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Effect of cyclodextrins on the degradation rate of benzylpenicillin

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The effect of cyclodextrin (CD) inclusion complexes on the degradation of benzylpenicillin in aqueous solutions was investigated at several different pH values and 37 °C. The effects of neutral as well as both positively and negatively charged CDs were evaluated; all together 13 different CDs. Kinetic studies with HPβCD and RMβCD at pH ranging from 1.2 to 9.6 showed that CDs have stabilizing effect on the β-lactam ring in aqueous acidic media but generally accelerated the hydrolytic cleavage of the β-lactam ring in neutral and basic media. At physiologic pH (pH 7.4) quaternary ammonium CD derivatives (i.e., positively charged CD derivatives) have the highest catalytic effect, resulting in 6- to 18-fold enhancement of hydrolysis rate, while both the neutral methylated CDs had much less effect, resulting in 2- to 3-fold enhancement, and the negatively charged CD derivatives, resulting in only about 1.1- to 1.2-fold enhancement in the hydrolytic cleavage of the β-lactam ring. Addition of water-soluble polymers to the aqueous reaction media containing CDs was shown to decrease the catalyzing effects of CDs on the β-lactam hydrolysis.

1. Introduction

1.1. Theoretical background

Benzylpenicillin (penicillin G) is a somewhat narrow spectrum, acid-labile β-lactam antibiotic that is generally administered intravenously. Although still used today the clinical usefulness of this first-generation penicillin is constantly decreasing due to increasing occurrence of resistant bacteria. Bacterial resistance to benzylpenicillin and other β-lactams is mainly due to modification of penicillin binding proteins (PBPs), that is due to alterations in the drug target, and secretion of β-lactamases that cleave the β-lactam ring, that is by deactivation of the drug molecule (Buynak 2006). Coadministration of β-lactamase inhibitors, such as clavulanic acid, sulbactam and tazobactam, can in some cases reduce bacterial resistance by making the β-lactamase inactive.

Various drug delivery systems for effective delivery and targeting of antibacterial agents have been developed. Examples of such delivery systems are antibiotic-containing nanocarriers formed by lipid bilayers and polymeric liposomes that deliver large amounts of drug molecules to bacterial cells saturating the secreted β-lactamases and, thus, delivering antibacterially effective amounts of the β-lactam drug to the bacteria (Buynak 2006; Tang et al. 2014). Another way is to prevent drug hydrolysis in aqueous media and protect the active molecule from the action of enzymes through complex formation with cyclodextrins (CDs). Nuclear magnetic resonance (NMR) spectroscopic studies, mass spectrometric (MS) and micro-calorimetric studies show that various β-lactam antibiotics form complexes with βCD and γCD derivatives in aqueous acidic media (Aki et al. 2009, 2004; Bisson-Boutelliez et al., 2010; Maffeo et al., 2006). The native αCD, βCD and γCD are cyclic oligosaccharides consisting six, seven or eight α-1,4-linked glucopyranose units, respectively. CDs have a unique cone shape with a somewhat hydrophobic central cavity and a hydrophilic outer surface. Thanks to this construction the CD molecule can be a "host" for a relatively hydrophobic "guest" molecule of the appropriate size (Loftsson 1995). Currently many CD derivatives are available on the market. Some are uncharged while others carry negative or positive charge, for example, uncharged methylated βCD, negatively charged sulfobutylether βCD and positively charged amine-derivatized CDs. These CDs are able to form complexes with a much greater range of compounds than the parent CDs (Loftsson and Duchêne 2007). In general, formation of CD complexes increases the aqueous solubility, stability and bioavailability of the encapsulated drug. However, in some cases the complexation has no effect and in other the complexation results in accelerated drug degradation. For example, CDs are known to accelerate chemical decomposition of β-lactam antibiotics (Loftsson and Ólafsdóttir 1991). In the present work the effect of CD complexation on the chemical stability of benzylpenicillin is evaluated. The observed first-order rate constants for the β-lactam hydrolysis were determined through kinetic studies of both pure aqueous benzylpenicillin solutions and benzylpenicillin in aqueous CD-containing systems under controlled pH and temperature conditions. In addition, impacts of thirteen CDs, mainly βCD and γCD derivatives on the benzylpenicillin stability at pH 7.4 are presented. The studies show the effects of neutral, anionic and cationic derivatives at several concentrations which allow determination of the stability constants of the benzylpenicillin/CD complexes and the pseudo-first-order rate constants for drug degradation within the CD complexes.

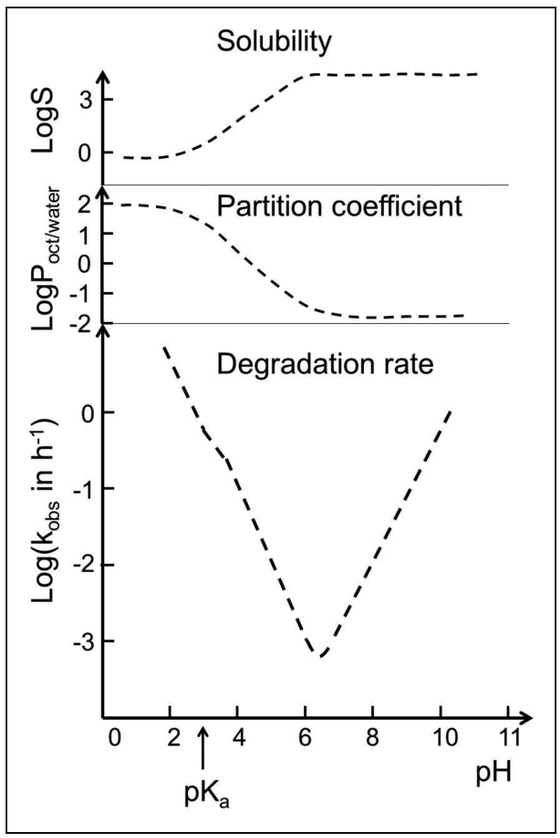


Fig. 1: Sketches showing the effect of pH on the physicochemical properties of benzylpenicillin: calculated solubility (S) in mg/ml at 25 °C; calculated octanol/water partition coefficient ($P_{oct/water}$) at 25 °C; observed degradation rate constant in aqueous 0.5 M sodium chloride solution (k_{obs}) in h^{-1} at 30 °C. Based on data from Brodersen (1947) and SciFinder of the Chemical Abstracts Databases (ACS 2015).

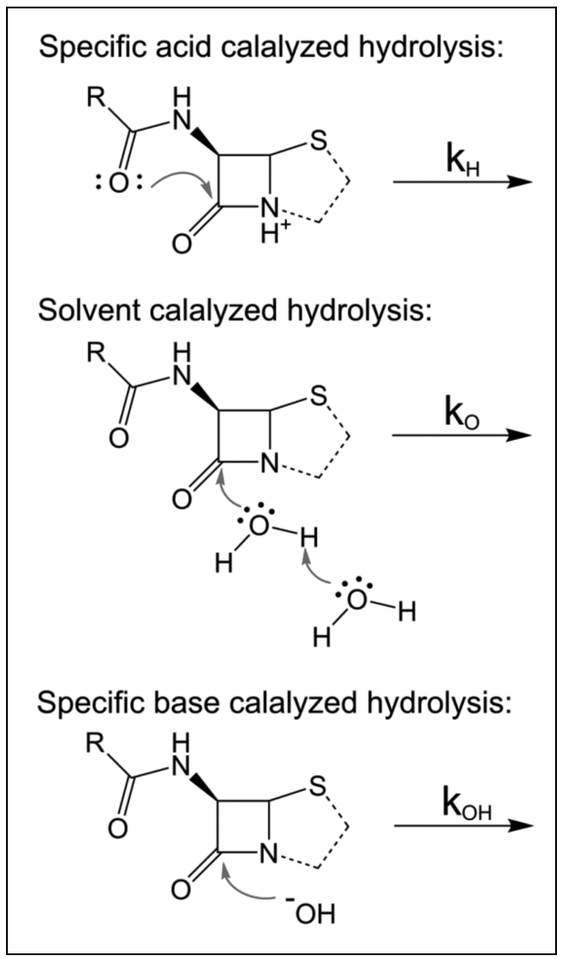


Fig. 3: Specific acid, solvent and specific base catalyzed hydrolysis of the β-lactam ring (López et al. 2000; Yamana et al. 1977).

The aim of this study was to determine which structural features of CDs are responsible for catalytic degradation of β-lactam antibiotics and explore the possibility of designing CDs that do not possess this catalyzing effect. The ultimate goal of this project is to design CD-based drug delivery systems for β-lactam antibiotics that do not catalyze their degradation in aqueous solutions and prevent deactivation of the drug molecule.

1.2. Chemical degradation of benzylpenicillin

Benzylpenicillin is an acid ($pK_a = 2.8$ at room temperature) that is lipophilic and poorly water soluble in its unionized form but hydrophilic and freely water soluble in its ionized form (Fig. 1). It undergoes pH-dependent hydrolysis of the β-lactam ring in aqueous solutions to form benzylpenicilloic acid and other degradation products with maximum stability at pH values between 6 and 7; specific acid catalyzed hydrolysis of the unionized drug and specific acid, solvent and specific base catalyzed degradation of the ionized drug (Figs. 1 and 2). The β-lactam ring is both responsible for the biological activity of the β-lactam antibiotics and their chemical instability in aqueous solutions. Due to its structural strain the four-membered lactam ring is relatively unstable and undergoes rapid hydrolytic cleavage. The specific acid catalyzed hydrolysis is initiated by N-protonation followed by intra-molecular catalysis of the ring-opening (Fig. 3) (Coll et al. 1998). Substituents (R in Fig. 3) that pull electrons away from the oxygen increase the

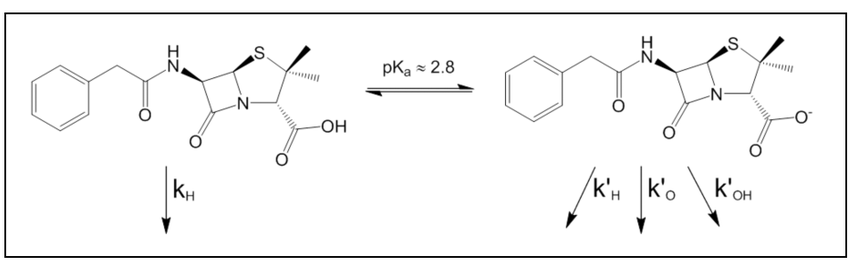


Fig. 2: Hydrolysis of benzylpenicillin in aqueous solution. The second-order rate constants for the specific acid catalyzed hydrolysis of the unionized (k_H) and the ionized (k'_H) drug, the first-order rate constant for the solvent catalyzed hydrolysis of the ionized drug (k'_o), and the second-order rate constant for the specific base catalyzed hydrolysis of the ionized drug (k'_{OH}).

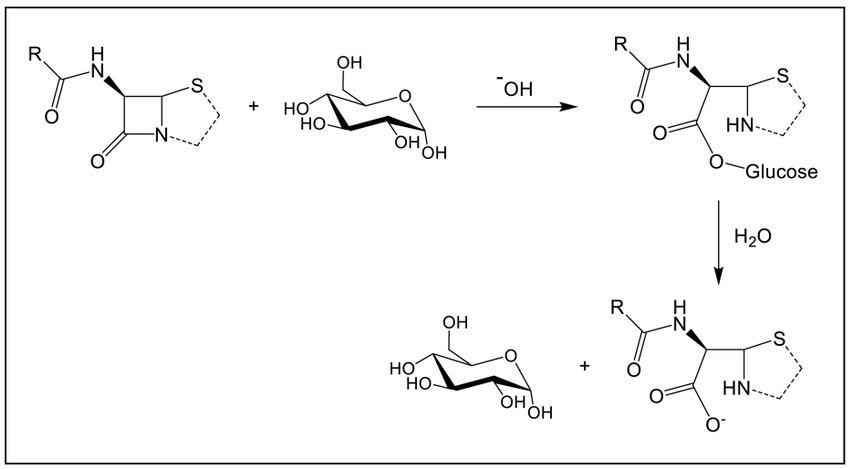


Fig. 4: Accelerated degradation of β-lactam antibiotics by glucose, sucrose and other carbohydrates appears to be due to alcoholysis of the β-lactam ring with formation of an unstable α-ester of penicilloic acid (Bundgaard and Larsen 1978, 1979; Hem et al. 1973; López et al. 2000).

chemical stability of β-lactams in acidic solutions. Thus, while benzylpenicillin is relatively unstable and rapidly hydrolyzed in the stomach (i.e. has low oral bioavailability) orally active penicillins (e.g., ampicillin and amoxicillin) having electron withdrawing substituents are relatively stable under acidic conditions. The solvent catalyzed hydrolysis most probably involves nucleophilic attack by a water molecule facilitated by neighboring water molecules (general base catalysis) to produce benzylpenicilloic acid (Fig. 3) (Yamana et al. 1977). The specific base catalyzed hydrolysis involves nucleophilic attack of OH^- on the carbonyl carbon (Fig. 3) (Yamana et al. 1977). The pH – rate profile (Fig. 1) can be described by the following equations:

$$-\frac{d[B]_T}{dt} = k_H[H^+][BH] + k'_H[H^+][B^-] + k'_{OH}[OH^-][B^-] \quad (1)$$

$$k_{obs} = k_H[H^+]f_{BH} + k'_H[H^+]f_B + k'_o f_B + k'_{OH}[OH^-]f_B \quad (2)$$

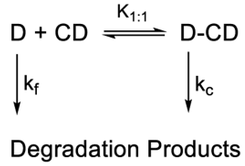
$$-\frac{d[B]_T}{dt} = k_{obs}[B]_T \quad (3)$$

where $[B]_T$ is the total concentration of benzylpenicillin (i.e., $[B]_T = [BH] + [B^-]$) in the reaction medium, [BH] is the concentration of unionized and $[B^-]$ the concentration of the ionized drug, respectively, k_H and k'_H are the second-order rate constants for the specific acid catalyzed hydrolysis of the unionized and ionized drug, respectively, k'_O is the first-order rate constant for the solvent catalyzed hydrolysis of the ionized drug and k'_{OH} the second-order rate constant for the specific base catalyzed hydrolysis of the ionized drug, f_{BH} and f_B the fractions of benzylpenicillin that are in the unionized and ionized form, respectively, and k_{obs} is the observed first-order rate constant for the hydrolysis (Loftsson 2014).

Alcohol-catalyzed hydrolysis (alcoholysis) of benzylpenicillin involves formation of penicilloyl ester intermediate (Fig. 4) (Davis et al. 1991). In the case of sucrose catalyzed degradation of benzylpenicillin formation of the benzylpenicilloyl sucrose ester intermediate can be detected in the reaction media at pH 7.00 and 8.48 (Bundgaard and Larsen 1978). Benzylpenicillin is also known to form ester intermediates with other sugars including glucose (Schneider and de Weck, 1969). Alcoholysis could be the first step in the reaction of β-lactam antibiotics with β-lactamase (Davis et al. 1991; Hem et al. 1973; López et al. 2000). In a previous study HPβCD, methylated βCD and HPγCD were shown to have stabilizing effect on cephalotin at pH 6.5 but destabilizing effect at pH 9.7 (Loftsson and Jóhannesson 1994).

1.3. Calculation of degradation rate constants

In general, one drug molecule interacts with one cyclodextrin molecule in dilute aqueous solutions to form 1:1 drug-CD complex in dilute aqueous solutions. The kinetic studies were performed in dilute aqueous solutions and, thus, it was assumed that only 1:1 complexes were formed:



where $K_{1:1}$ is the complex stability constant, k_c is the observed first-order rate constant for the drug degradation within the complex (D-CD) and k_f represents the observed first-order rate constant for the degradation of the free drug (D). Here D represents the unionized form (BH) and/or the ionized form (B^-) of benzylpenicillin. The observed first-order rate constant (k_{obs}) for the drug degradation is the weighted average of k_f and k_c:

$$k_{obs} = k_f \cdot f_f + k_c \cdot f_c \quad (4)$$

where f_f is the fraction of drug in solution that is unbound (i.e., free) and f_c is the fraction of drug in solution that is bound in a CD complex. Further manipulation of the mathematical equations (Loftsson 1995, 2014) gives:

$$k_{obs} = \frac{k_1 + k_c \cdot K_{1:1}[CD]}{1 + K_{1:1}[CD]} \quad (5)$$

where [CD] is the concentration of the free (i.e., unbound) CD in the aqueous medium. If the total CD concentration (i.e., $[CD]_T = [CD] + [D\text{-}CD]$) is much greater than the total drug concentration (i.e., $[D]_T = [D] + [D\text{-}CD]$) then $[CD] \approx [CD]_T$:

$$k_{obs} = \left(\frac{k_f + k_c \cdot K_{1:1}[CD]_T}{1 + K_{1:1}[CD]_T}\right) \quad (6)$$

k_f is determined in the CD free reaction medium and then k_c and $K_{1:1}$ can be obtained by determination of k_{obs} at various

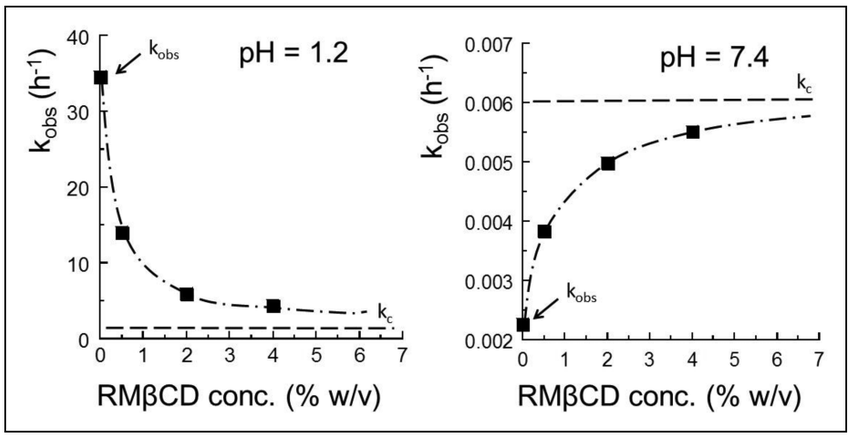


Fig. 5: The effect of increasing RMβCD concentration on the observed rate constant for benzylpenicillin hydrolysis in aqueous buffer solutions at pH 1.2 and pH 7.4 at 37.0 ± 0.1 °C. The initial benzylpenicillin concentration ($[D]_T$) in the aqueous reaction media was 2.46 mM and the RMβCD concentration ($[CD]_T$) ranged from 0.0 to 4.0% (w/v) (i.e., 0, 4, 15 and 30 mM).

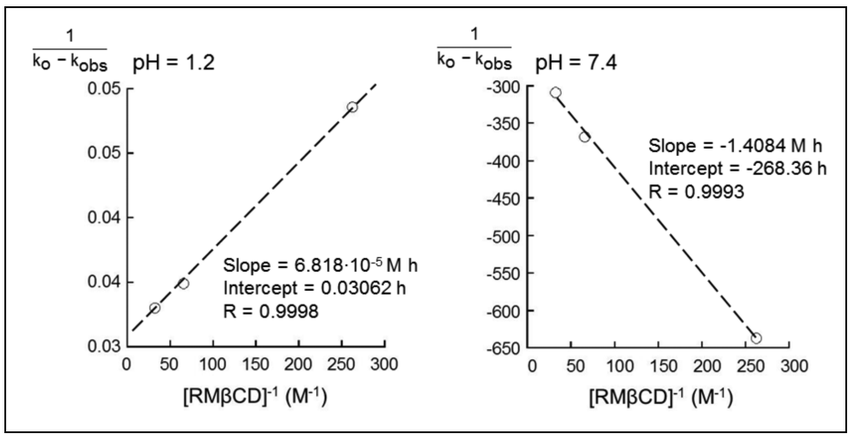


Fig. 6: Lineweaver-Burk plots for benzylpenicillin hydrolysis in aqueous buffered solutions at pH 1.2 and pH 7.4 at 37.0 ± 0.1 °C.

CD concentrations, either through non-linear fitting of Eq. (6) or linear plots such as of the Lineweaver-Burk plot where $(k_f - k_{obs})^{-1}$ *versus* $([CD]_T)^{-1}$ will give a straight line from which k_c can be obtained from the intercept and $K_{1:1}$ from the slope:

$$\frac{1}{k_f - k_{obs}} = \frac{1}{K_{1:1}(k_f - k_c)} \cdot \frac{1}{[CD]_T} + \frac{1}{k_f - k_c} \qquad (7)$$

Fig. 5 shows how increasing RMβCD concentration affects the observed rate constant (k_{obs}) for the benzylpenicillin hydrolysis. At pH 1.2 addition of RMβCD to the reaction medium decreases the observed degradation rate. Benzylpenicillin molecules bound to RMβCD are hydrolyzed at a slower rate than free benzylpenicillin molecules (i.e., $k_c < k_f$). However, at pH 7.4 addition of RMβCD accelerates the hydrolysis (i.e. $k_c > k_f$). Based on the $K_{1:1}$ value and the total concentrations of both benzylpenicillin and RMβCD only about 8 to 40% of RMβCD present in the reaction media is bound to the drug and, thus, we can assume that $[RM\beta CD] \approx [RM\beta CD]_T$ and use Eq. (7) to determine $K_{1:1}$ and k_c. From the linear Lineweaver-Burk plots (Fig. 6) the following values can be determined at pH 1.2:

$$K_{1:1} = \frac{Intercept}{Stope} = \frac{0.03062\,h}{6.818 \times 10^{-5} M\,h} = 450 M^{-1}$$

$$Intercept = \frac{1}{k_f - k_c} = \frac{1}{34.6\,h^{-1} - k_c}$$

$$= 0.03062\,h \Rightarrow k_c = 1.94\,h^{-1}$$

At pH 7.4 where RMβCD accelerates the degradation the following values are obtained:

$$K_{1:1} = \frac{Intercept}{Stope} = \frac{-268.36\,h}{-1.4084\,M\,h} = 190\,M^{-1}$$

$$Intercept = \frac{1}{k_f - k_c} = \frac{1}{2.27 \times 10^{-3} h^{-1} - k_c}$$

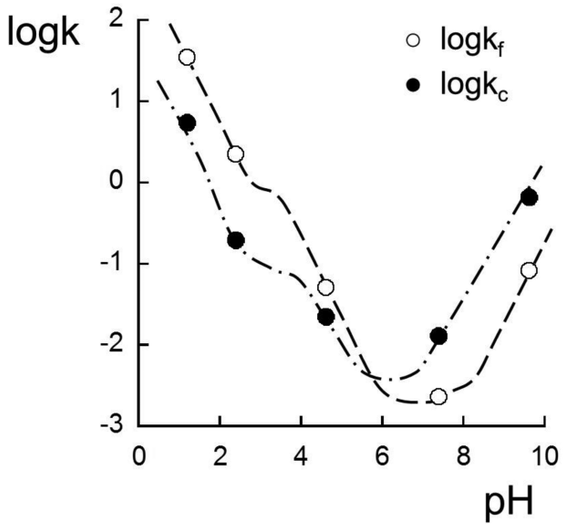
$$= -268.36h \Rightarrow k_c = 6.00 \times 10^{-3} h^{-1}$$

At pH 1.2 the k_c/k_f ratio is about 0.06 (i.e. k_f/k_c ratio is 18) indicating that the free drug is hydrolyzed about 18 times faster in the solution than bound drug within the complex. At pH 7.4 the k_c/k_f ratio is 2.6 indicating that the drug is hydrolyzed about 2.6-times faster within the complex than out in the solution.

2. Investigations, results and discussion

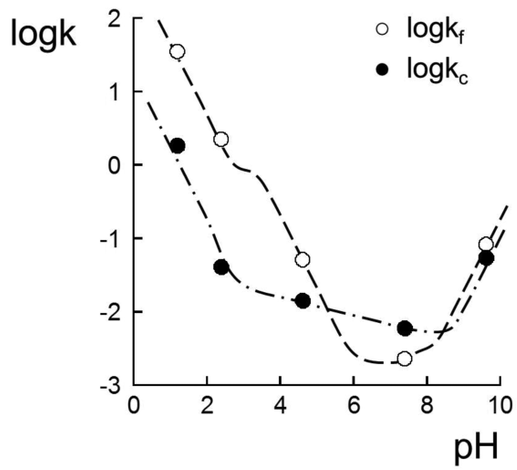
In general, CDs stabilize β-lactam antibiotics against hydrolytic degradation in aqueous acidic solutions but accelerate hydrolytic cleavage of the β-lactam ring of the penem ring structure in basic solutions. The differences in the CD effect may be explained through charge changes of carboxyl group ($pK_a = 2.8$) on the penem ring (Aki et al. 2009). The uncharged penem ring dominating at low acidic pH has greater affinity for the hydrophobic CD cavity than charged penem ring dominating at neutral and

Table 1: Values of k_c, k_f, and $K_{1:1}$ for benzylpenicillin in HPβCD solutions at pH 1.2, 2.5, 4.5, 7.4, 9.6



	HPβCD				
+pH	1.2	2.4	4.6	7.4	9.6
k_f (h^{-1})	34.6	2.28	5.08×10^{-2}	2.27×10^{-3}	8.22×10^{-2}
k_c (h^{-1})	5.44	1.98×10^{-1}	2.22×10^{-2}	1.28×10^{-2}	6.63×10^{-1}
k_c/k_f	0.16	0.09	0.44	5.6	8.1
$K_{1:1}$ (M^{-1})	480	150	330	61	32

Table 2: Values of k_c, k_f, and $K_{1:1}$ for benzylpenicillin in RMβCD solutions at pH 1.2, 2.5, 4.5, 7.4, 9.6



	RMβCD				
pH	1.2	2.4	4.6	7.4	9.6
k_f (h^{-1})	34.6	2.28	5.08×10^{-2}	2.27×10^{-3}	8.22×10^{-2}
k_c (h^{-1})	1.94	4.13×10^{-2}	1.44×10^{-2}	6.00×10^{-3}	5.48×10^{-2}
k_c/k_f	0.06	0.02	0.28	2.6	0.67
$K_{1:1}$ (M^{-1})	450	150	190	190	85

basic pH. The β-lactam ring of the drug can be protected through CD complexation when the penem structure is uncharged and relatively lipophilic. This is what indeed was observed in the case of benzylpenicillin and the neutral βCD derivatives HPβCD and RMβCD (Tables 1 and 2). In acidic media (pH 1.2 to 4.6) benzylpenicillin was hydrolyzed 2- to 10-fold slower within the HPβCD complex and 4 to 50 times slower within the RMβCD complex than out in the solution. In neutral to basic media (pH 7.4 and 9.6) benzylpenicillin was hydrolyzed 5- to 8-times faster within the HPβCD complex than out in the solution. However, in the case of RMβCD some stabilization was observed at pH 9.6 and in general this CD derivative displayed greater stabilizing effect in acidic solutions and less catalyzing effect in basic solution than HPβCD. Both CDs are highly water soluble

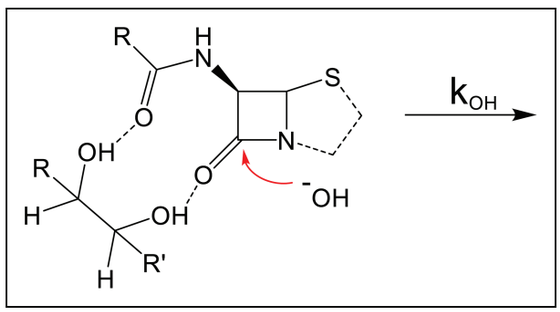


Fig. 7: Formation of hydrogen bonds between a polyhydric alcohol and a β-lactam molecule facilitating specific base catalyzed hydrolysis of the β-lactam ring. If the polyhydric alcohol is a CD then a non-inclusion β-lactam/CD complex is formed facilitating nucleophilic attack of the hydroxyl ion on the β-lactam ring. Sketch based on Fujiwara et al. (1985).

but RMβCD is somewhat more lipophilic and contains significantly (i.e., about 60%) fewer hydroxyl groups than the HPβCD molecule that can explain the observed differences in stabilizing and catalyzing effects on the benzylpenicillin hydrolysis. This is in agreement with a previous study where methylated βCD had a stabilizing effect on phenoxymethylpenicillin in aqueous solution at pH 9.8 while both HPβCD and HPγCD had a destabilizing effect (Loftsson and Ólafsdóttir 1991). Penicillins such as benzylpenicillin are known to form two co-existing types of drug-CD 1:1 complexes, one where the phenyl ring is inside the CD cavity and the other where the penem ring is inside (Maffeo et al. 2006). Tables 1 and 2 show that the unionized benzylpenicillin forms a more stable complex (i.e., displays higher $K_{1:1}$ value) than the more hydrophilic ionized form. Furthermore, ionization of the penem ring will reduce its ability to enter the lipophilic CD cavity and then formation of the phenyl ring/CD inclusion complex will be favored over the penem/CD inclusion complex. This will decrease the ability of CDs to protect the β-lactam ring against hydrolytic cleavage at $pH > pK_a$. Like other carbohydrates and some polyhydric alcohols CDs can catalyze β-lactam degradation in aqueous solutions (see Section 3). It is known that in aromatic solvents monohydric alcohols, like ethanol and isopropanol, form hydrogen bonds with the amide group of penicillins (Hatton and Richards 1960, 1962). Alcohols with two or more adjacent hydroxyl groups can form hydrogen bonds to both the amide group as well as to the β-lactam carbonyl (Fujiwara et al. 1985). Formation of such a non-inclusion complex between a β-lactam molecule and CD (i.e., where two adjacent hydroxyl groups form a complex with the β-lactam molecule) facilitates nucleophilic attack of a hydroxyl ion on the β-lactam ring accelerating the specific base cleavage of the ring (Fig. 7). Methylation of the hydroxyl groups reduces its ability to form hydrogen bonds and, thus, RMβCD has less destabilizing effect than HPβCD at pH 7.4 and even some stabilizing effect at pH 9.6 where HPβCD accelerated specific base cleavage of the β-lactam ring (Tables 1 and 2). At pH 9.6 enolization of the alpha-hydrogen to the penem carboxyl can occur.

In Table 3 the effects of neutral CDs as well as of positively and negatively charged CD derivatives on the stability of benzylpenicillin at physiologic pH (i.e., pH 7.4) and 37 °C are summarized. In all cases the CDs accelerate benzylpenicillin degradation in aqueous solution at this pH although to a different degree depending on their structure. For example, methylation of βCD and γCD decreases their catalyzing effect. Methylation of the hydroxyl group decreases their ability to form non-inclusion complexes with the β-lactam molecule (Fig. 7) and, thus, reduces their ability to catalyze the β-lactam ring cleavage. In this study the stability constants ($K_{1:1}$) of benzylpenicillin/CD complexes of neutral γCDs are smaller than those of corresponding βCDs. Substitution of CD neutral hydroxyl groups by quaternary ammonium groups enhanced the catalyzing effects of CDs by a factor of 2 to 5. However, substitution of CD neutral hydroxyl groups by negatively charged carboxymethyl groups decreased the catalyzing effects of γCD resulting in only about 10 to 20% rate increase (i.e., k_c/k_f is only 1.1 to 1.2, Table 3). In comparison the k_c/k_f ratio for the parent γCD was determined to be 6.6 (i.e., almost 600% increase) and that of the RMγCD 2.3 (i.e. about 130% increase). Of the CDs tested CMγCD had the least catalyzing effect of the benzylpenicillin degradation in aqueous solution at physiologic pH. The negative charge on the CMγCD molecule makes a nucleophilic attack by the negatively charged hydroxyl ions more difficult (i.e., due to repulsive force between the two negatively charged ions).

In general, addition of a small amount of a water-soluble polymer to the aqueous pH 7.4 benzylpenicillin solution increased slightly the degradation rate when no CD was present (Table 4). However, the polymers enhanced the stability of the benzylpenicillin/HPγCD complex (i.e., the $K_{1:1}$ value) from about unity to over 300 M^{-1} and decreased the value of k_c resulting in about four fold decrease in the catalyzing effect, that is the k_c/k_f ratio decreased from about 8 to about 2 upon addition of small amount of polymer to the reaction media at physiologic pH and 37 °C.

In conclusion, the effects of CDs on the hydrolytic cleavage of β-lactam antibiotics depend on both the pH of the aqueous reaction media and the CD structure. CDs have a stabilizing effect on β-lactams in acidic solutions (i.e., specific acid catalyzed degradation) but generally accelerate β-lactam degradation in neutral and basic media (i.e., specific base catalyzed degradation). However, the accelerating effect appears to depend on both the charge of the CD molecule and the number of available hydroxyl groups. Substitution of the hydroxyl groups on the CD molecules by negatively charged groups or methylation of the hydroxyl groups greatly reduces the catalyzing effect of CD. It is likely that the catalyzing effects of CDs on β-lactam degradation can be prevented by full substitution of the hydroxyl groups by neutral or negatively charged moieties.

3. Experimental

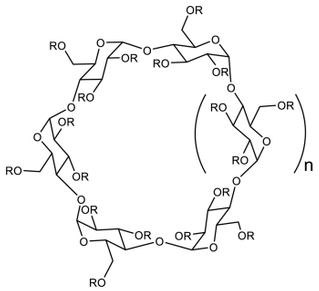
3.1. Materials

Penicillin G potassium salt was purchased from AppliChem (Dramstadt, Germany). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) molar substitution (MS) of 0.65 (Mw 1400 Da), randomly methylated β-cyclodextrin (RMβCD) with degree of substitution (DS) of 12.6 (Mw 1312 Da), γ-cyclodextrin (γCD), randomly methylated γ-cyclodextrin (RMγCD) with DS of 14.4 (Mw 1500 Da), and 2-hydroxypropyl-γ-cyclodextrin (HPγCD) with DS of 4.0-5.6 (Mw 1576 Da) was purchased from Wacker Chemie (Munich, Germany). Sulfobutyl ether γ-cyclodextrin (SBEγCD) (sodium salt) with DS of 4.8 was kindly donated by CyDex Pharmaceuticals (Lenexa, KS). (2-Hydroxy-3-N,N,N-trimethylamino)propyl βCD hydrochloride (HTMAPβCD) with DS of 3.2, (2-hydroxy-3-N,N,N-trimethylamino)propyl βCD hydrochloride – polymer (polymeric HTMAPβCD) (cross linked with epichlorohydrin) with DS of 2.3, 2-hydroxy-3-N,N,N-trimethylamino)propyl γ–CD (HTMAPγCD) chloride with DS of 4.1 and 4.5, carboxymethyl γCD (CMγCD) sodium salt with DS of 5.9 and 8.5, 2-hydroxy-3-N,N,N-trimethylamino)propyl αCD (HTMAPαCD) chloride with DS of 3.2 were kindly donated by CycloLab, Hungary. Carboxymethylcellulose sodium salt (CMC) with MW 90,000 Da, hexadimethrine bromide (HTMB) and poloxamer 407 (poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol)) were purchased from Sigma-Aldrich (St. Louis, MO). Milli-Q water (Millipore, Billerica, MA) was used for preparation of all solutions and the mobile phase for HPLC measurement. All other chemicals were commercially available products of special reagent grade.

3.2. Kinetic studies

All kinetic experiments were performed in aqueous CD buffer solutions containing from zero to 6.0% (w/v) CD. The buffer systems used were

Table 3: Values of k_c and $K_{1:1}$ of benzylpenicillin CD aqueous solution. k_f was determined to be 2.27×10^{-3} h^{-1} at pH 7.4 and 37 °C



Charge	Cyclodextrin	R is H or	k_c (h^{-1})	k_c/k_f	$K_{1:1}$ (M^{-1})
Neutral	RMβCD	$-CH_3$	6.00×10^{-3}	2.6	190
	RMγCD	$-CH_3$	5.24×10^{-3}	2.3	150
	HPβCD	$-CH_2CHOHCH_3$	1.28×10^{-2}	5.6	61
	HPγCD	$-CH_2CHOHCH_3$	1.82×10^{-2}	8.0	1
	γCD		1.49×10^{-2}	6.6	42
Positive	HTMAPβCD	$-CH_2CHOHCH_2N(CH_3)_3^+ Cl^-$	2.56×10^{-2}	11	64
	polymeric HTMAPβCD	$-CH_2CHOHCH_2N(CH_3)_3^+ Cl^-$	1.04	460	1100
	HTMAPγCD DS 4.1	$-CH_2CHOHCH_2N(CH_3)_3^+ Cl^-$	1.33×10^{-2}	5.9	220
	HTMAPγCD DS 4.5	$-CH_2CHOHCH_2N(CH_3)_3^+ Cl^-$	4.06×10^{-2}	18	28
	HTMAPαCD	$-CH_2CHOHCH_2N(CH_3)_3^+ Cl^-$	3.65×10^{-2}	16	28
Negative	CMγCD DS 5.9	$-CH_2COO^- Na^+$	2.55×10^{-3}	1.1	120
	CMγCD DS 8.5	$-CH_2COO^- Na^+$	2.66×10^{-3}	1.2	170
	SBEγCD	$-(CH_2)_4SO_3^- Na^+$	6.71×10^{-2}	30	5900

Table 4: Values of k_{obs}, k_c and $K_{1:1}$, of benzylpenicillin at 37 °C and pH 7.4 at several ternary complexes of water soluble polymers and HPγCD. k_f was determined to be 2.27×10^{-3} h^{-1} at pH 7.4 and 37 °C

polymer	k_{obs} (h^{-1})				k_c (h^{-1})	k_c/k_f	$K_{1:1}$ (Mk^{-1})
	0.0%	0.5% HPγCD	2.0% HPγCD	4.0% HPγCD			
No polymer	2.27×10^{-3}	2.70×10^{-3}	4.25×10^{-3}	5.10×10^{-3}	1.82×10^{-2}	8.0	1
0.25% Hexadimethrine bromide	3.23×10^{-3}	3.97×10^{-3}	5.03×10^{-3}	5.80×10^{-3}	6.14×10^{-3}	1.9	245
0.50% Hexadimethrine bromide	3.07×10^{-3}	3.93×10^{-3}	4.60×10^{-3}	5.30×10^{-3}	5.35×10^{-3}	1.7	364
0.25% CMC	3.00×10^{-3}	2.80×10^{-3}	3.77×10^{-3}	4.13×10^{-3}	5.48×10^{-3}	1.8	63
0.50% CMC	2.63×10^{-3}	3.20×10^{-3}	4.05×10^{-3}	4.70×10^{-3}	5.15×10^{-3}	2.0	150
0.25% Poloxamer	2.55×10^{-3}	3.27×10^{-3}	4.30×10^{-3}	5.03×10^{-3}	5.70×10^{-3}	2.2	129
0.50% Poloxamer	2.20×10^{-3}	3.10×10^{-3}	4.13×10^{-3}	4.40×10^{-3}	5.18×10^{-3}	2.4	127

0.2 M hydrochloric acid (pH 1.2), 0.1 M citric acid (pH 2.5), 0.1 M acetate (pH 4.5), phosphate-buffered saline (PBS) (pH 7.4) and 0.05 M borate buffer (pH 9.6). The ionic strength of the media was not adjusted. Prior to addition of drug the aqueous buffer solutions were equilibrated at 37.0 ± 0.1 °C in a water bath. Benzylpenicillin (10%) stock solution was prepared by dissolving the drug in aqueous 30% (v/v) methanol solution. The benzylpenicillin stock solution (55 µl) was added to the aqueous buffer solutions (6 ml) that resulted in less than 1% dilution of the buffer solution. The small amount of the volatile methanol (less than 0.3%) in the final reaction media did not affect the degradation kinetics. The initial benzylpenicillin concentration was 2.46 mM. The degradation rate was followed by monitoring the remaining drug concentration by reversed-phase high-performance liquid chromatographic (HPLC) method.

3.3. HPLC analysis

Quantitative determination of penicillin G was performed on a reversed-phase high-performance liquid chromatographic (HPLC) component system from Dionex Softron GmbH (Germany) Ultimate 3000 Series, consisting of a LPG-3400A pump with a built-in degasser, a WPS-3000-TSL autosampler column compartment, a VWD-3100 variable wavelength UV-Vis detector and Phenomenex Luna C18 150 mm × 4.60 mm, 5 micron column (Phenomenex, UK) with a matching HPLC KrudKatcher Ultra Column In-Line Filter (Phenomenex, UK). The mobile phase consisted of acetonitrile and aqueous 0.05 M KH_2PO_4 pH 5.1 solution (30:70 volume ratios). The flow rate was 1.0 ml/min, sample injection volume was 20 µL and the retention time was 2 min. Initially a system giving much longer drug retention was tested but it was redesigned to allow for rapid repeated injection during fast drug degradation. The degradation products of penicillin G are very hydrophilic and come out with the solvent front under these HPLC conditions. The degradation followed pseudo first-order kinetics and rate constants were determined from plots of the natural logarithm of the peak heights versus time plots (Loftsson, 2014). The correlation of the plots was close to unity and no interfering peaks were detected.

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Conflict of interest: There are no conflicts of interest.

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