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The Formation and Characterization of the Supramolecular Hydrogel based on Poly (Ethylene Glycol) Methyl Ether- α-Cyclodextrin Inclusion Complex Conjugated with Folic Acid

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Abstract: In this work, folic acid (FA) has been conjugated with the α-Cyclodextrin (α-CD) and then used for constructing novel supramolecular hydrogels together with Poly(ethylene glycol) methyl ether (MPEG) in aqueous solutions. This strategy can result in the formation of bioactive supramolecular hydrogels with multifunctional characteristics. Nuclear magnetic resonance (^{1}H NMR), and Fourier-transform infrared (FT-IR) spectroscopy were applied for the structural analyses of the functionalized cyclodextrin with FA. The formation of the supramolecular hydrogel was investigated using wide angle X-ray diffraction (WAXD) and FT-IR for α-CD-FA, MPEG, and freeze-dried hydrogel sample which formed in situ from 2 wt % of MPEG and 7 wt % of α-CD-FA.

Keywords: *Folic acid, supramolecular hydrogel, inclusion complex, α-Cyclodextrin, Poly(ethylene glycol) methyl ether.*

Introduction:

In recent years, supramolecular hydrogels have attracted considerable attention because of their potential applications in drug delivery and tissue engineering.[1-3] The design and construction of supramolecular hydrogels systems through host–guest interaction have been extensively studied.[4-6] Among all potential hydrogels, the supramolecular hydrogels, as a consequence of the inclusion complexation between α-CD and various guest polymers have sparked growing interest in recent years. For example, Yui et al. synthesized PEG/α-CD polyrotaxanes capped with amino acids, polypeptides and oligopeptides, which were used as biodegradable drug carriers or stimuli responsive hydrogels.[7] Huh et al. prepared the graft copolymers containing dextran as backbones and PEG or PPG as side chains, while the CDs were threaded into PEG or PPG chains to form hydrogels.[8,9] Zhu et al.[10] used the cisplatin-loaded block copolymer nanoparticles and α-CD to obtain the supramolecular hydrogels with a stepwise delivery property for cancer therapy.

Although supramolecular hydrogels with cyclodextrins as hosts possess interesting properties for the delivery of therapeutics with high pharmacological activity, low therapeutic index, and poor physicochemical properties, they cannot be used for active drug targeting because they are devoid of any specificity for biological structures. To exploit cyclodextrins as targetable drug delivery systems, the oligosaccharide structure should be properly functionalized with targeting moieties such as peptides, hormones, vitamins, antibody fragments, etc.

Folic acid is a small vitamin, which interacts specifically with the folate binding protein (FBP) located in the caveole-like invaginations on the cell surface receptor.[11,12] Upon receptor interaction, the folate acid-FBP complex is taken up by cells and moves through the many organelles involved in endocytotic trafficking, providing for cytosolic deposition.[11] The folic acid receptor is overexpressed by many types of tumor cells, including ovarian, endometrial, colorectal, breast, lung, renal, neuroendocrine carcinomas, and brain metastases.[13] The folate receptor mediated endocytosis has been largely investigated to expand the therapeutic value of drugs, by increasing delivery to the target tissue as well as the target/nontarget tissue ratio. When folic acid (FA) is attached to carboxyl site, the folate retains its normal receptor-binding affinity and therefore, can be internalized by receptor mediated endocytosis.[14] This principle has been exploited for the selective delivery of imaging agents,[15] gene[16] therapeutic agents,[17] micelles of block copolymers,[18] and other complexes of macromolecular[19] to tumor/cancer cells.

In this work, folic acid has been conjugated with the α-CD and then used for constructing novel supramolecular hydrogels together with Poly(ethylene glycol) methyl ether in aqueous solutions. This strategy can result in the formation of bioactive supramolecular hydrogels with multifunctional characteristics.

Materials and Methods:

Materials

Folic acid (FA), N-hydroxysuccinimide (NHS), 1-ethyl-3-3(3-dimethylaminopropyl) carbodiimide (EDC), Poly(ethylene glycol) methyl ether (MPEG) with molecular weight of 5000, were purchased from Sigma-Aldrich. α-Cyclodextrin was purchased from Acros. All other solvents and analytical reagents were purchased from commercial suppliers and used as received.

Methods

Conjugation of FA to α-CD (α-CD-FA)

NHS (0.001 g, 0.009 mmol) and EDC (0.0017 g, 0.009 mmol) were added to FA (0.004 g, 0.009 mmol) dispersed in 20 ml water. The mixture was stirred in the dark at room temperature for 12 h and then it was centrifuged and the obtained precipitate was added to an aqueous solution of α-CD (0.01 g in 10 ml water). The mixture was stirred at room temperature in the dark for at least 12 h to obtain a clear yellow-orange solution. This solution was then dialyzed in water to obtain the pure product. Its structure was characterized by ^{1}H NMR and FT-IR.

^{1}H NMR: δ=1.85–1.88 (m, 2H; H of FA), 2.25 (t, 2H; H of FA), 3.17–3.88 (m, 4H; H of C3, C5, C6, C2, C4 of α-CD), 4.26–4.37 (m, 1H; H of FA), 4.89–5.11 (m, 7H; H of C1 of α-CD), 6.77–6.79 (m, 2H; H of FA), 7.61–7.64 (d, 2H; H of FA), 8.7 ppm (m, 1H; H of FA);

Supramolecular hydrogelation & its characterization

Supramolecular hydrogels were prepared by the inclusion complexation of MPEG with α-CD-FA in an aqueous solution. In a typical experiment, the required amount of MPEG (1, 2 or 3 wt %) was dissolved in an aqueous solution and then mixed with an aqueous α-CD-FA solution (6, 7 or 8 wt %) at room temperature. The resultant mixture was stirred vigorously. Gelation resulted in a physical cross-linking network due to the supramolecuar self-assembly between MPEG and α-CD-FA. To confirm that the inclusion complex was formed from MPEG and α-CD-FA, WAXD and FT-IR measurements were performed.

Results and Discussion:

Preparation and characterization of MPEG/α-CD-FA supramolecular hydrogels

For preparation of MPEG/α-CD-FA supramolecular hydrogel, FA was first conjugated with α-CD by aqueous-phase ester activation chemistry. It was then subsequently used as the host molecule for the inclusion complexation with MPEG in their aqueous mixed system. ^{1}H-NMR and FT-IR spectroscopy were applied for the structural analyses of the functionalized cyclodextrin with FA.

Figure 1 shows the ^{1}H-NMR spectra of the α-CD, FA and FA functionalized α-CD recorded in D_2O. The ^{1}H-NMR spectrum of α-CD-FA contain similar peaks to those seen in the α-CD spectrum, which can be assigned to the cyclodextrin backbone. Therefore, the signals at 3.17–3.88 can be assigned to H-3, H-5, H-6, H-2, H-4 of α-CD and 4.89–5.11 for the H-1 of α-CD, respectively. Additional signals appearing at 8.7, 7.61, and 6.77 ppm indicate the presence of protons of the FA substituent. These signals are all shifted in comparison to the signals for the aromatic protons on free FA. Carboxylic hydrogen of FA appeared with a considerable shift at 2.25 ppm due to formation of ester linkage. This suggests successful bonding of FA to the α-CD molecule.

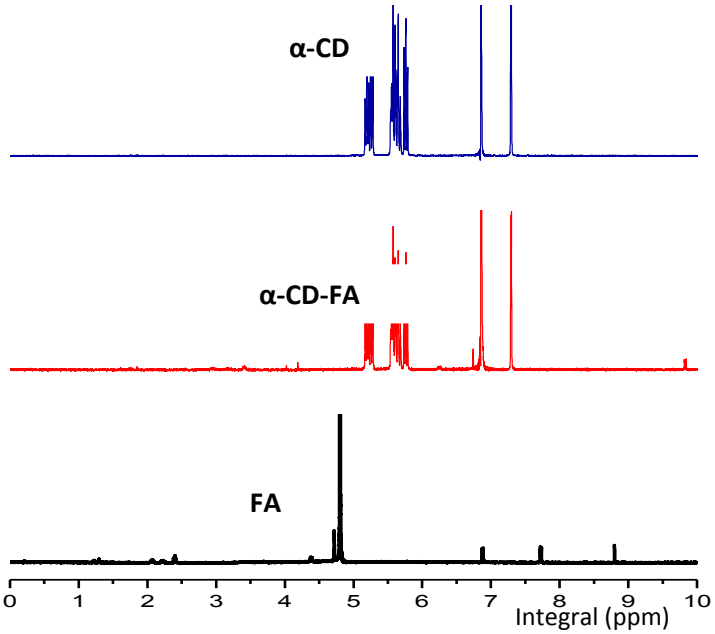

Figure 1. ^{1}H NMR spectrum in D_2O of α-CD-FA

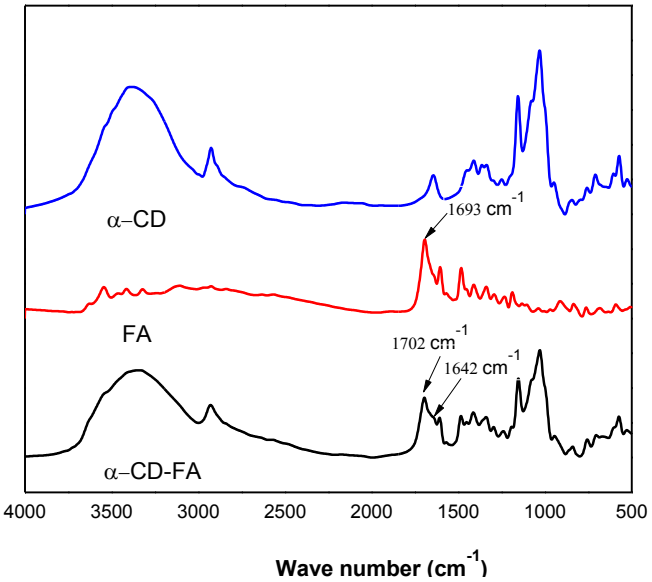

Figure 2. FT-IR spectra of α-CD-FA

Moreover, as shown in Figure 2, due to the complicated spectra obtained, it can be difficult to use vibrational spectroscopy for structural elucidation of derivatives of CDs. However, new bands are very apparent in the FT-IR of α-CD-FA particularly in the 1000-1700 cm^{-1} region. The band at 1702 cm^{-1} in the FT-IR spectrum of the final product can be assigned to a asymmetric vibration mode of C=O with shoulder at 1642 cm^{-1} can be assigned to O-H bending vibration. Meanwhile, carboxylic C=O vibration of the FA has appeared only peak at 1693 cm^{-1}. There is a shift in the bending vibration mode of the hydroxyl group in α-CD of 14cm^{-1} to a higher frequency, after functionalization. Moreover, all aromatic C=C vibration of the α-CD-FA shifted to a lower frequency when compared with the original

FA. These results indicate that FA was successfully conjugated with α-CD by the formation of ester linkage to form α-CD-FA.

We observed that such a α-CD-FA could be dissolved in water to form a homogeneous solution. In particular, in some cases, the aqueous α-CD-FA solution could be transformed into an invertible hydrogel with the introduction of MPEG, as shown in Figure 3. Depending on the amount of MPEG or α-CD-FA, this gelation could occur under mild conditions without high temperatures, the use of a chemical emulsifier or cross-linker. This phenomenon may be attributed to the formation of ICs between MPEG and α-CD-FA in their aqueous mixed system.

Figure 3 Photographs of the process of supramolecular hydrogel formation in an MPEG/α-CD-FA system.

In order to understand the chemical structure of the hydrogel, we measured the XRD patterns and FT-IR spectra of α-CD-FA, MPEG, and freeze-dried hydrogel sample which formed in situ from 2 wt % of MPEG and 7 wt % of α-CD-FA, as shown in Figure 4, 5. From the XRD patterns shown in Figure 4, the hydrogel sample is observed to have two characteristic diffraction peaks at 2θ = 19.8° (d = 4.44 Å) and 22.6° (d = 3.94 Å), which were not observed in the XRD patterns of MPEG and α-CD-FA. These two peaks could represent the channel-type structure of the MPEG/α-CD-FA.[20] These could be assigned to the {210} and {300} reflections from the hexagonal lattice with a = 13.6 Å.[21] The strong {210} reflection is shown as a typical peak observed for the polymer inclusion complexes with α-CD.[22] These results demonstrate the existence of the MPEG/α-CD-FA inclusion complexes, which would act as the physical cross-linking points or clusters and then connect to the supramolecular hydrogel networks spanning the whole sample.

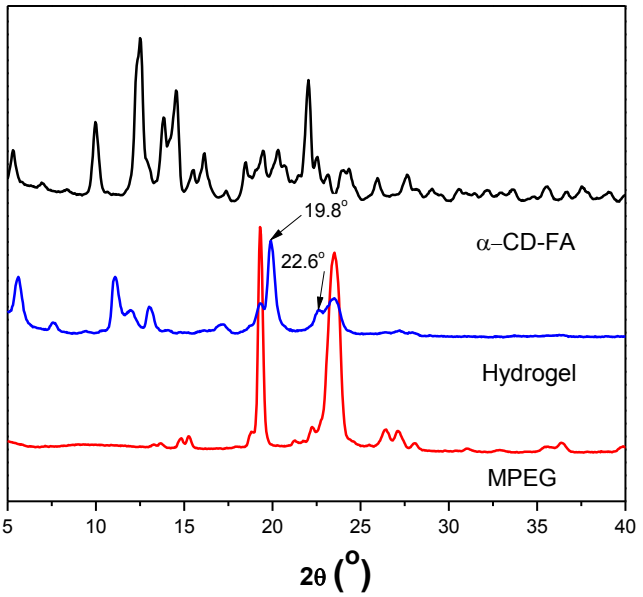

Figure 4 XRD patterns of MPEG, α-CD-FA and freeze-dried hydrogel sample formed from 2 wt% MPEG and 7 wt% α-CD-FA.

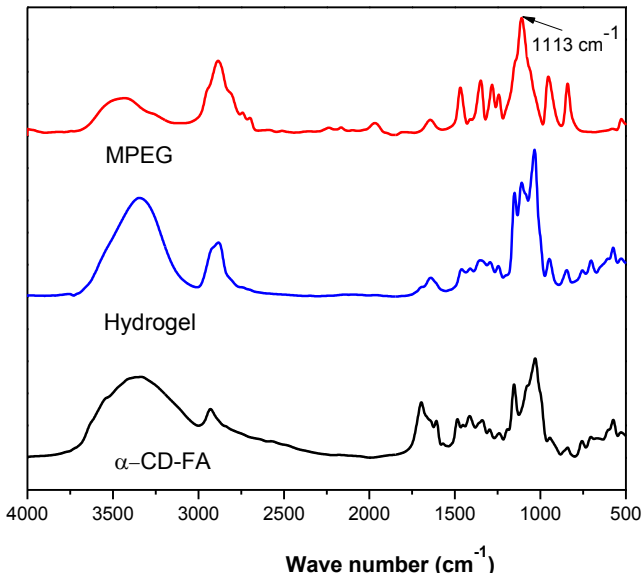

Figure 5 FT-IR spectra of MPEG, α-CD-FA and freeze-dried hydrogel sample formed from 2 wt% MPEG and 7 wt% α-CD-FA.

Moreover, both α-CD-FA and MPEG peaks are compiled in the FT-IR spectra of the freeze dried hydrogel with considerable bands shifts as shown in Figure 5. For example, symmetric vibration of the O-H has shifted toward a lower frequency of about 35 cm^{-1} compared with the α-CD-FA. Such a lower frequency shift can be attributed to intermolecular hydrogen bonding in IC. Similarly, the C-O-C (1113 cm^{-1}) linkage of MPEG has shifted to a lower frequency about 8 cm^{-1}, thus confirming the MPEG chains inside the α-CD cavity.

Conclusion:

A bioactive supramolecular hydrogel with multifunctional characteristics was prepared by the conjugation of the FA with α-CD and the interaction with MPEG in an aqueous solution. This gelation process could be carried out under mild conditions without requiring a high temperature or the use of chemical emulsifier or cross-linker. The hydrogel formation could be attributed to the inclusion complexation between MPEG and α-CD-FA. This study provides a novel drug-entrapment strategy for hydrophilic hydrogel-based carriers.

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