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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.

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.

2.9. Cell culture

The human embryonic kidney 293 (HEK293) cell line was purchased from the Korean Cell Line Bank (Seoul, Korea). The cells were maintained in Eagle's Minimum Essential Medium (MEM, WelGENE Inc., Daegu, Korea) supplemented with 10% heat-inactivated fetal bovine serum (FBS, WelGENE Inc., Daegu, Korea), 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37 °C in a humidified incubator containing 5% CO_2 and 95% air.

2.10. Cytotoxicity assay

The viability of HEK293 cells was measured by 4-[3-{4-iodophenyl}-2-{4-nitrophenyl}-2H-5-tetrazolio]-1.3-benzene disulfonate (WST-1) assay, which is based on the conversion of the tetrazolium salt WST-1 to formazan by mitochondrial dehydrogenases in viable cells (Scudiero et al., 1988). The assays were performed by incubating 5×10^3 cells/well with increasing concentrations (0, 5, 10, 50, 100, 500, and 1000 μM) of SGs in 96-well microtiter plates (Costar, Cambridge, MA, USA) at 37 °C in a humidified incubator containing 5% CO_2 for 24 h. Cells were then washed with PBS and 10 μl WST-1 (EZ-Cytox; Daeil Lab Service Co. Ltd., Seoul, Korea) reagent was added to each well. After 4 h of incubation at 37 °C (humidified, 5% CO_2), absorbance was determined using a SpectraMax 190 microplate reader (Molecular Devices, Corp., CA, USA) at 450 nm. Cell viability was expressed as a percentage of the untreated control. Experiments were carried out in triplicate.

2.11. Molecular modeling process

The Maestro program in Discovery Studio 2.5 was used as a computational tool for molecular dynamics (MD) and docking simulations. Initial structures of SG1 and PIN were prepared with the molecular editing module, followed by a conformational search tool in Dynamics (San Diego, CA, USA Accelrys Inc.). After the conformational search, distance-restrained MD simulation of the PIN/SG1 complex was performed for 10 ns under a CHARMM force at 300 K. Water molecules were implicitly treated using the generalized Born/surface area (GBSA) model (Hasel, Hendrickson, & Still, 1988).

The molecular docking simulation was performed independently using the Glide program (Friesner et al., 2004). The molecular grid was defined for the ligand binding site of SG1 using the receptor grid generation tool in this program. The size of the cubic grid box was 30 nm × 3 nm × 3 nm. The flexible-ligand docking simulation was carried out by the SP scoring mode. To obtain docking poses of PIN upon SG1, a maximum of 10,000 poses were kept for the initial phase of docking and 1000 poses were energy-minimized using the expanded sampling method (Choi et al., 2012).

3. Results and discussion

3.1. Solubility enhancement study and stoichiometry of PIN/SGs complexes

The purification of SGs isolated from *S. meliloti* Rm 1021 was performed by size exclusion and anion exchange chromatography. The molecular structures of SGs are shown in Fig. 1a. The SGs were classified into three types (SG1, SG2, or SG3) based on the DS(suc) (Wang et al., 1999). Each SG structure was confirmed by MALDI-TOF MS (data not shown) (Kwon, Lee, & Jung, 2011).

The effect of SGs on the solubilization of PIN was investigated using phase solubility diagram methods (Fig. 2a). The UV absorbance gradually increased as the host (SGs) concentration increased (0–10 mM), which indicated complex formation between PIN and SGs. As shown in Fig. 2a, the solubilizing ability of SG1 was the greatest among the PIN/SGs complexes. It was found that PIN solubility was increased by 18-fold in the presence of a 10-mM SG1 solution. In the phase solubility diagram study, linear correlations were obtained ($r^2 > 0.99$), predicting the formation of 1:1 inclusion complexes. According to Higuchi and Connors, all of the SGs complexes formed A_L-type curves (Higuchi, 1965). The stoichiometry between the PIN and SGs was also confirmed by Job's plot. As shown in Fig. 2b, the highest point of curve was centered on the 0.5 abscissa, thus confirming 1:1 stoichiometry for the PIN/SG1 complex, which was consistent with the estimation by the phase solubility diagram (Gil & Oliveira, 1990). The formation constants

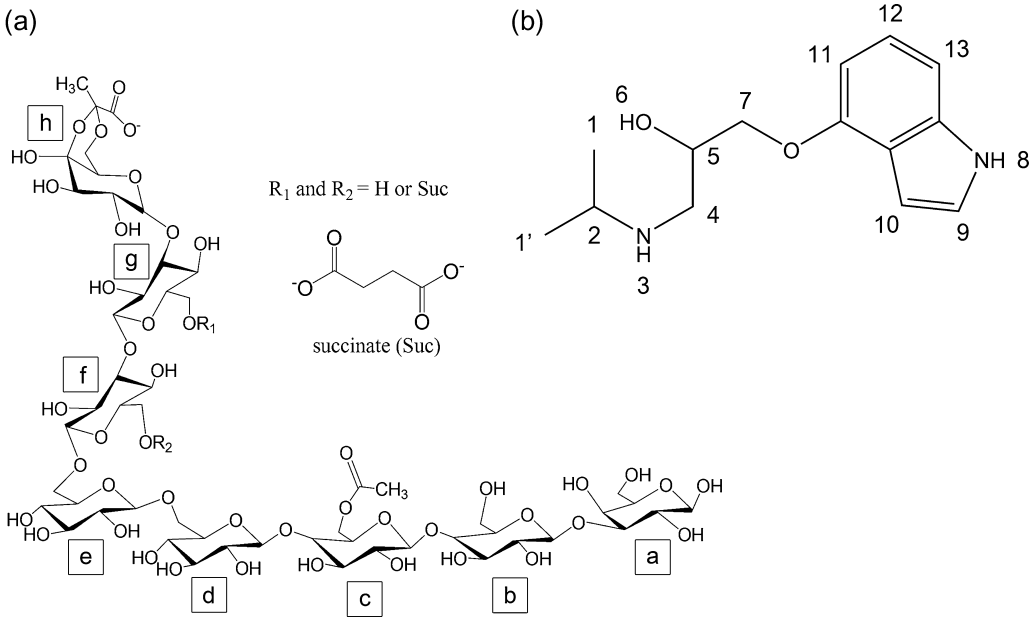

Fig. 1. Molecular structures of SGs (a) and PIN (b). SGs were named SG1, SG2, or SG3 according to the DS(suc) (0–2) in the molecule.

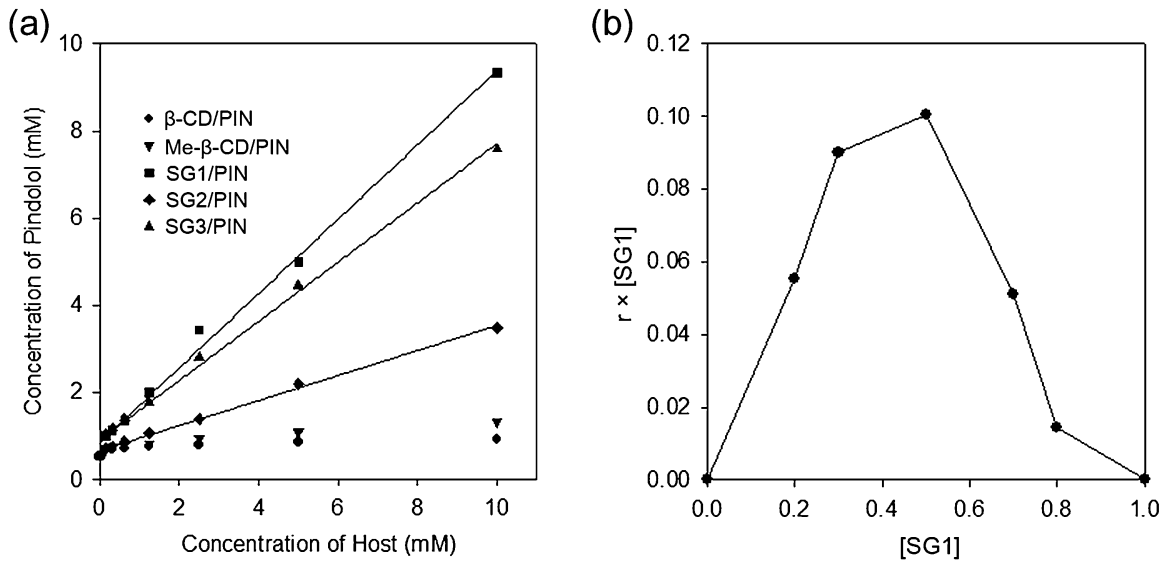

Fig. 2. Phase solubility diagram of PIN/SG1 complex (a), and continuous variation plots (Job's plots) of PIN with SG1 (b) using UV spectroscopy.

Table 1
Formation constants for PIN/SGs.

SGs	PIN *Kc* (M^{-1})
SG1	16683.9
SG2	814.1
SG3	5123.9

(Kc) were calculated using Eq. (1) using the slope and the intercept determined from phase solubility diagrams. The formation constants obtained for the PIN/SGs complexes are listed in Table 1, and the order was SG1 (16683.9 M^{-1}) > SG3 (5123.9 M^{-1}) > SG2 (814.1 M^{-1}) > Me-β-CD > β-CD. The solubility curves of Me-β-CD and β-CD could be classified as the B_S type, indicating that the complex showed higher solubility than control, but CDs at concentrations over 2 mM produced no further enhancement of solubility. Although linear glycans have no cavity for guest molecules, they are amphiphilic because of the hydroxyl groups and methines on either side of the glucose chain backbone (Sivakama Sundari, Raman, & Balasubramanian, 1991). Depending on the overall sugar structure and linkage types, linear glycans possess hydrophobic surfaces, enabling complex formation with various non-polar compounds.

3.2. NMR spectroscopy

For NMR spectroscopic analysis, PIN and SGs were mixed in aqueous solution, and then the soluble filtrates were lyophilized

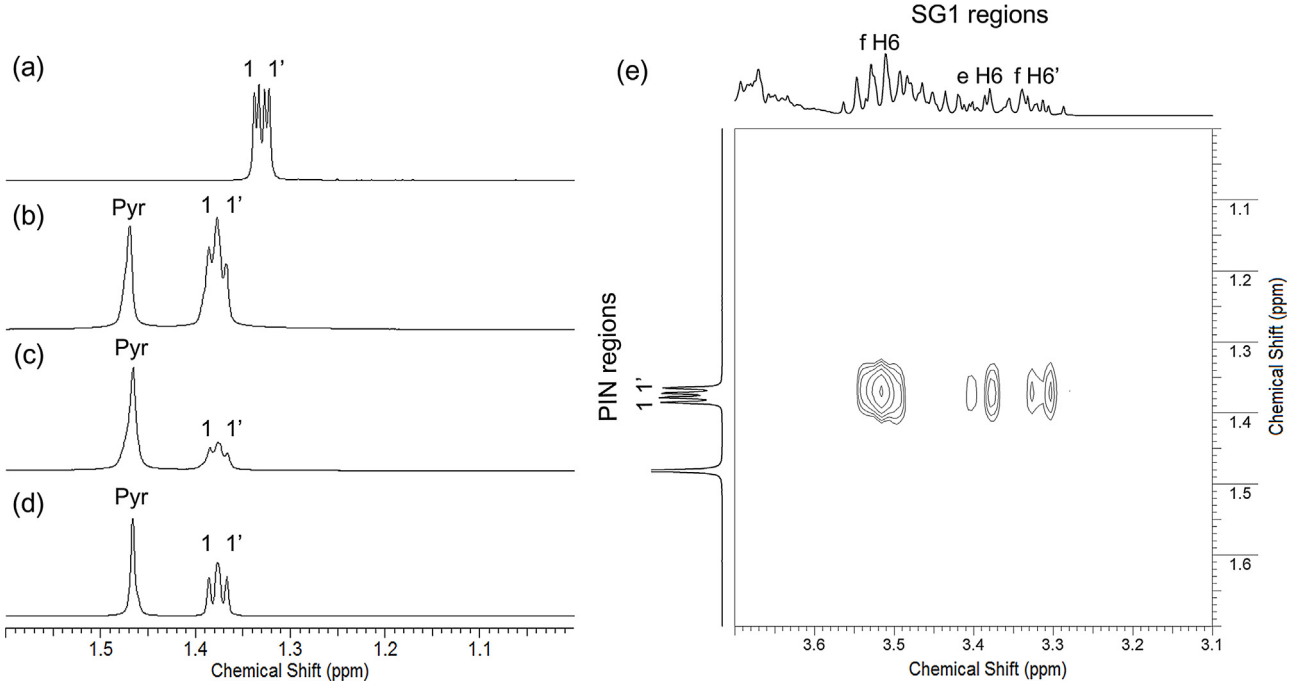

Fig. 3. Partial ^{1}H NMR spectra of PIN (a) in the presence of SG1 (b), SG2 (c), or SG3 (d) and partial nuclear overhauser effect spectroscopy spectrum for the PIN/SG1 complex (e).

Table 2
^{1}H chemical shifts of PIN alone, and in the presence of SG1 (a), SG2 (b), or SG3 (c).

PIN		(a)		(b)		(c)	
Position	Chemical shift (ppm)	Chemical shift (ppm)	$\lvert\Delta\delta\rvert$ (ppm)	Chemical shift (ppm)	$\lvert\Delta\delta\rvert$ (ppm)	Chemical shift (ppm)	$\lvert\Delta\delta\rvert$ (ppm)
H-1	1.336	1.381	0.045	1.381	0.045	1.381	0.045
H-1′	1.325	1.372	0.047	1.371	0.046	1.372	0.047
H-2–6	–	–	–	–	–	–	–
H-7	4.355	4.387	0.032	4.388	0.033	4.388	0.033
H-9	6.654	6.656	0.002	6.655	0.001	6.654	0.000
H-10	7.192	7.198	0.006	7.196	0.004	7.197	0.005
H-11	7.151	7.155	0.004	7.153	0.002	7.153	0.002
H-12	7.305	7.311	0.006	7.309	0.004	7.309	0.004
H-13	6.633	6.638	0.005	6.636	0.003	6.630	0.003

–: not measured by overlapped peaks.

and dissolved in D_2O. ^{1}H NMR spectroscopy is a common technique for the estimation of non-covalent interactions in host-guest complexation (Schneider, Hacket, Rudiger, & Ikeda, 1998), which can cause chemical shift changes in the host or guest molecule. It could be seen that almost all PIN's aliphatic protons were affected in the presence of SGs. These chemical shifts and their changes in the complex were summarized in Table 2, and the characteristic partial ^{1}H NMR spectra of PIN and PIN/SGs complexes were shown in Fig. 3. The branched methyl groups of PIN (1 and 1′) appear in the region of 1.325–1.336 ppm (Fig. 3a) with a doublet of doublets, and the addition of SGs resulted in downfield shifts (1.372–1.381 ppm) with a triplet pattern (Fig. 3b–d). After complexation, the de-shielded protons of PIN indicated their proximity to an electronegative oxygen atom in the neighboring SGs, and the observed peak broadening may reflect restricted motion of PIN following complexation with SGs (Pirnau, Floare, & Bogdan, 2012). These observations clearly demonstrated that PIN formed complexes with all three SGs, SG1, SG2, and SG3. Furthermore, the observed peak intensities of PIN were increased due to the solubilizing effects of SGs, and the enhanced order was in accordance with the formation constants described above. The differences between the chemical shift changes were trivial in the three complexes including SG1, SG2, or SG3.

The complex formation between PIN and the SGs was further investigated by NOESY spectra, since their cross-peaks were expected for protons closer than 0.5 nm (Correia, Bezzenine, Ronzani, Platzer, Beloeil, & Doan, 2002). If PIN was effectively bound with SG1, NOE correlations between protons within PIN and SG1 would be observed in the corresponding NOE cross peaks. The partial NOESY spectrum of the PIN/SG1 complex is shown in Fig. 3e and cross peaks were observed. This spectrum clearly demonstrated the intermolecular interactions between the protons of branched methyl groups in PIN and the protons of e H6, f H6, and f H6′ in SG1. This result strongly indicated that the branched methyl groups of PIN mainly participated in complex formation with the e and f glucose units of SG1.

3.3. DSC analysis

DSC analysis was performed to elucidate the interaction between PIN and SGs (Figueiras, Ribeiro, Torres-Labandeira, & Veiga, 2007). Fig. 4a presents the thermograms of the PIN/SG1 complex, the physical mixture, SG1, and PIN. PIN showed a sharp endothermic peak at 171 °C, whereas the SG1 contained a broad exothermic peak around 220 °C. The thermal curve of the SG1 and PIN physical mixture also exhibited a small PIN endothermic peak. In contrast, after complexation of PIN with SG1, the peak corresponding to the melting point of PIN had completely disappeared. This change provided clear evidence of successful complex formation.

3.4. FT-IR spectroscopic analysis

Intermolecular interaction between a drug and a solubilizer often leads to changes in the FT-IR spectra of complexes (Stancanelli

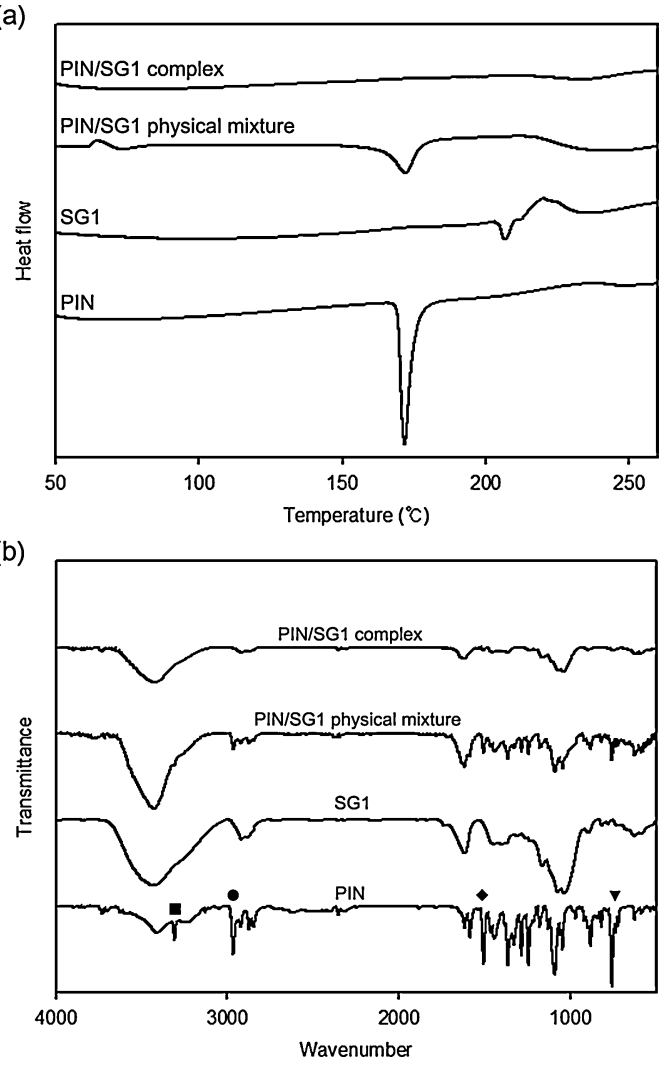

Fig. 4. DSC curves for PIN, SG1, the PIN/SG1 physical mixtures and the PIN/SG1 complex (a) and FT-IR spectra of PIN, SG1, PIN/SG1 physical mixture, and the PIN/SG1 complex (b).

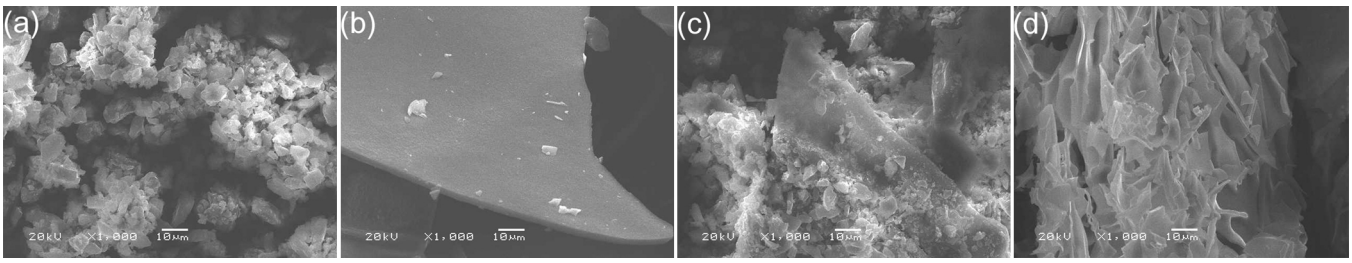

Fig. 5. SEM images of PIN (a), SG1 alone (b), the PIN/SG1 physical mixture (c), and the PIN/SG1 complex (d).

et al., 2008). In an attempt to investigate the PIN/SGs complex in greater detail, FT-IR spectra of the PIN/SG1 complex, the physical mixture, SG1, and PIN were compared (Fig. 4b). The FT-IR spectrum of PIN showed characteristic absorption bands at 3309 cm^{-1} (■) corresponding to N—H stretching (Fig. 1b, 11), at 2966–2844 cm^{-1} (•) corresponding to C—H aliphatic stretching, at 1504 cm^{-1} (♦) corresponding to N—H bending in plane (Fig. 1b, 3), and at 883 cm^{-1} (▼) corresponding to N—H bending out of plane (Fig. 1b, 3) (Castro, Canotilho, Nunes, Eusebio, & Redinha, 2009), whereas the spectrum of SG1 showed a broad absorption band at 3486 cm^{-1} for O—H stretching vibrations and absorption at 2920 cm^{-1}, which was attributed to C—H stretching. The peaks shown at 1629 cm^{-1} and 1080 cm^{-1} correspond to the symmetric stretching of carboxyl groups and the asymmetric C—O stretch, respectively (Simook Kang, Kyung, & Jung, 2006). In the physical mixture, these significant absorption bands of PIN remained. However, all of these characteristic PIN absorption bands were absent in the complex, providing further evidence of successful molecular complex formation.

3.5. SEM analysis

SEM provided a useful tool to examine the microscopic aspects of complex formation (Sathigari et al., 2009). The morphology of PIN, SG1, the physical mixture, and the PIN/SG1 complexes are shown in Fig. 5. PIN was composed of crystalline particles (Fig. 5a), whereas the characteristic planar shape of SG1 can be observed in Fig. 5b. The SEM image of the physical mixture system clearly showed the characteristic PIN particles, which were attached to the surface of SG1 (Fig. 5c). However, a prominent change in surface morphology was observed in the case of PIN/SG1 complex (Fig. 5d) where the original PIN and SG1 morphologies had disappeared, and only amorphous morphology was present. These morphological changes indicated the formation of a new complex.

3.6. Cytotoxicity assay

The effect of SGs on the viability of HEK293 cells was assessed by WST assay, a commonly used method for quantifying cytotoxicity (Hilmi et al., 2003). Fig. 6 shows HEK293 cell viabilities in the presence of various concentrations of each SGs. As shown in Fig. 6, none of the SGs induced any significant decrease in cell viability at concentrations up to 1000 μM, and some even produced positive effects on viability. This demonstrated that the toxicity of SGs to HEK293 cells was low enough to warrant further study, and that these SGs were suitable for enhancement of the solubility of PIN or its analogs.

3.7. Molecular modeling process

To investigate the binding mode of PIN with SG1, molecular docking simulations were carried out. Interatomic distances of protons between b and h residue in SG1 (a), a and d residue in SG2 (b), and a and g residue in SG3 (c) were restrained to be within 5 Å, based on the experimental ROESY data (Fig. 7). Peaks marked with arrow (→) are cross peaks due to the adjacency of protons in each residue.

In the case of SG1, examination of the obtained simulation data suggested that the interaction between the f glucose unit in SG1 and the methyl groups of PIN was the most critical driving force to stabilize the complex. Coulomb energy also supported an interaction between hydrogens of the SG1 and the PIN. For the interaction between PIN and the SG1, total van der Waals and Coulomb energies were −16.217 and −14.188 kcal mol^{-1}, respectively. It was concluded that the formation of this hypothetical complex between SG1 and PIN was theoretically possible from the present molecular docking simulations. This proposed docked pose was in agreement with the NMR data (Fig. 8).

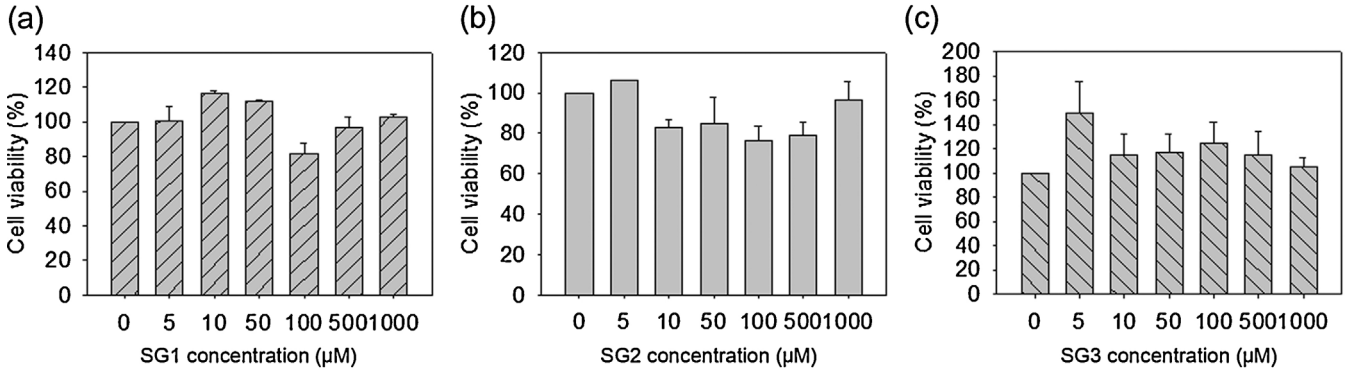

Fig. 6. HEK293 cell viability following exposure to SG1 (a), SG2 (b), or SG3 (c) at concentrations of 0, 5, 10, 50, 100, 500, 1000 μM ($n = 3$).

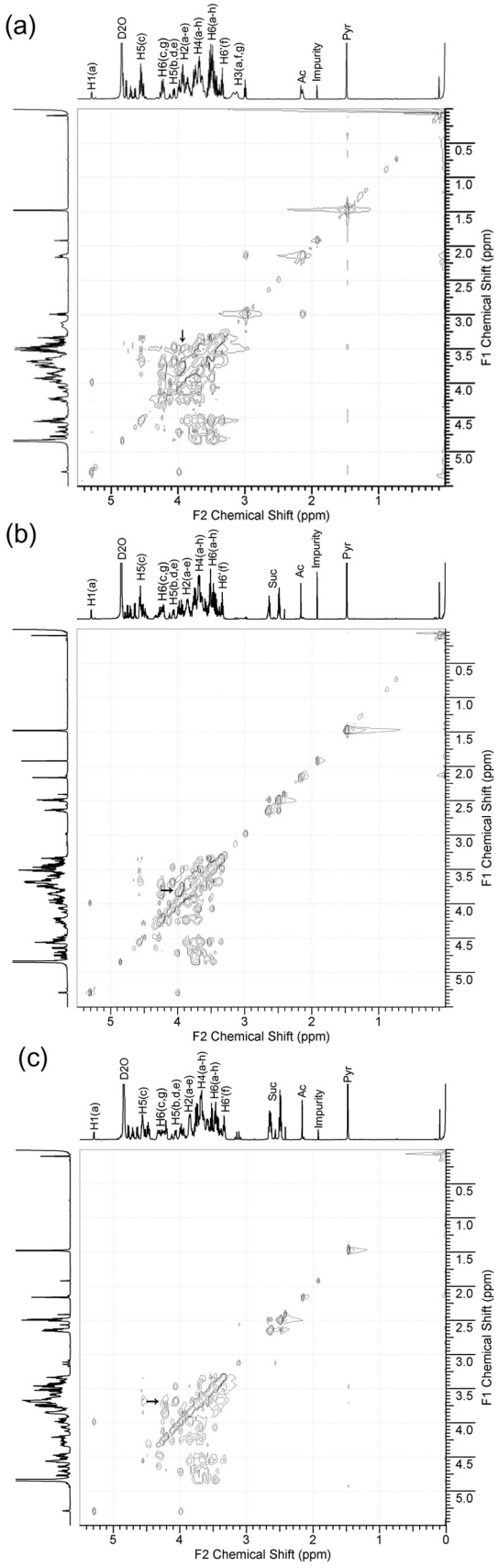

Fig. 7. Rotating-frame nuclear overhauser effect correlation spectra for the SG1 (a), SG2 (b), and SG3 (c).

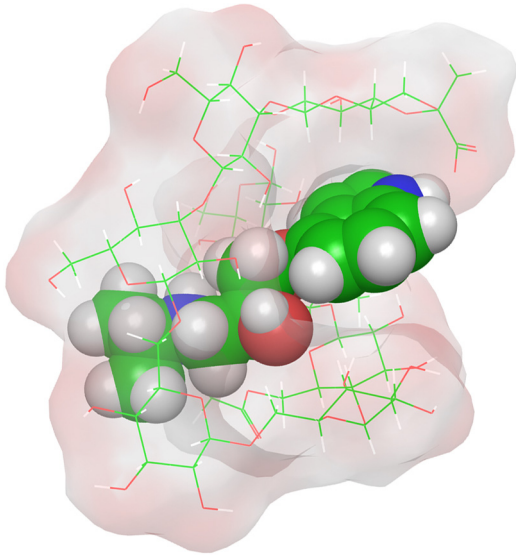

Fig. 8. Lowest energy docked model of the complex between SG1 and PIN. PIN was represented as a space-filling model.

4. Conclusion

In this study, SG1, SG2 and SG3 were isolated and purified by chromatography. Phase solubility studies, Job's plot, NMR, FT-IR, DSC, and SEM were applied to characterize PIN/SGs complex formation. These SGs, even though it has linear structure, showed much better solubilization ability comparing with Me-β-CD, a well-known solubilizer for PIN. Particularly, SG1 was found to have the highest PIN solubilization ability of the 3 SGs. The solubility of pindolol was increased up to 18-fold in the presence of 10 mM SG1 compared with PIN only, and 7-fold compared with 10 mM Me-β-CD reported as the best solubilizer of PIN (Gazpio et al., 2005). The apparent formation constants (K_C) for PIN/SGs complexes were 16683.9 M^{-1} for PIN/SG1, 814.1 M^{-1} for PIN/SG2, and 5123.9 M^{-1} for PIN/SG3. A 1:1 complex stoichiometry was confirmed by Job's plot. ^1H and NOESY NMR spectroscopy indicated that the aliphatic chain of PIN interacted with SGs in the complex and the optimal PIN/SG1 hypothetical model was in agreement with these findings. Based on the results of the WST cytotoxicity assay, we suggest that preparation of PIN/SGs complexes represented an efficient method for improving solubility with negligible cytotoxicity. Thus, complexation with SGs from *S. meliloti* could help improve the solubility-related bioavailability limitations for PIN. Throughout the present study, linear SGs showed excellent complexation ability with a poorly soluble drug, PIN, suggesting that they may be a novel potential solubilizer for various other poorly soluble drugs.

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