Synthesis of Oligomeric Microbial Cyclosophoraoses as Novel Complexation Agents

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Cyclosophoraoses (Cys) are synthesized by all members of the family Rhizobiaceae. Cys are a class of unbranched cyclic oligosaccharides composed glucose residues linked solely by β-1,2-glycosidic bonds, and the predominant ring size distribution is between 17 and 25 glucose residues.[1] The biological activity of Cys has been studied in the peas, clovers, and beans (*Phaseolus vulgaris* L.) root-nodule bacteria *Rhizobium leguminosarum bv. phaseoli*. These molecules have been shown to provide two independent functions; namely, they are involved in the formation of nitrogen-fixing root nodules[2,3] and promote the osmoadaptation of bacterial cells.[4] Recent reports have shown that Cys forms inclusion complexes with a variety of hydrophobic guest molecules such as amphotericin B, fluorescein, flurbipropen, indomethacin, paclitaxel and vitamins.[5] Much attention has thus been focused on their potential ability to form inclusion complexes with other lipophilic molecules as well as on their biological functions. To further applications of Cys, the cyclic oligosaccharides were modified with various functional groups such as carboxymethyl,[6] sulfonyl,[7] and succinyl[8] groups. However, no attempts have yet been made a polymerization or oligomerization of Cys to improve the inclusion complexation ability. Similarly, cyclodextrins (cyclic β-1,4-glycans)-based polymers are of interest due to their merits compared to parent CDs, such as high solubility in water and capability to solubilize a number of drugs, those with large molecular structures in particular. CD polymers can also increase the drug bioavailability.[9-12] Therefore, the synthesized Cys polymers or oligomers would be used as efficient hosts for the solubility enhancement of hardly soluble drugs as well as for the bioavailability increase of natural compounds.

Ibuprofen and naproxen are non-steroidal anti-inflammatory (NSAID), analgesic and antipyretic agent having main mechanism of action through block a group of enzymes called COX (cyclo-oxygenase) enzymes. They reduce pain as analgesic drugs, high temperatures as antipyrexial drugs, and swelling as anti-inflammatory drugs and make the blood less likely to clot as antithrombolic drugs.[19] However, they have a very low solubility in water and when administered orally, it causes gastrointestinal side-effects, drowsiness and dizziness. Therefore, improvement of their solubility is challenging and rational.[16]

In the study, Cys were isolated from *Rhizobium phaseoli*, which is a member of the family Rhizobiaceae by ethanol precipitation and purified by various chromatographic techniques.[15] An oligomeric cyclosophoraoses (oligo-Cys) were synthesized from cyclosophoraose (Cys), epichlorohydrin (EP) and choline chloride (CC) through a one-step polymerization procedure.[12,13] The physicochemical properties of the modified oligo-Cys were characterized using various techniques including MALDI-TOF MS and ^{1}H NMR spectroscopic analysis and the effects of the degree of Cys oligomerization on their drug inclusion performance was investigated by ultraviolet (UV)-visible spectroscopy. The formation of their inclusion complexes also was confirmed by fourier transform infrared spectroscopy (FT-IR).

Preparation of water soluble oligo-Cys was achieved by the reaction of Cys, isolated and purified from *Rhizobium phaseoli*, with EP and CC in an alkaline medium by a one step procedure (Scheme 1).[12,13] The reaction mixture was separated by Bio-Gel P-6 depends on degree of oligomerization. Fractionation of the reaction mixture on a gel filtration column of Bio-Gel P-6 yielded three major carbohydrate peaks (Fig. 1). In the MALDI-TOF MS spectra, the ring sizes of the Cys, which ranged from 17 to 24, were confirmed by MALDI-TOF MS spectrometry (Fig. 2(a)). Based on the MALDI-TOF mass spectrometric analysis,[18] the average molecular weight (M_n) of Cys was determined as 3078 Da.
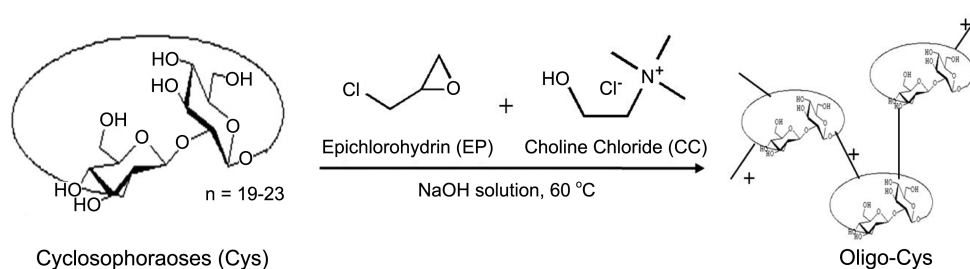
Scheme 1. Reaction scheme of polycondensation of the Cys with EP and CC.
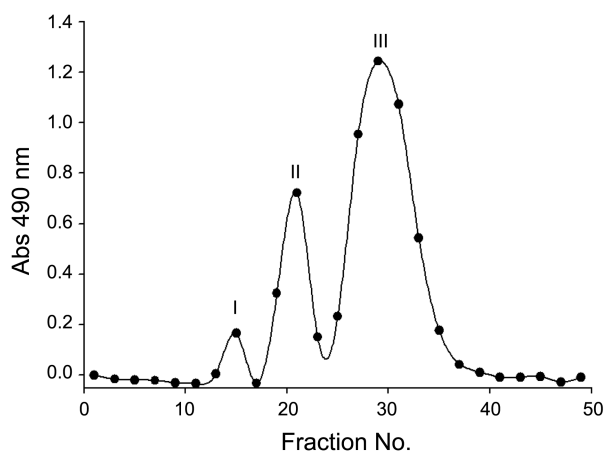
Figure 1. Gel filtration chromatography of the synthetic cationic oligo-Cys fractionation on Bio-Gel P-6.

Through MALDI-TOF MS spectroscopic analysis, the lower molecular weight fraction (peak III) revealed that the peak III is a mixture of different isomers of Cys glyceryl ethers and just modified Cys by EP and CC, because its average molecular weight (M_n) is slightly increased as about 3586 Da (Fig. 2(b)). The other peaks supposed real oligomer fractions (peak I and II) which consist of one and two Cys compound interconnected with longer or shorter glyceryl ether chains. Through MALDI-TOF MS analysis (Fig. 2(c) and (d)), higher molecular weight fraction appeared mostly at a average molecular weight of 7537 Da and 11323 Da,

respectively, and suggested as the oligomer fractions consistent by with the expected molecular weight of dimer and trimer of Cys, respectively. Thus, the peak II and I were presumed to be Cys dimer and trimer, respectively, connecting as a glyceryl bridge by EP and CC. In the addition, the ^{1}H NMR spectra (Fig. 3) were shown one peak around 5 ppm assigned to the C-l proton of the glucose unit, two broadened peaks between 3 and 4ppm corresponding to protons from C-2, 3, 4, 5 and 6 of the pyranose rings of Cys, respectively. The peaks were detected at around 3.1 ppm is assigned to the methyl groups of CC, indicating the successful introduction of quaternary ammonium group. The peak assigned to the proton of 2-hydroxypropyl ether segments also is assigned at about 3 ppm, reflecting the chemical shift caused by the introducing of EP. The NMR spectra of different molecular weight fractions are shown in Figure 3. An apparent peaks at about 3 ppm, was present in the modified Cys or oligo-Cys fractions (Fig. 3(b), (c) and (d)), whereas these peaks was absent in only Cys (Fig. 3(a)).

Figure 4 shows the UV absorption spectra of ibuprofen and naproxen in aqueous solution with Cys and synthesized Cys (modified Cys, Cys dimer and Cys trimer) where the absorption intensity of dissolved ibuprofen and naproxen is increased by the addition of Cys dimer and Cys trimer. In case of the absorption of naproxen, the addition of Cys dimer and trimer shows similar effective UV absorbance increase than other addition. However, the addition of Cys trimer shows more effective UV absorbance increase than
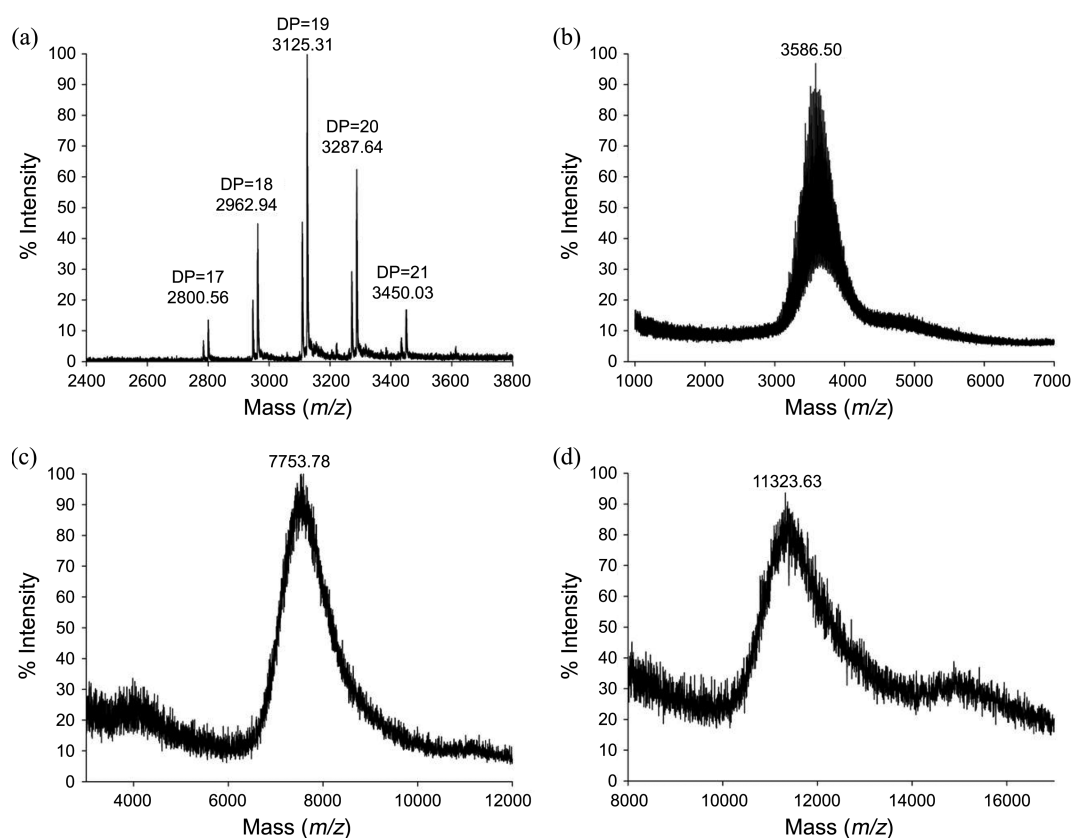

Figure 2. MALDI-TOF mass spectra of native Cys (a) and modified Cys (b) acquired in positive ion reflector mode, and MALDI-TOF mass spectra of Cys dimer (c), Cys trimer (d) also acquired in positive ion linear mode.
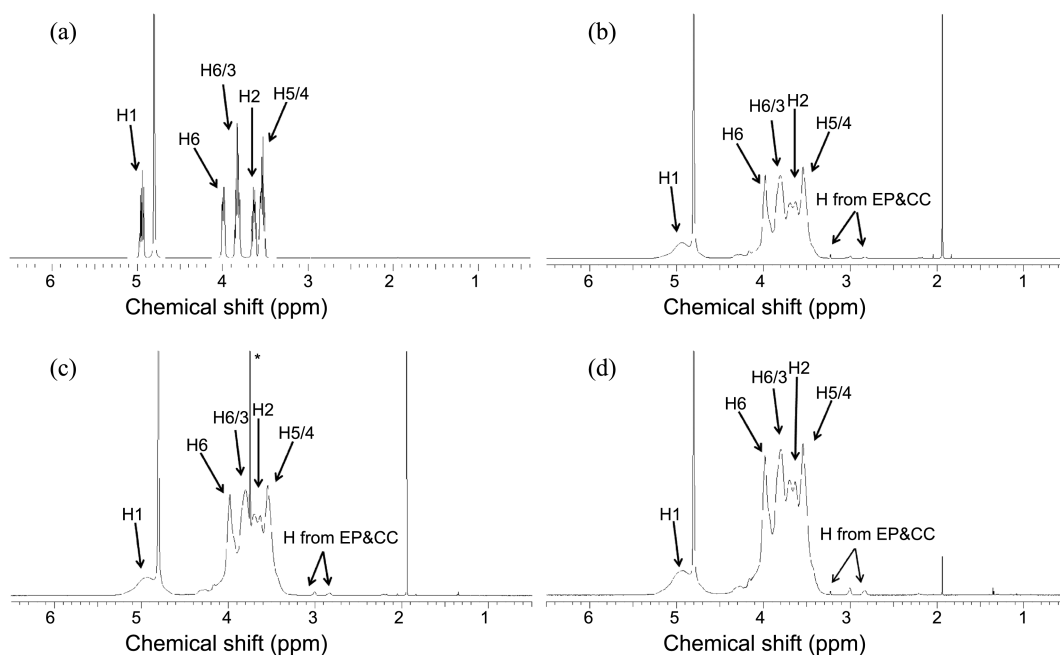
Figure 3. ^{1}H NMR spectra of native Cys (a), modified Cys (b), Cys dimer (c), Cys trimer (d). *These peak represents an unknown contaminant.

Cys dimer and others in case of the absorption of ibuprofen. The UV absorbance increase suggests that these oligo-Cys may become novel useful compexation agents of low water-soluble compounds such as ibuprofen and naproxen. It can be concluded that free ibuprofen and naproxen are effectively included in the cavities of Cys dimer or trimer compared with Cys and modified Cys.
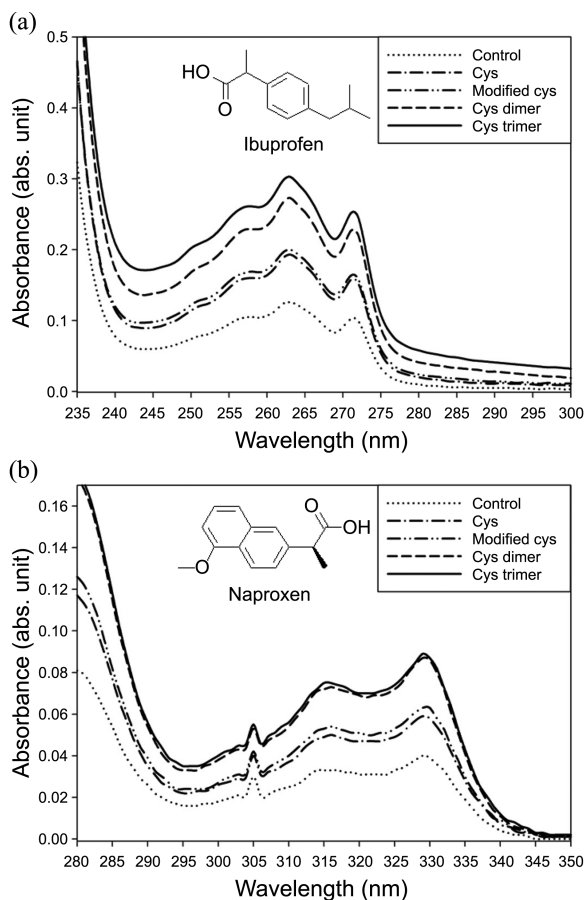
FT-IR spectra provide further evidence on the formation of inclusion complexes between drugs and oligo-Cys. Because FT-IR spectra of Cys dimer are observed very similar curves compared with these of Cys trimer, FT-IR spectra of drug/Cys trimer inclusion complexes are included as shown in Figure 5. Ibuprofen and naproxen are characterized by peaks appearing between 1750 and 650 cm^{-1} (Fig. 5(a)). They are different from the strong peaks of Cys trimer at about 1074 cm^{-1} (Fig. 5(b)). The peak at 1727 cm^{-1} and 1718 cm^{-1} of

Figure 4. UV-visible spectra of ibuprofen (a) and naproxen (b) in the absence and presence of host molecules (1 mM).
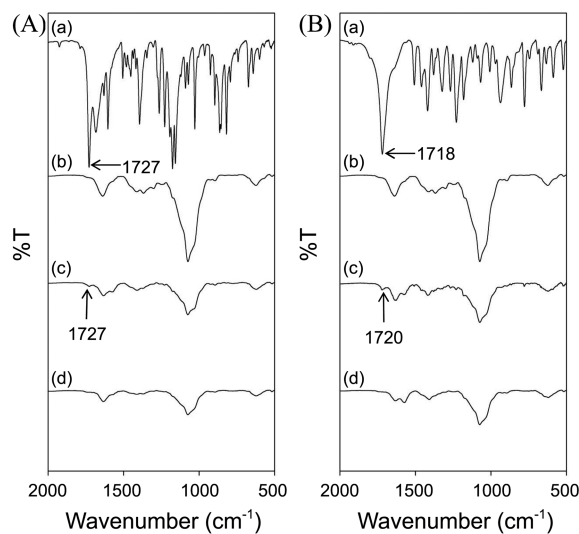

Figure 5. FT-IR spectra of ibuprofen (A) and naproxen (B) of drug crystalline (a), Cys trimer (b), drug/Cys trimer physical mixture (c), and drug/Cys trimer inclusion complex (d).

the C=O stretching of the carboxylic groups is the important characteristic band of ibuprofen and naproxen, respectively. The carbonyl stretching vibration band of ibuprofen and naproxen observed at 1727 cm^{-1} and 1720 cm^{-1}, respectively, remained unchanged in the physical mixture (Fig. 5(c)) but disappear after inclusion complexation with Cys trimer (Fig. 5(d)). These results indicate the modification of environment of drugs due to the formation of drugs/oligo-Cys complex. If it were not so, then the spectra would resemble that of a physical mixture of drugs and oligo-Cys.

In the study, novel water-soluble oligomeric microbial cyclosophoraoses (oligo-Cys) was synthesized by EP and CC as a crosslinking agent from microbial cyclosophoraoses (Cys), isolated from *R. phaseoli*. The MALDI-TOF MS and ^{1}H NMR study revealed that the oligo-Cys, synthesized by the crosslinking reaction, composed of dimer or trimer cross-linking Cys. Cys oligomer was investigated inclusion com-plexation as a novel host molecule for poor soluble drugs such as ibuprofen and naproxen. In UV-visbles spectroscopic study for solubility enhancement, the absorption intensity of dissolved ibuprofen and naproxen is effectively increased by the addition of Cys dimer and Cys trimer. FT-IR spectra also supported the observation of inclusion complex formation between drugs and Cys trimer.

Experimental section

Meterials. Epichlorohydrin (EP) and choline chloride (CC) were purchased from TCI korea (Tokyo Chemical Industry. Co., Ltd. Seoul, South Korea). Ibuprofen and naproxen were purchased from Sigma Chemical Company. Other materials and solvent were of analytical reagent grade. Double distilled-deionized water was used throughout.

Preparation of Cyclosophoraoses (Cys). The *R. phaseoli* produces Cys. Isolation and purification of Cys were carried out as described in the previous reports.[14,15]

MALDI-TOF MS and NMR Spectroscopic Analysis of Oligo-Cys. For the identification of oligo-Cys, NMR spectro-scopic analysis was performed on a Bruker (AMX, Germany) spectrometer (600 MHz) with deuterium water (D$_2$O; 99.9 at.% D). All NMR measurements were performed with 0.7 mL samples in 5 mm NMR tubes. Tetramethylsilane (TMS, Me$_4$Si) was used as an external reference. MALDI-TOF ex-periments were essentially performed using a Voyager-DE^{TM} STR BioSpectrometry (PerSeptive Biosystems, Framingham, MA, USA) in positive ion reflector mode or linear mode. Samples were prepared according to the dried droplet method[17] by using DHB (2,5-dihydroxybenzoic acid, Aldrich-Sigma), in 10 mg/mL in H$_2$O. The number-average molecular weights, Mn, of the oligo-Cys sample were determined in the linear mode.

Solubility of Drug Complexes. Solubility study of ibuprofen and naproxen were carried out by adding 10 mg of drug to 1 mM of aqueous solution of the synthesized oligo-Cys to a sealed glass container. The mixture was sonicated for 10 min in water bath and then magnetically stirred for 24 hr at room temperature, shielded from light to prevent any degradation of the molecules. After equilibrium, the suspen-sions were filtered with 0.2 μm filter. The amount of ibupro-fen and naproxen dissolved was analyzed with a UV-vis spectrophotometer.

Fourier Transform Infrared Spectroscopy (FT-IR). Fourier transform infrared spectroscopy was performed on Bruker IFS-66/Sspectrometer (AMX, Germany), running from 4000 to 500 cm^{-1} using zinc selenide as a sample holder. The blend of ibuprofen and naproxen with trimeric Cys, respectively, at 1:1 (mol/mol) ratio was manually ground using a mortar with a pestle for 10 min. 1.5-2.0 mg of four different samples, drug (ibuprofen or naproxen), trimeric Cys, physical mixture and Drug/monomers complex, were mixed with a KBr pellet.

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