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Research Article

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Biocompatible and biodegradable *β*-cyclodextrin conjugate of butanoic acid: A potential formulation for colon health

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ABSTRACT

Butanoic acid is beneficial in the maintenance of colon health, and is the preferred energy substrate for the cells in the colon. Here, butanoic acid was covalently attached to β-cyclodextrin through ester linkage using sodium hydride as the deproton reagent, and the preliminary release behavior of butanoic acid in the colonic contents of rats was investigated at 37 °C. Butanoic acid was almost released 57.3% from the β-cyclodextrin conjugate after 12 h in the colonic contents of rats, via the biodegradation by glycosidases and hydrolases in the colon. This fact demonstrates that β-cyclodextrin could act as a carrier for an orally administered colon-targeting delivery of butanoic acid as a nutrient.

Keywords: Colon-targeting delivery, Nutrition, Butanoic acid, Cyclodextrin, Release behavior.

INTRODUCTION

Short-chain fatty acids (SCFA) are beneficial substrates of human nutrition owing to their various roles in health aspects, such as the functionality of carbohydrate and calorie conservation [1, 2]. Butanoic acid (BA), an important member of SCFA, is thought to play a significant role in the maintenance of mucosal health, and is the preferred energy substrate for the cells in the colon [3, 4]. As a matter of fact, butanoic acid is liquid, acrid taste, and has an unpleasant smell. Butanoic acid enemas smell bad, and patients are not always willing to undergo the treatment. Another problem with enemas is that butanoic acid doesn't stay in the colon for very long [5]. Therefore, a colon-targeting delivery system of butanoic acid is expected to be a promisingly potential formulation for the nutrition of colon.

β-Cyclodextrin (*β*-CyD) is a well-known macrocyclic oligosaccharide consisted of 7 *α*-1, 4-linked D-glucopyranose units (Figure 1). *β*-CyD is hardly hydrolyzed and only slightly absorbed through the stomach and small intestine, but fermented into small saccharides by colonic microflora. Thus *β*-CyD is absorbed as small saccharides in the large intestine [6], and this biocompatibility makes *β*-CyD particularly useful in the pharmaceutical and food industries. In the pharmaceutical industry *β*-CyD is used as a complexing agent for improving some properties of drugs, such as solubility, stability, absorption and/or bioavailability, by forming the inclusion complexes [7-10]. However the complexes are in equilibrium with guest and host molecules. When a complex is orally applied, it readily dissociates in the gastrointestinal fluid, depending on the magnitude of the stability constant. This indicates that *β*-CyD complexes are not suitable for colon-targeting delivery [11]. One of the methods to circumvent the dissociation is to bind a drug covalently to *β*-CyD. In recent years, several drug/*β*-CyD conjugates and their pharmaceutical properties have been reported [12-17].

Here the authors report the preparation of BA/*β*-CyD conjugate, attempting to construct a colon-targeting delivery of BA as a nutrient. The preliminary release behavior of BA in the colonic contents of rats was investigated.

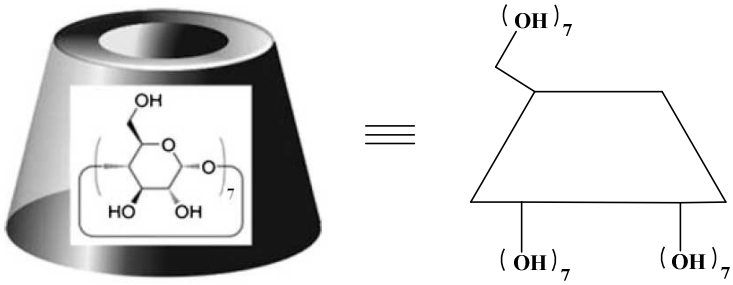


Fig. 1. The chemical structure of *β*-CyD

EXPERIMENTAL SECTION

Materials

β-CyD was recrystallized twice from distilled water and dried under reduced pressure at 110 °C for 24 h before use. *N*, *N*-dimethylformamide (DMF) was freshly distilled over CaH_2 and stored over 4A molecular sieves. Dichloromethane (DCM) was dried by $CaCl_2$ for 12 h and distilled prior to use. All other chemicals were of commercial grade without further purification.

Analytical methods

FT-IR spectra were recorded on a PerkinElmer Spectrum 100 Series FT-IR spectrometer. The HPLC assays were performed on a Perkin-Elmer Series 200 HPLC system using a Kromasil 100-10-C18 column (4.6 mm×250 mm); flow rate: 1.0 cm^3/min; detection wavelength: 220 nm; the mobile phase: methanol-0.05 M phosphate buffer (pH 2.0, 20:80 v/v).

Preparation of the BA/β-CyD conjugate

To a solution of butanoic acid (0.62 g, 7.05 mmol) in 60 cm^3 DCM, oxalyl chloride (1.80 cm^3) was added at room temperature. After the addition of five drops of DMF, the mixture was stirred overnight with a reflux condenser. After completion of the reaction, the excess oxalyl chloride was removed under reduced pressure. Thus, the crude butanoyl chloride was obtained and dissolved in DMF (10.0 cm^3), which was used in the next step.

NaH (60% in mineral oil, 0.284 g, 7.05 mmol) was added to a solution of *β*-CyD (2.0 g, 1.76 mmol) in DMF (80 cm^3) at 0 °C, and the mixture solution was stirred overnight. The above butanoyl chloride in DMF was added, and the mixture was stirred while allowing it to stand at room temperature for 10 h. It was evaporated under reduced pressure to a volume of ca. 5 cm^3, and acetone (300 cm^3) was added to precipitate the *β*-CyD derivatives. The precipitate was filtered and dried. The crude products were isolated by an open RP-18 column using H_2O-MeOH (10%-20%-60%-100%) as eluents. Thus, **1** was obtained in 32.2% yields (0.74 g), x = 2.4 (Average degree of substitution).

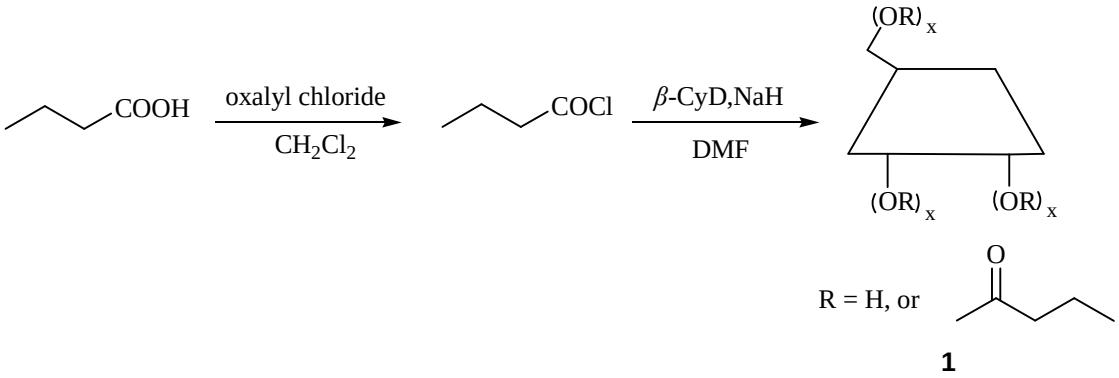
Hydrolysis of the BA/β-CyD conjugate incubated with the colonic contents of rats

The hydrolysis behavior, incubated with the colonic contents of male Kunming rats, was performed at 37 °C according to a literature procedure [17], i.e. male Kunming rats (200 ± 10 g) were anesthetized by diethyl ether and midline incisions were made. Contents of colon were collected, and diluted to half concentration with isotonic phosphate buffer (pH 6.8). Then the dispersions of contents were filtered through a gauze to remove large particles. The conjugate solution (10.0 cm^3, 4.0×10^{-3} M in the corresponding isotonic buffer) was added to the filtrate (10.0 cm^3) in air-tight vessels and incubated at 37 °C. The pH of incubation solutions was adjusted to 6.8 by the addition of small amounts of 0.1 M NaOH. Every one or two hours, an aliquot (1.0 cm^3) of the reaction solution was adjusted to pH 2.0 by the addition of 1.0 M HCl, and BA was extracted out by diethyl ether (3×3.0 cm^3). Then, the combined organic phases were evaporated under reduced pressure, and the residue was dissolved in methanol (0.1 cm^3). The concentration of BA was determined by HPLC.

RESULTS AND DISCUSSION

Chemistry

According to the methods for functionalization of *β*-CyD with sodium hydride [18], the BA/*β*-CyD conjugate was prepared in two steps as shown in Scheme 1. In the first step, butanoyl chloride was prepared using oxalyl chloride as a chlorinating agent. In the second step, the coupling of BA to *β*-CyD was accomplished in basic media using NaH as the deproton reagent. Therefore, BA was bonded to *β*-CyD through ester linkage. The IR spectrum of **1** showed the obviously characteristic bands, i.e. it was the absorption band of ester group at 1746 cm^{-1}.



Scheme 1. The preparation routes of the BA/*β*-CyD conjugate

Hydrolysis of the conjugate in the colonic contents of rats

The preliminary release behaviors were carried out for the BA/*β*-CyD conjugate. As shown in Figure 2, BA was nearly released 57.3% from the conjugate after incubated with the colonic contents of rats for 12 h, and merely released 18.6% after incubated without the colonic contents. Moreover, the release rate of BA in the colonic contents of rats was faster than that without the colonic contents. This fact indicates that the BA/*β*-CyD conjugate could release BA in the colon after oral administration, and its rate was relatively slow.

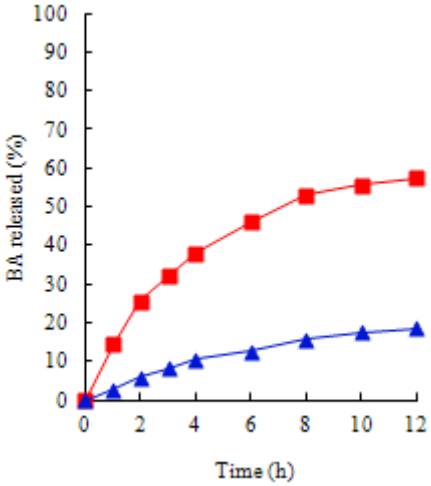


Fig. 2. The release behaviors of BA from the BA/*β*-CyD conjugate in the absence (▲) and presence (■) of rat colonic contents (25%, w/v) in phosphate buffer (pH 6.8) at 37 °C

CONCLUSION

Butanoic acid is the preferred energy substrate for the cells in the colon, and a colon-targeting delivery system of it is expected to be a promising formulation for the nutrition of colon. Here, butanoic acid was covalently attached to *β*-cyclodextrin through ester linkage, and butanoic acid was nearly released up to 57.3% from the conjugate after incubated with the colonic contents of rats for 12 h, via the biodegradation by glycosidases and hydrolases in the colon. This fact demonstrates that *β*-cyclodextrin could act as a carrier for an orally administered colon-targeting delivery of butanoic acid as a nutrient.

Acknowledgments

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