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Rapid Communication

Efficient Side-chain Modification of Dextran *via* Base-catalyzed Epoxide Ring-opening and Thiol-ene Click Chemistry in Aqueous Media*

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Abstract In this study, a novel approach by combining base-catalyzed epoxide ring-opening and thiol-ene click chemistry is presented for the side-chain modification of dextran. The vinyl-modified dextran is prepared by a basic epoxide ring opening reaction of allyl glycidyl ether in 0.1 mol/L NaOH, followed by thiol-addition click reaction of three model sulfhydryl compounds using water-soluble Irgacure 2959 as the photoinitiator, leading to side-chain functionalized dextran modified with carboxyl, bidentate dicarboxyl or amino groups. This is the first example of combining epoxide ring-opening and thiol-ene click chemistry for side-chain modification of dextran in aqueous media. Importantly, it may also be extended as a convenient and efficient method for the side-chain modification of other polysaccharides.

Keywords: Functionalization; Thiol-ene click chemistry; Dextran; Sulphur compounds; Green chemistry.

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Dextran (Dex) is one of the most abundant and most commonly used natural polysaccharide, which has been widely applied in biotech and biomedical fields, including medicine and pharmacy (especially for blood plasma substitute)[1], tissue engineering[2, 3], drug delivery[4–7], gene therapy[8], coating[9], surface modification[10] and bioseparation[11]. It has been approved by United States Food and Drug Administration (FDA) for parenteral use, indicating good safety and great application potential. Its excellent aqueous solubility, wide availability, biocompatibility and nonfouling properties make it highly promising as a safe and reliable biomaterial for clinical use. Moreover, the natural origin makes it attractive in an environmental and commercial point of view[12]. However, unlike other polysaccharides, such as chitosan, alginate, chondroitin sulfate, heparin and hyaluronic acid, which have various functional groups (*e.g.*, amino, carboxyl or sulfo groups), dextran only has hydroxyl groups, which may hamper its widespread applications. Thus, incorporation of other functional groups *via* chemical modification of dextran is highly desirable to fully realize the potential of dextran and its derivatives in biomedical research.

Side-chain modification of dextran, a simple and general method to obtain components with new or improved properties, is primarily achieved by esterification, etherification and reductive amination[13–15]. Click chemistry, introduced by Sharpless and co-workers in 2001, focuses on easy-to-make chemical compounds and

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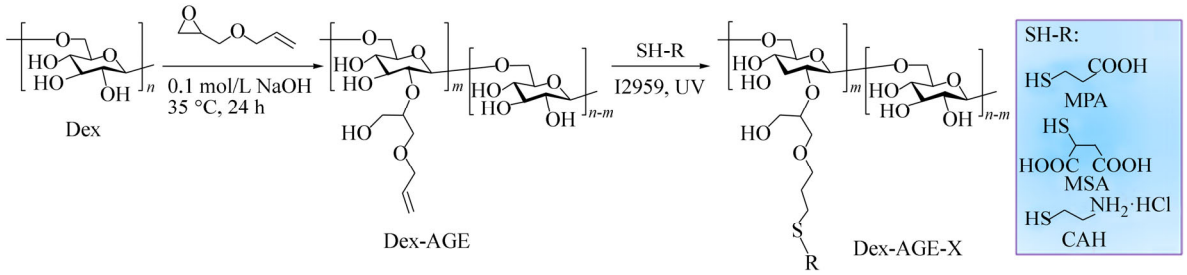
materials derived from modular blocks, and has advantages of being fast, effective, reliable and selective[16]. Recently, copper-mediated Huisgen 1,3-dipolar cycloaddition reaction, one of the most popular click chemistry reactions, has been developed for hydrophobic modification of dextran[17, 18]. However, the long reaction time and high reaction temperature, in particular for macromolecular systems, may be a limitation of this technique. On the other hand, the residual copper catalyst may cause potential deleterious effects and hamper the subsequent biological applications[19]. The major challenge in the exploitation of Huisgen 1,3-dipolar cycloaddition reaction in the modification of polysaccharides is the difficulty of introducing the azide or alkyne group[20]. In most cases, the reactions must be performed in rigorously anhydrous dimethyl sulfoxide or dimethyl formamide[18, 21–23].

Alternatively, thiol-based click reactions, such as thiol-ene[24], thiol-yne[25], thio-epoxy[26] and thiol-isocyanate[27] have been proved to be a versatile approach for engineering multifunctional materials and surfaces in a modular fashion without the use of metal catalyst. The thiol-click reactions have certain inherent benefits as compared to other types of click reactions. They can proceed at moderate temperature with high efficiency and rapid kinetics, in the presence of water, without expensive and potentially toxic catalysts, and are highly tolerant of a wide range of functional groups[28]. Recently, considerable efforts have been made to develop new materials based on thiol-click reactions[29]. However, there have been only a few reports regarding the functionalization of polysaccharides *via* thiol-based click reactions. Auzély-Velty and co-workers have reported the first example of modular functionalization of hyaluronic acid and dextran *via* radical thiol-addition[12]. This group synthesized functional polysaccharides by esterification of the hydroxyl groups with pentenoic anhydride and subsequent modification through photo-initiated radical thiol-ene chemistry. However, organic solvent dimethyl formamide must be used as cosolvent in this process.

Herein, we report a new strategy for side-chain modification of dextran, utilizing base-catalyzed epoxide ring-opening reaction and photo-initiated thiol-ene click chemistry in aqueous media. In this approach, ring opening of functional epoxide (allyl glycidyl ether, AGE) by hydroxyl group was performed in alkaline aqueous solution. The vinyl-modified dextran was then reacted with three model sulfhydryl compounds using water-soluble Irgacure 2959 (I2959) as the photoinitiator, leading to side-chain functionalized dextran modified with carboxyl, bidentate dicarboxyl or amino groups. It should be noted that the whole synthetic procedure can be carried out in aqueous medium without the use of toxic reagents and organic solvents, at ambient reaction temperature, thus representing an environmental friendly polysaccharide modification approach. The obtained dextran derivatives were characterized by nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) and gel permeation chromatography (GPC). To the best of our knowledge, this was the first report on the side-chain modification of dextran by combining epoxide ring-opening reaction and thiol-ene click chemistry in aqueous media.

As shown in Scheme 1, the Dex-AGE conjugate was conveniently prepared by treating dextran with AGE in 0.1 mol/L NaOH. In this way, the vinyl groups necessary for the subsequent thiol-ene click reaction were introduced in a relatively simple manner, avoiding the use of any other toxic reagents or organic solvents in the synthesis. The quantitative ^{1}H-NMR spectra of Dex and Dex-AGE recorded in DMSO-d_6 were displayed in Fig. 1 with the relevant signals labeled. The actual degree of substitution (DS, defined as the ratio of AGE units to anhydroglucosidic units) was determined to be 22.9% from the relative integrations of AGE proton peak (peak 10, $-OC\underline{H_2}CH-$, 2H) into carbohydrate proton peaks (peak 1 + 7, δ = 4.80–4.36), which was lower than the predesigned value of 100.0%. This could be explained by the partial hydrolysis of AGE in aqueous medium[30]. Higher degree of modification could be achieved by increasing the ratio of AGE. As shown in Fig. S1, the appearance of three new peaks (g, h and a′) also demonstrated that the functional vinyl ligands were successfully grafted on to the dextran backbone. The DS of AGE was calculated to be 21.7% based on the ratio of integration at peak g and peak a + a′. Moreover, the relative integration of peak a′ was very close to the relative integration of peak g or h, which demonstrated that hydroxyl group on the C2 position was the most reactive group[31]. There was no significant difference in FTIR spectra between Dex and Dex-AGE (Fig. 2A),



Scheme 1 Synthetic routes for the preparation of Dex-AGE, Dex-AGE-MPA, Dex-AGE-MSA and Dex-AGE-CAH

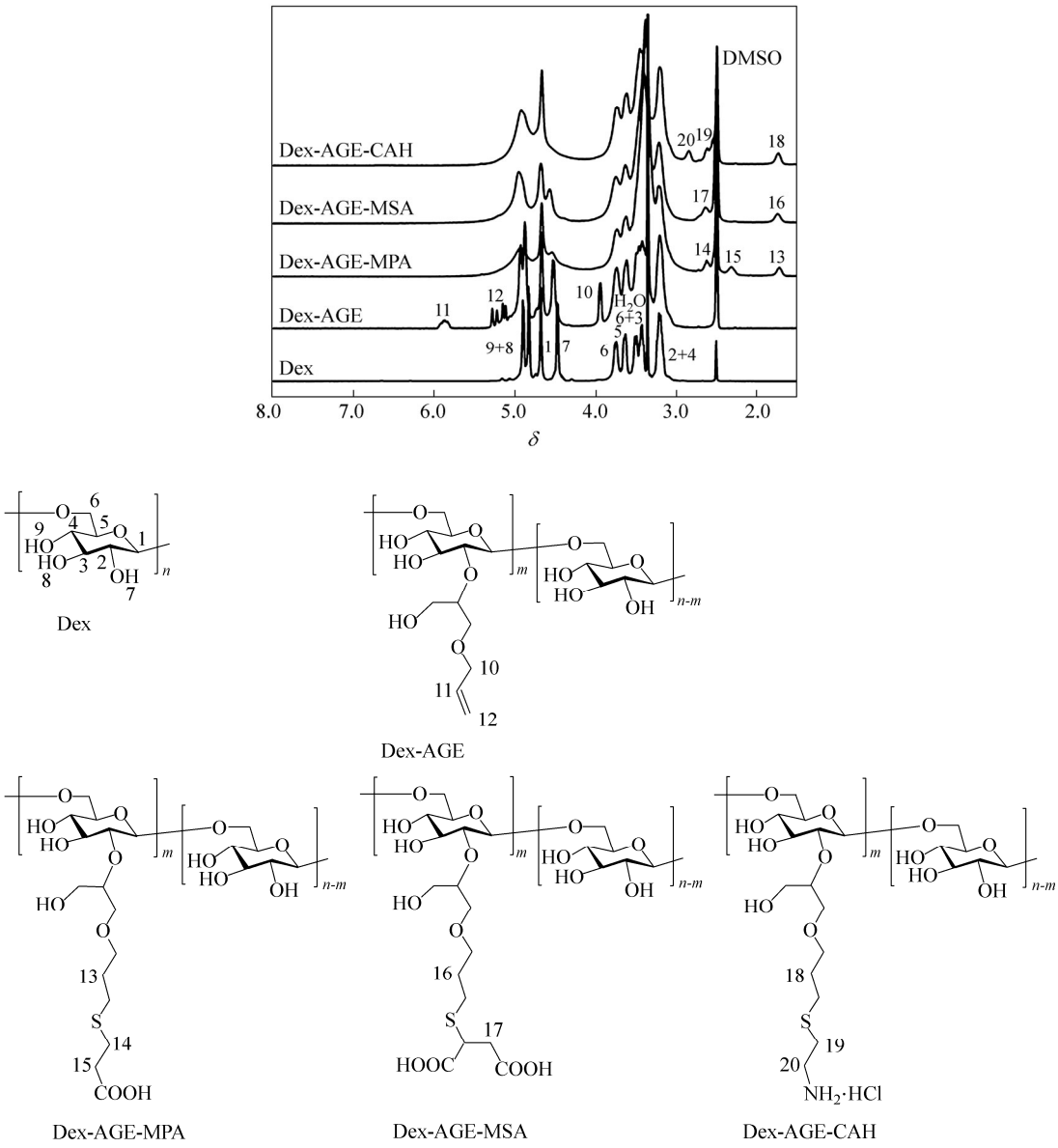


Fig. 1 Chemical structures and ^{1}H-NMR spectra of Dex, Dex-AGE, Dex-AGE-MPA, Dex-AGE-MSA and Dex-AGE-CAH

but nonetheless further evidence of successful functional reactions could also be proved by GPC. The GPC trace of Dex-AGE (Fig. 2B) was monomodal and quite symmetric, revealing the weight average molecule weight (M_w) of 2.71 × 10^4 g/mol and polydispersity index (PDI, M_w/M_n) of 1.95. In comparison with those of dextran (M_w = 2.56 × 10^4 g/mol, PDI = 1.91), GPC trace of Dex-AGE exhibited a moderate shift to the higher molecule weight region, indicating that the functional vinyl ligands were successfully grafted on to the dextran backbone.

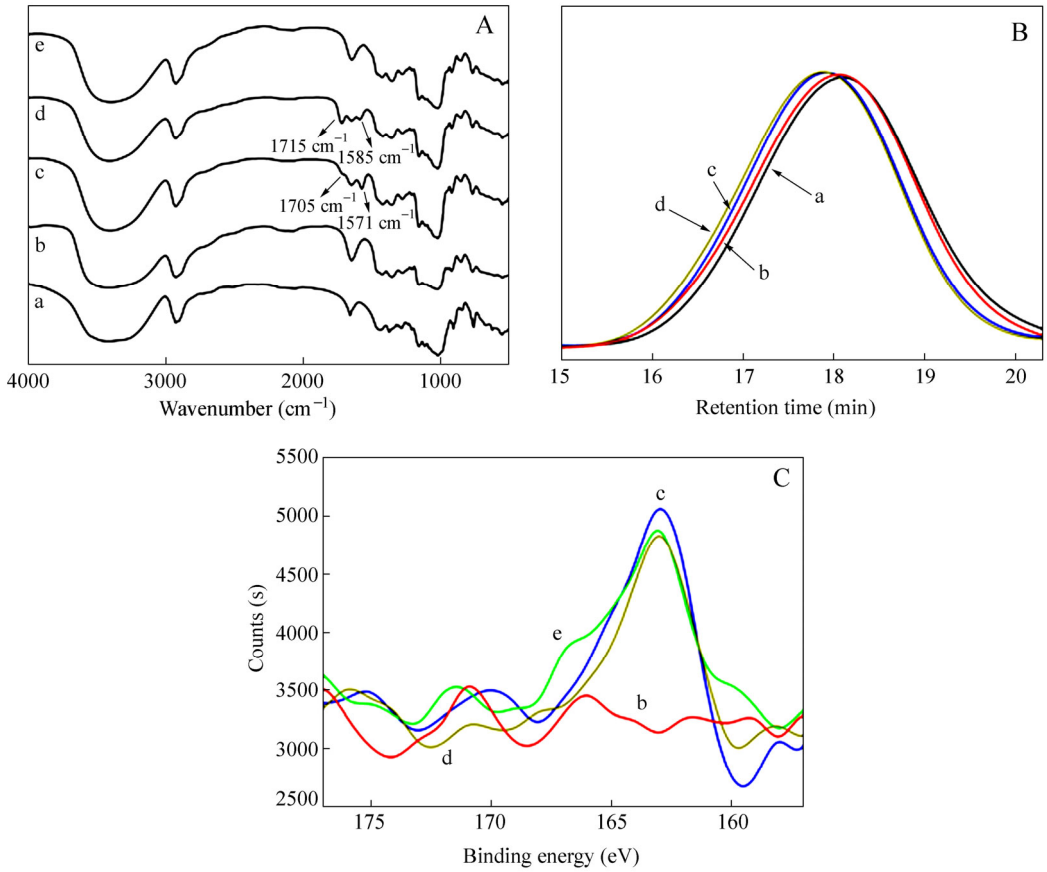


Fig. 2 (A) FTIR, (B) GPC traces, and (C) XPS spectra recorded for (a) Dex, (b) Dex-AGE, (c) Dex-AGE-MPA, (d) Dex-AGE-MSA and (e) Dex-AGE-CAH

To prepare the side-chain functionalized dextran modified with carboxyl, bidentate dicarboxyl or amino groups, 3-mercaptopropionic acid (MPA), mercaptosuccinic acid (MSA) and cysteamine hydrochloride (CAH) were selected as the model mercaptans for click conjugation (Scheme 1). MPA was chosen due to ease of characterization by FTIR, compared with the initial polysaccharide precursor[12]. On the other hand, the introduction of carboxyl groups into the backbone of dextran would facilitate the covalent conjugation with other functional components containing amino groups. MSA containing the neighboring carboxyl groups was selected because of the great potential of its relevant dextran derivative for efficient encapsulation and intracellular delivery of anticancer drugs, such as doxorubicin and cisplatin[19, 32]. CAH containing the amino group was chosen on account of its versatility to convert an electrophilic vinyl (C=C) into a nucleophilic amine (−NH_2). Moreover, the pendant amine groups on linear polysaccharide could potentially serve as reaction points for carbodiimide-activated amidation and the reductive amination of aldehydes or ketones, both of which could be carried out in aqueous solutions.

The thiol-ene coupling reactions were photochemically induced by 365 nm UV light in aqueous solutions in the presence of the water-soluble photoinitiator I2959, which initiated homolytic cleavage of S−H bonds

resulting in the formation of thiyl radicals. An intermediate carbon-centered radical was generated after the addition of thiyl radical across the C═C bond, followed by chain transfer to a second thiol molecule to yield the thiol-ene addition product and a new thiyl radical[33]. The reaction solution was dialyzed against deionized water to remove the excess reactants and the small-molecule impurities, and followed by lyophilization, affording the desired product as a fluffy powder.

The structures of Dex-AGE-MPA and Dex-AGE-MSA were confirmed by ^{1}H-NMR, FTIR and aqueous GPC analysis. The quantitative ^{1}H-NMR spectra of Dex-AGE-MPA and Dex-AGE-MSA were displayed in Fig. 1. The complete disappearance of the signals of the protons associated with the double bond (peaks 10, 11 and 12) and the appearance of the characteristic proton signals corresponding to the grafted thiol (Dex-AGE-MPA: peaks 13, 14 and 15; Dex-AGE-MSA: peaks 16 and 17) demonstrated the successful conjugation of MPA and MSA to the backbone of dextran. The actual graft ratios of MPA and MSA were determined to be 95% and 93%, respectively, by comparing integration areas of MPA or MSA proton peaks in the range of δ = 1.83–1.62 with those of carbohydrate proton peaks in the range of δ = 5.25–4.32. The FTIR spectra of Dex-AGE-MPA and Dex-AGE-MSA (Fig. 2A) clearly revealed the presence of absorbance peaks at 1705 cm^{-1} and 1715 cm^{-1} characteristic of carboxylic acid ($\nu_{C=O}$) and absorbance peaks at 1571 cm^{-1} and 1585 cm^{-1} characteristic of carboxylate ($\nu_{C=O}$). GPC analyses (Fig. 2B) indicated a clear shift to the higher molecular weight region (Dex-AGE-MPA: M_w = 3.18 × 10^4 g/mol, PDI = 1.86; Dex-AGE-MSA: M_w = 3.35 × 10^4 g/mol, PDI = 1.89), further confirmed that MPA and MSA were successfully grafted on to Dex-AGE *via* click chemistry. Besides, it's worthy of note that Dex-AGE did not exhibit a clear shift to short retention time in comparison with dextran. It was because branching leads to a contraction of the polymer chain in terms of the hydrodynamic volume[34]. However, both Dex-AGE-MPA and Dex-AGE-MSA exhibited an obvious shift to short retention time, which could be explained by the increase of hydrodynamic volume owning to the increase of molecular weight and intramolecular electrostatic repulsion of the ionic groups along the polymer chain caused expansion of the random coil[35]. A combination of NMR, FTIR, and GPC verified that the thiol-ene reaction proceeded rapidly and completed in 60 min, yielding Dex-AGE-MPA and Dex-AGE-MSA with high grafting efficiency.

The neutral clickable polysaccharide Dex-AGE was converted to positively charged Dex-AGE-CAH consisting of primary amine. The FTIR spectrum of Dex-AGE-CAH did not show any significant changes compared with Dex-AGE and Dex, but the ^{1}H-NMR spectrum confirmed the successful conjugation of CAH to Dex-AGE, with a graft ratio of 99%. As shown in Fig. S2, GPC trace of Dex-AGE-CAH (M_n = 1.59 × 10^4 g/mol, M_w = 3.25 × 10^4 g/mol, PDI = 2.04) exhibited a clear shift to the higher molecule weight region, in comparison with that of dextran (M_w = 2.55 × 10^4 g/mol, PDI = 2.03) and Dex-AGE (M_w = 2.75 × 10^4 g/mol, PDI = 2.15). Moreover, the existence of sulfur in Dex-AGE-MPA, Dex-AGE-MSA and Dex-AGE-CAH after thiol-addition was verified by XPS analysis. As shown in Fig. 2(C), the binding energy at 163.2 eV was attributed to the S 2p[36]. It also demonstrated the successful thiol-addition click reaction.

In summary, we have developed a novel, simple, aqueous phase two-step synthesis route to prepare side-chain functionalized dextran with carboxyl, bidentate dicarboxyl or amino groups. One of the biggest advantages of this method, as compared with the previous studies, is that the whole synthetic procedure can be carried out in aqueous medium without the use of toxic reagents and organic solvents, thus representing a green chemistry approach. Compared with the classical copper-catalyzed azide-alkyne cycloaddition reaction, it's more effective, time saving and environmentally friendly. Moreover, the presented combination of epoxide ring-opening and thiol-ene click chemistry in aqueous media may also be extended as an easy and gentle strategy for the modification of other polysaccharides, such as pullulan, starch and lentinan.

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