Synthesis, Cytotoxicity, and Phase-Solubility Study of Cyclodextrin Click Clusters

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Received 24 February 2014; revised 28 June 2014; accepted 8 July 2014

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24107

ABSTRACT: To explore the possibility of cyclodextrin click clusters (CCCs) as a new cyclodextrin-based excipient, we prepared three different CCCs; heptakis{6-(4-hydroxymethyl-1H-[1,2,3]triazol-1-yl)-6-deoxy}-β-cyclodextrin (HT-β-CD), heptakis{6-(4-hydroxymethyl-1H-[1,2,3]triazol-1-yl)-6-deoxy}{2,3-di-*O*-methyl}-β-cyclodextrin (HT-β-CD(OMe)$_2$), and heptakis{6-(4-sulfonylmethyl-1*H*-[1,2,3]triazol-1-yl)-6-deoxy}-β-cyclodextrin (ST-β-CD). The CCCs were prepared using copper(I)-catalyzed azide-alkyne cycloaddition from 6-azido-6-deoxy-β-CD and their water solubility, cytotoxicity, and drug-solubilizing effect were investigated. Water turbidity testing of the CCCs showed that the minimum water solubility of the CCCs is at least 20 times higher than that of β-CD. An MTT cell viability assay performed on HeLa cells demonstrated a low cytotoxicity of the CCCs compared with 2,6-dimethyl-β-cyclodextrin. HT-β-CD(OMe)$_2$ and ST-β-CD did not demonstrate any cytotoxicity within the experimental concentration (~5 mM) like 2-hydroxypropyl-β-CD. A phase-solubility study of prednisolone with the CCCs suggested that CCCs showed increased solubility of prednisolone in the presence of increasing concentrations of the CCCs. The comparison between the conventional CD derivatives and CCCs on solubility, cytotoxicity, and binding property implies that CCCs are alternative cyclodextrin derivatives useful for overcoming the restrictions of conventional cyclodextrin chemistry. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: cyclodextrins; complexation; excipients; solubility; encapsulation; copper(I)-catalyzed azide-alkyne cycloaddition; cyclodextrin click cluster; cytotoxicity

INTRODUCTION

Cyclodextrin click clusters (CCCs) are a kind of chemically modified cyclodextrin (CD) derivative, in which all the primary alcohols of CDs are transformed to triazole units by copper(I)-catalyzed azide-alkyne cycloaddition (CuAAc) (Scheme 1a). The second face of CDs can be also modified through CuAAc, but the cases of secondary face modification are relatively rare.[1] The CuAAC reaction is modular, specific, wide in scope, and provides high yields. The two reaction counterparts, azide and alkyne, are inert to most biological and organic conditions, including highly functionalized biological molecules, water, and the majority of common reaction conditions in organic synthesis.[2] Thus, the CuAAC reaction was adapted by many fields of organic chemistry.[3] In particular, the CuAAC reaction was quickly introduced to the chemistry of CDs, and since 2007, the amount of work published dealing with CDs and CuAAC has increased greatly.[4] In addition to the synthetic advantages, CCCs have unique, structural characteristics including well-defined structures, precise molecular weights, and multivalent functionalization sites. CCCs have been extensively exploited in polymers,[5,6] nucleic acid carriers,[7–9] sensors,[10] glycoconjugates,[11–13] multivalent scaffolds,[14–16] and magnetic resonance imaging probes.[17,18]

Several CDs are used industrially in pharmaceutical and allied applications.[19] The ability to utilize the hydrophobic cavity of CD to encapsulate bioactive molecules in water has drawn tremendous interest from the pharmaceutical industry because encapsulation improves the stability and bioavailability of cargo compounds.[20]

Natural α-, β-, γ-CD, which consist of six, seven, or eight glucose units, were widely used, but the chemically modified CD derivatives, like hydroxypropyl-β-CD, hydroxypropyl-γ-CD, sulfobutyl ether-β-CD, methyl β-CD (Scheme 1b) were already marketed as excipients in the pharmaceutical formulation.[21]

Chemically modified CD derivatives have merits beyond natural CDs in some applications. For example, sulfobutyl ether-β-CD can solve the nephrotoxicity of β-CD observed in parenteral injection. β-CD itself is the most commonly used CD, although it is the least soluble (solubility in water at 20°C; 1.85 g/100 mL). When parenterally administered, β-CD is not metabolized but accumulates in the kidneys as insoluble cholesterol complexes, resulting in severe nephrotoxicity.[22] Sulfobutyl ether-β-CD has been developed as a means for solving the solubility problem observed with parenteral β-CD administration.[23]

However, the conventional modification methods of CDs have a few drawbacks. The common products of conventional synthesis are not homogeneous; they are mixtures of compounds with different degrees of substitution.[24] This heterogeneity of modified β-CD derivatives makes it difficult for researchers to precisely investigate the inclusion phenomena at a molecular level. Usually, the reagents and conditions of conventional CD modification are limited to either propylene oxide ring opening for 2-hydroxypropyl CD or 1,4-butane sultone ring opening for 4-sulfobutyl ether CD. The reactions offer low efficiency and

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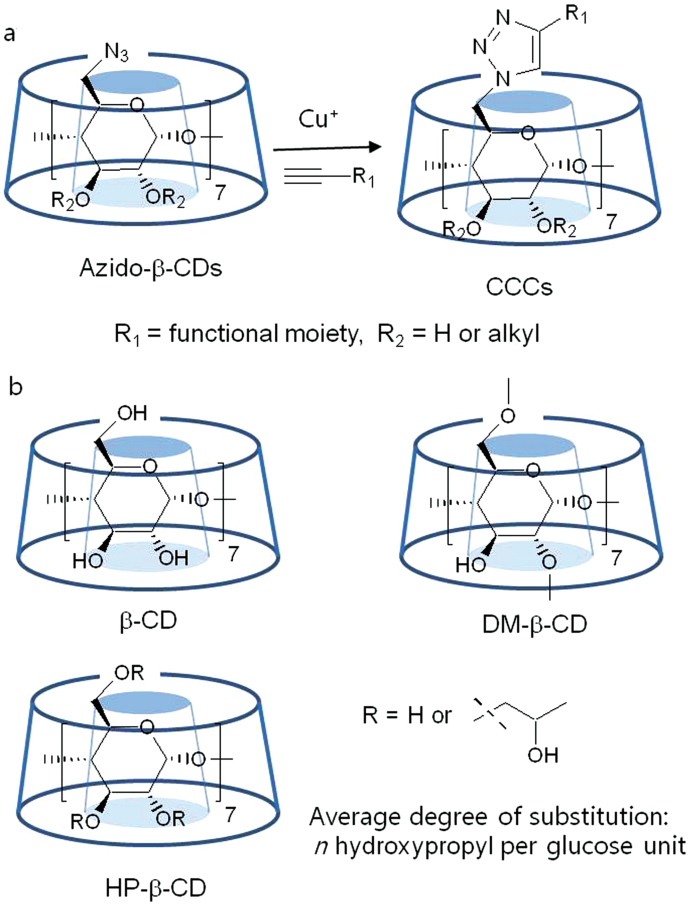
This article contains supplementary material available from the authors upon request or via the Internet at http://wileylibrary.com.

Journal of Pharmaceutical Sciences



Scheme 1. (a) General molecular structure of cyclodextrin click clusters. (b) Molecular structures of β-CD and two reference cyclodextrin derivatives.

are difficult to precisely control. In addition, they would not be suitable for the incorporation of further functional groups on CDs. The bioorthogonality of the CuAAC reaction gives a distinct advantage over traditional CD modification to CCCs.[25]

In order to apply CCCs to the field of drug, food, and cosmetic formulation, three important considerations should be taken into account; CCC synthesis, cellular safety, and cargo encapsulation ability. Unfortunately, CCC studies in the pharmaceutical field are rare. To answer these questions, we prepared three CCC derivatives and investigated water solubility, cytotoxicity, and the drug inclusion properties of each derivative, which were compared with the properties of β-CD and two conventional CD derivatives.

EXPERIMENTAL

Materials

β-Cyclodextrin and prednisolone were purchased from TCI PN C0777and P0637 (Seoul, South Korea). 2-Hydroxypropyl-β-CD (HP-β-CD) and heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) were purchased from Aldrich PN 389145, H0513 (Yongin, South Korea). All purchased reagents were used without purifications. ^{1}H, ^{13}C NMR (nuclear magnetic resonance), HH COSY (homonuclear correlation spectroscopy), and CH HSQC (heteronuclear single quantum coherence) spectra were measured on a Bruker Advance III 600 NMR spectrometer (Seoul, South Korea) equipped with a PABBO BB-1H Z GRD probe head. The other NMR spectra were measured on a JNM-AL300 (JEOL) spectrometer (Seoul, South Korea). Chemical shifts were reported as δ in units of parts per million (ppm), and *J*-values were noted in Hz. MALDI–MS (matrix-assisted laser desorption/ionization mass spectrometry) spectra were measured on a Voyager-DE™ STR Biospectrometry Workstation (Applied Biosystems supplied in Seoul, South Korea). A CEM Discover™ microwave reactor was applied for the microwave-assisted organic synthesis.

Heptakis-(6-chloro-6-deoxy)-β-CD (Cl-β-CD), heptakis-(6-azido-6-deoxy)-β-CD (azido-β-CD), heptakis{6-(4-hydroxymethyl-1H-[1,2,3]triazol-1-yl)-6-deoxy}-β-CD (HT-β-CD), heptakis{6-(4-sulfonylmethyl-1H-[1,2,3]triazol-1-yl)-6-deoxy}-β-CD (ST-β-CD), heptakis(2,3-di-O-methyl)-(6-azido-6-deoxy)-β-CD (azido-β-CD(OMe)$_2$), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA), and sodium propargyl sulfonate were prepared via literature procedures, and the ^{1}H and ^{13}C NMR spectra of the compounds were consistent with the literatures (Supporting Information S1).

The synthesis and NMR characterization of heptakis{6-(4-hydroxymethyl-1H-[1,2,3]triazol-1-yl)-6-deoxy}{2,3-di-*O*-methyl}-β-CD (HT-β-CD(OMe)$_2$) were reported in Supporting Information S1 and S2.

Turbidity Measurement

To each CD, 150 μL of deionized distilled water was added and the mixtures were sonicated (Hwa Shin Power Sonic® 410) for 30 min. The mixture was vigorously vortexed and 120 μL of the mixture was quickly transferred into a 96-microwell plate. The visible absorption at 595 nm was measured by microplate reader (PerkinElmer VICTOR™ 3, photometry mode, supplied in Seoul, South Korea).

MTT Assay

HeLa (human cervical adenocarcinoma cell line) cells were seeded in 96-well culture plate at 2 × 10^4 cells/well and incubated in DMEM (Gibco/BRL, Dulbecco's Modified Eagle's Medium) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) antibiotics (Gibco/BRL) for 24 h. The stock solutions (10 mM) of CDs were prepared in DMEM medium. HeLa cells on a 96-well plate were treated with a series of CD solutions (0, 0.01, 0.1, 1, 2, and 5 mM) and the plate was incubated in a CO_2 incubator at 37°C for 1 day. To each well, 100 μL of MTT was added [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5 mg/mL Stock in PBS, Sigma M5655]. After a 2-h incubation at 37°C, the medium was removed. To dissolve the formazan crystals, 100 μL of dimethyl sulfoxide (Sigma–Aldrich 472301) was added and mixed well via pipette. Then, the absorbance values at 570 nm were measured by microplate reader (PerkinElmer VICTOR™ 3, photometry mode).

Phase Solubility Studies

HPLC was performed on an Agilent 1100 series liquid chromatography system with a diode array detector interfaced with an Agilent Chem Station for data analysis. The column was a 4.6 mm ID × 150 mm Extend-C18 column (5 μm pore size; Hewlett Packard, CA, Seoul, South Korea) with a Security Guard™ guard cartridge (3.0 × 4.0 mm, Phenomenex, CA, Seoul, South Korea). The mobile phase consisted of

 DOI 10.1002/jps.24107