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Synthesis and characterization of hyperbranched poly (sulfone-amine) modified β-cyclodextrin

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Abstract: An economical strategy to prepare hyperbranched poly(sulfone-amine) modified β-cyclodextrins (HPSA-m-CDs) from natural β-cyclodextrin (β-CD) and other commercially available materials has been reported. The final product has many good properties of hyperbranched poly(sulfone-amine)s (good solubility, low viscosity etc.), while its inclusion ability can also be well kept. It is a feasible approach to prepare functionalized modified cyclodextrin at very low cost, and may have potential applications in the fields of catalysis, drug delivery, food additives, etc.

Introduction

Cyclodextrins (CDs) have been widely investigated due to their well-defined cavity structures and abilities to recognize and embrace various molecule guests [1-5]. One of its most important applications is in the pharmaceutical field [6-10]. In their parent state, CDs have a cavity to form complexes with drug molecules; however, its weak biological recognizability restrains the utility in drug release system, especially, cyclodextrin-based controlled drug release system. Hence, the construction of biocompatible modified cyclodextrins with high performance is an important research subject. In most cases, the modification of CDs takes place in the hydroxyl groups at 2, 3 and 6 positions of the D-glucopyranose units. The primary hydroxyl groups at 6 positions (located in the narrow side of the cavity) display greater reactivity than the secondary hydroxyl groups located in the wide side of the cavity under certain circumstances (weakly alkaline, strong steric effect, etc.), which makes selective modification feasible. These selectively modified CDs can keep the ability to complex with drug molecules while the "new" groups in 6 positions improve their properties greatly to meet the demands of being functionalized drug carriers. Many researchers have reported the synthesis of modified CDs which have potential utility in drug delivery [9-13]. One example is the β-CD conjugate with opioid peptide that may be used as brain target drug carrier [11, 12]. Some neoglycoconjugates based on cyclodextrins also exhibit good biological recognizability [13]. However, the synthesis

of these glycoconjugates usually requires multi-step reaction, and the productivity is rather low. Moreover, Newkome et. al. have reported the synthesis of β-CD-based dendrimers which exhibit good molecular recognition properties [9]. Despite their delightful properties, the dendrimer modified-CDs are not readily prepared since the original material: 6-heptaamino-modified-β-cyclodextrins and combinatorial-based dendrimers are very expensive, which greatly limits its wide application.

Hyperbranched polymers are popular subjects of polymer science in recent years; they have numerous potentialities in many fields, of course, including in pharmacy [14-21]. On one hand, the favourable properties of dendrimers are due to the highly branched structure; on the other hand the one-step polymerization route and high productivity make them suitable substitutes for dendrimers in mass production. Recently, our research group put forward a new strategy to prepare hyperbranched polymers from commercially available A_2 and BB'_2 type monomers [22, 23]. The rapid reaction between A and B groups forms dimers which can be regarded as AB'_2 type monomers, and further polymerization of these "new" monomers results in hyperbranched polymers [22, 23].

In the present work, we combine this strategy with selective modification of CDs to prepare hyperbranched poly(sulfone-amine) modified β-cyclodextrin from natural β-cyclodextrins. The synthetic cost jumps down greatly, while a considerable productivity can be acquired. Furthermore, natural CDs are obtained on an industrial scale, readily available and affordable; so such a route to synthesize functionalized materials from natural CDs is quite practical and economical.

Results and discussion

Selective Modification of β-CD by HDI

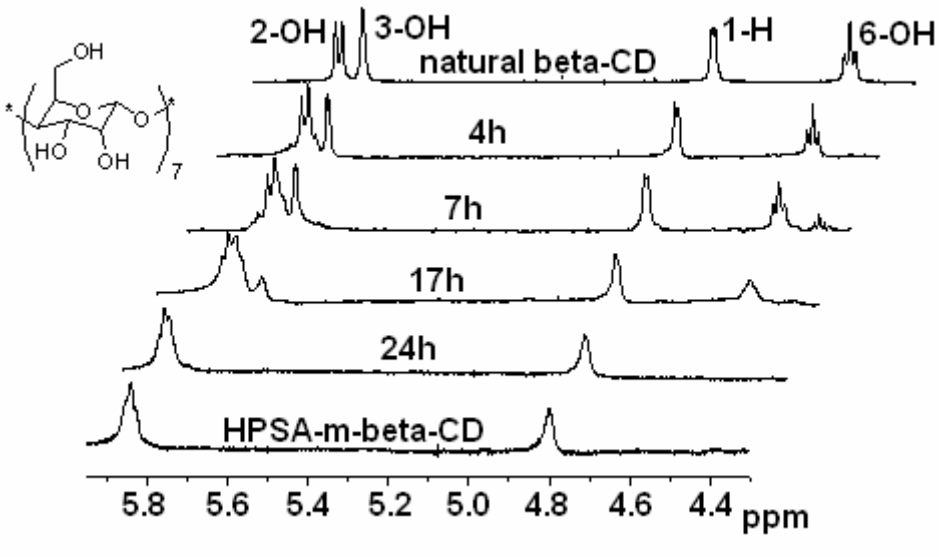


Fig. 1. ^{1}H NMR spectra of the selective modification procedure (d_6-DMSO, 400 MHz).

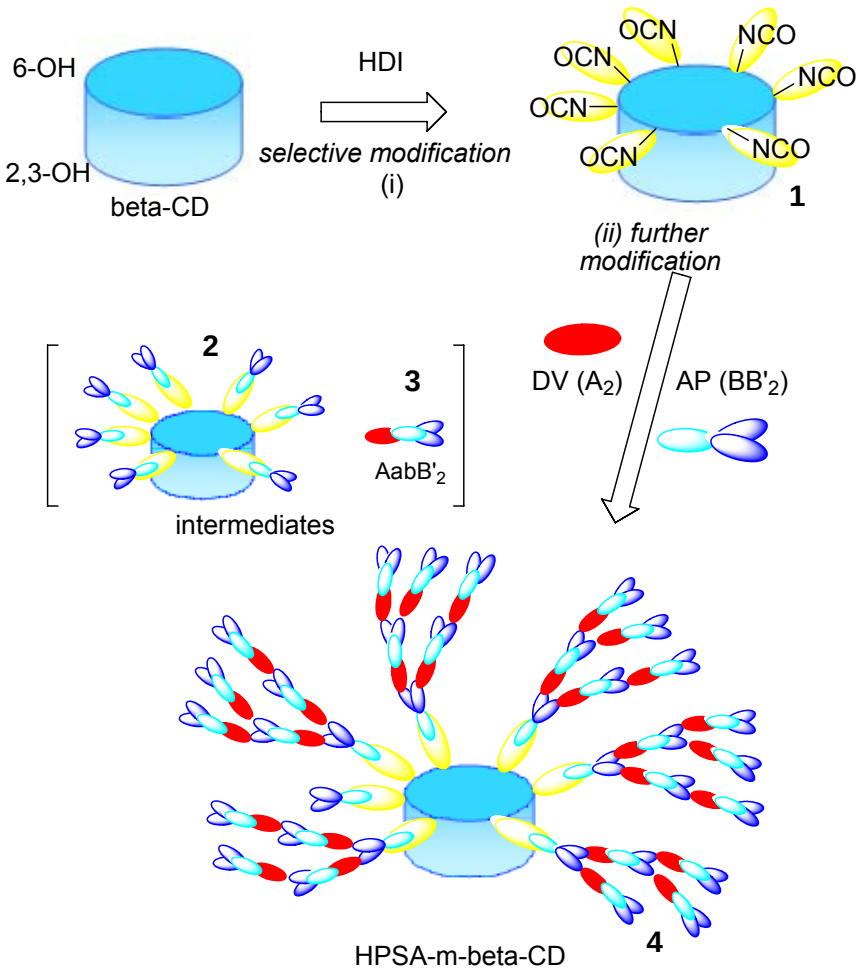
1,6-Diisocyanathohexane (HDI) is a commercially available reagent with high reactivity. One of its isocyanate groups can react with hydroxyl and introduce a "new" active isocyanate group, which may act as the starting point in further modification.

The reaction between HDI and β-CD was investigated by in-situ ^{1}H NMR. NMR spectra collected at various periods are illustrated in Fig. 1.

As shown in Fig. 1, the signal at 4.46 ppm (assigned to primary hydroxyl groups at 6 positions) decreases notably with reaction time, and eventually vanishes after reaction for 24 h. At the same time, signals assigned to secondary hydroxyls, at 5.73 and 5.68 ppm becomes wider and wider, and finally merge into one big peak. However, the total intensity of secondary hydroxyl groups remains almost unchanged during the reaction process. The ratio of area integration of 1C-H (4.81 ppm) and secondary OH is approximately 1:2 at any time. It reveals that primary hydroxyls in CDs have priority in reaction with the isocyanate group. 6-OH substituted β-CDs are prepared while 2,3-OHs at the wider side of CDs are well preserved, so that guest molecules can access the cavity of modified-CDs.

Preparation and Characterization of HPSA-m-β-CD

The synthesis of HPSA-m-β-CD is depicted in Scheme 1. It consists of two steps: (i) selective modification of natural cyclodextrins by 1,6-diisocyanathohexane (HDI); (ii) further modification to form hyperbranched polymer.



Scheme 1. Synthesis of HPSA-m-β-CD.

Since isocynate groups are more reactive than vinyl groups in divinyl sulfone (DV), the precursor **1** obtained in the first step can be easily attached to 1-(2-aminoethyl)

piperazine (AP) to form intermediate **2**, which may further copolymerize with dimer **3** (formed by AP and DV) and result in hyperbranched poly(sulfone-amine) modified β-CDs **4**. After reaction at 50 ^{o}C for 72 h, the solution is still transparent and no gelation occurs.

As shown in step (ii) of Scheme 1, the dimers **3** will copolymerize with precursors **2** to form HPSA-m-CDs 4. On the other hand, the dimers **2** itself can homopolymerize into hyperbranched poly(sulfone-amine) without CD centers. HPLC was performed on C18 reverse phase column to remove the byproduct. The final product has a molecular weight M_n of 2.5 X 10^4 and M_w of 3.3 X 10^4 (GPC, calibrated by linear PEO), and shows outstanding water solubility. (0.05 mol HPSA-m-β-CDs /kg H_2O vs 0.014 mol β-CDs /kg H_2O)

Degree of branching (DB) of the substituted poly(sulfone-amine) arms can be calculated by the DB definition proposed by Fréchet et al. [24]:

$$DB = \frac{N_B + N_T}{N_B + N_T + N_L} \quad (1)$$

In Equation (1), N_B, N_T and N_L are numbers of branched units, terminal units and linear units respectively. Traditionally, DB can be determined by 1D NMR spectrum. However, for hyperbranched poly(sulfone-amine), the complicated structure and severe overlapping NMR signals hinder it from being determined by this conventional method.

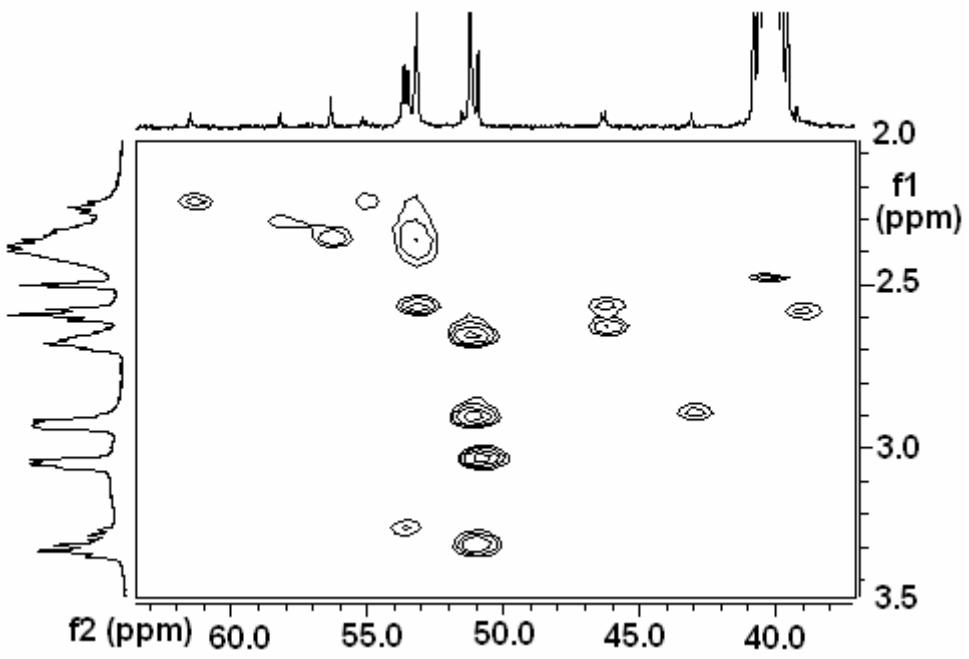


Fig. 2. ^{13}C, ^{1}H-HSQC of HPSA (d_6-DMSO, 400 MHz for proton and 100 MHz for ^{13}C).

As can be seen in Fig. 2, there are severe overlaps in both ^{1}H NMR (2.5-2.7 ppm) and ^{13}C NMR (51 ppm) spectra. Therefore, it is hard to determine DB of HPSA by only 1D NMR. However, according to our recent work, with the help of 2D NMR techniques, DB of complicated system can be elucidated [25, 26]. The DB of HPSA arms in HPSA-m-β-CD is calculated to be 0.41 based on Equation (1).

In Fig. 3, signals assigned to secondary hydroxyls are well kept, while the signal of 6-OH disappears. The ratio between integrals of 2, 3-OH and 1-H is equal to 2:1, further supporting the selective modification.

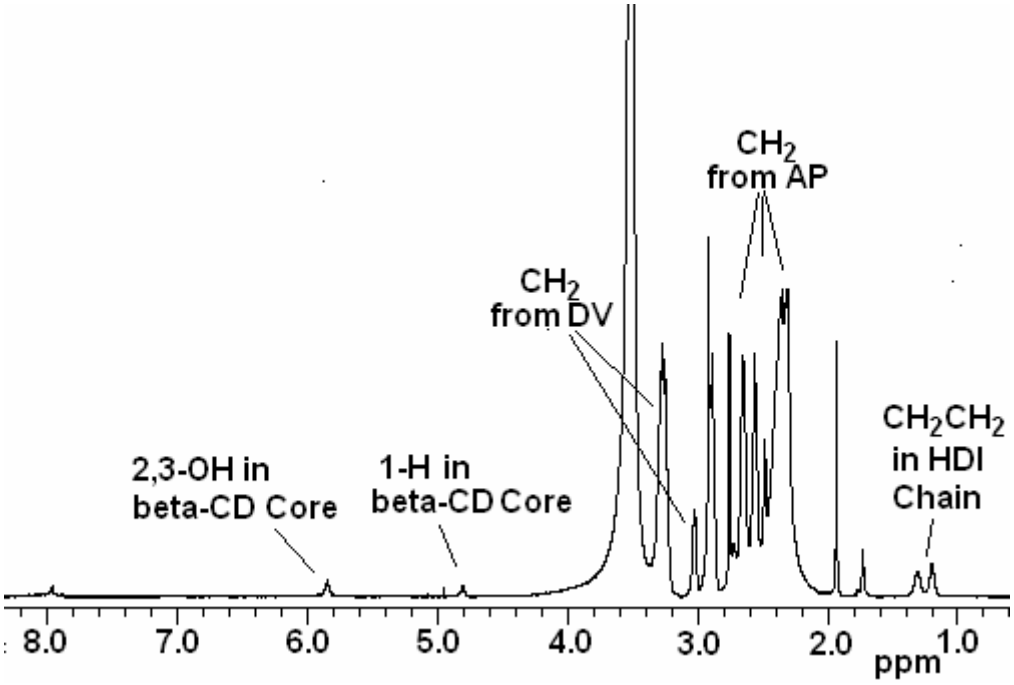


Fig. 3. ^{1}H NMR of HPSA-m-β-CD (d_6-DMSO, 400 MHz).

The Integrals of various units are listed in Tab. 1 (Integrals are normalized by Integral of 1-H in β-CD core).

Tab. 1. NMR quantitative analysis of HPSA-m-β-CD.

Proton	1-H in β-CD core	2,3-OH in β-CD core	2,3,4,5-CH_2 from HDI	CH_2 from AP	CH_2 from DV
Monomer	β-CD	β-CD	HDI	AP	DV
Number of protons	7	14	8	12	8
Integral	1	1.98	7.69	216.9	*
Relative Amount of monomer	1	1	6.7 =0.96X7	126 =18.8X6.7	126 =18.8X6.7

* Integral of DV is unavailable, since its signal is overlapped with that of H_2O.

Tab. 1 reveals that 96% of 6-OH has been modified by HDI. Moreover, in further modification process, there are about 18 AP molecules in each branched arm. Since AP and DV can form intermediate **3** quickly [22, 23], it can be assumed that there are almost the same number of DV in each arm. Therefore, the structure of HPSA-m-β-

CD is as follows: In one m-CD molecule, there is one core (1 β-CD), 6.7 arms with about 18 AP and 18 DV in each arm. Correspondently, the molecular weight can be deduced:

$M_{n,\,NMR} = 3.2 \times 10^4$ (2)

This value agrees well with molecular weight determined by of GPC.

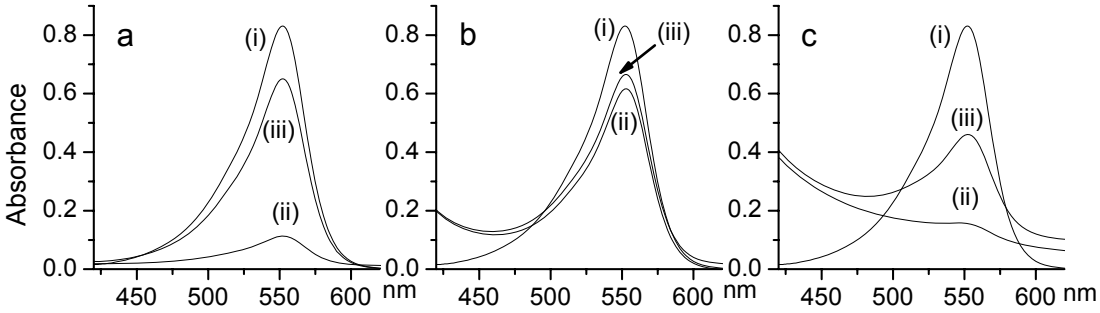


Fig. 4. UV-VIS spectra of alkaline phenolphthalein solutions (pH = 10.5) (i) phenolphthalein only; (ii) addition of **a**- natural CDs; **b**- HPSA without CDs; **c**- HPSA-m-β-CDs; (iii) further addition of adamantan-2-amine hydrochloride.

The inclusion ability of **4** was measured by UV-vis spectrometry. Alkaline phenolphthalein solution (pH = 10.5) has a strong absorbance at 550 nm. Upon the addition of natural CDs, a dramatic drop in absorbance at 550 nm occurs due to the specific host-guest inclusion interaction between phenolphthalein and the hydrophobic cavity of β-cyclodextrin [9, 27]. After the addition of non-UV-active adamantan-2-amine that is known to bind strongly to β-CD cavity, a re-enhancement of absorbance at 550 nm can be observed (see Fig. 4a). Fig. 4b indicates that if the hyperbranched poly(sulfone-amine) without CD center is added into alkaline phenolphthalein solution, only a small decrease in absorbance at 550 nm can be seen due to the association between hyperbranched polymer and phenolphthalein. Adding the adamantan-2-amine, the absorbance intensity is almost unchanged. Apparently, the difference in Fig. 4a and Fig. 4b is caused by the inclusion ability of cyclodextrin's cavity [9, 27]. Fig. 4c testifies to the inclusion ability of HPSA-m-β-CDs. It can be seen that the complexation behaviour of HPSA-m-β-CDs is similar to that of natural cyclodextrins. Comparison of Fig. 4a and Fig. 4c also implies that the complexation of phenolphthalein with HPSA-m-β-CDs is not as complete as that of pure β-CD.

Conclusions

The merits of this synthetic route can be concluded as follows:
Natural CDs and HDI are readily available and relatively affordable materials. The low cost of these materials and simple synthetic route make the preparation and utility of functionally modified CDs no longer limited in laboratory.
CD-based hyperbranched poly(sulfone-amine) has a good water solubility, which is a favourable property for drug carriers and other applications.
Various kinds of functionalized drug carriers based on modified cyclodextrins can be easily obtained by changing the sorts and size of the hyperbranched arms.

The inclusion ability of HPSA-m-β-CDs can be well kept.
This reaction has a high yield compared with those multi-step reactions. (In the case of poly (sulfone-amine) modified β-CD, the yield is 73%.)

Experimental part

Materials and measurements

β-cyclodextrin (β-CD, Yunan Cyclodextrin Company, P. R. China) was recrystallized from water and dried at 80 ^{o}C under vacuum. 1,6-Diisocyanathohexane (HDI, Acros), adamantan-2-amine chloride (Aldrich) and 1-(2-aminoethyl)-piperazine (AP, Aldrich) were used without further purification.

Divinyl sulfone (DV, Aldrich) was purified by vacuum distillation before use. N,N-dimethylacetamide (DMA, Shanghai Reagent Co., P. R. China) was distilled before use.

NMR measurements were performed on a 400 MHz Varian NMR spectrometer with d_6-DMSO as solvent. High-performance liquid chromatograms were obtained on a Waters 515 HPLC with C-18 column as immobile phase and water and methanol (95:5) as mobile phase. The molecular weight of product was measured on a HP 1100 gel permeation chromatograph (GPC) with water as solvent and PEO as standards. UV-vis measurements were carried out on a PE LAMBDA 20 spectrometer.

In-situ investigation of selective modification by HDI

β-CD (11.5 mg, 0.01 mmol) was dissolved in 1 mL d_6-DMSO. A ^{1}H NMR spectrum was taken before addition of HDI, and then another spectrum was recorded right after HDI (14 mg, 0.1 mmol) was added into the NMR tube. Then the tube was immersed in a water bath kept at 50 ^{o}C. NMR spectra were recorded at the same temperature (20 ^{o}C) every hour.

Synthesis of hyperbranched poly(sulfone-amine) modified β-CD (HPSA-β-CD)

β-CD (113.5 mg, 0.1 mmol) was dissolved in 20 mL DMA; then HDI (140.1 mg, 1 mmol) was added under stirring. The mixture was stirred at 50 ^{o}C for 24 h. After cooling to room temperature, AP (642 mg, 5 mmol) and DV (591 mg, 5 mmol) were charged. The solution was heated to 50 ^{o}C under nitrogen atmosphere, and then kept for another 120 h. The reaction mixture was poured into methanol. The precipitate was washed with cold methanol three times, then vacuum dried at 100 ^{o}C for 24 h.

Synthesis of hyperbranched poly(sulfone-amine) without β-CD

A three-necked flask was charged with 2.363 g (20 mmol) of DV, 2.584 g (20 mmol) of AP, and 15 mL of chloroform. The mixture was stirred and reacted at 40 °C for 120 h under an atmosphere of nitrogen, and then poured into 500 mL of methanol. The precipitate was collected by filtration and washed with methanol and acetone several times before drying under vacuum at 80 °C for 24 h to yield a white powder product.

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