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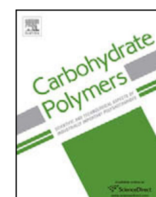

Intermolecular complexation of low-molecular-weight succinoglycans directs solubility enhancement of pindolol

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ABSTRACT

The low-molecular-weight succinoglycans isolated from *Sinorhizobium meliloti* are repeating octasaccharide units consisting of monomers, dimers, and trimers. Pindolol is a beta-blocker used to treat cardiovascular disorders. We investigated the formation of complexes between pindolol and low-molecular-weight succinoglycan monomers (SGs). Even though SGs have a linear structure, the solubility of pindolol in the presence of SGs was increased up to 7-fold compared with methyl-β-cyclodextrin reported as the best solubilizer of pindolol. Complexation of SGs with pindolol was confirmed by nuclear magnetic resonance, Fourier-transform infrared spectroscopy, differential scanning calorimetry, and scanning electron microscopy. Formation constants of complexes were determined from phase solubility diagrams. Conformation of complex was suggested based on a molecular docking study. The present study indicated that formation of pindolol/SGs complexes not only resulted in increased pindolol solubility but also could be useful for improving its clinical application as it did not affect cell viability.

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1. Introduction

Limited water solubility of a drug is a frequently encountered problem at the discovery and preclinical stages of drug development. It is estimated that development and clinical application of about 40% of drug candidates are limited by poor solubility (Hauss, 2007; Li & Zhao, 2007; Lipinski, 2000; Neslihan Gursoy & Benita, 2004). One of the most commonly used strategies to overcome this problem is drug complexation with various solubilizers, such as cyclodextrins (CDs). As CDs have torus-shaped structures with an ability to incorporate hydrophobic guests in the internal cavity, they have been widely utilized for solubilization of hydrophobic compounds (Craig, 2002; Del Valle, 2004; Kim, Choi, & Jung, 2009; Yongeun Kwon, Park, & Jung, 2010). However, since the internal cavity sizes of CDs are fixed and the intrinsic solubility is limited, various derivatives or substitutes for CDs has been studied as a means of expand their complexing capabilities.

The soil bacterium *Sinorhizobium meliloti* has a symbiotic association with the leguminous plant *Medicago sativa* (alfalfa) through the formation of nitrogen-fixing root nodules. *S. meliloti* produces succinoglycan, an acidic exopolysaccharide (EPS), which has an important role in root nodulation (Battisti, Lara, & Leigh, 1992). EPS is composed of octasaccharide repeating units (degree of polymerization (DP) = 8) containing one galactose at the reducing end and seven glucoses modified with acetyl (Ac), succinyl (Suc), and pyruvyl (Pyr) groups (Chouly, Colquhoun, Jodelet, York, & Walker, 1995; Reinhold, Chan, Reuber, Marra, Walker, & Reinhold, 1994). EPS can be classified into two categories: low-molecular-weight (LMW) succinoglycans and high-molecular-weight (HMW) succinoglycans The LMW succinoglycans consist of monomers, dimers, and trimers of the octasaccharide repeating unit, whereas the HMWs can contain hundreds of these units (Wang, Wang, Pellock, & Walker, 1999). There is heterogeneity within the LMW succinoglycan monomers (SGs) group in terms of the degree of substitution (DS) of succinyl moieties. Depending on the number of these, the SGs are further

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classified into SG1 (DP 8, DS(Suc) 0, DS(Pyr) 1, DS(Ac) 1), SG2 (DP 8, DS(Suc) 1, DS(Pyr) 1, DS(Ac) 1), or SG3 (DP 8, DS(Suc) 2, DS(Pyr) 1, DS(Ac) 1), respectively.

Pindolol (PIN), 1-(indol-4-yioxy)-3-isopropylaminopropan-2-ol, is a non-cardioselective β-adrenergic blocking drug commonly used for treatment of hypertension, angina pectoris, and glaucoma (Quyyumi, Wright, Mockus, & Fox, 1984). However, PIN has lipophilic characteristics and it is practically insoluble in water (<0.1%, w/v at neutral pH) (Perlovich, Volkova, & Bauer-Brandl, 2007). To solve this problem, it has been previously reported that the complexation of PIN with CD and their derivatives improved its solubility and transcorneal permeability (Gazpio et al., 2005; Knapp, 2000).

In a previous study, we demonstrated that the LMW succinoglycan dimers in the linear hexadecasaccharide (DP = 16) provided a hydrophobic environment enabling solubilization of insoluble flavonoids or drugs through an induced-fit type adjustment (Cho, Choi & Jung, 2013; Cho, Cho, Kim, Lee, & Jung, 2011; Choi, Kim, Cho, Choi, Lee, & Jung, 2012). In the present study, a linear SGs isolated from *S. meliloti* were investigated as a novel complexation agent for PIN. The PIN/SGs complex formation was confirmed using nuclear magnetic resonance (NMR) spectroscopy, differential scanning calorimetry (DSC), Fourier transform spectroscopy (FT-IR) assay and scanning electron microscopy (SEM) imaging.

2. Experimental

2.1. Chemicals

PIN, β-cyclodextrin (β-CD), and methyl-β-cyclodextrin (Me-β-CD) were purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). D_2O (99.96% at D), and CD_3OD (99.8% at D), were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Other chemicals used were of analytical reagent grade.

2.2. Bacterial culture and purification of SGs

The isolation of SGs from *S. meliloti* was carried out as described previously (Wang et al., 1999; Zevenhuizen & van Neerven, 1983). *S. meliloti* strain Rm 1021 was grown in a glutamate mannitol salt (GMS) medium for 5 d at 30 °C. Cells were removed by centrifugation, and the supernatant was concentrated to one-fifth of its original volume using a rotary evaporator. After adding 3 volumes of ethanol, the precipitated HMW succinoglycans were removed by centrifugation. The supernatant was again concentrated to one-fifth of its original volume. Seven volumes of ethanol were added and LMW succinoglycans in the pellet were collected by centrifugation. LMW succinoglycans were subjected to Bio-gel P6 chromatography with 0.5% of acetic acid to separate LMW succinoglycan monomers from dimers and trimers. The LMW succinoglycan monomers were then separated into SG1, SG2, and SG3 using a DEAE sephadex A-25 column with a KCl gradient from 5 to 250 mM in 10 mM MOPS buffer. Each SG fraction was desalted using Bio-gel P2 and they were confirmed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (Voyager-DE^{TM} STR BioSpectrometry, PerSeptive Biosystems, Framingham, MA, USA) in the negative-ion mode using 2,5-dihydroxybenzoic acid (DHB) as the matrix.

2.3. Preparation of complexes of PIN with SGs

Excess PIN (2.5 mg) was mixed with 1 ml of distilled water containing various concentrations of SGs (0–10 mM). The mixtures were stirred with a magnetic bar at 25 °C for 24 h to equilibrate them, and then filtered with a 0.2-μm syringe filter (PTFE syringe filter, Advantec), and lyophilized prior to investigation of complex formation between SGs and PIN.

2.4. Phase solubility study and complex stoichiometry study'

Phase solubility studies of SGs with PIN were carried out using ultraviolet–visible (UV–vis) spectrophotometry (UV 2450, Shimadzu Corporation) (Higuchi, 1965). Spectra were obtained over the 200–300 nm range, and the complex formation constants were determined from the phase solubility diagram using the following Eq. (1).

$$K_c(M^{-1}) = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

The continuous variation method (Job's plot) was used to determine complex stoichiometry (Job, 1928). UV–vis absorbance spectra of a series of PIN/SGs mixtures were assessed. The difference of λ_{max} at 219 nm in the presence and absence of SGs was plotted against the molar fraction (r), where r = [PIN]/{[PIN] + [SGs]}. Each complex was mixed using the same proportions while varying the molar fraction (r = 0, 0.2, 0.3, 0.5, 0.7, 0.8, and 1.0) and keeping the total concentration constant (2 mM).

2.5. NMR spectroscopy

^{1}H NMR spectra were recorded using a Bruker Avance 600 (AMX, Germany) spectrometer. The samples were dissolved in deuterated water (D_2O, 99.96%). The chemical shift displacements were obtained based on Eq. (2), where $\delta_{(free)}$ was the chemical shift of PIN without SGs, and $\delta_{(complex)}$ was the chemical shift of PIN with SGs.

$$\Delta\delta = \delta_{(complex)} - \delta_{(free)} \quad (2)$$

For the rotating-frame nuclear overhauser effect correlation spectroscopy (ROESY) and nuclear overhauser effect spectroscopy (NOESY) spectra, the time domain was zero, filled to 2048 points in F2 and 256 points in F1. The NOESY data for the complex were recorded in a spin-lock field with a mixing time of 600 ms. The ^{1}H and ^{13}C peaks of SGs were assigned by heteronuclear single quantum coherence (HSQC) and distortionless enhancement by polarization transfer (DEPT) NMR Spectroscopy techniques (data not shown).

2.6. Differential scanning calorimetry (DSC)

The DSC thermograms of PIN and complexes were recorded on a DSC 7020 (SEICO INST., Japan) with a thermal analyzer. The thermal behaviors of samples were examined by heating the samples in a sealed aluminum pan from 30 to 300 °C at a rate of 10 °C/min under nitrogen gas, using a sealed empty pan as reference.

2.7. FT-IR spectroscopic analysis

The FT-IR analysis was conducted using a Bruker IFS-66/S (AMX, Germany) infrared Fourier transform spectrometer using KBr pellets as support. Scans were performed at a resolution 0.1 cm^{-1} from 4000 to 500 cm^{-1}.

2.8. SEM analysis

SEM images were acquired on a JSM-6380 (Jeol, Tokyo, Japan) scanning electron microscope. Images were acquired using 1 kV accelerating voltage. To fix the samples on a brass stub, double-sided adhesive carbon tape was used. The samples were coated by a thin gold layer at 30 W for 30 s in a vacuum.

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