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Rapid Communication

Stabilization of aspartame by cyclodextrins

Marcus E. Brewster [1,2], Thorsteinn Loftsson [3], Jóhanna Baldvinsdóttir [3] and Nicholas Bodor [2]

[1] *Pharmatec, Inc., Alachua, Florida (U.S.A.),* [2] *Center for Drug Discovery, University of Florida, J. Hillis Miller Health Center, Gainesville, Florida (U.S.A.) and* [3] *Department of Pharmacy, University of Iceland, Reykjavik (Iceland)*

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Summary

The primary and secondary hydroxyl groups on the β-cyclodextrin molecule were selectively substituted by 2-hydroxypropyl ether groups. The influence of 2-hydroxypropyl-β-cyclodextrin, substituted mainly at the primary or the secondary hydroxyl groups, on the hydrolysis rate of aspartame was investigated and compared to the effects of 2-hydroxypropyl-β-cyclodextrin (Encapsin ™) from Janssen, a mixture of maltosyl- and dimaltosyl-β-cyclodextrin, and 2-hydroxyethyl-β-cyclodextrin. All the cyclodextrin derivatives decreased the rate of hydrolysis of aspartame in aqueous solution. The distribution of the 2-hydroxypropyl substituent on the β-cyclodextrin molecule affected its stabilizing effect.

Cyclodextrins are cyclic oligosaccharides with hydroxyl groups on the outer surface and a void cavity in the center. Their outer surface is hydrophilic, and therefore they are usually soluble in water, however, the cavity has a lipophilic character. Cyclodextrins are known to form non-covalent inclusion complexes with a wide variety of guest molecules, both in the solid state and in aqueous solutions (Loftsson et al., 1990, 1991; Szejtli, 1991a, b). Substitution of the primary and secondary hydroxyl groups on the cyclodextrin molecule usually results in mixtures of isomers (Pitha and Pitha, 1985). One such cyclodextrin derivative is 2-hydroxypropyl-β-cyclodextrin which is formed by treating a base-solubilized solution of β-cyclodextrin with propylene oxide (Pitha et al., 1986). Recently, it has been shown that the basicity of the reaction medium affects the regioselectivity (Pitha et al., 1990). The secondary hydroxyl groups on the cyclodextrin molecule, i.e. OH-2 and OH-3 on the glucosepyranose units of the molecule, are more acidic than the primary hydroxyl groups, i.e. OH-6. Thus, alkylation of OH-6, the most accessible hydroxyl group, was favored in a strong basic solution and alkylation of 2-OH, the most acidic hydroxyl group, was favored in a weak basic solution.

Aspartame (*N*-L-α-aspartyl-L-phenylalanine 1-methyl ester) is an artificial sweetener commonly used as a food additive and also in some drug formulations, but its instability in aqueous solutions has limited its usage. In this communication, we report our study on the effects of three different 2-hydroxypropyl ethers of β-cyclo-

Correspondence: T. Loftsson, Department of Pharmacy, University of Iceland, IS-101 Reykjavik, Iceland.

dextrin, as well as of a mixture of maltosyl- and dimaltosyl-β-cyclodextrin, and of 2-hydroxyethyl-β-cyclodextrin on the stability of aspartame.

Aspartame was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), β-cyclodextrin (β-CD) from Chinoin Pharmaceutical and Chemical Works Co. Ltd (Budapest, Hungary), 2-hydroxyethyl-β-cyclodextrin (HE-β-CD) from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD; Encapsin™) from Janssen Biotech N.V. (Olen, Belgium) and a mixture of maltosyl- and dimaltosyl-β-cyclodextrin (3:7) (M/DM-β-CD) from Ensuiko Sugar Refining Co. (Yokohama, Japan). Standard preparations of various HP-β-CD isomers were supplied by the courtesy of Dr. Josef Pitha (National Institutes of Health, NIA/GRC, Baltimore, MD, U.S.A.). All other chemicals used in this study were commercially available products of special reagent grade.

The primary (P-HP-β-CD) and secondary (S-HP-β-CD) 2-hydroxypropyl-β-cyclodextrin derivatives were synthesized according to a method previously described by Pitha and co-workers (1990). Briefly, β-CD was dissolved at 60 °C by stirring in aqueous solution containing 30% (P-HP-β-CD) or 1.5% (S-HP-β-CD) sodium hydroxide. Then the solution was cooled in an ice bath and treated with propylene oxide. After stirring overnight, first in an ice bath and then at room temperature, the solution was neutralized with sulfuric acid and evaporated under vacuum. The residue was extracted with ethanol, the extracts evaporated, and after dissolution in water dialyzed for about 3 h against water. Finally, the aqueous solution was lyophilized. The two products were characterized by high-field ^{1}H- and ^{13}C-NMR as well as fast atom bombardment (FAB) mass spectrometry, and compared to standards. The molecular substitution (MS) of P-HP-β-CD was determined to be 1.7 with 60% of the 2-hydroxypropyl groups located at the 6-position, i.e. at the primary 6-O. The MS of S-HP-β-CD was determined to be 0.9 with over 50% of the 2-hydroxypropyl groups located at the 2-position, i.e. one of the two possible secondary positions, and less than 10% at the 6-position. The HP-β-CD obtained from Janssen had MS of 0.45.

The quantitative determination of aspartame was performed on a high-performance liquid chromatographic (HPLC) equipment consisting of a Milton Roy ConstaMetric 3000 solvent delivery system, a Rheodyne 7125 injector, a Beckman Ultrasphere ODS 5 μm (150 × 4.6 mm) column and a Spectra-Physic SP8450 UV/Vis detector operated at 252 nm. The mobile phase consisted of methanol and aqueous 0.05 M citrate buffer, pH 4.7 (4:6). The retention time was 2.4 min at 1.50 ml/min flow rate (U.S.P., 1990).

The kinetic experiments were carried out in aqueous 0.1 M hydrochloric acid solution (pH 1.05), 0.2 M acetate buffer solution (pH 5.49), or 0.065 M phosphate buffer solution (pH 7.72) containing various amounts of P-HP-β-CD, S-HP-β-CD, HP-β-CD, M/DM-β-CD or HE-β-CD. The ionic strength of the reaction medium was not adjusted. Stock solution of aspartame in water was added to the aqueous reaction media, previously equilibrated at the desired temperature in a water bath, and mixed thoroughly. The initial aspartame concentration was 1.7×10^{-4} M. 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 constant (k_{obs}) determined from the disappearance of the drug by linear regression of natural logarithm of the peak height against time plots. Each experiment was repeated at least three times and the values reported are the means ± S.D.

At pH 7.72 the rate of hydrolysis of aspartame was determined in aqueous buffer solutions containing four different P-HP-β-CD or S-HP-β-CD concentrations [CD]. The stability constant (K_c) for the cyclodextrin complexes and the pseudo-first-order rate constants (k_c) for degradation of the drugs within the cyclodextrin 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-cyclo-

TABLE 1

Observed first-order rate constants for the degradation of aspartame in aqueous solution containing various β-cyclodextrin derivatives

pH	Temperature (°C)	Cyclodextrin [a]	$k_0 \pm$ S.D. ($\times 10^2$) (min^{-1})	$t_{1/2}$ (min)
1.05	75.0	No cyclodextrin	0.261 ± 0.006	266
		2.0% P-HP-β-CD	0.199 ± 0.007	348
		2.0% S-HP-β-CD	0.225 ± 0.012	308
5.49	80.0	No cyclodextrin	0.311 ± 0.018	223
		5.0% P-HP-β-CD	0.276 ± 0.045	251
		5.0% S-HP-β-CD	0.213 ± 0.021	325
7.72	55.0	No cyclodextrin	6.50 ± 0.13	10.7
		1.0% P-HP-β-CD	4.95 ± 0.04	14.0
		1.0% S-HP-β-CD	5.63 + 0.11	12.3
		1.0% HP-β-CD	5.21 ± 0.09	13.3
		1.0% M/DM-β-CD	5.30 ± 0.06	13.1
		1.0% HE-β-CD	5.21 ± 0.05	13.3

[a] Concentration is in % (w/v).

dextrin complex were calculated from the intercept and the slope of a linear plot obtained when $k_0/(k_0 - k_{obs})$ was plotted vs the reciprocal total cyclodextrin concentration (1/[CD]).

All the cyclodextrins tested stabilized aspartame in aqueous solutions, increasing its half-life ($t_{1/2}$) from 13 to almost 50% (Table 1). In a basic solution at pH 7.72, the greatest stabilizing effect was obtained with P-HP-β-CD. At that pH the P-HP-β-CD-aspartame complex had a much larger stability constant (K_c) than that of the S-HP-β-CD-complex, but S-HP-β-CD had a much better stabilizing effect on the drug located within the complex, i.e. lower k_c (Table 2).

Cyclodextrins are torus-shaped molecules with a hydrophobic cavity in the center and hydroxyl groups on the edges. The secondary hydroxyl groups are located on the wider side and the primary hydroxyl groups on the narrow side. If the aspartame molecules form complexes with the β-CDs by moving into the cavity from the wider side of the torus, then hydroxypropyl groups located on that side, i.e. hydroxypropyl ethers of the secondary hydroxyl groups, would result in steric hindrance on complex formation. This could explain the lower K_c of S-HP-β-CD in Table 2. At the same time the hydroxypropyl groups would provide extra protection of the aspartame molecules located within the complex against hydroxyl ions, i.e. specific base-catalyzed hydrolysis.

In conclusion, β-CDs have a stabilizing effect on aspartame in aqueous solutions. Regioselective substitution of the hydroxyl groups on the β-CD can result in derivatives possessing different complexing properties.

TABLE 2

First-order rate constants and stability constants for the degradation of aspartame in aqueous pH 7.72 phosphate buffer solution at 55.0 °C

Cyclodextrin	k_0 ($\times 10^2$) (min^{-1})	k_c ($\times 10^2$) (min^{-1})	K_c (M^{-1})
P-HP-β-CD	6.50	3.46	181
S-HP-β-CD	6.50	2.34	34

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