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Solubilization of haloperidol by acyclic succinoglycan oligosaccharides

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ABSTRACT

The isolated succinoglycan octasaccharide dimers isolated from *Sinorhizobium meliloti* 1021 have unique acyclic structures, displaying amphipathic properties against water. Thus, their potential usage as solubilizers of various water-insoluble drugs through non-covalent complexation are possible. In this study, we examined the solubility of a poorly water-soluble drug, haloperidol, in the presence of the acyclic form of succinoglycan dimers, and demonstrated that its solubility was increased up to 87 fold, Interestingly, the level of its solubility was even 7–10 fold higher than that achieved with β cyclodextrin or its derivatives that are cyclic forms, which is possibly due to the molecular flexibility of the acyclic structure of the dimers as well as the hydrophobic nature. Analyses of the stoichiometry and the stability constants for each complex were performed using phase solubility method, respectively. Additional analyses were also performed to confirm the formation of succinoglycan–drug complexes. Furthermore hypothetical 3-dimesional conformation of the complex was estimated through molecular docking simulations. Upon cytotoxicity test with a human cell line, the succinoglycan dimers displayed little effect up to 1000 μM, suggesting their potential usage to improve solubility and bioavailability of poorly soluble therapeutic agents.

1. Introduction

The nitrogen fixation soil bacteria *Sinorhizobium meliloti* (Rm 1021) produce acidic exopolysaccharide (EPS), which may play a crucial role in the development of the root nodule symbiosis between bacterium and legumes of Alfalfa (Battisti, Lara, & Leigh, 1992; Brewin, 1991; Gonzalez, Reuhs, & Walker, 1996; Gonzalez, York, & Walker, 1996.). Succinoglycan octasaccharides secreted by *Sinorhizobium meliloti* consists of a β-1,3, β-1,4, and β-1,6 linked octasaccharide subunit containing one galactose at the reducing end and seven glucose residues, one to two succinyl group located at the C-6 position of the seventh sugar residue and a pyruvyl group linked to the eighth sugar residue through a 4, 6-ketal linkage (Fig. 1) (Chouly, Colquhoun, & Jodelet, 1995; Reinhold et al., 1994).

Haloperidol (HAL) is 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one, which is a butyrophenone antipsychotic that has been used for the treatment of schizophrenia, delusions and hallucinations. (Bernard & Simona, 2005) However, this compound is practically insoluble in water (1.4 mg 100 mL^{-1}) (Remington, 1990) and has a relatively low oral bioavailability (60%) (Drug Information, 2008). A well known approach for the solubilization of HAL is complexation with cyclodextrins (CDs) and its derivatives. HAL solubility was increased 20-fold in the presence of a 10-fold excess of methyl β CD (Me β-CD) and 12-fold in the presence of a 10-fold excess of 2-hydroxypropyl β-CD (HP β-CD). The stoichiometries and stability constants of the two β-CD complexes, HAL–Me β-CD (1:1 and 2345 M1 at 27 °C) and HAL–HP β-CD (1:1 and 2112 M1 at 27 °C), have been previously determined. (Loukas, Vraka, & Gregoriadis, 1997).

Despite the well-known propensity of CDs to include various drugs in their cavities, it is plausible that acyclic oligosaccharides could function as complexing agents as well as since their conformations may be able to accommodate a wider part of haloperidol when compared to the CD inclusion complex. Although the elucidation of the presence of the complexation event or the mechanism by which acyclic oligosaccharides interact with specific target molecules is important to understand the biological role of the oligosaccharides (Eggens et al., 1989), very little work has been reported regarding the molecular complex formation with acyclic oligosaccharides (Kano, Minami, Horiguchi, Ishimura, & Kodera, 1995). We previously demonstrated that succinoglycan dimers can provide hydrophobic space as a linear form, suggesting that succinoglycan dimers might undergo an induced-fit type adjustment. (Cho, Choi, Kim, Lee, & Jung, 2011) Herein, highly water soluble acyclic succinoglycan dimers were tested as novel

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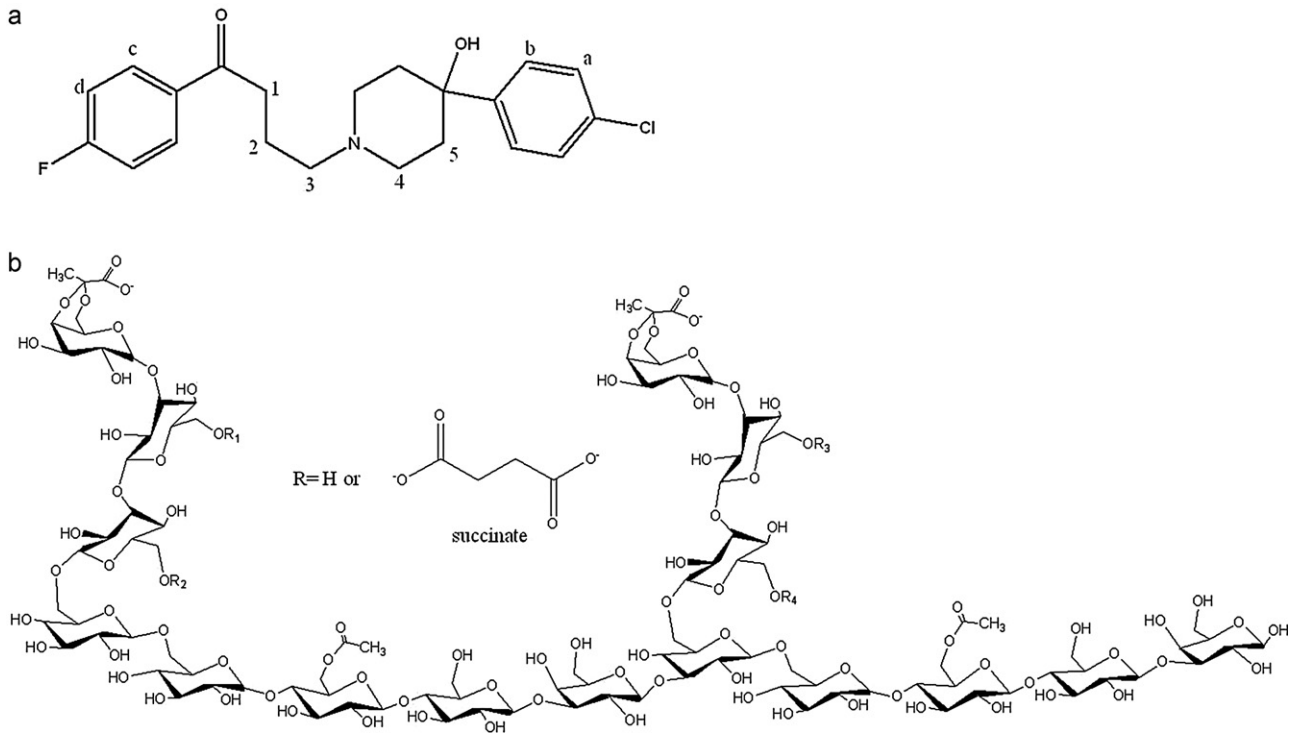

Fig. 1. Structure of (a) haloperidol and (b) the succinogylcan dimer. R_1 and R_2 indicate the sites for the succinyl group.

solubilizers for the first time to see whether these oligosaccharides with acyclic forms could increase the solubility of haloperidol. The data from the phase solubility diagram demonstrated the ability of acyclic succinoglycans to increase the solubility of haloperidol. We also confirmed the result with additional data from the Nuclear Magnetic Resonance Spectroscopy (NMR), Differential scanning calorimetry (DSC) and FT-IR spectra analyses.

2. Experimental

2.1. Materials

Haloperidol (>98.0% (LC) (T)) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) β-cyclodextrin and 2-hydropropy-β-cyclodextrin (HP-β-CD) were purchased from Sigma–Aldrich Chemicals Co. (St. Louis, MO, USA). D_2O (99.9% at D) and dimethyl sulfoxide-d6 (D, 99.9%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). They were employed without further purification.

2.2. Preparation of succinoglycan dimers

The isolation and purification of succinoglycan dimers from *S. meliloti* were carried out as described previously (Wang, Wang, Pellock, & Walker, 1999). *S. meliloti* was cultured in 500 ml of GMS medium at 30 °C and 150 rpm for 5 days. Cells were removed by centrifugation (8000 × *g* for 10 min). After centrifugation, the supernatant was concentrated by a factor of five relative to the original volume by rotary evaporation. The supernatant contained exopolysaccharide (EPS) and EPS was precipitated from the concentrated supernatant by adding three volumes of ice-cold ethanol and then the sample was subjected to centrifugation (8000 × *g* for 10 min). After removing the EPS, the supernatant was again concentrated by a factor of five relative to its original volume. Succinoglycan was then precipitated by adding another seven volumes of ice-cold ethanol and then subjecting the sample to centrifugation. The precipitate was dissolved in distilled water. Chromatography was the performed for further purification. Samples were applied to a column of Bio-Gel P6 (2.5 cm × 145 cm), which was eluted at room temperature with 0.5% AcOH. The monomer fraction was pooled and concentrated. The concentrated sample was separated into four fractions (D1–D4) using DEAE Sephadex A-25 (1.5 cm × 48 cm), according to succinate moiety (Fig. 1). The samples were eluted with KCl in an MOPS buffer using linear gradients of 5–400 mM KCl. Fractions were collected and subsequently desalted using a Bio-Gel P2 (2 cm × 48 cm) column. The purified succinoglycan dimers were confirmed through MALDI-TOF mass spectrometry (Voyager-DE^{TM} STR Bio-Spectrometry, Applied Biosystems, Framingham, MA, USA) in the negative ion mode using 2,5-dihydroxybenzoic acid (DHB) as the matrix (Kwon, Lee, & Jung, 2011). The mass spectra were recorded in DHB at a molar ratio of 10^{-3} with a total loading of around 1 μg of sample.

2.3. Phase solubility analysis

Phase solubility studies were performed using the method described by Higuchi and Connors (Higuchi & Connors, 1965). An excess amount of HAL (50 mM) was added to various concentrations of succinoglycan dimers (D1–D4) in capped vials and the solution was dispersing using a sonicator for 30 min. Vials were sealed to avoid changes due to evaporation, magnetically stirred for 24 h at 30 °C and shielded from light to prevent degradation of the molecules. After reaching equilibrium, the samples were filtered through a 0.2 μm PVDF filter. Each sample was analyzed using a UV–vis spectrophotometry (UV 2450, Shimadzu Corporation) from 220 to 350 nm at 30 °C to evaluate the concentration of the dissolved HAL. Phase solubility diagrams were determined to estimate the complex formation constants of the complexes. The equilibrium constant (K) for the formation of $[S_mL_n]$ can be represented by:

$$K_{1:1} = \frac{[SL]}{[S][L]} \quad (1)$$

$$K_{1:2} = \frac{[SL_2]}{[SL][L]} \quad (2)$$

The solubility (S_{eq}) of HAL in aqueous solutions of variable concentrations is given by:

$$S_{eq} = S_o + [SL] + [SL_2] = S_o + K_{1:1}S_o[L] + K_{1:1}K_{1:2}S_o[L]^2 \quad (3)$$

where S_o and [L] donate the concentrations of free HAL and succinoglycan dimers, respectively, whereas [SL] and [SL_2] represent the concentrations of the 1:1 and 1:2 HAL/dimers complexes, respectively. A nonlinear regression of experimental data corresponding to each phase diagram was conducted to estimate the complex formation constants (K_{ij}). The results of this analysis indicated that HAL/dimer ratios were 1:1 and 1:2. (Martin & Valle, 2004).

2.4. FT-IR spectroscopic analysis

Fourier-transform infrared spectra were obtained on a Bruker IFS-66/Sspectrometer (AMX, Germany). 1.5–2.0 mg of four different samples, HAL, dimers, physical mixture and HAL/dimer complex, were mixed with a KBr pellet.

2.5. Differential scanning calorimetry

The physical properties of the succinoglycan dimers and HAL were measured by differential scanning calorimetry DSC Q200 V24.4 (TA Instruments, USA). Approximately 5 mg of HAL, dimers, physical mixture and HAL/dimer complex sample was heated in a sealed aluminum pan, using an empty sealed pan as a reference, over the temperature range of 30–300 °C at a rate of 10 °C min^{-1}. An indium standard was used to calibrate the temperature scale.

2.6. 2D nuclear overhauser effect spectroscopy (NOESY)

2D NMR spectroscopic analysis was carried out on a Bruker 500 MHz spectrometer (AMX, Germany). The samples were dissolved in 80% dimethyl sulfoxide-d_6 (D, 99.9%) and 20% deutrated water (D_2O, 99.96%).

2.7. Scanning electron microphotographs (SEM)

SEM photographs were acquired on a JSM-6380 scanning electron microscope. The powders were previously fixed on a brass stub using double-sided adhesive carbon tape and then were made electrically conductive by coating a thin layer of gold on the surface for 30 s at 30 W in vacuum. The pictures were taken at an excitation voltage of 20 kV.

2.8. Computational method

The atomic level structure of the D3 succinoglycan was built using Conformation Search module in MacroModel software (Schrodinger Inc.). The initial structure of D3 was energy-minimized and subjected to conformational searching. The global energy-minimum for the D3 was calculated with the OPLS2001 force field using the mixed torsional/low-mode sampling method. The resultant D3 structure was further energy-minimized and then used as a receptor structure for the haloperidol molecule.

Molecular docking simulations were performed using the Glide module (Friesner et al., 2004) with Maestro 9.2 software (Schrodinger Inc.) in flexible mode. The starting structure of haloperidol was prepared using the Builder module of the Maestro software. The molecular grid was defined for the D3 using the Receptor Grid Generation tool in Glide with a cubic box 30 Å in each dimension. Molecular docking was conducted under standard precision *SP* mode using the Glide XP 5.0 scoring function to obtain reasonable binding mode and affinity data. In this docking mode, a 0.5 kcal/mol energy window and distance-dependent dielectric constant ($\varepsilon = 1$) were applied to the sampling process. To generate the initial docking pose of haloperidol upon D3, a maximum of 100,000 poses were kept for the initial phase of docking and 1000 poses were energy-minimized using the expanded sampling method. 30 docked poses were recorded throughout the docking process. The 1:2 binding model between D3 and haloperidol was constructed using the combination of 2 haloperidols that did not violate the steric constraints of each other.

2.9. Cell culture

The Human Embryonic Kidney 293 (HEK293) cell lines were purchased from Korean Cell Line Bank (Seoul, Korea). Cells were maintained in Minimum Essential Medium Eagle (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.

2.10. Cytotoxicity assay

MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) method was used to determine the viability of HEK293 cells (Mosmann, 1983). HEK293 cells were seeded at a final density of 5×103 cells/well in 96-well microtiter plates (Costar, Cambridge, MA, USA) and incubated at 37 °C in a humidified incubator containing 5% CO_2 overnight. After the incubation cells were treated with different concentrations (0, 5, 10, 50, 100, 500, and 1000 μM) of succinoglycan dimers and incubated at 37 °C in a humidified incubator containing 5% CO_2 for 24 h. After 24 h incubation cells were washed with PBS and the 20 μl MTS (CellTiter 96® AQueous One Solution; Promega, Madison, WI) solution was added to each well incubate the plate at 37 °C for 4 h in a humidified, 5% CO_2 atmosphere. After 4 h of incubation, record the absorbance at 490 nm using a 96-well plate reader (Zenyth 1100; ANTHOS, Austria). Cell viability was expressed as the percentage of the untreated control. Experiments were carried out in triplicate.

3. Results and discussion

3.1. Phase solubility studies

The phase soulubility diagram of HAL/dimers (D1–D4) and methyl-β-CD are shown in Fig. 2. As shown in Fig. 2, all of the dimer complexes formed A_P-type curves, which suggest the formation of higher order complexes with higher HAL concentrations. These results indicate that HAL formed 1:2 complexes with the succinoglycan dimers. It appears that the solubilizing effect of D3 was the greatest among the complexes (Fig. 2). The stability constants, *K*, of the complexes were calculated using Eq. (3) and are listed in Table 1.

Table 1
Stability constant of HAL and dimers complexes.

Succinoglycan oligosaccharides	Apparent stability constant, $K_{m:n}$ (M^{-1})	
	$K_{1:1}$	$K_{1:2}$
D1	994	1493
D2	2957	5
D3	6857	494
D4	1920	286

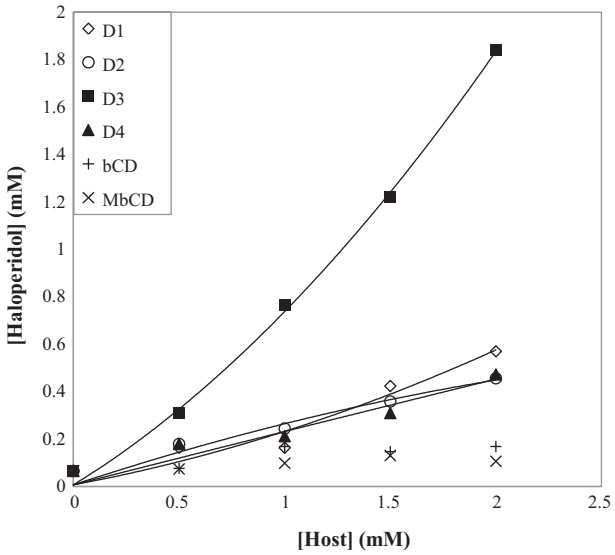

Fig. 2. Phase solubility diagram of HAL at various concentrations (0, 0.5, 1, 1.5, 2.0 mM) of D1(◊), D2(○), D3(■), D4(▲) and methyl β CD(×) with the complexes in water at 30 °C.

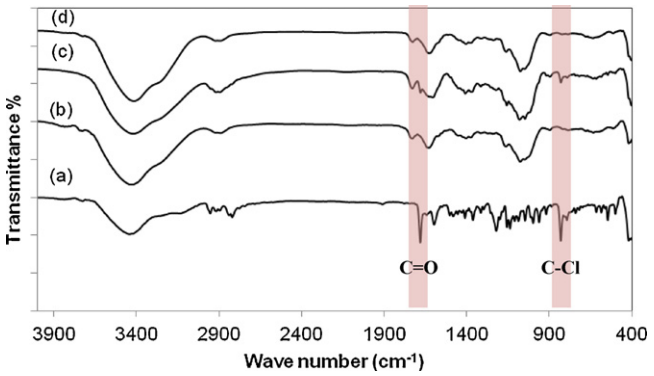

Fig. 3. FT-IR spectra of (a) HAL, (b) D3, (c) physical mixture and (d) HAL/D3 complex. Spectra were acquired between 4000 and 400 cm^{-1}.

3.2. FT-IR spectroscopic analysis

The FT-IR spectra of D3 (Fig. 3a), HAL (Fig. 3b), their physical mixture (Fig. 3c), and their complex are shown in (Fig. 3d). The spectrum of HAL contained the characteristic absorption band for carbonyl stretching vibration at 1681, C—F, C—N, C—O, and C—Cl stretching vibration at 1361, 1220, 1135 and 829 cm^{-1} (Fig. 3a), whereas the D3 spectrum contained carbonyl stretching vibration bands at 1729 and 1629. The FT-IR spectrum of the physical mixture contained absorption peaks with reduced intensity at the same position as the C—Cl peak of pure HAL, which likely resulted from the addition of HAL and D3. However, the characteristic absorption peaks disappeared in the complex products, suggesting that the drug environment was modified. The disappearance of the stretching vibration peak of C—Cl indicated the presence of intensive molecular interactions in the complex, which can be attributed to the formation of hydrogen bonds.

3.3. Differential scanning calorimetry (DSC)

The thermal properties of the dimers, HAL, physical mixtures, and complexes were investigated by DSC (Fig. 5). There was a sharp endothermic peak in the DSC curve of HAL at 150 °C, which corresponded to the melting point of HAL. In contrast, the DSC curves of the dimers contained an exothermic peak around 220 °C (Fig. 4a–d), respectively. In the physical mixture of HAL and dimers, the endothermic peaks around 150 °C and 220 °C were due to HAL and the dimers, respectively. However, in the DSC curves

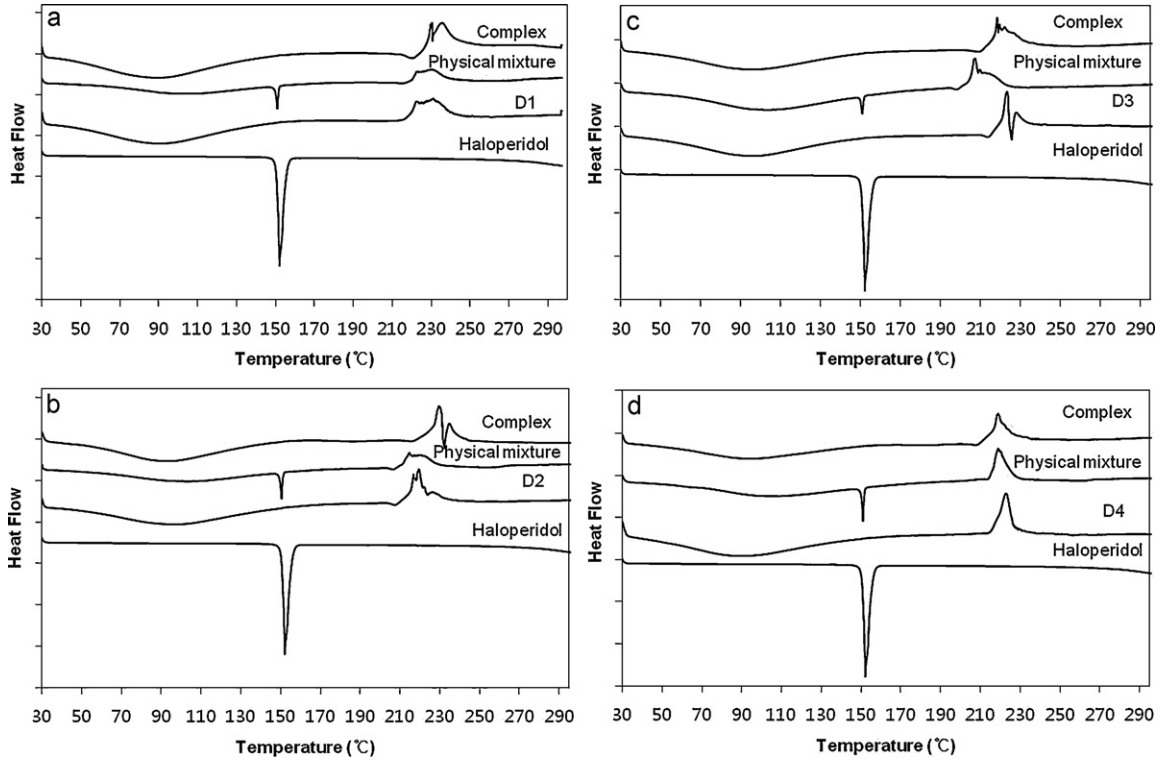

Fig. 4. DSC curves of HAL, dimers, equivmolar ratio of physical mixtures (10 mM) and equivmolar complexes (10 mM) (a) D1, (b) D2, (c) D3 and (d) D4.

of the HAL/Dimer complexes, the endothermic peaks at about 150 °C, which corresponded to the free HAL, disappeared and only the exothermic peak around 220 °C was present (Fig. 4a–d). These results indicate that HAL formed complexes with the dimers.

3.4. 2D nuclear overhauser effect spectroscopy (NOESY) analysis

Nuclear overhauser effect spectroscopy (NOESY) provides important information on the interaction between HAL and dimers atoms by measuring the intermolecular dipolar cross-correlations (Yang et al., 2011). When the two protons are located close in space, a nuclear overhauser effect (NOE) cross-correlation occurs in the NOE spectroscopy (NOESY). The NOE cross-peaks between protons from two species can be elucidated based on the spatial contacts within 0.4 nm (Correia et al., 2002). The NMR signal of D3 was assigned by ^{1}H and ^{13}C, HMBC (Heteronuclear Multiple Bond Correlation), and HSQC (Heteronuclear Single Quantum Coherence) NMR Spectroscopy techniques (data not shown.). Correlations between proton d of HAL with *f* H-4, *h*H-6 and *a*H-6 protons of D3, as well as self correlations of HAL of H-1, 2, 3, 4 with C protons and H-2, and5 with a, b, and d protons were observed in the NOESY spectrum of the HAL/D3 complex (Fig. 5).

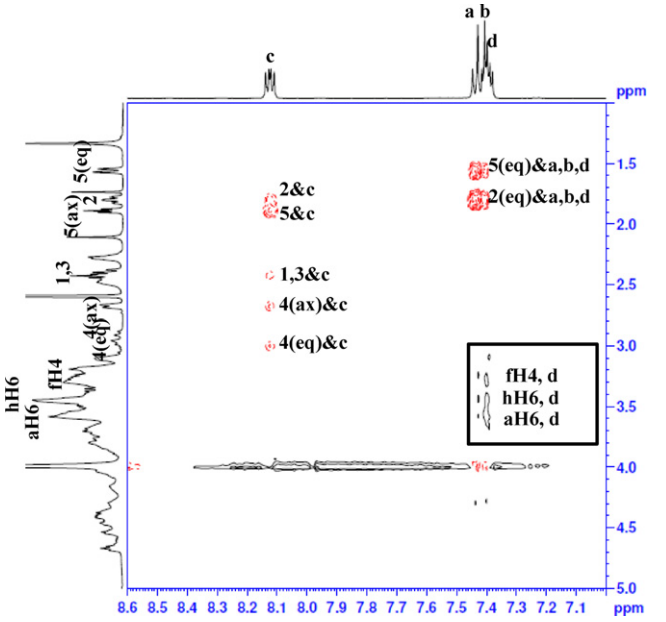

Fig. 5. NOESY spectrum of a solution containing 80% dimethyl sulfoxide-d6 (D, 99.9%) and 20% deutrated water (D_2O, 99.96%) and equivmolar amounts of HAL (10 mM) and D3 (10 mM).

3.5. Scanning electron microscope (SEM) analysis

Scanning electron microscopy (SEM) is a qualitative method used to study the structural aspects of raw materials and drugs or the products obtained by different methods of preparation, such as physical mixing, solution complexation, and others (De Araujo et al., 2008; Duchêne, 1987). SEM photographs of HAL, D3 and HAL/D3 complex are shown in Fig. 6. The regular crystal shape of HAL was observed in many different sizes (Fig. 6a). In addition, D3 appeared as irregular-shaped crystal particles with large dimensions (Fig. 6b). However, the morphology of the HAL/D3 complex appeared as amorphous particles and was quite different from the sizes and shapes of both HAL and D3 (Fig. 6c), which further confirmed the formation of the complex.

3.6. Computational analysis

Fig. 7 is a snapshot of the docked poses of the two haloperidol molecules upon D3 succinoglycan. The Glide docking scores for a pose-1 and a pose-2 were ranked −5.474 and −4.943, respectively.

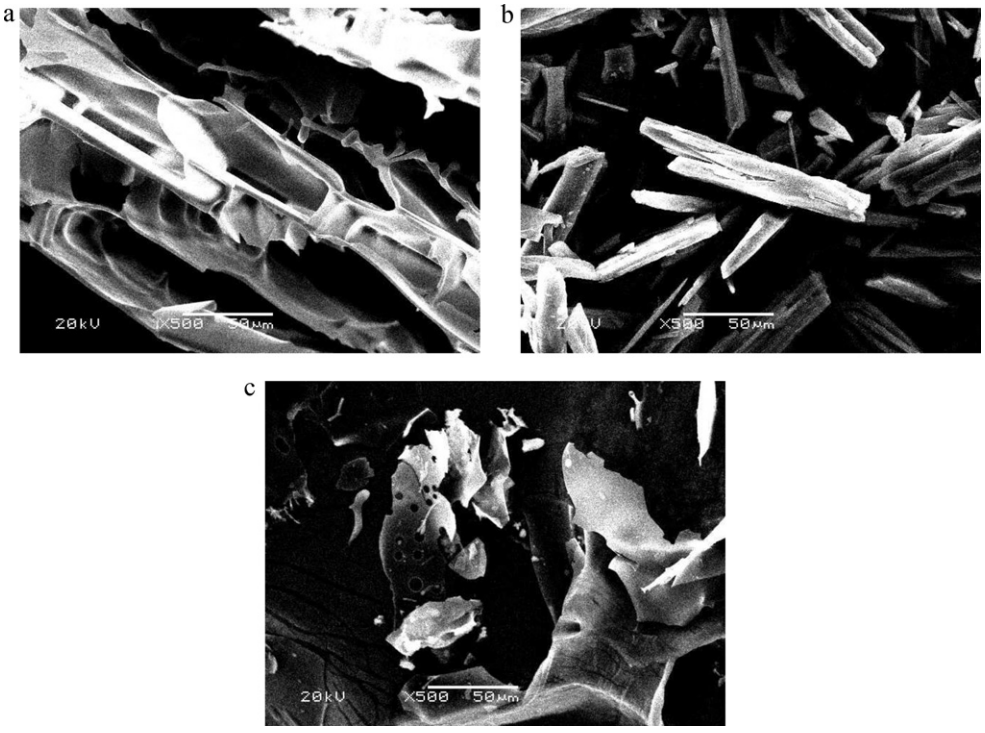

Fig. 6. Scanning electron micrographs of (a) HAL, (b) D3, and (c) complex 1:1, molar ratio, 500× magnification, bar = 50 µm.

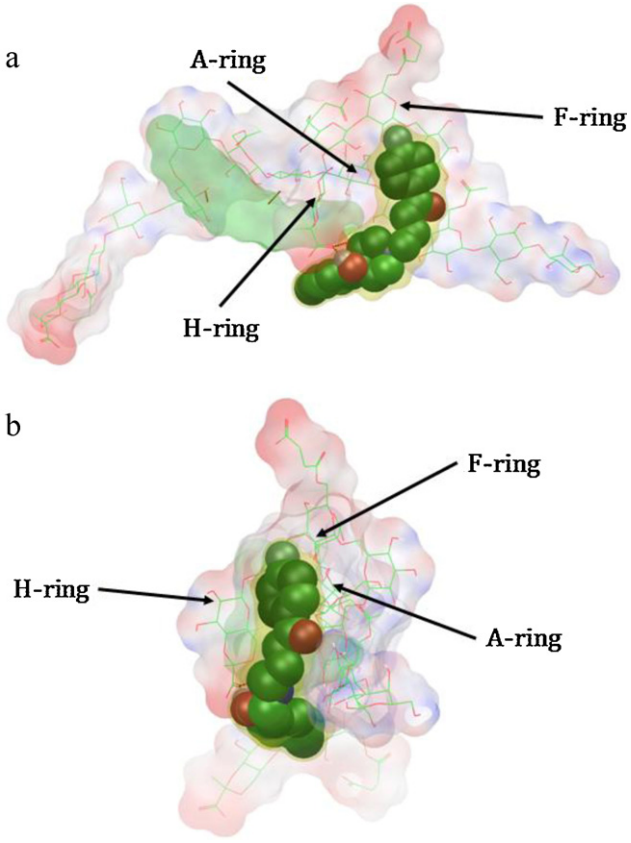

Fig. 7. Front (A) and rear view (B) of the 1:2 binding model of the complex between D3 and haloperidols. The pose-1 structure of haloperidol was represented as a space-filling model, and the pose-2 structure is shown as a solvent accessible surface area in shadow.

The docking scores for each pose of haloperidol showed fine correlation with apparent stability constants, $K_{1:1}$ and $K_{1:2}$ for the complex between D3 and haloperidols. Two haloperidols were bound to the molecular surface of D3 using atomic interactions between both haloperidol-D3 and haloperidol-haloperidol. The fluorophenyl and piperidyl residues of two haloperidol molecules had close contacts with each other. Stable pose-1 structure of haloperidol made close contacts with the *f*- and *h*-sugar ring of D3, while the pose-2 showed contacts with the *a*-sugar ring of D3. Overall, the docked features closely corresponded to the NMR-derived structural characteristics of the complex between D3 and haloperidol molecules. It was concluded that the formation of both the 1:1 and 1:2 complex between D3 and haloperidol was theoretically possible from the present molecular docking simulations.

3.7. Cytotoxicity assay

The colorimetric MTS test, based on the selective ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide into purple formazan, relies on intact metabolic activity of cells, and is frequently used for screening of cytotoxicity (Mosmann, 1983). Thus, we examined the cytotoxicity of the succinoglycan dimers by the MTS assay using a human cell line, HEK 293, which is commonly used in toxicity studies to assess the effect of drug candidates (Hettiarachchi et al., 2010). Fig. 8 showed cytotoxicity curves for the of succinoglycan dimers. The percentages of viable HEK293 cells were plotted against various concentrations of the 4 succinoglycan dimers, respectively. Although the cell viability in the treatment of the D4 dimer had a tendency to decline at the higher concentrations above 500 μM, all the 4 succinoglycan dimers did not cause any significant cytotoxicity upon treating the cells up to 1000 μM, and even showed positive.

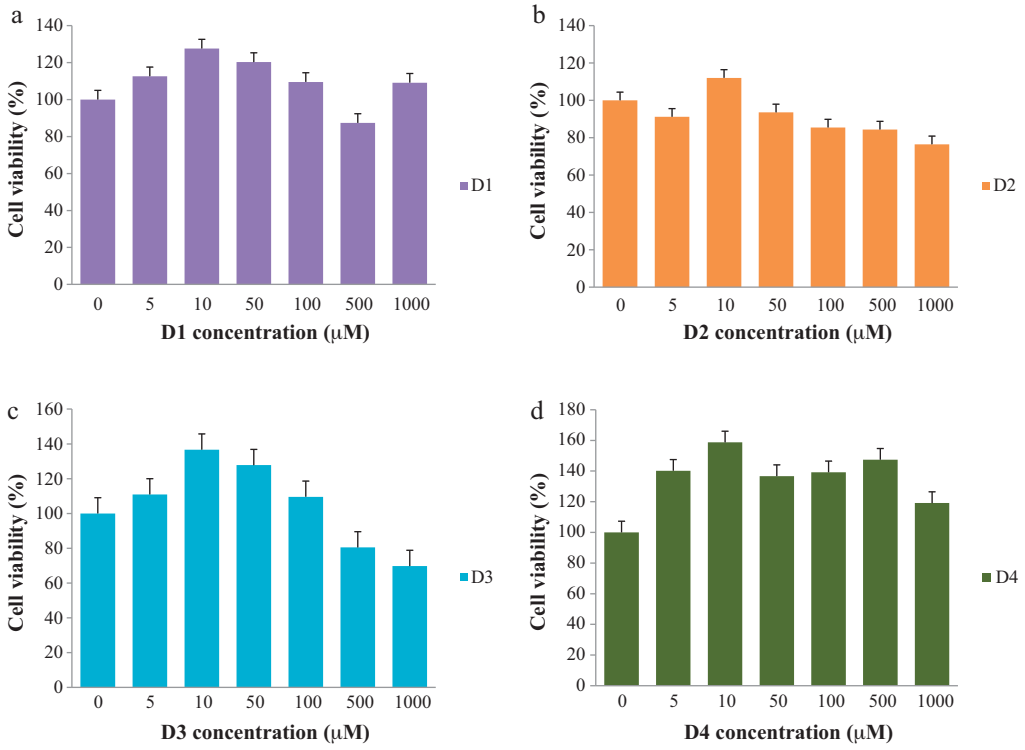

Fig. 8. Cytotoxicity of succinoglycan dimers (a) D1, (b) D2, (3) D3, and (d) D4 at the concentration of 0, 5, 10, 50, 100, 500, 1000 μM against HEK293 cells ($n = 3$).

4. Conclusions

In this study, the complexation behavior, characterization, binding ability, solubilization and stability of succinoglycan dimers isolated from *S. meliloti* with haloperidol were investigated. The results showed that succinoglycan dimers enhanced the water solubility as well as the stability of HAL. The HAL/D3 complex was more stable than the other HAL/dimers (D1, D2, and D4) in terms of the stability constants. The FT-IR, DSC, and SEM results also confirmed effective formation of acyclic succinoglycan dimer complexes with haloperidol. In the NOESY experiments, intermolecular cross-peaks were observed between HAL and D3, suggesting that the complex between HAL and D3 formed both 1:1 and 1:2 complexes. These results were also observed in the molecular docking study. Therefore, we can conclude that the binding ability of dimers for complexation are influenced by the three-dimensional structures of dimers, and the dimers can regulate the efficiency of complexation, which is due to the differential charge distribution of the carboxyl groups within the succinoglycan dimer. Based on these results, the acyclic glycan originated from microbial organisms can be used to enhance the solubility and stability of hydrophobic drugs and their derivatives. Conversion of MTS by the mitochondria of living cells is one of the assays currently used to evaluate the cell viability (Jos et al., 2009). The results of these studies succinoglycan dimers cytotoxicity were not specific to the HEK293 cell. It was suggested that the succinoglycan dimers might be a safe biomaterial, which can be used in therapeutic agents.

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References

Battisti, L., Lara, L., & Leigh, J. A. (1992). Specific oligosaccharide form of the *Rhizobium meliloti* exopolysaccharide promotes nodule invasion in alfalfa. *Proceedings of the National Academy of Sciences*, *89*, 5625–5629.

Bernard, G., & Simona, A. (2005). The haloperidol story. *Annals of Clinical Psychiatry*, *17*, 137–140.

Brewin, N. J. (1991). Development of the legume root nodule. *Annual Review of Cell Biology*, *7*, 191–226.

Cho, E., Choi, J. M., Kim, H., Lee, I. S., & Jung, S. (2011). Hydrophobic interactions of succinoglycan dimers isolated from *Sinorhizobium meliloti* with hydrophobic fluorescence probes, 8-anilino-1-naphthalenesulfonate and 6-p-toluidino-2-naphthalenesulfonate. *Bulletin of the Korean Chemical Society*, *32*, 4071.

Chouly, C., Colquhoun, I. J., & Jodelet, A. (1995). NMR studies of succinoglycan repeating-unit octasaccharides from *Rhizobium meliloti* and *Agrobacterium radiobacter*. *International Journal of Biological Macromolecules*, *17*, 357–363.

Correia, I., Bezzenine, N., Ronzani, N., Platzer, N., Beloeil, J. C., & Doan, B. T. (2002). Study of inclusion complexes of acridine with β- and (2,6-di-O-methyl)-β-cyclodextrin by use of solubility diagrams and NMR spectroscopy. *Journal of Physical Organic Chemistry*, *15*, 647–659.

De Araujo, D. R., Tsuneda, S. S., Cereda, C. M., Del, G. F., Carvalho, F., Preté, P. S., et al. (2008). Development and pharmacological evaluation of ropivacaine-2-hydroxypropyl-beta-cyclodextrin inclusion complex. *European Journal of Pharmaceutical Sciences*, *33*(1), 60–71.

Drug Information, A.H.F.S. Haloperidol. www.ashp.org/mngrphs/ahfs/a382159.htm (2008).

Duchêne, D. (1987). *Cyclodextrins and their industrial uses*. Paris: Editions de Santé.

Eggens, I., Fenderson, B., Toyokuni, T., Dean, B., Stround, M., & Hakomori, S. (1989). Specific interaction between Lex and Lex determinants. A possible basis for cell recognition in preimplantation embryos and in embryonal carcinoma cells. *Journal of Biological Chemistry*, *264*, 9476.

Friesner, R. A., Banks, J. L., Murphy, R. B., Halgren, T. A., Klicic, J. J., Mainz, D. T., et al. (2004). Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *Journal of Medicinal Chemistry*, *47*, 1739–1749.

Gonzalez, J. E., Reuhs, B. L., & Walker, G. C. (1996). Low molecular weight EPS II of *Rhizobium meliloti* allows nodule invasion in *Medicago sativa*. *Proceedings of the National Academy of Sciences*, *93*, 8636–8641.

Gonzalez, J. E., York, G. M., & Walker, G. C. (1996). *Rhizobium meliloti* exopolysaccharides: Synthesis and symbiotic function. *Gene*, *179*, 141–146.

Hettiarachchi, G., Nguyen, D., Wu, J., Lucas, D., Ma, D., Isaacs, L., et al. (2010). Toxicology and drug delivery by Cucurbit[n]uril type molecular containers. *PLoS One*, *5*(5), e10514.

Higuchi, T., & Connors, K. A. (1965). Phase-solubility techniques. *Advances in Analytical Chemistry and Instrumentation*, *4*, 117–212.

Jos, A., Pichardo, S., Puerto, M., Sánchez, E., Grilo, A., & Cameán, A. M. (2009). Cytotoxicity of carboxylic acid functionalized single wall carbon nanotubes on the human intestinal cell line Caco-2. *Toxicology in Vitro*, *23*, 1491–1496.

Kano, K., Minami, K., Horiguchi, K., Ishimura, T., & Kodera, M. (1995). Ability of non-cyclic oligosaccharides to form molecular complexes and its use for chiral separation by capillary zone electrophoresis. *Journal of Chromatography A*, *694*, 307–313.

Kwon, C., Lee, S., & Jung, S. (2011). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric behavior of succinoglycan monomers, dimers, and trimers isolated from *Sinorhizobium meliloti* 1021. *Carbohydrate Research*, *346*, 2308–2314.

Loukas, Y. L., Vraka, V., & Gregoriadis, G. (1997). Novel non-acidic formulations of haloperidol complexed with beta-cyclodextrin derivatives. *Journal of Pharmaceutical and Biomedical Analysis*, *16*(2), 263.

Martin, E. M., & Valle, D. (2004). Cyclodextrins and their uses: A review. *Process Biochemistry*, *39*(9), 1033–1046.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55–63.

Reinhold, B. B., Chan, S. Y., Reuber, T. L., Marra, A., Walker, G. C., & Reinhold, V. N. (1994). Detailed structural characterization of succinoglycan, the major exopolysaccharide of *Rhizobium meliloti* Rm1021. *Journal of Bacteriology*, *176*, 1997–2002.

Remington, J. P. (1990). *Remington's Pharmaceutical Sciences* (18th ed.). Easton: Mack Pub. (pp. 1086.)

Yang, L. J., Chen, W., Ma, S. X., Gao, Y. T., Huang, R., Yan, S. J., et al. (2011). Host–guest system of taxifolin and native cyclodextrin or its derivative: Preparation, characterization, inclusion mode, and solubilization. *Carbohydrate Polymers*, *85*(3), 629–637.

Wang, L. X., Wang, Y., Pellock, B., & Walcker, G. C. (1999). Structural characterization of the symbiotically important low-molecular-weight succinoglycan of *Sinorhizobium meliloti*. *Journal of Bacteriology*, *181*, 6788.