Cyclodextrin-Catalyzed Deacetylation of Spironolactone is pH and Cyclodextrin Dependent

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ABSTRACT: The complexation of spironolactone (SP) with cyclodextrins (CDs) and the effect of pH on the CD catalyzed deacetylation of SP was studied in the presence of β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin ($[SBE]_{7m}$-β-CD), γ-cyclodextrin (γ-CD), and sulfobutylether γ-cyclodextrin (SBE-γ-CD). The complexation of SP with β-CD and the mechanism of deacetylation was confirmed using NMR. The complexation of SP with CDs was determined by means of the phase-solubility method at pH 2, in which chemical degradation was minimal. The phase-solubility diagrams were classified as A_L-type and the apparent stability constants ($K_{1:1}$) for 1 : 1 inclusion complex were calculated to be 9939 M^{-1}, 10,976 M^{-1}, 15,816 M^{-1}, 4792 M^{-1} and 4118 M^{-1} for β-CD, HP-β-CD, $(SBE)_{7m}$-β-CD, γ-CD, and SBE-γ-CD, respectively. The effect of pH on the degradation rate of SP was studied in the presence and absence of 4.4 m*M* CD solutions at pH 4, 5, 6, 7, and 8 (25°C). The stability studies showed that CD-catalyzed degradation of SP can be decreased by lowering the pH. The pH-rate profiles of SP degradation with different CDs gave slopes of 1.0. Because no buffer catalysis was observed, the reaction appears to be specific-base catalyzed. The catalytic activity of CDs was as follows: SBE-γ-CD < $(SBE)_{7m}$-β-CD < HP-β-CD ≈ γ-CD < β-CD. NMR studies confirmed that SP forms an inclusion complex with β-CD and complexation occurs by means of the secondary face. The NMR studies also showed that during the deacetylation of SP, the secondary hydroxyl groups of β-CD at the 2- and 3-position were acetylated. The decrease of catalytic activity of CDs at low pH values and the CDs differing ability to catalyze the degradation of SP correlated qualitatively with the ionization state of the CD hydroxyl groups, which were lower in SBE-CDs. The site of binding differences and the number of hydroxyl groups present probably also contribute to the differences. J Pharm Sci 89: 241–249, 2000

Keywords: spironolactone; cyclodextrins; solubility; stability

INTRODUCTION

Cyclodextrins (CDs) are a group of cyclic oligosaccharides that have been shown to improve pharmaceutical properties of drugs, such as solubility and stability, by forming inclusion complexes.[1,2] The complexation with CDs has also destabilized some drugs.[3–5] The ability to stabilize some drugs while destabilizing others has been explained by the different structures of the formed inclusion complexes.[6] The degradation rate of the drug may increase if the hydrolytically labile part of the molecule localizes in the vicinity, for example, of the hydroxyl groups of the CD. However, if the penetration of the drug is deeper into the cavity,

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the labile part of the molecule may be protected by the CD, resulting in stabilization.

Spironolactone (SP, Fig. 1) is a synthetic aldosterone antagonist that has been shown to form inclusion complexes with CDs. In earlier studies, CDs have been shown to increase aqueous solubility, dissolution rate, and bioavailability of SP.[7–14] However, studies had also shown that the complexation of SP with β-CD[15,16] and some commonly used CD-derivatives[17] increases the deacetylation of SP, which brings into question the use of CDs in SP formulations. In the Kaukonen et al. study,[17] it was observed that CD derivatives showed a differing ability to catalyze the deacetylation of SP. However, the Kaukonen et al. study was performed in water without pH control.

Here, the complexation of SP with CDs and the effect of pH on the degradation rate of SP was studied with β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether β-cyclodextrin ($[SBE]_{7m}$-β-CD, with an average degree of substitution of seven), γ-cyclodextrin (γ-CD) and sulfobutylether γ-cyclodextrin (SBE-γ-CD). In addition, complexation of SP with β-CD and the mechanism of cyclodextrin catalyzed degradation of SP was studied using nuclear magnetic resonance spectrometry (NMR).

MATERIALS AND METHODS

Chemicals

SP was purchased from Aldrich Chemical Co. (Milwaukee, WI) and diacetylated SP, 7α-thiospironolactone (TSP), was obtained from G. D. Searle (Skokie, IL). $(SBE)_{7m}$-β-CD (Captisol®, mw = 2249) was obtained from CyDex Inc. (Overland Park, KS), SBE-γ-CD (mw = 2827, degree of molar substitution 9.7) was from Center for Drug Delivery Research (Lawrence, KS), and HP-β-CD (Encapsin HPB™, mw 1411, degree of molar substitution 4.8) from Janssen Biotech (Olen, Belgium). β-CD and γ-CD were obtained from Wacker Biochem Corp. (Adrian, MI), and glucose and sodium azide (NaN_3) from Fisher Scientific (Pittsburgh, PA). Deuterium oxide (D_2O) and deuterium chloride (DCl) were obtained from Aldrich Chemical Co. All other reagents were of analytical grade.

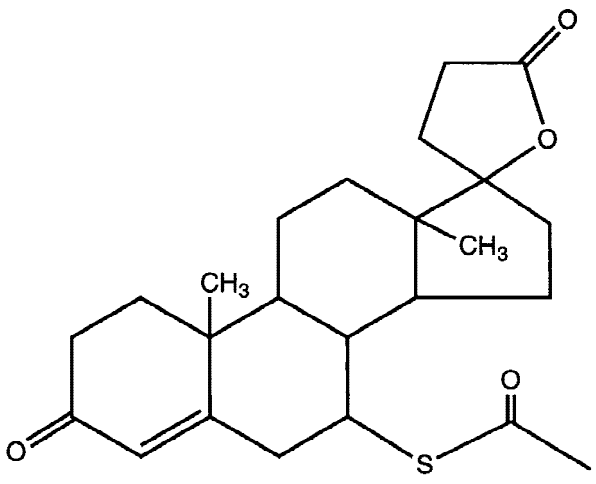


Figure 1. The chemical structure of spironolactone (SP).

Apparatus

High performance liquid chromatography (HPLC) was performed with a Shimadzu instrument consisting of a Shimadzu LC-6A solvent delivery module, a Shimadzu SPD-6A UV-spectrophotometric detector (set at 238 nm), a Shimadzu SCL-6A system controller, a Shimadzu C-R6A Chromatopac integrator (Shimadzu Corp., Kyoto, Japan), and a Rheodyne loop injector (Rheodyne, Cotati, CA). A Phenyl Hypersil (15 cm × 4.6 mm id, 5 μm) HPLC-column was used for separations. The injection volume was 20 μL and flow rate was 1.5 mL/min. The mobile phase used consisted of 48% water in methanol, which gave 13-minute and 11-minute retention times for SP and TSP, respectively.

NMR spectra and kinetics experiments were performed on a Bruker AM-500 operating at 500.13 MHz for ^1H. Selective ROESY and 1-D experiments were performed on a Bruker Avance 400 operating at 400.13 MHz for ^1H.

Solubility Studies

The complexation of SP with CDs was determined using the phase-solubility method of Higuchi and Connors.[18] An excess amount of SP was added to 0.01 *M* HCl solution (pH 2, μ = 0.15 with NaCl) containing 0 to 8.8 m*M* CDs. The suspensions were shaken at 25°C for 24 hours, and after equilibration they were centrifuged, and the supernatant was diluted and analyzed by HPLC. Preliminary experiments showed that all the CDs studied did not degrade SP at pH 2.0 over the time frame of the study, and 24 hours was sufficient to reach equilibrium.

Stability Studies

The effect of pH on the CD-catalyzed degradation of SP was studied in the presence and absence of 4.4 m*M* CD at pH 4, 5, 6, 7, and 8. At pH 4 and 5, studies were performed in 25 m*M* citrate buffer (μ = 0.15), and at pH 6, 7, and 8, 25 m*M* phosphate buffer (μ = 0.15) was used. In addition, the effect of CD concentration (0.044–4.4 m*M*) and phosphate buffer concentration (10 m*M* and 25 m*M*) on the degradation of SP was studied at pH 7. The solutions were prepared by adding 100 μL of SP/methanol solution (2.5 mg/mL) to the 10.0-mL aqueous buffer solution containing CD. Because of problems with bacterial growth in buffers and buffer/CD solutions and the effect of bacteria on SP deacetylation, 0.02% NaN_3 was added to the solutions to inhibit microbiologic growth. Validation studies showed that NaN_3 (0.02%) and methanol (100 μL in 10 mL) has no effect on the degradation kinetics of SP. The vials were placed in a constant temperature bath (25°C) and samples were removed at appropriate intervals. The reaction was quenched by the addition of 15 μL 1 *M* HCl, and the samples were analyzed by HPLC. The pseudo–first-order rate constants (k_{obs}), half-lives ($t_{1/2}$), and $t_{90\%}$ for overall degradation of SP were determined from the linear semilogarithmic plots of remaining SP versus time.

NMR Studies

The complexation of SP with β-CD was studied in solutions containing 3.0 m*M* SP and 8.8 m*M* β-CD. The solution was prepared by dissolving 1.25 mg of SP and 10 mg of β-CD in 1.0 mL of D_2O (pD 2, pD adjusted beforehand with DCl). A kinetic study was performed with initial SP and β-CD concentrations of 3.2 m*M* and 8.8 m*M,* respectively. At the beginning of the experiment, 2.0 mg of SP was added to the 1.5 mL of D_2O buffer solution (20 m*M* phosphate buffer, pD 7.0) containing 15 mg β-CD. The acetylation of β-CD was followed for 10 hours by NMR. The freeze-dried solution containing SP's degradation products was prepared from the SP suspension in which initial SP and β-CD concentrations were both 18 m*M*. The suspension was prepared by adding 15 mg of SP and 41 mg of β-CD to 2.0 mL of D_2O. The suspension was shaken at 25°C for 24 hours and after equilibration and filtration (45-μm filter), the supernatant was isolated and freeze-dried. The freeze-dried material was dissolved to 0.5 mL of dimethyl sulfoxide (DMSO) and analyzed by NMR. The ionization of β-CD and $(SBE)_{7m}$-β-CD at high pH values was studied in 0.001 *M* (pH 11), 0.01 *M* (pH 12), 0.1 *M* (pH 13), and 1.0 *M* (pH 14) KOH solutions. The solvent in these solutions was a mixture of H_2O (90 %) and D_2O (10 %), and the CD concentration was 4.4 m*M*.

RESULTS AND DISCUSSION

Solubility Studies

The complexation of SP with CDs was studied at pH 2 to decrease the CD-catalyzed degradation of SP during equilibration. Because SP is non-ionizable, the solubility of SP and complexation of SP with CDs is not pH dependent. With all CDs, the phase-solubility diagrams (Fig. 2) were classified as A_L-type,[18] and the stability constants for 1 : 1 inclusion were calculated by using eq. (1):

$$K_{1:1} = \frac{\text{Slope}}{S_0\,(1 - \text{Slope})} \qquad (1)$$

where $K_{1:1}$ is the stability constant for the complex, S_0 is the intrinsic solubility of SP in the absence of CD, and slope is the slope from the phase-

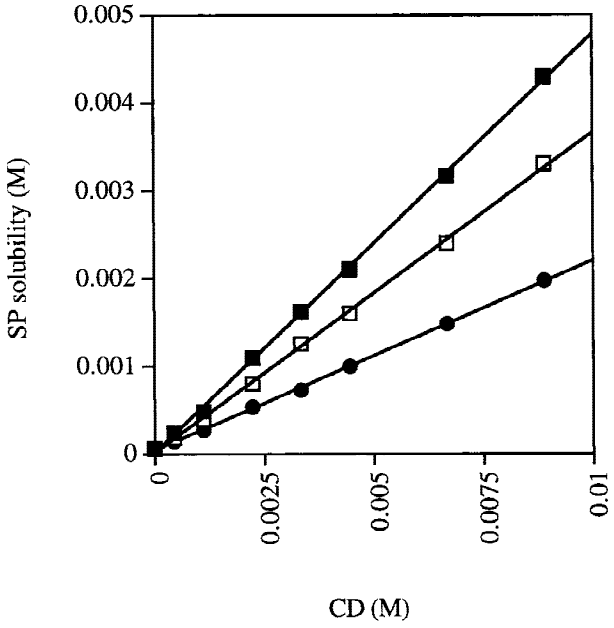


Figure 2. The phase solubility diagram of SP with $(SBE)_{7m}$-β-CD (■), β-CD (□), and γ-CD (●) at pH 2 (25°C).

solubility diagram. The solubility (S_0) of SP in 0.01 *M* HCl was determined to be 24 μg/mL, which is close to previously reported values of 18 μg/mL[17] and 28 μg/mL.[19] The calculated stability constants for complexation of SP with the different CDs are shown in Table I.

The phase-solubility studies showed that SP forms significantly stronger inclusion complex with β-CD and β-CD derivatives than with γ-CD and SBE-γ-CD, consistent with the earlier findings by others.[14,15] These results support the conclusion that the size of the β-CD cavity is more suitable for complex formation with SP. With β-CD and β-CD derivatives, the rank order of the stability constants was as follows: $(SBE)_{7m}$-β-CD > HP-β-CD > β-CD. These results are in good agreement with earlier studies that have shown that neutral and positively charged drugs form strong inclusion complexes with SBE-β-CDs.[20,21] These results suggest that SP may interact with the hydroxypropyl and sulfobutylether side chains. However, SBE-γ-CD was found to have a slightly weaker interaction with SP compared with γ-CD.

Contrary to earlier studies,[8,10,11,13,14] β-CD and γ-CD formed A_L-type phase-solubility diagrams with SP. In those earlier studies, SP has been reported to follow B-type phase solubility behavior with β- and γ-CD. However, the studies were performed in water without pH control, and SP has probably partially or fully degraded during equilibration (see Stability Results). In addition, the analytical method used to determine SP solubility was nonspecific; ultraviolet-spectrophotometric methods were used without separation of SP from its main degradation product, 7α-thiospironolactone (TSP). Furthermore, in our studies, the phase-solubility behavior of TSP in the presence of β-CD followed B-behavior (data not presented), consistent with the fact that in previous studies the phas-solubility diagram was not produced by SP alone. Sezetjli[15] had noted previously that a TSP/β-CD solid complex was formed when SP was equilibrated with β-CD.

Table I. The Stability Constants for 1:1 Inclusion Complex Formation of Spironolactone with Different CDs at pH 2.0 (0.01 *M* HCl, μ = 0.15) Determined by Phase-Solubility

CD	$K_{1:1}$ (M^{-1})
β-CD	9,939
HP-β-CD	10,976
$(SBE)_{7m}$-β-CD	15,816
γ-CD	4,792
SBE-γ-CD	4,118

Stability Studies

The stability of SP was studied at pH 4, 5, 6, 7, and 8 in 4.4 m*M* CD solutions (corresponding to a 1% $[SBE]_{7m}$-β-CD solution). In addition, the effect of CD and buffer concentration on the CD-catalyzed degradation of SP was studied at pH 7.0. In all studies the degradation of SP followed pseudo–first-order kinetics, and the main degradation product was TSP. The results correspond very well with those of Kaukonen et al.,[17] who showed TSP was the main degradation product of SP in the presence of CDs. Without CD, SP has been shown to be quite stable, and the degradation product was proposed to be canrenone.[22]

Table II shows the effect of phosphate buffer concentration on the half-life of SP at pH 7.0 in the presence of HP-β-CD and $(SBE)_{7m}$-β-CD. Phosphate buffer concentration had no effect on the CD-catalyzed degradation of SP. Pramar and Gupta[22] had shown that phosphate and citrate buffers significantly catalyze the degradation of SP in the absence of CDs. However, the increase in degradation rates with buffers seen by Pramar and Gupta was substantially lower than the catalysis observed, in our study, in the presence of CDs. Table II also shows the effect of CD concentration on the half-life of SP at pH 7.0. With all CDs, the degradation rate of SP increases with CD concentration. However, the increase in CD concentration from 0.44 m*M* to 4.4 m*M* increases the degradation rate of SP much less than the increase of CD concentration from 0.044 m*M* to 0.44 m*M*.

Scheme 1 is an adequate model that has been used to describe the overall degradation of drug (D) forming a 1 : 1 inclusion complex (DCD) with CDs. In Scheme 1, k_f and k_c are the rate constants for degradation of free and complexed drug, respectively. The degradation of the drug can be described with eq. (2) when $[CD]_T >> [D]_T$

$$k_{obs} = \frac{k_f + k_c K_{1:1} [CD]_T}{1 + K_{1:1} [CD]_T} \quad (2)$$

where k_{obs} is the observed rate constant for degradation of the drug under pseudo–first-order conditions, $[CD]_T$ is the total CD concentration,

Table II. The Effect CD and Buffer Concentration to the Half-Life of SP at pH 7.0 (25°C)

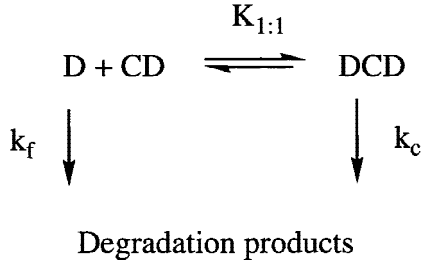
		Half-life (h)		
		CD Concentration		
Buffer	CD	0.044 m*M*	0.44 m*M*	4.4 m*M*
25 m*M* phosphate buffer (μ = 0.15)	β-CD	2.3	0.5	0.4
	γ-CD	16.7	2.4	1.7
	HP-β-CD	10.6	2.3	1.9
	$(SBE)_{7m}$-β-CD	209.1	40.2	31.6
	SBE-γ-CD	379.9	101.3	55.8
10 m*M* phosphate buffer (μ = 0.15)	HP-β-CD	10.3	2.1	1.8
	$(SBE)_{7m}$-β-CD	209.3	38.3	28.3

and $K_{1:1}$ is the equilibrium constant for inclusion complex formation. Eq. (2) can be linearized at fixed conditions of temperature, pH, and so forth, and k_c and $K_{1:1}$ can be determined by curve fitting. In this case, k_f can be considered negligible in the presence of 4.4 m*M* CDs ($[CD]_T >> [D]_T$) and large values of $K_{1:1}$ ($\approx 10^4\ M^{-1}$), allowing the following approximation [eq. (3)] to be made.

$$k_{obs} \approx k_c \tag{3}$$

Thus, in 4.4 m*M* CD solutions, the observed degradation rate constant for SP is approximately equal to the theoretical degradation rate constant for SP in the inclusion complex. The same conclusion (i.e., that most of the SP is in its complexed state) can be reached from the estimates of the stability constants determined from the phase-solubility experiments.

The effect of pH on the CD-catalyzed degradation of SP was studied in 4.4 m*M* CD solutions to ensure that all (most) of the SP molecules were in the complexed form. Table III shows the $t_{90\%}$ values of SP at the pH values studied. The results show that with all CDs, the degradation of SP is base dependent. The best stability was achieved with SBE-CDs at low pH-values. The pH-rate profiles (log k_{obs} versus pH, Fig. 3) of SP with CDs form parallel lines with slopes very close to 1.0 again, suggestive of base catalysis. The catalytic activity of CDs is as follows: SBE-γ-CD < $(SBE)_{7m}$-β-CD < HP-β-CD ≈ γ-CD < β-CD.



Scheme 1.

CD-catalyzed degradation of drugs has usually been explained by the interaction of drugs with the hydroxyl groups of CDs during complexation. Fromming and Szejtli[6] classified the CD-catalyzed degradation into covalent and noncovalent catalysis. In covalent catalysis, often the drug is thought to react with the ionized or un-ionized hydroxyl group of the CD, resulting in a covalent tetrahedral intermediate that breaks down to degradant and derivatized CD. In noncovalent catalysis, drug degradation is increased without covalent intermediates, and the degradation rate of drug increases, for example, because of hydrogen bonding with the CD.[2] CDs have been shown to catalyze the degradation of aztreonam and cephalothin at pH values at which specific base-catalyzed degradation of the drug dominates.[5] In addition, CDs have been shown to increase the stability of prostaglandin E_2[23] and aspirin[24] at low pH values but catalyze the degradation of these drugs at higher pH values.

The NMR studies described in the following section will show that CDs probably catalyze the degradation by direct reaction of the CDs with SP, resulting in acetylation of the secondary hydroxyl groups of the CD. The apparent base catalysis

Table III. The Shelf-Life ($t_{90\%}$) of SP in the Presence and Absence of 4.4 m*M* β-Cd, HP-β-CD, $(SBE)_{7m}$-β-CD, γ-CD, and SBE-γ-CD (25 m*M* buffer, μ = 0.15) at various pH values (25°C)

	$t_{90\%}$ (h)					
pH	Without CDs	β-CD	HP-β-CD	$(SBE)_{7m}$-β-CD	γ-CD	SBE-γ-CD
4.0	320.4	37.9	200.6	—[a]	169.6	—[a]
5.0	127.2	4.6	22.1	370.5	23.7	499.8
6.0	92.4	0.46	2.30	42.3	2.31	71.8
7.0	61.4[b]	0.06	0.29	4.81	0.26	8.49
8.0	20.4[b]	0.01	0.03	0.52	0.02	0.82

[a] Less than 5% of SP was degraded over 50 days.
[b] Values were estimated after 10% degradation of SP.

observed (Fig. 3) would be consistent with the following modification of Scheme 1 (Scheme 2) where CD-H, CD^-, DCD-H, and DCD^- represent the un-ionized and ionized forms of the CD and drug/CD complex, respectively, Ka and K′a are the dissociation constants of the CD and drug/CD complex, respectively, and k_c and k_c' are the rate constants for the degradation of the drug in the un-ionized and ionized drug/CD complex, respectively. By assuming that equilibrium defined by $K'_{1:1}$ is negligible at pH values around neutral or below and that, $k_c' >> k_c$ (only base catalysis was observed, see Fig. 3), Scheme 2 and be simplified to Scheme 3.

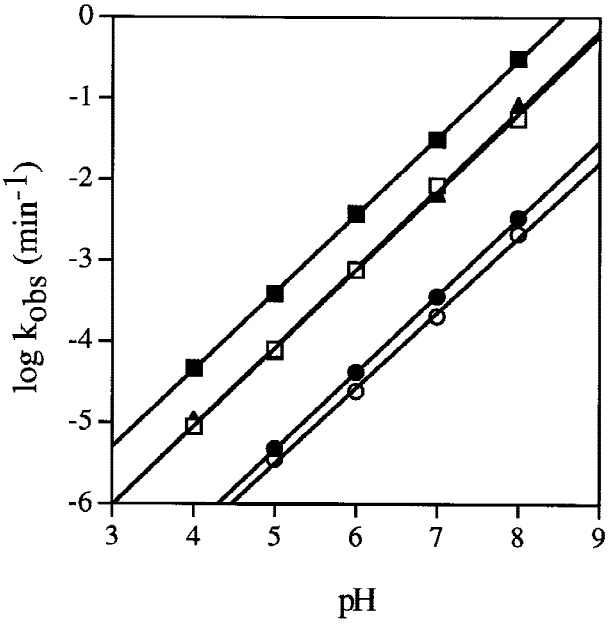


Figure 3. The pH rate profiles of SP in 4.4 m*M* β-CD (■), HP-β-CD (□), γ-CD (▲), $(SBE)_{7m}$-β-CD (●), and SBE-γ-CD (○) solutions (25 m*M* buffer, μ = 0.15) at 25°C.

By assuming that k_f is negligible at high CD concentrations, the degradation of SP can be described by eq. (4)

$$-\frac{d[D]_T}{dt} \approx -\frac{d[DCD^-]}{dt} = k_c'\,[DCD^-] \qquad (4)$$

When $[H^+] >> Ka$, $[DCD^-]$ is related to total complex concentration ($[DCD]_T$) by eq. (5)

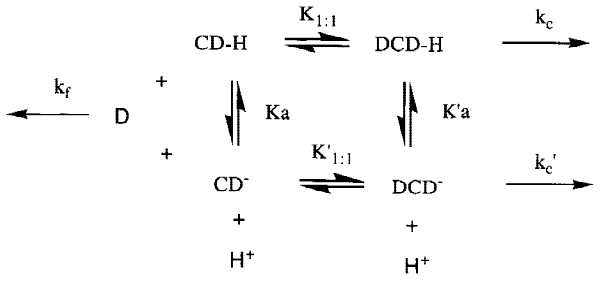
$$[DCD^-] = \frac{K'a[DCD]_T}{K'a + [H^+]} \approx \frac{K'a\,[DCD]_T}{[H^+]} \qquad (5)$$

Substituting eq. (5) into eq. (4) gives eq. (6)

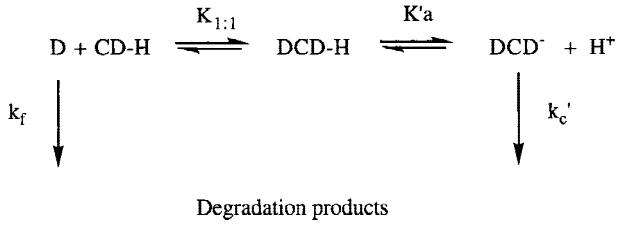
$$-\frac{d[D]_T}{dt} \approx \frac{k_c'\,K'a[DCD]_T}{[H^+]} \qquad (6)$$

For large values of $K_{1:1}$ and where $[CD] >> [D]_T$, and substituting $K_w/[OH^-]$ for $[H^+]$, eq. (7) results.

$$-\frac{d[D]_T}{dt} \approx \frac{k_c'K'a}{K_w}\,[OH^-]\,[D]_T \qquad (7)$$



Scheme 2.



Scheme 3.

Therefore, at fixed pH values and when the other assumptions described previously are valid, the observed pseudo–first-order rate constant for the degradation SP in the presence of excess CD concentration can be described by eq. 8

$$k_{obs} \approx \frac{k_c' K' a}{K_w} [OH^-] \quad (8)$$

This equation adequately describes the data seen in Figure 3. The lack of buffer catalysis confirms that the reaction is specific-base catalyzed and suggests that the reactivity of SP in the presence of the CDs will be a function of k_c' and the dissociation constant (K′a) of the drug/CD complex.

NMR Studies

In an attempt to learn more about the degradation of SP in the presence of CDs, a number of NMR experiments were performed. NMR was used to study the complexation of SP with CDs and to determine the probable mechanism of CD-catalyzed degradation of SP. The studies were performed with β-CD and SP because the side chain of the CD derivatives makes the interpretation of NMR spectra more complicated.

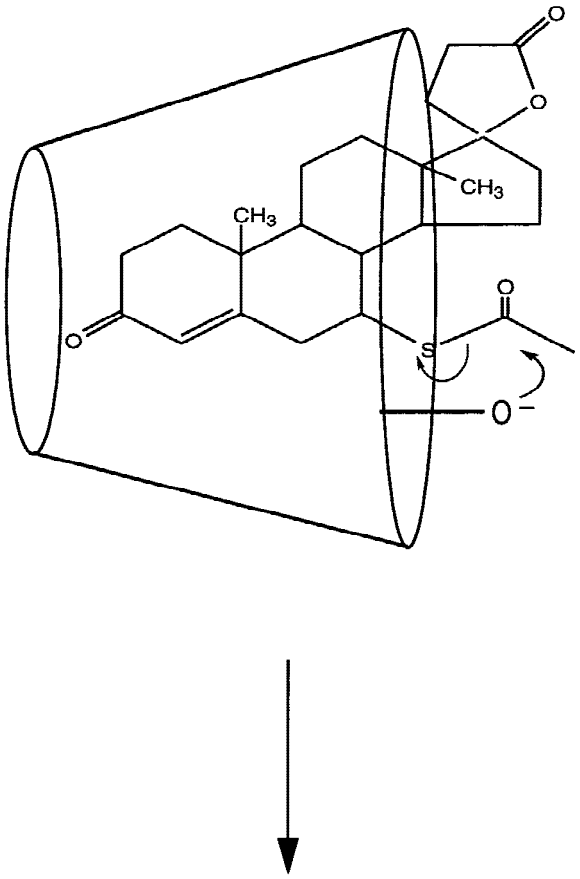
In a previous study, Wouessidjewe et al.[16] noted that complexation of SP results in chemical shift changes at H-7 and H-9 of SP. These authors proposed a 2 : 1 β-CD:SP complex. NMR experiments to assess which parts of the SP molecule actually entered the β-CD cavity, and from which face, were performed in this study. The study was done at pH 2 to eliminate the degradation of SP caused by complexation with β-CD. A 1-D ROESY experiment (to detect short-range through space interaction between protons) showed prominent enhancements from the β-CD sugar signals between 4.0 and 3.6 δ to the SP protons at H-4 and H-2, and the 21- and 22-methyl groups. Each of these signals was then checked in turn for enhancements back to β-CD protons because SP signals are also found between 4.0 and 3.6, causing intramolecular ROEs to be observed as well. For SP, only the H-4 protons enhanced β-CD protons, which were identified as H-1 and H-5 of β-CD, both close to the primary face of the β-CD. These results demonstrate secondary face complexation because only the H-4 end of the molecule can be localized in the β-CD cavity, and the perturbed signals at H-7 and H-9 are adjacent to it. The NMR data (and all the other data from this study) are consistent with 1 : 1 complexation in solution. It is likely that the chemical shift perturbations are due to the proximity of H-7 and H-9 to the secondary hydroxyl groups; this also positions the thioacetate group at the reactive site (see observations following).

A kinetic experiment was performed to follow the degradation of SP with time in β-CD solution by NMR. The study was performed at pH 7.0 where the half-life of SP was determined to be 0.4 hours. The experiment showed disappearance of the thioacetate group of SP and simultaneous appearance of two new acetate signals consistent with acetylated CD, plus some free acetate. One isomer was dominant early in the experiment, but with time, both the second isomer and free acetate signals increased. This suggests that one hydroxyl group is initially acetylated, followed by an intramolecular acetyl migration, which is frequently a facile process in carbohydrates. Subsequently, the acetylated β-CD hydrolyzes to release free acetate. The two acetylated isomers were not separated or rigorously identified, but the mixture was recovered from water by freeze-drying and by redissolving at higher concentration in DMSO. A DEPT spectrum of the sample showed no detectable acetylation at C-6, which would have been apparent as a methylene carbon shifted downfield from the normal position of 60 δ to ca. 68 δ for the C-6 carbon. It is therefore likely that the isomers present are acetylated at C-2 and C-3 on the secondary face of the CD, both positioned near the thioacetate in the complex; acetyl migration could readily convert one to the other.

The NMR studies reveal that β-CD catalyses the degradation of SP by covalent catalysis. As discussed earlier, according to this model, SP reacts with CD's ionized hydroxyl groups, and the stability of SP increases at lower pH values because less hydroxyl groups are in their ionized form. This shows how a small concentration of ionized hydroxyl groups in CD molecules can dramatically increase the degradation rate of drugs

that place reactive groups such as the thioacetate group of SP near the secondary face. The model consistent with this is shown in Figure 4.

The pKa values of drug/CD complexes are unknown. The pKa value of β-CD itself, however, has been reported to be 12.20.[6] Thus, at pH 7.0 only ≈ 1/10^{5}th of the hydroxyl groups of the β-CD molecule are in their ionized form. However, it should be pointed out that thiol esters are more susceptible to nucleophilic attack than ordinary esters,[25] which may also explain the high catalytic activity of the ionized hydroxyl groups with SP. Assuming a value of 2.86×10^{-5} min^{-1} for k_f at pH 7.0 (value estimated from the hydrolysis of SP at pH 7.0 in the absence of CD), k_{obs}/k_f is ≈ 1.1×10^3. That is, β-CD provides 10^3 fold catalysis for SP degradation, a value that would do justice to some enzymatic reactions.



TSP + Acetyl-CD

Figure 4. The model describing the degradation of SP by a hydroxyl anion on the secondary face of a cyclodextrin molecule.

The ionization of β-CD and $(SBE)_{7m}$-β-CD was studied by NMR in an attempt to explain if the lower catalytic activity of $(SBE)_{7m}$-β-CD compared with β-CD could be explained by differences in K′a values rather than k_c' values, the assumption being that values of Ka will reasonably reflect relative K′a values. NMR samples were prepared at pH 11, 12, 13, and 14. No changes in chemical shift of any protons were observed in $(SBE)_{7m}$-β-CD as the pH was raised. At both pH 11 and 12, no changes were noted with β-CD; however, at pH 13, the H-2, H-3, and H-4 signals of β-CD all shifted significantly downfield, suggesting a change in the ionic state of β-CD. There was also a small but significant shift of the C-2 signal in the ^{13}C NMR spectrum. No change in any chemical shift for $(SBE)_{7m}$-β-CD was observed even at pH 14. The higher apparent pKa value of SBE-β-CDs may be due to the presence of seven negatively charged sulfobutyl groups inhibiting the ionization of the nonalkylated hydroxyl groups. Thus, it appears that the ability of CDs to catalyze degradation of SP may be related (in part) to the ionization of the secondary hydroxyl groups.

CONCLUSIONS

β-CDs form stronger inclusion complexes with SP than γ-CDs consistent with some earlier findings, but γ-CDs have a lower catalytic effect on the deacetylation of SP. The CD-catalyzed degradation of SP can be decreased by decreasing solution pH. The best stability of SP can be achieved with SBE-CDs. NMR studies showed that during degradation, the hydroxyl groups of β-CD at the 2- and 3- position are acetylated. The study with β-CD and $(SBE)_{7m}$-β-CD shows that the differing ability of CDs to catalyze the degradation of SP may be related to the pKa values of the hydroxyl groups. Kaukonen et al.[17] also proposed that an increased degree of hydroxyl group derivatization may decrease CD-catalyzed degradation of SP. This is clearly possible because Kaukonen et al.[17] showed that dimethyl-β-CD did not catalyze SP deacetylation. In addition, small differences in the site of binding may affect the proximity of the thioacetate group relative to the hydroxyl groups. These differences would manifest themselves in eq. (8) as differences in k_c' values. The lower catalytic activity of the γ-CDs is consistent with this

because the site of binding could be different between γ-CDs and β-CDs. Most probably, the relative catalytic activity of CDs is a combination all three of these effects; the state of ionization, site of binding, and the number of hydroxyl groups available for reaction.

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