrin against pH are shown in Fig. 3. Fig. 3. indicates that a fair regularity does not exist between the ionization of betacyclodextrin and the hydrolysis rate decrease, but the hydrolysis reaction is gradually accelerated in the region of high pH value.

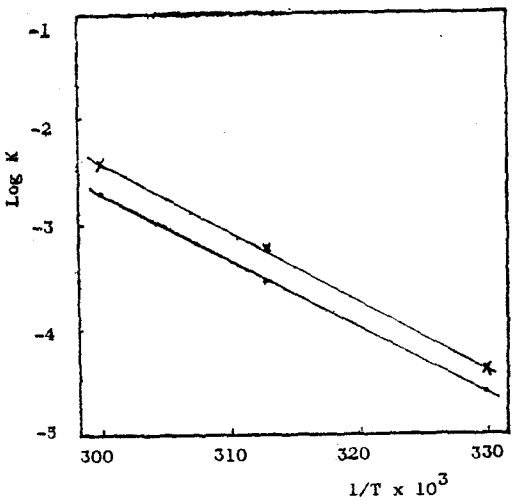

Fig. 2—Arrhenius plot of hydrolysis of phenobarbital at different temperature. Key: x, 1.00% betacyclodextrin, •, 0.00% betacyclodextrin.

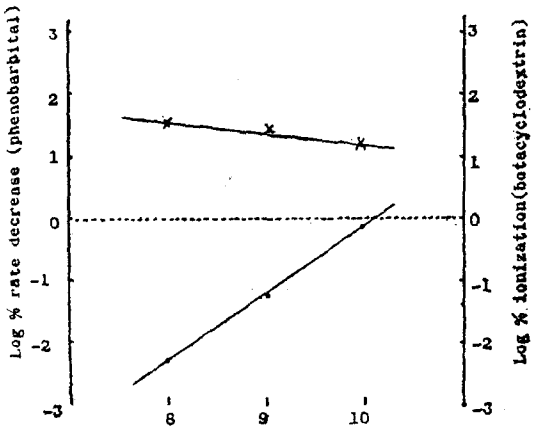

Fig. 3—A plot of log percent of hydrolytic rate decrease of phenobarbital hydrolysis (x)in the system containing betacyclodextrin against pH(•) and log percent of ionization of betacyclodextrin against pH at 45°.

CONCLUSION

The effect of betacyclodextrin on the alkaline hydrolysis of phenobarbital has been studied from the standpoint of chemical kinetics in an accelerated condition. The betacyclodextrin inhibited the hydrolysis of phenobarbital up to 1.5 fold in the system containing 1% betacyclodextrin.

The decreasing tendency of the phenobarbital hydrolysis is larger at the low pH than at the high pH. From this fact it is reasonable to expect that complex formation would provide a better shield for the phenobarbital from hydroxyl ion attack.

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