

Synthesis of Microbial Cyclosophoraose Derivatives Grafted Magnetic Nanoparticles

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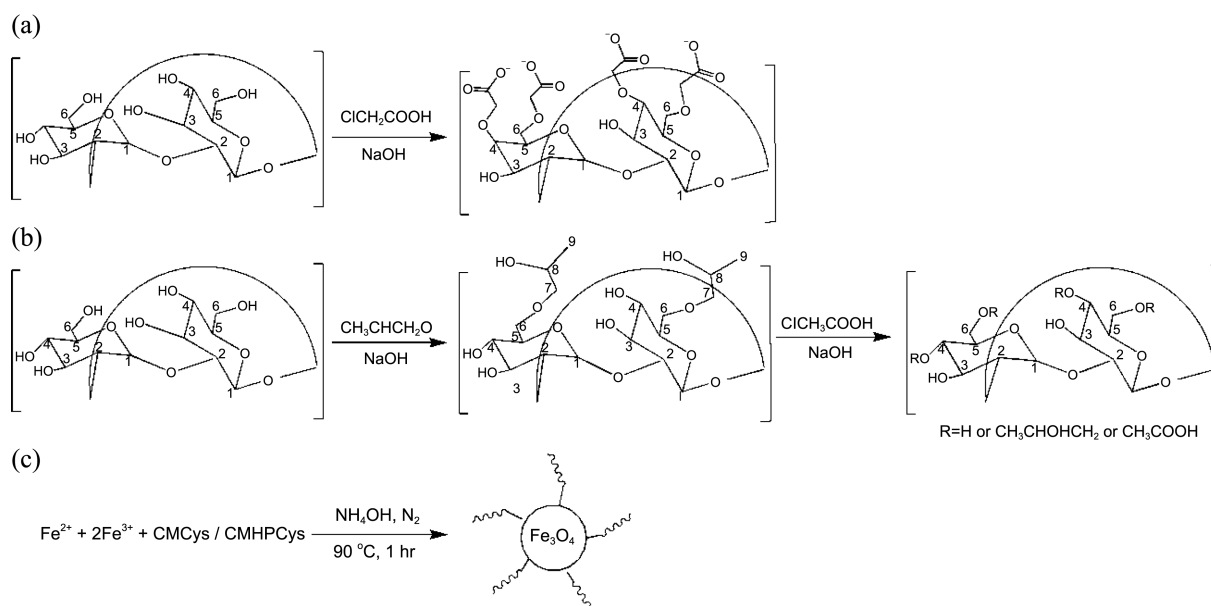
Rhizobium leguminosarum biovar *viciae* VF-39 were isolated from nodules of *Vicia faba*.² The Rhizobiaceae are a family of gram-negative bacteria that infect and enter into symbiosis with legume plants for the fixation of nitrogen molecules into ammonia. The type of carbohydrate found on the cell surface during this process is related to the specificity of initial recognition and nodule development.¹ Cyclosophoraoses (Cys) are a class of unbranched cyclic oligosaccharides isolated from *R. leguminosarum* biovar *viciae* VF-39.² Cys are composed of glucose residues linked by β -1,2-glycosidic bonds, with a degree of polymerization (DP) varying from 17 to 23.

Cys form complexes with various hydrophobic guest molecules, such as the cancer chemotherapy drug paclitaxel and the non-steroidal anti-inflammatory drug indomethacin.^{3,4} This complex-forming ability helps overcome the extremely low aqueous solubility of these drugs, making Cys extremely useful for clinical applications. To increase the variety of molecules with which they can interact, Cys have been chemically modified with functional moieties such as butyryl, methyl, and carboxymethyl groups.⁵⁻⁷ The modifications enhance the ability of Cys to form complexes with flavonoids, hydrobenzoin, and *N*-acetyltryptophan, as

compared to that of the unmodified Cys. Carboxymethylated Cys enhanced the solubility of hydrobenzoin, and *N*-acetyltryptophan about 5.1- and 299-fold, respectively.⁷ However, both Cys and modified Cys can also function as chiral selectors in capillary electrophoresis for several flavonoids-naringenin, hesperetin, and taxifolin.⁸ Furthermore, they also functioned as catalytic carbohydrates for methanolysis by reducing the free energy of activation and thus stabilizing the transition state of some reactions.⁹

Commercially available carbohydrates, *e.g.*, cyclodextrin- and modified cyclodextrin-bonded magnetic nanoparticles (MNPs), applied as a matrix can enhance the encapsulation of guest molecules, efficiently regulate the drug release rate and drug targeting, act as an adsorption reagent for chiral aromatic amino acids, and remove heavy metals from industrial waste water.¹⁰⁻¹² In addition to their complex-forming properties, carbohydrate-bonded MNPs have the advantage of being reusable.¹³ This paper describes the synthesis of MNPs by novel functionalization using Cys derivatives.

First, the isolation and purification steps of Cys were conducted. The average molecular weight of Cys has been confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and



Scheme 1. Synthesis of (a) CM-Cys, (b) CM-HP Cys, and (c) CM-Cys/CM-HP Cys-grafted Fe_3O_4 MNPs.

structural confirmation was obtained using nuclear magnetic resonance (NMR) spectroscopy.¹⁴ Carboxymethyl Cys (CM-Cys) was produced by modifying Cys with monochloroacetic acid under alkaline conditions (Scheme 1(a)). The reaction was monitored by thin layer chromatography (TLC), and the products were characterized by MALDI-TOF MS, NMR spectroscopy, and Fourier transform infrared (FTIR) spectroscopy. The NMR data showed that carboxymethyl groups replaced the 4-OH and 6-OH of each glucose unit of Cys (data not shown), as previously described.⁹ CM hydroxypropyl cyclodextrin (CM-HP Cys) was prepared by a two-step method involving the synthesis of HP Cys, followed by the addition of carboxymethyl groups to HP Cys (Scheme 1(b)).¹⁷ The products obtained at each step were characterized by NMR spectroscopy and MALDI-TOF MS. The NMR data showed that HP groups were conjugated at the 6-OH position of each glucose unit of Cys, while the 4-OH and 6-OH of glucose were substituted by carboxymethyl groups. MS data indicated that the average molecular weight of CM-HP Cys was 3683.9 (data not shown).

CM-Cys and CM-HP Cys were grafted onto MNPs. In this one-step reaction, the carboxyl groups of CM-Cys and CM-HP Cys reacted directly with the hydroxyl groups on the MNP surface to form Fe-carboxylate.¹⁵ The FTIR spectra of bare MNPs and of CM-Cys- and CM-HP Cys-grafted MNPs confirmed the conjugation of CM-Cys and CM-HP Cys to the MNP surface (Figure 1). The characteristic adsorption band for Fe-O bonds was observed at 613 cm^{-1} . The C-O stretching bands for CM-Cys and CM-HP Cys were seen at 1085 cm^{-1} . The peaks at 1446 cm^{-1} and 1652 cm^{-1} corresponded to $-\text{COO}-\text{Fe}$ groups.^{12,15} For CM-Cys and CM-HP Cys MNPs, the band corresponding to Fe-O decreased, while the intensities of the adsorption peaks corresponding to C-O stretching and the carboxylate and $-\text{COO}-\text{Fe}$ groups were enhanced compared to those for bare MNPs, indicating that CM-Cys and CM-HP Cys were successfully functionalized onto the surface of Fe_3O_4 MNPs.

Uncoated and Cys derivative-coated MNPs were examined by transmission electron microscopy (TEM). Densely

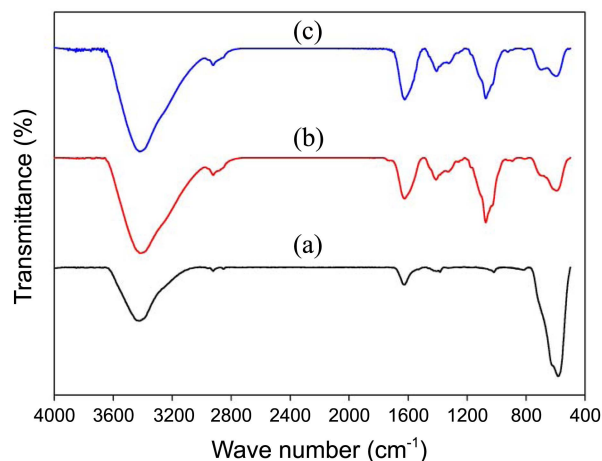


Figure 1. (a) FTIR spectra of a bare MNP, (b) a CM-Cys-grafted MNP, and (c) a CM-HP Cys-grafted MNP.

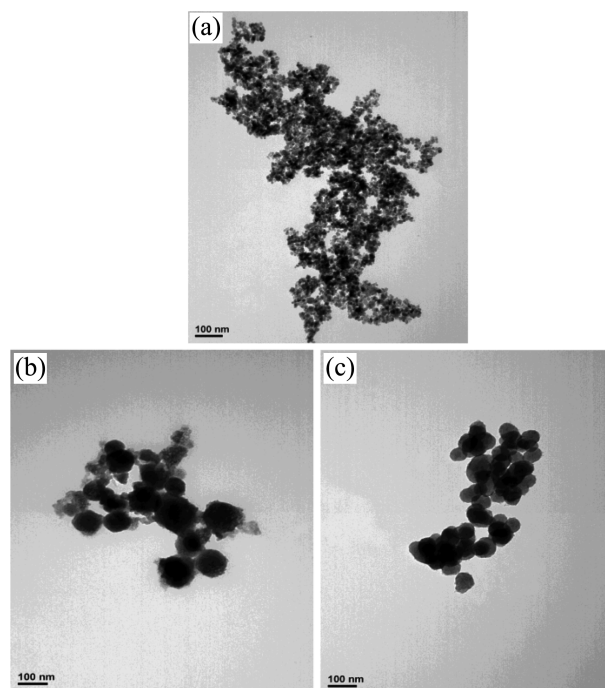


Figure 2. Transmission electron micrographs of (a) a bare MNP, (b) a CM-Cys-grafted MNP, and (c) a CM-HP Cys-grafted MNP.

packed spherical clusters were observed in all samples (Figure 2). The MNP size increased upon grafting of the modified microbial carbohydrates, as determined by dynamic light scattering (DLS). The mean diameters of the bare MNPs and the CM-Cys- and CM-HP Cys-grafted MNPs were approximately 19.3, 40, and 30.4 nm, respectively (Figure 3). The incremental increase in the hydrodynamic diameter could be attributed to carbohydrate functionalization.

The unmodified and Cys-modified particles were also assessed by thermogravimetric analysis (TGA). Weight re-

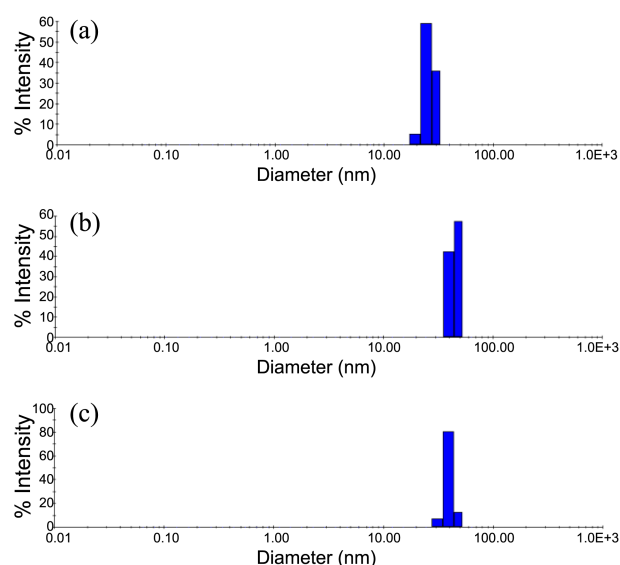


Figure 3. DLS data for (a) a bare MNP, (b) a CM-Cys-grafted MNP, and (c) a CM-HP Cys-grafted MNP.

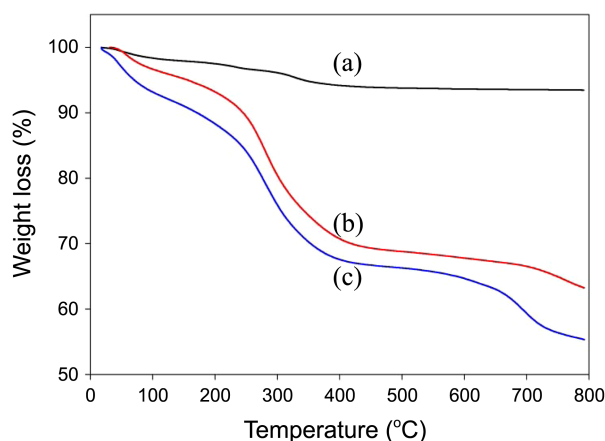


Figure 4. (a) TGA curves of a bare MNP, (b) a CM-Cys-grafted MNP, and (c) a CM-HP Cys-grafted MNP.

ductions of 1.7%, 3.3%, and 6.9% were observed for bare MNPs, CM-Cys-grafted MNPs, and CM-HP Cys-grafted MNPs, respectively (Figure 4) at around 100 °C, likely due to the loss of water from the samples.¹⁵ The weight decreased continuously in the range of temperatures from 200 °C to 400 °C for CM-Cys- and CM-HP Cys-grafted MNPs, which could be attributed to the decomposition of the conjugated carbohydrate moieties.^{12,15} The total weight losses for CM-Cys- and CM-HP Cys-MNPs were estimated at 29.3% and 32.5%, respectively. Thus, the TGA curves confirmed the successful conjugation of CM-Cys and CM-HP Cys molecules onto the MNPs.

In the present study, the microbial carbohydrate Cys was successfully isolated and modified with carboxymethyl and carboxymethyl hydroxypropyl groups, as confirmed by NMR spectroscopy and MALDI-TOF MS. These Cys derivatives were grafted onto the surface of Fe₃O₄ MNPs, as verified by FTIR spectroscopy, DLS, and TGA. The morphological features of the modified MNPs were visualized by TEM. Taken together, these data demonstrate the successful conjugation of microbial carbohydrate derivatives to MNPs. Therefore, these novel agents will be potentially used in a wide range of biotechnological applications such as chiral separation, catalytic carbohydrates, molecular imaging and nanobiosensor with the advantages of magnetism and reusability of microbial carbohydrates-directed MNPs.

Experimental Section

Isolation of Cys. *R. leguminosarum* biovar *viciae* VF-39 was grown in 500 mL GMS medium, supplemented with 5 g mannitol and 150 mM NaCl, for 14 days at 25 °C. The carbohydrate Cys was isolated by ethanol precipitation method as previously reported,¹⁴ and purified by size chromatography. The structure composition and DP were confirmed by NMR spectroscopy (Bruker 500 MHz spectrometer; AMX, Germany) and MALDI-TOF MS (Voyager-DETM STR spectrometer; Applied Biosystems, Framingham, USA).

Synthesis of CM-Cys and CM-HP Cys. CM-Cys was prepared according to a previously reported method (Scheme

1(a)).⁷ We added 16.3% monochloroacetic acid solution (3.3 mL) to a mixture of Cys (200 mg) and NaOH (1.12 g) in water (3 mL). After the mixture was stirred for 4 h at 50 °C, it was neutralized with 6 N HCl, precipitated by adding 5× volume MeOH, and left overnight at 4 °C. After centrifugation, the precipitate was desalted using Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, USA). The product was monitored by TLC (ethanol:buthanol:water = 5:5:4) and then identified by NMR spectroscopy and MALDI-TOF MS.

CM-HP Cys was synthesized in a two-step reaction (Scheme 1(b)). HP Cys was prepared as follows. NaOH (147.8 mg) was dissolved in 822 μL distilled water, and Cys (200 mg) was added to the solution with stirring until completely dissolved. Propylene oxide (100 μL) was added dropwise to the mixture at the freezing point with stirring. After 24 h at room temperature, the reaction was terminated by adding 5 N HCl (pH = 7.0).¹⁶ A Bio-Gel P-4 column (Bio-Rad) was used to desalt the mixture, and the identity of the product was confirmed by NMR spectroscopy and MALDI-TOF MS. The carboxymethyl group was then conjugated to HP Cys, as described above, for the synthesis of CM-Cys.

Synthesis of CM-Cys- and CM-HP Cys-grafted MNPs. CM-Cys- and CM-HP Cys-grafted MNPs were synthesized using a one-step co-precipitation method (Scheme 1(c)).¹⁵ Maintaining a molar ratio of Fe²⁺:Fe³⁺ = 1:2 (17.2 mg FeCl₂·4H₂O and 47.2 mg FeCl₃·6H₂O), 83.7 mg CM-Cys / 92.6 mg CM-HP Cys were dissolved in 800 μL of distilled water with continuous stirring. After the solution was heated to 90 °C, 100 μL NH₄OH (25%) was added. The reaction proceeded for 1 h at 90 °C with constant stirring in an N₂ environment. The MNPs were washed with distilled water several times to remove any unreacted chemicals and were dried in a vacuum oven.

Characterization of Microbial Carbohydrate-grafted MNPs. TEM (JEOL JEM-2010; JEOL Ltd., Japan) was performed at a voltage of 80 kV to examine the morphological features of MNPs. TEM samples were prepared by coating a Formvar/carbon 200-mesh copper grid with one drop of diluted particle suspension. The grid was dried at room temperature for 24 h prior to analysis.¹² The nanoparticle size was determined by DLS. FTIR spectroscopy (AMX) was performed in the range of 500–4000 cm^{−1} in a KBr matrix to characterize all MNPs. TGA was performed on a TG-DTA 2000SA thermogravimetric analyzer (Bruker), with a heating rate of 10 °C/min from room temperature to 800 °C under N₂.¹⁵

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