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Preparation of a novel organo-soluble chitosan grafted polycaprolactone copolymer for drug delivery

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ABSTRACT

The unsatisfactory solubility of chitosan has been a key barrier to its modification and application. To improve its solubility, a novel organo-soluble glycidol grafted chitosan was developed as a potential material for bio-related applications. The hydrophilic glycidol was grafted onto the amino groups of chitosan via a facile and "green" aqueous reaction. The grafting levels of glycidol strongly influenced the organo-solubility and water-solubility of the chitosan due to the inhibition of its intermolecular interactions. The resulting glycidol grafted chitosan (Gly-HCS and Gly-LCS) was directly dissolved in dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). Taking advantage of the improved organo-solubility, an amphiphilic Gly-LCS grafted polycaprolactone (GC-g-PCL) copolymer and its assembly were prepared as an efficient anti-tumour doxorubicin (DOX) carrier. The DOX loaded GC-g-PCL assembly exhibited a control release profile and anti-tumour activity. Acting as a basic substrate, this organo-soluble chitosan derivative has the potential to meet a wide range of requirements in the field of biological macromolecules.

1. Introduction

Biological macromolecules, such as polysaccharides, proteins and peptides, have been employed as indispensable biomaterials for decades. In particular, polysaccharides, such as chitosan [1–4], dextran [5,6] and alginate [7,8], have been highlighted as a promising materials due to their superior properties such as biocompatibility, chemical activities, abundant reservation and low cost. Chitosan, which is the deacetylated product of chitin, is a typical cationic amino-containing polysaccharide that has been widely used for biomedical applications [9–11] and environmental treatment [12,13]. However, the unsatisfactory solubility of chitosan has been a major barrier for its modification and application, i.e., chitosan is only soluble in acidic aqueous solutions. Restricted by its solubility, researchers can only modify chitosan in an acidic aqueous solution or via a heterogeneous process [14–17], which significantly hampered its potential application.

To circumvent this barrier, a series of protection/deprotection protocols were developed for preparing organo-soluble chitosan derivatives [18–21]. On the strength of these protocols, some chitosan-based amphiphilic graft copolymers were successfully prepared and applied as a drug delivery system [22,23]. Phthaloylated chitosan was one of the most successful intermediates for dissolving the chitosan in organic solvents, such as DMF [18,21]. With improved solubility, a number of hydrophobic polymeric moieties were conjugated onto the chitosan backbone [24–26]. Then, the de-phthaloylation was achieved via treatment with alkali and reducible hydrazine. However, the severe condition of the protection/deprotection led to degradation and undesired side reactions. Cai et al. applied anionic surfactant sodium dodecyl sulphate (SDS) to obtain a dimethyl sulfoxide (DMSO) soluble electrostatic complex under mild conditions [19,20]. After modification, the SDS was removed through competitive absorption with a Tris solution. The weak binding of the complex restricted the reaction in a neutral environment. In addition, trimethylsilylated chitosan [27] and methanesulfonic acid/chitosan [28,29] were also successful examples of the protection/deprotection protocols. However, some restrictions and disadvantages exist for the modification and application. In previous chitosan applications, the high "performance-to-price ratio" played an important role in its applications. The structure and impurities of chitosan and other natural macromolecules are not perfectly well-defined. Therefore, such a complicated protection/deprotection procedure, which leads to structure deterioration and high costs, might not be appropriate for

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the modification of chitosan. It is essential to improve the solubility of chitosan with facile, appropriate and minimum modifications.

The strong intermolecular interactions of chitosan, such as hydrogen bonding, results in its poor solubility and processability [1]. In previous reports, some examples of N-substituted chitosan, such as half-acetylated and PEGlated chitosan [30–32], exhibited improved solubility in both water and organic solvents due to the reduction of their intermolecular interactions [33,34]. These phenomena inspired us to optimise the organo-solubility of chitosan via facile modifications of the chitosan backbone with biocompatible small molecules, and the resulting organo-soluble chitosan derivative was used directly.

Herein, organo-soluble glycidol grafted chitosan (Gly-CS) was investigated in detail. The resulting chitosan derivative exhibited good solubility in DMSO as well as dimethylformamide (DMF). Taking advantage of this organo-solubility, a novel amphiphilic graft copolymer, that is, Gly-CS-grafted-polycaprolactone (Gly-CS-g-PCL), and its assembly were homogeneously prepared. Doxorubicin (DOX) was loaded into the assembly as a model hydrophobic anti-tumour drug, and its drug release profile and anti-tumour activity were also investigated in vitro using the HepG2 cell line.

2. Materials and methods

2.1. Materials

Low molecular weight chitosan (LCS) (viscosity-average molecular weight (MW) of 15 kDa, molecular distribution of 2.1–2.3 and degree of deacetylation (DD) of 90%) was produced by degrading the high MW chitosan (Aoxing Corporation, Zhejiang, China, with a pristine MW of 150 kDa and DD of 88%) through hydrogen peroxide treatment [23,35]. High-quality chitosan with a molecular weight (MW) ranging from 150 kDa to 250 kDa, stannous octanoate ($Sn(Oct)_2$) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were obtained from the Sigma–Aldrich Corporation. Hexamethylenediisocyanate (HMDI) was purchased from Alfa Chemicals. Caprolactone (CL) was purchased from Acros Chemicals, and dried with CaH_2 for weeks prior to distillation under vacuum. Doxorubicin hydrochloride (DOX·HCl) was purchased from Adamas Chemicals. Dimethylsulfoxide (DMSO) was used after being dried by CaH_2. All others chemicals were used without further purification. HepG2 cells were obtained from Prof. Youqing Shen's group (Department of Chemical Engineering, Zhejiang University). The culture media, Dulbecco's Modified Eagle's Medium (DMEM), and foetal bovine serum were purchased from Haotian Corporation, China.

2.2. Characterisation

^{1}H-NMR spectra were recorded on a Bruker DMX-500 NMR spectrometer operating at 500 MHz. The fluorescence spectra were recorded on a PerkinElmer LS55 spectrometer, and the UV–vis spectra were recorded on a Shimadzu UV-1800 spectrometer. Transmission electron microscopy (TEM) images were captured on a JEOL JEM-1230 electron microscope operating at an acceleration voltage of 80 kV. The micelle size and zeta potential were analysed on a Malvern Zetasizer nano 90. Each analysis involved five runs of 1 min at 25 °C. The micelle solutions had a final polymer concentration of approximately 2 mg/ml. For the light scattering measurements, all of the sample solutions were filtered through a 0.45 μm cellulose membrane. The potentiometric titration was performed with a UB-7 pH meter (Denver Instrument).

3. Experimental

3.1. Synthesis of Glycidol-high molecular weight chitosan (Gly-HCS) and Glycidol-low molecular weight chitosan (Gly-LCS)

First, high-quality chitosan was dissolved in a 1% acetic acid solution to form a 2.5 wt.% solution. Then, a certain amount of glycidol was added to the solution under stirring at 50 °C. The reaction was maintained for three days, and dialysed against deionised water for another three days using a dialysis tube with a molecular weight cut-off (MWCO) of 3500. The dialysed solution was concentrated and freeze-dried to yield a sponge-like solid. A similar process was also used to produce Gly-LCS, where the concentration of LCS was increased to 5 wt.% and the reaction duration was reduced to 24 h.

3.2. Potentiometric titration of Gly-HCS and Gly-LCS

The Gly-HCS or Gly-LCS (0.1 g) sample was completely dissolved in 10 ml of 0.1 M standard hydrochloric acid. Then, the sample solution was titrated by a 0.1 M standard sodium hydroxide solution. During each injection, the pH value was recorded on the pH meter after the value stabilised.

3.3. Synthesis of isocyanate terminated-polycaprolactone (PCL–NCO)

Polycaprolactone (PCL) was prepared via traditional ring-opening polymerization. Briefly, $Sn(Oct)_2$ and benzyl alcohol were used as the catalyst and initiator, respectively. The apparent efficiency of the initiator was approximately 80% under our conditions, and the MW of PCL was set as 2500 in this study. Then, a certain amount of CL, initiator and catalyst were charged into a flask under vacuum. The polymerization was performed at 140 °C for 12 h, and then, the product was dissolved in chloroform and precipitated by methanol. The white powder of PCL was obtained after vacuum drying at 40 °C. To prepare PCL–NCO, hexamethylene diissocynate (HMDI) was employed as a functional capped reagent. PCL with hydroxide terminal groups was dissolved in chloroform, and 10 mole equiv. of HMDI were charged into the system under an Ar atmosphere. The reaction was maintained at 35 °C overnight followed by precipitation into excess ether and vacuum dried.

3.4. Synthesis of amphiphilic Gly-LCS-grafted-polycaprolactone (GC-g-PCL)

The selected Gly-LCS was dissolved into dehydrated DMSO at a concentration of 2.5 wt.%. After complete dissolved, one equiv. by weight of PCL–NCO was added to the solution. The reaction was maintained at 40 °C for two days. Then, the admixture was precipitated by an ether/methanol (1:1) mixed solution. To completely remove the free PCL–NCO, the resulting crude product was further purified through Soxhlet extraction by methylene chloride for two days. A slight yellow copolymer was produced after being vacuum dried.

3.5. Micellization of GC-g-PCL and critical association concentration (CAC) measurement

The micelle solutions were prepared by the dialysis procedure described by Eisenberg et al. [36]. Briefly, GC-g-PCL was dissolved in DMSO at a concentration of 5 mg/ml. The solution was loaded into a dialysis tube with a MWCO of 3500 and dialysed against PBS (pH 7.4, 20 mM) for three days. The CAC was measured using a pyrene probe [36], and the micelle solutions with different concentrations