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Rapid Communication

Cyclodextrin-accelerated degradation of β-lactam antibiotics in aqueous solutions

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Summary

The catalytic effect of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, 2-hydroxypropyl-γ-cyclodextrin and glucose on the degradation of aztreonam and phenoxymethylpenicillin in aqueous buffer solutions has been studied. All three cyclodextrin derivatives and glucose increased the rate of degradation of aztreonam. The two hydroxypropyl cyclodextrin derivatives also accelerated the degradation of phenoxymethylpenicillin. This effect of cyclodextrins on β-lactam antibiotics will limit their usage in pharmaceutical preparations containing these drugs.

Aztreonam was the first totally synthetic monocyclic β-lactam (monobactam) antibiotic (Sykes et al., 1981). It is active against Gram-negative aerobic bacteria, including *Pseudomonas aeruginosa*, and has a high degree of resistance to hydrolysis by β-lactamases. It must be administered parenterally when used to treat systemic infections, since its absolute bioavailability is only about 1% after oral administration (Brogden and Heel 1986). Aztreonam undergoes hydrolysis of the β-lactam ring in aqueous solutions. The pH of maximum stability is 6.0 and at this pH and 35 °C the half-life is approx. 130 days (Pipkin, 1986).

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It is well known that cyclodextrins, which are cyclic oligosaccharides with a void cavity in the center, are capable of forming inclusion complexes with many drugs. The complexation usually alters the physicochemical properties of the drug molecule, such as the aqueous solubility and stability (Loftsson and Bodor, 1989; Loftsson et al., 1989; Duchêne and Wouessidjewe, 1990). Generally, the stability of the drugs in aqueous solutions has been improved by this type of complexation due to slower degradation of the drug within the complex than out in the solution. However, addition of some β- and γ-cyclodextrin derivatives to aqueous buffer solutions containing aztreonam had destabilizing effects on the drug. We also found that these same cyclodextrin derivatives affected the rate of degradation of phenoxymethylpenicillin. In this paper, we report our findings and

compare them with the destabilizing effects of glucose and metal ions.

Aztreonam was supplied by courtesy of the Squibb Institute of Medical Research, U.S.A. Phenoxymethylpenicillin was obtained from Apodan, Denmark. The following cyclodextrins (CDs) were used as supplied, without further purification: heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD, Sigma, U.S.A.), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, Pharmatec, U.S.A.), and 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD, Pharmatec). All other chemicals used in this study were commercially available products of special reagent grade.

The quantitative determination of aztreonam was performed on a high performance liquid chromatographic (HPLC) equipment consisting of a Waters model 6000A pump, Waters model U6K injector, Beckman Ultrasphere ODS 5 μm column (150 × 4.5 mm) and Waters model 440 absorbance detector operated at 254 nm. The mobile phases consisted of methanol, water and acetic acid (25 : 73 : 2) and acetonitrile and 0.02 M phosphate buffer, pH 7.74 (27 : 73) for aztreonam and phenoxymethylpenicillin, respectively. For aztreonam the retention time was 2.4 min at 1.0 ml/min flow rate, and for phenoxymethylpenicillin, 2.1 min at 1.5 ml/min flow rate.

The kinetic experiments were carried out in aqueous 0.23 M Tris or 0.05 M carbonate buffer solutions containing various amounts of DM-β-CD, HP-β-CD, HP-γ-CD, glucose or metal ions. In some cases the disodium salt of EDTA was added to the reaction medium. The ionic strength of each buffer solution was adjusted to 0.5 by addition of a calculated amount of sodium chloride. Stock solution of the drug in methanol was added to the aqueous buffer solutions, previously equilibrated at the desired temperature in a water bath, and mixed thoroughly. The initial concentration of aztreonam was 4×10^{-2} mg/ml and that of phenoxymethylpenicillin 0.2 mg/ml. All reactions were run under pseudo-first-order conditions. Aliquots (20 μl) were injected into the column at various time intervals, and the pseudo-first-order rate constants (K_{obs}) determined from the disappearance of the drug by linear regression of natural logarithm of the peak height vs time plots.

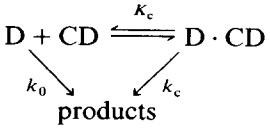
The rate of degradation of aztreonam and phenoxymethylpenicillin was determined in aqueous buffer solutions containing five different CD concentrations. The stability constant (K_c) for the CD complexes and the pseudo-first-order rate constants (k_c) for degradation of the drugs within the CD complexes were calculated from Lineweaver-Burk plots assuming formation of 1 : 1 complex:

$$\frac{k_0}{k_0 - k_{obs}} = \frac{k_0}{k_c(k_0 - k_c)[CD]} + \frac{k_0}{(k_0 - k_c)}$$

where k_0 represents the pseudo-first-order rate constant for the degradation of the free drug. The values of k_c and K_c for a given drug-CD complex were calculated from the intercept and the slope

TABLE 1

The first-order rate constants and the stability constants for the degradation of aztreonam and phenoxymethylpenicillin in aqueous pH 9.8 carbonate buffer solutions



Cyclo-dextrin	Aztreonam				Phenoxymethylpenicillin			
	$k_0(\times 10^2)$ (min^{-1})	$k_c(\times 10^2)$ (min^{-1})	K_c (M^{-1})	T (°C)	$k_0(\times 10^2)$ (min^{-1})	$k_c(\times 10^2)$ (min^{-1})	K_c (M^{-1})	T (°C)
HP-β-CD	9.3	12.3	74.68	65	13.8	25.0	50.24	65
HP-γ-CD	9.3	22.3	16.70	65	13.8	25.0	40.51	65
DM-β-CD	1.0	162	1.96	45	2.00	0.94	156.5	45

TABLE 2

Observed first-order rate constants for the degradation of aztreonam in aqueous pH 7.73 and 7.99 carbonate buffer solutions and pH 7.21 and 8.15 Tris buffer solution containing various amounts of glucose at 75°C

% (w/v) glucose	k_{obs} ($\times 10^2$) (min^{-1})			
	pH 7.21	pH 7.73	pH 7.99	pH 8.15
0	0.860	1.19	2.38	3.74
5	1.88	5.73	8.18	12.6
10	2.75	8.87	3.30	18.8

of a linear plot obtained when $k_0/(k_0 - k_{obs})$ was plotted against the reciprocal total CD concentration (1/[CD]).

Both aztreonam and phenoxymethylpenicillin degraded according to pseudo-first-order kinetics. The rate of degradation of aztreonam was significantly faster within the cyclodextrin complex than outside it in the solution (Table 1). For the hydroxypropyl derivatives (HP-β-CD and HP-γ-CD) the rate was 1.3–2.4-times faster, but for DM-β-CD it was much more rapid. Similar results were obtained from phenoxymethylpenicillin, although DM-β-CD had some stabilizing effect on the drug (Table 1). Glucose (Table 2) also had a destabilizing effect on aztreonam, but the transition-metal ions had insignificant effects (Table 3). Thus, it appears that the monocyclic β-lactam antibiotic aztreonam behaves towards carbohydrates in a manner similar to that of penicillins and cephalosporins (Page, 1987). Further, the CDs tested generally have an accelerating effect on the rate of degradation of the two β-lactam antibiotics in alkaline solutions and in this way behave like carbohydrates, such as glucose and dextrans, and other polyhydric alcohols with adjacent hydroxy groups (Bundgaard and Larsen, 1978, 1983; Fujiwara et al., 1985). This will limit the usage of CDs in pharmaceutical formulations containing β-lactam antibiotics.

TABLE 3

Observed first-order rate constants for the degradation of aztreonam in aqueous pH 7.21 Tris buffer solution containing various metal ions at 75°C (metal ion concentration, 1.0×10^{-4} M; EDTA concentration, 1.0×10^{-3}M)

Metal ion	k_{obs}($\times 10^3$) (min^{-1})
None	8.6
None with EDTA	8.6
Co(II)	9.2
Cu(II)	9.3
Fe(II)	9.3
Fe(III)	12.3
Ni(II)	9.3

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