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The effect of cyclodextrins on the aqueous stability of cyclopentolate hydrochloride

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Abstract

Cyclopentolate hydrochloride (Cy.HCl) undergoes degradation to give α-(1-hydroxycyclopentyl)-benzeneacetic acid (a ß-hydroxy acid, by the expected normal ester hydrolysis pathway) and phenylacetic acid (by an unusual cyclic mechanism). These two products are formed simultaneously by parallel mechanisms. The interaction between cyclopentolate (Cy) and cyclodextrins (CDs) results in the formation of a 1:1 stoichiometric complex. ß-CD and its substituted analogs exhibit the greatest degree of stabilization of Cy against hydrolysis. In the presence of CDs, the parallel mechanism still operates, but the proportion of the reaction proceeding by the two pathways is altered. For the ß-hydroxy acid formation pathway, the complexed drug degrades at a rate $\approx 1/10$ as rapid as that of the uncomplexed drug in solution, while the decrease in rate constant for the phenylacetic acid formation pathway is substantially less. Apart from the stabilization effect, evidence for complex formation is available from NMR and molecular modeling studies. The optimum structure of the complex is obtained when the aromatic ring of the Cy molecule enters the CD cavity. When this occurs, the rate of normal ester hydrolysis is reduced. The hydroxycyclopentyl group apparently does not penetrate deeply into the CD cavity because of the presence of an intramolecular bond which can form between the hydroxyl group and the carbonyl oxygen. When interaction with CD occurs on the hydroxycyclopentyl end of the molecule the cyclic hydrolysis mechanism is affected, but not appreciably.

Keywords: Cyclodextrins; Kinetics; Hydrolysis; Parallel mechanism; Stabilization

1. Introduction

Cyclopentolate hydrochloride, a cycloplegic and mydriatic agent, is widely used for diagnostic purposes for its shorter duration of action (Opthalmic Drug Facts, 1989). The kinetics and mechanism of hydrolysis of cyclopentolate (Cy) in

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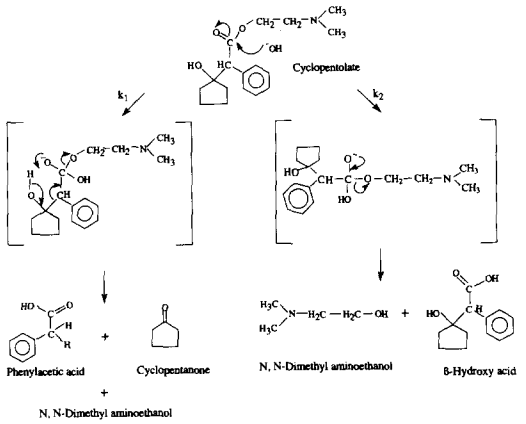

Fig. 1. Proposed parallel reaction mechanism for formation of phenylacetic acid and the ß-hydroxy acid in alkaline solution.

alkaline solutions have been described in a previous report (Roy and Guillory, 1995). The proposed mechanism of hydrolysis is illustrated in Fig. 1.

Cyclodextrins are cyclic oligosaccharides having a hydrophilic outer surface and a lipophilic central cavity. These structural characteristics allow various types of drug molecules ('guests') to interact to a greater or lesser extent, forming non-covalently bonded inclusion complexes either in the solid phase or in aqueous solutions. The incorporation of a drug molecule into the CD cavity can affect many of its physico-chemical properties and can result in increased aqueous solubility and stability. In some instances, CDs improve the stability of compounds by denying access to a catalytic species which must attack a particular reaction center. Alternatively, they may sterically hinder the substrate from undergoing the structural changes that result in degradation.

In the case of ester hydrolysis, complexation with CDs can have an accelerating or a decelerating effect (Anderson and Bundgaard, 1984; Siegel and Breslow, 1975). Inhibition of hydrolysis occurs when the ester function resides deep within the CD cavity, for the rate of hydrolysis depends then on the amount of uncomplexed ester present in the solution. When the ester is partially enclosed inside the CD cavity, a decrease in the hydrolysis rate can be due to a steric effect.

Kearney et al. (Kearney et al., 1992) studied the effect of cyclodextrins on the intramolecular lactamization of gabapentin in aqueous solution. The enhanced rate obtained in the presence of cyclodextrins was attributed to complexation-induced conformational changes in the reactive moieties of gabapentin. The geometrical conformation imposed upon gabapentin as a result of the inclusion process facilitated the approach of the amino group to the carboxyl group by effectively locking the conformation of gabapentin to a reactive conformation. In the present investigation, it was of interest to determine the extent to which CDs would influence the parallel pathways leading to the hydrolysis of the cyclopentolate molecule.

2. Materials and methods

Cy.HCl, phenylacetic acid and cyclopentanone were obtained from Sigma Chemicals. N, N-dimethylaminoethanol was obtained from Aldrich (Roy and Guillory, 1995). α-, ß- and γ-Cyclodextrin (α-, ß- and γ-CD) were obtained from Sigma Chemicals. 2-Hydroxypropyl-ß-cyclodextrin (HP-ß-CD) was obtained from Pharmatec, heptakis(2,6-di-O-methyl) ß-cyclodextrin (DIMEB), heptakis(2,3,6-tri-O-methyl) ß-cyclodextrin (TRIMEB) and hydroxyethyl-ß-cyclodextrin (HE-ß-CD) were obtained from Aldrich Chemical Co. All other chemicals and buffers were reagent grade and were used without further purification. All solutions were prepared using double-distilled water. A borate buffer system was used for pH 8.5 and pH 10.0 condition. The buffer solutions were adjusted to a constant ionic strength of 0.2 M with sodium chloride.

The HPLC instrumentation, ^{1}H NMR, molecular modeling, pH readings and kinetic studies were conducted using instruments described earlier (Roy and Guillory, 1995).

The HPLC mobile phase consisted of 80% v/v, 50 mM acetate buffer, pH 4.7, and 20% v/v acetonitrile. The flow rate was 2.0 mL /min and the wavelength for detection was set at 254 nanometers. In preliminary studies, it was shown that the reaction followed first-order kinetics for

Table 1
The effect of various cyclodextrins (1:20 molar ratio) on the pseudo-first-order rate constant for the hydrolysis of cyclopentolate at 50°C, I = 0.2 M

CD	pH 8.5			pH 10.0		
	k^*_{obs} (h^{-1})	$t_{1/2}$ (h)	k_o/k_{obs}	k^*_{obs} (h^{-1})	$t_{1/2}$ (h)	k_o/k_{obs}
—	0.52 (k_o)	1.33	—	0.86 (k_o)	0.81	—
α-CD	0.52	1.33	1.00			
ß-CD	0.35	1.98	1.49	0.58	1.20	1.48
γ-CD	0.40	1.73	1.30			
DIMEB	0.22	3.15	2.36	0.29	2.39	2.97
TRIMEB	0.50	1.39	1.04			
HE-ß-CD	0.33	2.10	1.58	0.47	1.48	1.83
HP-ß-CD	0.31	2.24	1.68	0.44	1.58	1.96

* $n = 2$.
The study at pH 10.0 was done using those cyclodextrins that showed the greatest degree of stabilization at pH 8.5.

3–4 half-lives and no significant change in pH (ΔpH < 0.2) was found at the end of kinetic runs. Samples periodically withdrawn during a kinetic run were quenched using an acetate buffer solution (pH 4.7) and then stored at −20°C.

Molecular mechanical calculations were performed with the program SYBYL (version 5.3), which uses the Tripos force field (version 5.2) on a Silicon Graphics 4D120GTX Graphics workstation (SYBYL, 1989). Energy minimizations were carried out using the MAXMIN 2 energy minimizer with its default values. The structure was optimized until the energy change from one iteration to the next was less than 0.05 kcal.

3. Results and discussion

The rate of degradation of Cy in the presence of cyclodextrins was measured in aqueous buffered solutions at 50°C, at pH 8.5 and pH 10.0. The various cyclodextrins were employed at a drug to cyclodextrin molar ratio of 1:20 (Table 1). HPLC analysis revealed that the hydrolysis of Cy in the presence of CDs resulted in the same degradation products as were formed in their absence (phenylacetic acid and ß-hydroxy acid being the predominant degradation products). This indicates that the interaction of Cy with various CDs does not alter the mechanism of degradation, which proceeds by two parallel pathways. It is evident from Table 1 (pH 8.5) that α-CD and TRIMEB have little or no stabilizing effect on the hydrolysis of Cy under the experimental conditions employed. ß-CD and its alkyl derivatives have a significant stabilizing effect on Cy. Of the CDs studied, the structural characteristics of Cy and ß-CD allow for maximum attractive interactions between the hydrophobic and hydrophilic portions of both entities upon inclusion. In contrast, the smaller cavity diameter of α-CD may not permit the same depth of penetration of Cy, resulting in less attractive interactions between Cy and α-CD, whereas the larger cavity diameter of γ-CD should allow for a greater depth of penetration. However, in the case of γ-CD, the larger cavity size may not allow for the same degree of interaction between Cy and the methylene groups comprising the cyclodextrin cavity. Similar results were also obtained at pH 10.0 using those cyclodextrins that showed the greatest degree of stabilization at pH 8.5.

ß-CD and its alkylated derivatives also were found to have superior complexing ability for a number of other compounds described in the literature, including chlorambucil, melphalan (Green, 1988), indomethacin (Backensfeld et al., 1990), salbutamol (Ueda and Nagai, 1980) and gabapentin (Kearney et al., 1992).

The stability constant for the cyclodextrin inclusion complex of Cy with HP-ß-CD was evaluated by the kinetic method (Szejtli, 1982). The

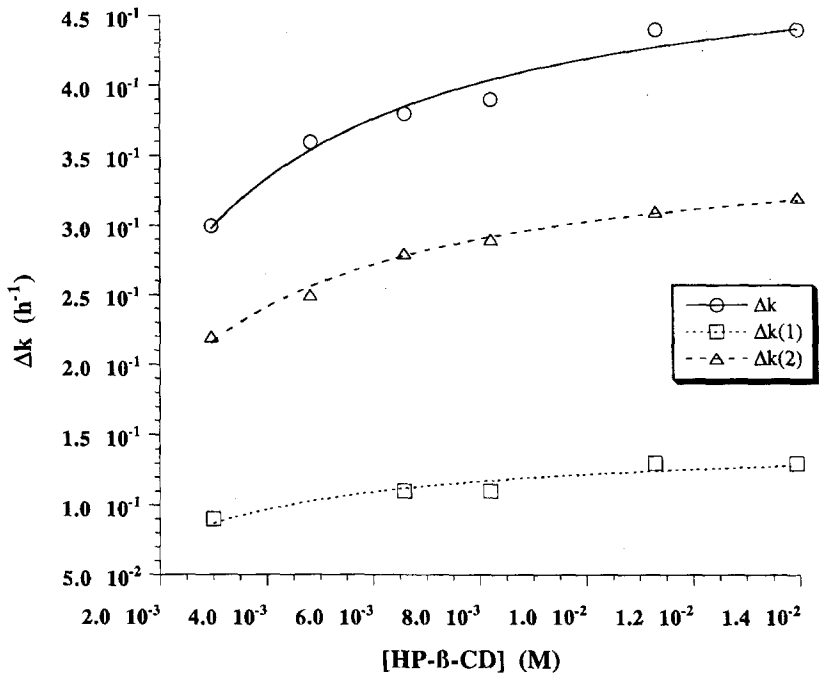

Fig. 2. Change in rate constant, Δk, in the presence of HP-ß-CD at pH 10.0, 50°C according to Eq. 1. Key: (O) hydrolysis of cyclopentolate; (□) formation of phenylacetic acid; (Δ) formation of the ß-hydroxy acid.

binding isotherm used to describe a 1:1 model is given below:

$$\frac{\Delta k}{K_o} = \frac{q_{11}K_{st}(L)}{1 + K_{st}(L)} \quad (1)$$

where $q_{11} = 1 - \frac{k_c}{K_o}$ and $\Delta k = k_o - k_{obs}$,

k_o is the rate constant for decomposition of uncomplexed substrate (S), k_c is the rate constant for the decomposition of the complex (SL) and K_{st} is the stability constant for complex formation and (L) is the ligand used.

Fig. 2 illustrates the non-linear curve fit of the binding isotherm where Δk ($\Delta k = k_o - k_{obs}$) is plotted versus the concentration of the cyclodextrin, [HP-ß-CD]. The kinetic parameters for this system are presented in Table 2. It is evident from Fig. 2 that Δk is not a linear function of the concentration of the added cyclodextrin, rather it asymptotically approaches a maximum value. This saturation behavior is characteristic of reactions that proceed through complex formation prior to the rate-determining step (Szejtli, 1982). The agreement observed between the experimental data and the calculated curves demonstrates that such an isotherm adequately describes the degradation kinetics, and thus the interaction between Cy and CDs is due to the formation of 1:1 stoichiometric complex.

As parallel mechanisms operate independently of one another, the binding isotherm was fitted to the degradation kinetics for each of the parallel pathways (Fig. 2, where $\Delta k = k_{o(1)} - k_1$ or $k_{o(2)} - k_2$, $k_{o(1)}$ is the rate constant obtained for the formation of phenylacetic acid and $k_{o(2)}$ is the rate constant obtained for the formation of the ß-hydroxy acid in the absence of HP-ß-CD; all the rate constants being obtained by the fit of the parallel rate law to the data (Roy, 1993)). The non-linear fit of the binding isotherm gave K_{st} values that are comparable (480 and 490) (Table 2). Similarity in the binding constant values provides added evidence that, in both cases, complex formation occurs prior to the rate-determining step. For the ß-hydroxy acid formation pathway, the complexed drug degrades at a rate $\approx 1/10$ as rapid as

Table 2
Kinetic parameters for cyclopentolate hydrolysis in the presence of increasing concentrations of HP-ß-CD at 50°C, pH 10.0, I = 0.2 M

Type of reaction	K_{st} [a](M^{-1})	k_o^b (h^{-1})	$t_{1/2}$ (h)	k_c^a (h^{-1})	$t_{1/2}$ (h)
Hydrolysis of cyclopentolate	490	0.86		0.35	1.98
Phenylacetic acid formation pathway	480	0.48	1.44	0.33	2.09
ß-Hydroxy acid formation pathway	490	0.40	1.73	0.04	17.3

[a] Model Derived.
[b] Experimental.

degradation of the uncomplexed drug in solution, while the decrease in rate constant for the phenylacetic acid formation pathway is substantially less. To further investigate the difference in ester stabilization achieved by the two parallel pathways, the structure of the complex was studied using ^1H NMR and molecular modeling.

Fig. 3 shows the ^1H NMR spectra of the phenyl signal of Cy in the presence and absence of equimolar amounts of HP-ß-CD in D_2O. In the presence of HP-ß-CD, the signals of the aromatic protons are shifted upfield, indicating greater shielding of the protons when the aromatic ring resides inside the cyclodextrin cavity (predominantly hydrophobic interaction) (Ueda and Nagai, 1980).

Fig. 4 shows the NMR signal(s) for the N, N-dimethyl group attached to the nitrogen atom of the Cy molecule, in the absence and in the presence of HP-ß-CD, DIMEB or α-CD. This signal integrates for six protons, and this is attributed to the protons of the two methyl groups. In the absence of any cyclodextrin, the methyl groups are magnetically equivalent (or in a time averaged environment due to rapid rotation of the C-N bond), and thus only one peak is observed. In the presence of the substituted ß-CD's, two singlets of equal intensity are observed since the protons of the two methyl groups are non-equivalent. From molecular modeling experiments, dis-

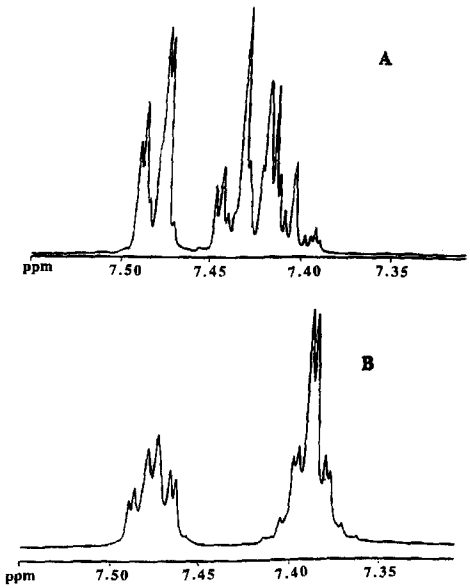

Fig. 3. ^1H-NMR spectra of cyclopentolate at 25°C in the absence (A) and in the presence of an equimolar concentration of HP-ß-CD (B); changes observed in the aromatic region.

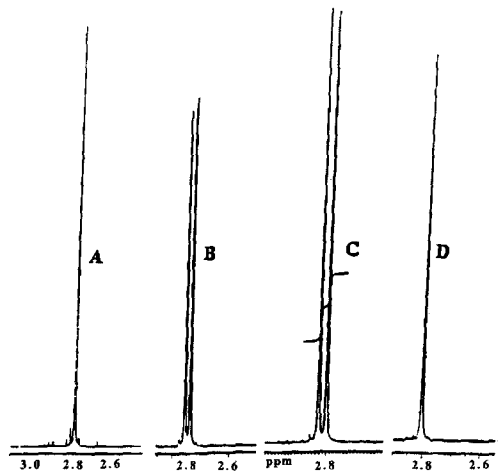

Fig. 4. ^1H-NMR spectra of the N, N-dimethyl groups of cyclopentolate at 25°C in the absence (A) and in the presence of an equimolar concentration of HP-ß-CD (B), DIMEB (C) or α-CD (D).

cussed later, we conclude that the tail of the cyclopentolate molecule is not protruding out of the cavity, but lies somewhat flat on the surface of the cyclodextrin ring. The two methyl groups are in different environments and this can give rise to two singlets. On the other hand, only one peak (singlet) is obtained in the presence of α-CD indicating that the cyclopentolate ring does not enter deep into the cavity to affect the N, N-dimethyl groups. Molecular modeling was performed with cyclopentolate and α- and ß-CDs. Apparently, no X-ray structures for complexes of cyclopentolate and cyclodextrin have been reported. Molecular modeling studies were done using the ß-CD molecule as no crystallographic data exists for HP-ß-CD. It is assumed that the mechanism of inclusion formation is similar for HP-ß-CD and ß-CD. This is a reasonable assumption since NMR experiments demonstrated that, in the presence of HP-ß-CD and DIMEB, the same functional groups of the Cy molecule were being affected. The principal goal of this molecular modeling experiment was to obtain a reasonable representation of the structure of the complex formed, and to correlate this finding with results obtained using NMR data.

Fig. 5A illustrates the global minimum energy conformation of the cyclopentolate-ß-CD complex. The optimum structure of the complex is obtained when the aromatic ring of the Cy molecule enters the cyclodextrin cavity. Results of molecular modeling studies indicate that Cy is more likely to enter the larger cavity side of ß-cyclodextrin molecule. This correlates well with the NMR data where the greatest effect is obtained in the aromatic region of the spectrum. In the case of Cy, due to the unusual conformation of the hydroxycyclopentyl group, the extent of penetration is small. The hydroxycyclopentyl group is bulkier than an aromatic ring, and thus the extent of penetration is significantly less compared with that observed for adiphenine (Tong, 1991).

As can be seen in Fig. 5B, a hydrogen bond can exist between the hydroxyl group of the cyclopentyl moiety and the carbonyl oxygen giving a six-membered ring which also lowers the degree of penetration of the cyclopentolate molecule into

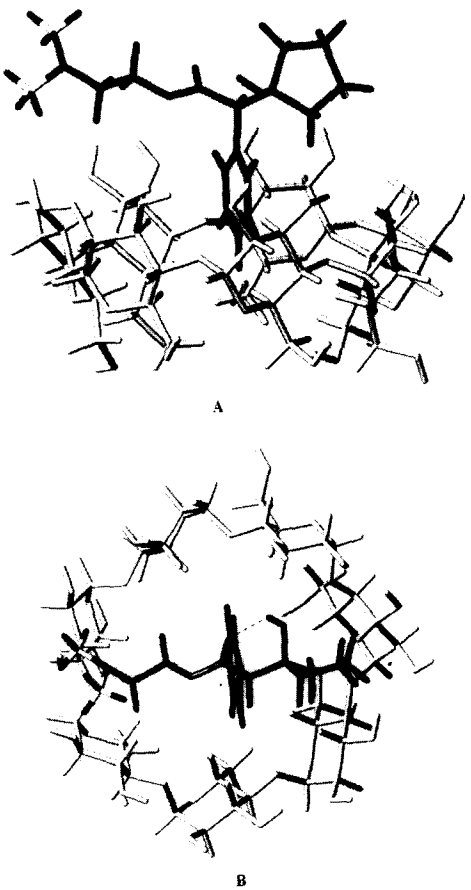

Fig. 5. Computer-generated minimal energy structures of the cyclopentolate-ß-CD complex: (A) side view and (B) orthogonal view.

the cavity. Kinetic studies have shown that cyclodextrin affects the ß-hydroxy acid formation pathway more than the phenylacetic acid formation pathway. A possible explanation for this selective stabilization of one of the pathways may be found in the conformation of the Cy molecule in the CD cavity. In the case of the phenylacetic acid formation pathway, at pH 8.5 or 10.0, the attack of hydroxide ion and the formation of the six-membered transition state (since Cy resides primarily on the outside of the cavity) are not appreciably affected by the presence of CD, and thus there is not a significant decrease in rate constant. In the case of the ß-hydroxy acid formation pathway, CD can affect the formation of the tetrahedral intermediate due to steric interactions, and substantially decrease the rate of the reaction.

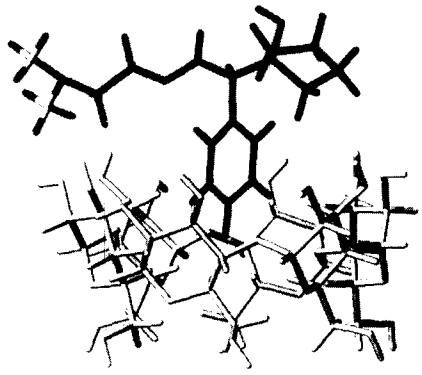

Fig. 6. Computer-generated minimal energy structure of the cyclopentolate-α-CD complex.

A space-filling model of the cyclopentolate-ß-CD complex shows a tight, snug fit indicative of strong van der Waals interactions which hold the Cy molecule strongly in the CD cavity. Fig. 6 shows the minimum energy structure of the cyclopentolate-α-CD complex. With α-CD, the degree of penetration is low due to the smaller ring size of the cyclodextrin cavity. The carbonyl group is essentially open to attack by hydroxide ions. Kinetic data also showed no stabilization being achieved by the α-CD molecule.

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