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# Biosynthesis of oligodextrans with different $M_w$ by synergistic catalysis of dextransucrase and dextranase

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ABSTRACT

In this study, we have investigated various adding times of dextranase to the dextransucrase system to reveal the synergistic process of dextransucrase and dextranase. Dextranase added into the dextransucrase–sucrose system at different times gave rise to different main dextran products. The results showed that dextranase added into sucrose system at the same time with dextransucrase synthesized low molecular weight ($M_w$) dextran targeted to 5 kDa, while dextranase added during the reaction process of dextransucrase directionally prepared dextran with medium $M_w$ of 10 kDa and 20 kDa. Fourier-transform infrared (FTIR) spectroscopy and $^1$H Nuclear magnetic resonance (NMR) exhibited that the synthesized oligodextrans were mainly composed of α-1,6-glycosidic linkages (dextran 6587 Da, 96.82%; dextran 22,521 Da, 96.03%) and low α-1,3-glycosidic branch. The research established the relationship between the synergistic catalysis of the double enzymatic system and the synthesis of oligodextrans with different $M_w$.



## 1. Introduction

Dextran, a chain polysaccharide, is generally composed of α-1,6- and α-1,3-glycosidic linkages based on special dextransucrase-producing strains (Kothari & Goyal, 2013; Vettori, Mukerjea, & Robyt, 2011). Given their different $M_w$ and branching degrees, this biopolymer has different medical values. For instance, dextran with a relatively lower $M_w$ (6 kDa to 8 kDa) can form a complex with iron, which is very important for treating severe anemia (Hussain et al., 2013). In addition, it has been reported that dextrans with relatively low $M_w$ (20, 40, and 70 kDa) are excellent plasma extenders (Bark & Grande, 2014; Spence, Higgins, & Kimmel, 1952). To date, oligodextrans are mainly synthesized industrially by *Leuconostoc mesenteroides* dextransucrase catalyzing sucrose, and acid hydrolysis is subsequently conducted. However, the yield of industrial production method should be improved further due to the considerable loss of the original dextran. In addition, the formed dextran often contains high amounts of chlorides, which seriously affect the purity and quality of the products. Industrial oligodextrans have broad $M_w$ distribution because of the random disorder of acid hydrolysis. Therefore, enzymatic synthesis of dextran is increasingly favored by researchers.

Currently, researchers mainly use dextransucrase to generate dextrans with different $M_w$ via adjusting the temperature and concentration of sucrose and dextransucrase. Kim, Robyt, Lee, Lee, and Kim (2003) showed that dextransucrase reacting with high sucrose concentration formed low $M_w$ dextrans (<10 kDa) with a relatively high yield of 69.9%. Dextransucrase remains covalently connected to the reducing ends of dextran (Bounaix et al., 2010; Shukla et al., 2014), resulting in difficulties in the separation and purification of dextrans. Dextranase, obtained from several microorganisms such as Streptococcus, Bacillus, and Penicillium, can catalyze the hydrolysis of consecutive α-1,6-glycosidic linkages of dextrans to form oligodextrans (Erhardt, Stammen, & Jordening, 2008; Khalikova, Susi, Usanov, & Korpela, 2003; Yang et al., 2013). Glucansucrase produced from *Streptococcus mutants* was combined with dextranase to investigate the prohibition of dental plaque (Ito et al., 2011). Kim and Day (1994) used a mixed culture of *L. mesenteroides* and *Lipomyces starkeyi* to produce dextranase to synthesize clinical dextrans. This method is appropriate to improve dextran production. However, it is not suitable to purify products because multiple substances are present in the mixed culture broth. Goulas, Cooper, Grandison, and Rastall (2004) synthesized isomaltooligosaccharides and oligodextrans by combining dextransucrase and dextranase. They reported that the oligosaccharide product, which mainly contains sugar (36%), had a degree of polymerization (DP) ranging from 10 to 60 together with some higher or lower $M_w$ dextrans. The products have a broad distribution of

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$M_w$ with relatively lower yield of oligodextrans. Therefore, a new synthesis process of oligodextrans needs to be studied.

The synergistic catalysis of dextransucrase and dextranase is a biochemical reaction process, which involves the synthesis and degradation of biopolysaccharides for the production of oligodextrans with different $M_w$ in the sucrose solution system. To perfect the biopolysaccharides' catalytic theory and control the synergistic catalytic process in a double enzyme system, the elucidation of the synergistic reaction of the combined dextransucrase and dextranase would be of great scientific significance. Previously, we have successfully produced dextransucrase from the engineered strain *Escherichia coli* BL21 (DE3)/pET28-dexYG constructed in our own laboratory (Zhang, Hu, Zhu, Zhu, & Wang, 2008). Besides, we also have screened and purified two dextranase-producing strain—*Hypocrea lixii* and *Penicillium aculeatum* (Zhang, Wu, Huang, Hu, & Wang, 2011). Based on the above researches, in this study, we have investigated different adding times of dextranase to the dextransucrase system so as to reveal the synergistic process of dextransucrase and dextranase. Several oligodextrans ($M_w$ ranging from 5 kDa to 20 kDa) was synthesized through different combinations of dextransucrase and dextranase in relatively high total yields. Fourier-transform infrared (FTIR) spectroscopy and $^1$H NMR exhibited that the synthesized oligodextrans were mainly composed of α-1,6-glycosidic linkages (dextran 6587 Da, 96.82%; dextran 22,521 Da, 96.03%) and low α-1,3-glycosidic branch. COSY and HSQC spectra clearly showed the internal H—H and C—H correlations. The results established the relationship between the synergistic process of the double enzymatic system and the synthesis of oligodextrans with controllable $M_w$.

## 2. Materials and methods

### 2.1. Material

Dextransucrase was produced from the engineered strain *E. coli* BL21 (DE3)/pET28-dexYG constructed in our own laboratory (Zhang et al., 2008). The BL21 (DE3)/pET28-dexYG strain was inoculated into Luria Bertani medium containing 50 μg/mL of kanamycin in a fermentation condition of 37 °C and 250 rad/min. IPTG (1 mM) was added to the fermentation liquid when $OD_{600}$ reached 0.6 to induce the expression of dextransucrase. The induction was continued for 5 h, and then the culture broth was centrifuged at 12,000 × *g* and 4 °C for 5 min (Malten, Hollmann, Deckwer, & Jahn, 2005) to harvest the cells. Lastly, the bacterial pellet was disrupted by pulse sonication in a 0.02 M acetic acid–sodium acetate buffer (pH 5.4) as crude dextransucrase. The dextransucrase activity was determined by measuring the initial rate of fructose liberation. The enzymatic reaction was carried out at 25 °C in 0.02 M acetic acid–sodium acetate buffer (pH 5.4) containing 0.02 M $Ca^{2+}$ with magnetic stirring. Samples were centrifuged for 10 min at 12,000 × *g* before the absorbance of the supernatant was determined. One unit of dextransucrase activity was defined as 0.1 mg fructose liberated per hour under the aforementioned condition (Zhang et al., 2008).

Dextranase came from a new dextranase-producing strain, which was identified as *P. aculeatum* screened from soil samples by our own members (Zhang et al., 2011). The strain was cultured in a medium for 7 days at 28 °C and 200 rad/min. The medium contained 1.0% dextran (70 kDa), 0.3% peptone, 0.1% $K_2HPO_4{\cdot}3H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$, 0.001% $FeSO_4{\cdot}7H_2O$, and 0.05% KCl (w/v). The broth was centrifuged at 6000 × *g* and 4 °C for 15 min. The obtained supernatant was crude dextranase. The dextranase activity was determined by Kim's method (Kim et al., 2011). The reaction was carried out at 35 °C in 0.02 M acetate buffer (pH 5.0) containing 4 mL of 3% dextran (70 kDa) and 1 mL of appropriately diluted crude dextranase solution. One unit of dextranase activity was defined as the amount of enzyme that hydrolyzed dextran to yield reducing sugars equivalent to 1 μM glucose per min under the aforementioned conditions.

A series of dextran standards (dextran 1400, 670, 410, 270, 150, 50, 25, 12, and 5 kDa) for the calibration curves of $M_w$ was purchased from Sigma. Other chemicals were of analytic grade.

### 2.2. Effect of sucrose and dextransucrase concentrations on the yield of high-$M_w$ dextran

Sucrose concentrations ranged from 5% (50 g/L) to 45% (450 g/L) with an increasing unit of 50 g. Sucrose was dissolved in a 0.02 M acetic acid–sodium acetate buffer (pH 5.4) containing 0.02 M $Ca^{2+}$, which was important to stabilize dextransucrase activity. After the sucrose solutions (100 mL) were equilibrated to the reaction temperature at 25 °C, dextransucrase (0.2, 0.5, 1.0, and 2.0 U/mL) was added according to the sucrose solution. The reaction occurred in 200 mL Erlenmeyer flasks (120 rpm). A volume of 300 mL ethanol was added to the reaction solution after 24 h to precipitate the high molecular dextrans, and this precipitation step was repeated three times. The precipitated dextrans were then placed in an oven (40 °C) for 24 h. The experiment was repeated three times and the result data was the average value of three parallel experiments.

### 2.3. Characteristics of the preferential degradation of high-$M_w$ dextran by dextranase

#### 2.3.1. Determining different $K_m$ of dextranase reacting with different $M_w$ of dextran

In this section, dextranase solution was obtained by separation and purification. The centrifuged supernatant of the culture broth underwent ammonium sulfate precipitation and Sepharose 6B gel filtration chromatography.

$K_m$ can be used to illustrate the affinity of enzyme to substrate and is inversely proportional to the affinity. The initial reaction rate was determined by choosing six concentrations (from 5 mg/mL to 80 mg/mL dissolved in 0.02 M acetic acid–sodium acetate buffer; pH 5.0) of dextrans (70, 40, and 20 kDa dextran synthesized in our laboratory) to react with purified dextranase solution at 35 °C. The experiment was repeated three times. The $K_m$ can be calculated from the Lineweaver–Burk plots:

$$\frac{1}{v} = \frac{\left(K_m/V_{\max}\right)1}{[s] + 1/V_{\max}}$$

where $V$ is the reaction velocity (the reaction rate), $K_m$ is the Michaelis–Menten constant, $V_{\max}$ is the maximum reaction velocity, and $[s]$ is the substrate concentration.

#### 2.3.2. Effect of dextranase with different $M_w$ of dextran at different intervention times

Dextransucrase catalyzes sucrose to form dextran, and the $M_w$ of dextran increases with time. Therefore, the degrading rate of dextranase varies with different $M_w$ of dextran at a range of intervention times. In this section, three parallel groups of dextransucrase and sucrose were selected and different intervention times of dextranase added to these groups were investigated (Table 1). For the three groups, the reactions were performed at 25 °C and 120 rpm in a shaker. The samples were placed in a boiling water bath for 6 min at an interval of 1 h after dextranase was added and diluted to 3 mg/mL.

Diluted samples that passed through a mixed cellulose ester film (a filter membrane with 0.22 μm aperture and 25 mm diameter) were analyzed by high-performance liquid chromatography (HPLC), in which a TSK gel G4000XL (7.8 mm × 30 cm) column was used. Chromatography was operated at 60 °C with a flow rate of 0.6 mL/min connected to a differential refractive index detector.

**Table 1**
Different intervention time of dextranase added to the sucrose–dextransucrase system.

| | Concentration of sucrose (mg/mL | Concentration of dextransucrase (U/mL) | Intervention time of dextranase[a] (h) | Concentration of dextranase (U/mL) |
|---|---|---|---|---|
| Group 1[b] | 100 | 2.0 | 6 | 2.5 |
| Group 2[b] | 100 | 2.0 | 12 | 2.5 |
| Group 3[b] | 100 | 2.0 | 18 | 2.5 |

[a] The intervention time was the reaction time of dextransucase when the dextranase was added. Took group 1 as an example, when the dextransucrase catalyzed sucrose for already 6 h, dextranase was added to the group and the reaction continued for the last 18 h.
[b] The three parallel groups were composed of same concentration of sucrose and dextransucrase.

Calibration curves of the retention time and $M_w$ were prepared for dextran standards (Sigma): 1400, 670, 410, 270, 150, 50, 25, 12, and 5 kDa dextran.

#### 2.3.3. Dextranase reacting with double substrate

We selected two dextrans (A: $M_w$ 48,231 Da, B: $M_w$ 5717 Da), which can be excellently separated by HPLC, as substrates to demonstrate whether *P. aculeatum* dextranase preferably degrades high-$M_w$ dextran or not. The same amount (0.3 g) of dextran A and B was dissolved in 30 mL of 0.02 M acetic acid–sodium acetate buffer (pH 5.0). Dextran A and B were synthesized in our laboratory. Approximately 2.5 U/mL of dextranase was added when the double substrate solution was equilibrated to 35 °C. The samples were placed in a boiling water bath for 6 min at an interval of 3 min, diluted to 3 mg/mL, and analyzed by HPLC as described in Section 2.3.2.

### 2.4. Synthesis of oligodextran by four different combination manners of dextransucrase and dextranase

#### 2.4.1. Simultaneous addition of dextransucrase and dextranase to the sucrose solution

We selected a two-enzyme system. After 10% sucrose solution (dissolved in 0.02 M acetic acid–sodium acetate buffer; pH 5.4) was equilibrated to 25 °C, dextransucrase (2.0 U/mL) and dextranase (2.5 U/mL) were simultaneously added to the solution (Fig. 1). The reaction occurred in a beaker (2000 mL) with a mechanical stirring device, and temperature was controlled at 25 °C in a water bath. The $M_w$ was measured by HPLC as previously described.

#### 2.4.2. Dextranase degradation at 12 h

We used group 2, which is described in Section 2.3.2, with a mechanical stirring device instead of a shaker. After dextransucrase reacted with sucrose for 12 h, dextranase was added to the system, and the reaction continued for 12 h. Fig. 1 shows the subsequent treatment of the reaction solution.

#### 2.4.3. Dextranase degradation at 18 h

We used group 3, which is described in Section 2.3.2, with a mechanical stirring device instead of a shaker. Fig. 1 shows the subsequent treatment of the reaction solution.

#### 2.4.4. Dextranase degradation after dextransucrase polymerization

In this section, 2.0 U/mL of dextransucrase was added to 10% sucrose solution and catalyzed for 24 h at 25 °C controlled in a water bath. At the first 4 h, reaction occurred in a mechanical stirring device and then continued to react for the next 20 h without stirring. Afterward, the water bath temperature was adjusted to 35 °C, which was the optimum temperature of *P. aculeatum* dextranase. Dextranase (2.5 U/mL) was added to the reaction solution and degraded high-$M_w$ dextrans synthesized by dextransucrase for 5 h. Fig. 1 shows the subsequent treatment of the reaction solution.

### 2.5. Structural and qualitative analysis of dextrans with different $M_w$

#### 2.5.1. Qualitative analysis of dextrans

Dextran powders synthesized according to previously described methods were dissolved in distilled water (3 mg/mL to 6 mg/mL), passed through a mixed cellulose ester film (a filter membrane with 0.22 μm aperture and 25 mm diameter), and analyzed by HPLC as described in Section 2.3.2.

#### 2.5.2. Analysis of functional groups by FTIR spectroscopy

KBr was selected as the solid diluent to mix with dextran powder, which was then pressed in a slicer and analyzed by FTIR (Thermo Nicolet67, America) operated at 25 °C from 4000 $cm^{-1}$ to 400 $cm^{-1}$ with an instrumental resolution of 0.09 $cm^{-1}$. The experimental data were processed in Origin drawing software.

#### 2.5.3. NMR analysis of glycoside linkages and H—H/C—H correlations

Dextrans with different $M_w$ were dissolved in pure $D_2O$ (30 mg/mL) and then loaded into NMR tubes. $^1H$ NMR, $^{13}C$ NMR, COSY, and HSQC spectra were obtained from Agilent Technologies (America) superconducting NMR spectrometer VNMRS600. The experiments were operated at 600 MHz and 30 °C using

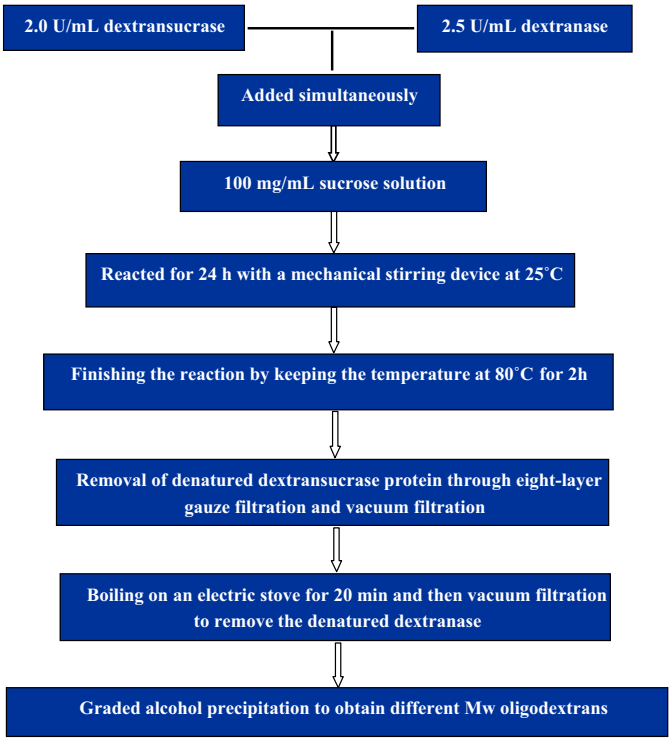


**Fig. 1.** The synthesis route of oligodextrans when dextransucrase and dextranase were added simultaneously. The ethanol concentration was measured by an alcohol meter.

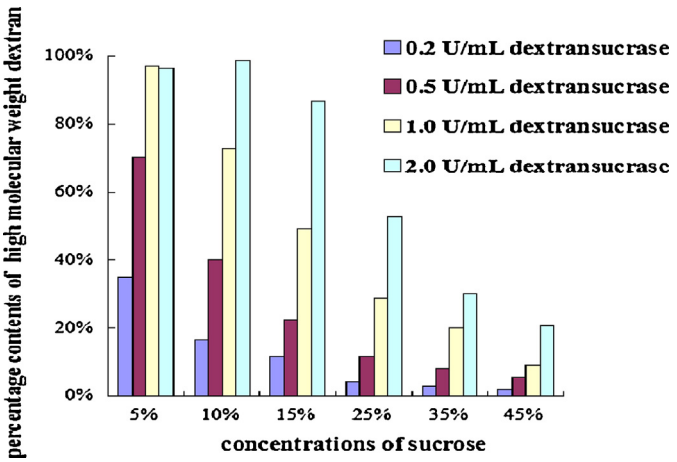


**Fig. 2.** Yields of high $M_w$ dextrans in the condition of different concentrations of sucrose and dextransucrase. The concentrations of sucrose were range from 50 g/L (5%) to 450 g/L (45%); The concentrations of dextransucrase were range from 0.2 U/mL to 2.0 U/mL; Yields of high $M_w$ dextran were calculated according to the following formula: [weight of dry dextran]/[0.5 × sucrose content] × 100%; the result data were the average value of three parallel experiments.

tetramethylsilane as internal standard. The spectra were analyzed using the MestReNova software.

## 3. Results and discussion

### 3.1. Effect of sucrose and dextransucrase concentrations on the yield of high-$M_w$ dextran

Fig. 2 indicates that the yield of high-$M_w$ dextran was inversely proportional to sucrose concentrations but directly proportional to dextransucrase concentrations. However, as dextransucrase concentration increased, the yield of high-$M_w$ dextrans decreased when the sucrose concentration was relatively lower (for example, 5%); this decrease is due to the reaction mechanism of dextransucrase (Robyt, Yoon, & Mukerjea, 2008). The result showed that 2.0 U/mL of dextransucrase could catalyze 10% sucrose as completely as possible with a 98.8% yield. Therefore, we selected 10% sucrose solution as substrates for the subsequent reactions.

### 3.2. P. aculeatum dextranase preferentially degraded high-$M_w$ dextran

Fig. 3A shows three Lineweaver–Burk plots of dextranase reacting with 70, 40, and 20 kDa dextran. We obtained three $K_m$ values based on the three linear equations (Fig. 3A and Table 2). $K_m$ was inversely proportional to the $M_w$ of dextran, indicating that the affinity of *P. aculeatum* dextranase to dextrans was proportional to the $M_w$.

Fig. 3B shows the change in $M_w$ of dextran synthesized by dextransucrase and dextranase added at different times. With the passage of time, the $M_w$ of dextran showed a decreasing trend at different intervention times of *P. aculeatum* dextranase. Dextran was gradually degraded with a decreasing $M_w$ when the sucrose solution was mixed with dextransucrase for 6 h and dextranase

**Table 2**
$K_m$ values of *P. aculeatum* dextranase reacting with three dextran.

| | $K_m$ (mol/L) |
|---|---|
| Dextran 70 kDa | 0.202 × 10−3 |
| Dextran 40 kDa | 0.501 × 10−3 |
| Dextran 20 kDa | 1.75 × 10−3 |

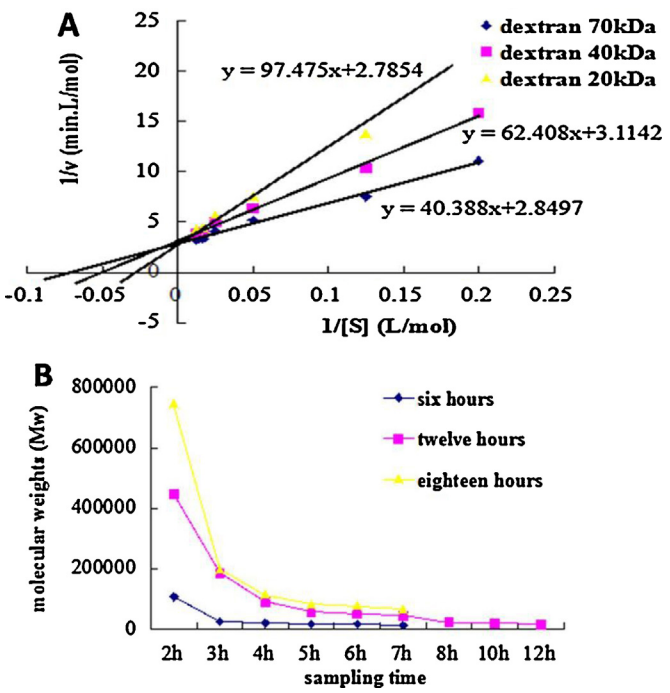


**Fig. 3.** (A) Lineweaver–Burk plots of dextranase reacting with different $M_w$ dextrans. The data were the average value of at least three parallel experiments. (B) Effect of dextranase at different intervention times on the $M_w$ of dextrans

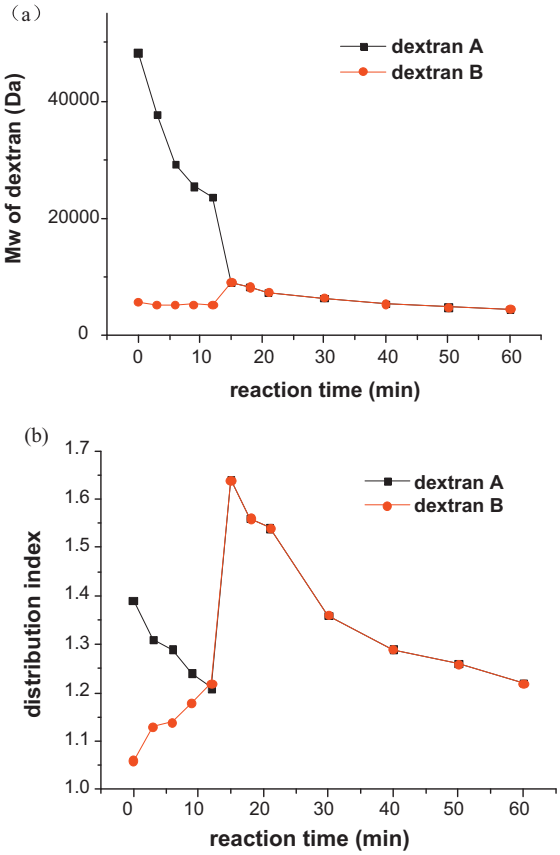


**Fig. 4.** (a) the $M_w$ change of both dextran A and dextran B with the time passage in the condition of dextranase catalyzing; (b) the change of distribution index of $M_w$ of dextran A and dextran B with the time passage. The distribution index was the ratio of weight average $M_w$ ($M_w$) to number average $M_w$ ($M_w$n). It reflected the component of dextrans and was measured by the HPLC connected to a differential refractive index detector.

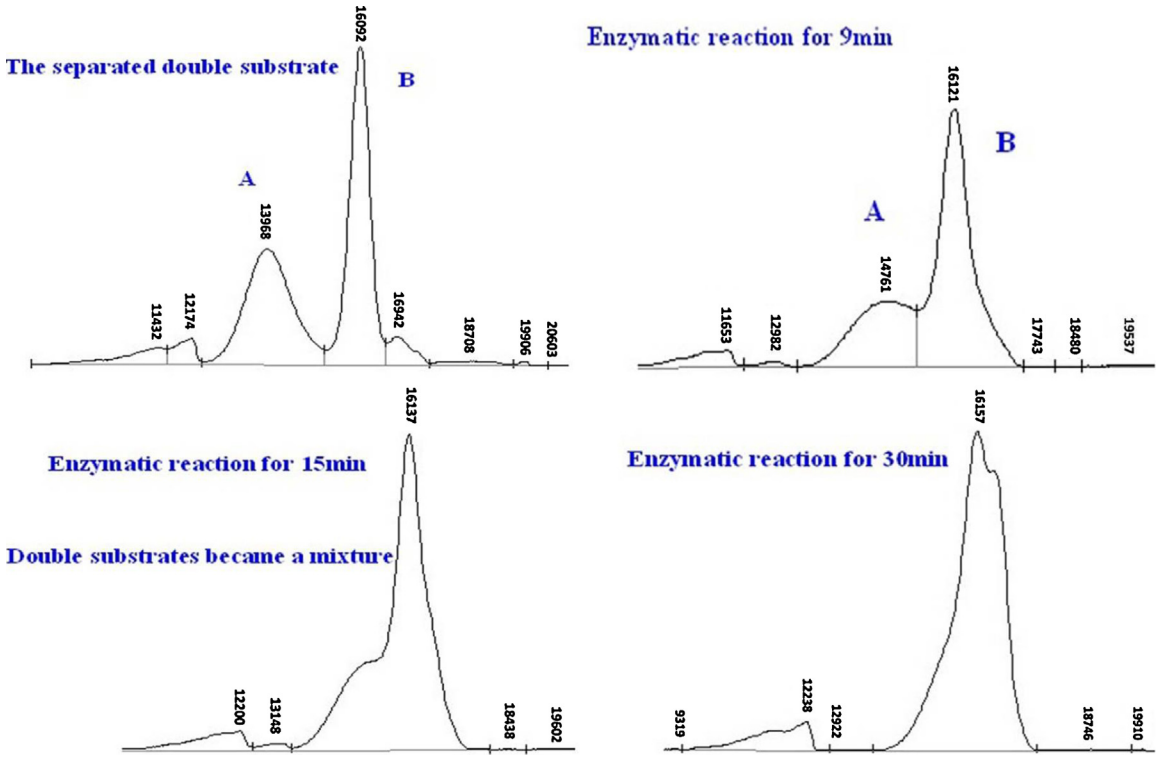


**Fig. 5.** The HPLC result of the dextranase' reaction with double substrate for different time.

was added at this time. After 3 h, the $M_w$ reached 30 kDa and the degradation rate became slower. The $M_w$ was maintained at 10 kDa after 7 h. This result was due to the lower affinity between dextranase and degraded dextran, which accordingly weakened the inhibition to dextransucrase, resulting in a considerable synthesis rate and degradation rate. Dextranase added at this time could quickly degrade dextran with a relatively higher affinity when dextransucrase reacted with sucrose for 12 h. The $M_w$ of dextran thus significantly decreased, and this decreasing trend was observed for 12 h. The $M_w$ of dextran was maintained at 20 kDa after degradation for 6 h when dextransucrase was mixed with sucrose solution for 18 h although dextranase added at this moment could rapidly degrade dextran. The slope of the curve of the $M_w$ indirectly indicated the catalytic efficiency of dextranase. Fig. 3B exhibits the late entry of dextranase in the dextransucrase–sucrose solution. The faster change of dextrans $M_w$ indicated that the affinity of *P. aculeatum* dextranase to dextrans was proportional to the $M_w$.

Dextran products with a series of $M_w$ are a combination of many homologs. Thus, the $M_w$ cannot represent an accurate figure; instead, this value provides the range of pharmacopoeia prescription. In this experiment, dextran A ($M_w$ 48,231 Da) and B ($M_w$ 5717 Da) can be excellently separated in the GPC column, and both of A and B exhibited a low distribution index (dextran A and B were 1.31 and 1.06, respectively). This result indicated that both of the two substrates had uniform compositions. When *P. aculeatum* dextranase was added, two completely separated peaks were observed in the HPLC at the first 15 min. The $M_w$ of dextran A rapidly decreased whereas that of B slightly changed at approximately 5300 Da (Fig. 4a). However, the composition of dextran B became increasingly uneven (Fig. 4b). This finding is consistent with that shown in Fig. 3A, which indicated that the affinity of *P. aculeatum* dextranase to dextrans was proportional to the $M_w$. Therefore, *P. aculeatum* dextranase would initially degrade dextran A with a relatively higher $M_w$ when dextran A and B were simultaneously presented in the solution. Dextran A and B became a mixture and could not be separated after 15 min (Fig. 5). At this time, the distribution index of dextrans reached the maximum (D 1.64), indicating that the compositions were mostly uneven. Then, the distribution index became smaller on the continuing dextranase degradation. The change of distribution index and $M_w$ jointly confirmed that *P. aculeatum* dextranase initially reacted with dextran A. This phenomenon demonstrated that *P. aculeatum* dextranase preferentially degraded dextran with a high $M_w$.

### 3.3. Dextrans with different $M_w$ by graded alcohol precipitation

Dextransucrase can catalyze sucrose to form dextran with increasing $M_w$ and transfer D-glucopyranosyl group from sucrose to acceptors presented in the reaction solution (Diez-Municio et al., 2012; Kim et al., 2003). Therefore, *P. aculeatum* dextranase added in the catalytic process of dextransucrase can release isomaltose and isomaltotriose as well as dextran chains (Zhang et al., 2011) as acceptors of dextransucrase. In the dual enzyme system, various and complex dextran products exist because of the dextransucrase's acceptor reaction and the hydrolytic activity of dextranase. Thus, oligosaccharide separation was difficult (Bennett, 2014; Ma, Cao, & Yu, 2013). However, oligodextrans can be separated by ethanol precipitation, and the yields can be controlled by adjusting the reaction conditions. Twelve kinds of oligodextrans were obtained in this study with relatively high total yields (Table 3). One main product existed in every method (5241, 10,941, 22,521, and 9821 Da dextran in methods 1, 2, 3, and 4, respectively). The results showed that dextranase added into sucrose system at the same time with dextransucrase synthesized low $M_w$ dextran targeted to 5 kDa, and dextranase added during the reaction process of dectransucrase directionally prepared dextran with medium $M_w$ of 10 kDa and 20 kDa. This was due to *P. aculeatum* dextranase's confirmed preferential degradation of high $M_w$ dextran, leading to the enrichment of medium and low $M_w$ dextrans.

**Table 3**
Dextrans with different $M_w$ obtained by the combined use of dextransucrase and dextranase with different intervention time.

| | Method 1[a] | Method 2 | Method 3 | Method 4 |
|---|---|---|---|---|
| Dextran I | $M_w$ 19,374 Da 7.3%[b] | $M_w$ 19,101 Da 13% | $M_w$ 22,521 Da 42.83% | $M_w$ 19,852 Da 18.7% |
| Dextran II | $M_w$ 8295 Da 18.78% | $M_w$ 10,941 Da 39.8% | $M_w$ 9547 Da 26.1% | $M_w$ 9821 Da 45.5% |
| Dextran III | $M_w$ 5241 Da 35.5% | $M_w$ 6587 Da 22% | $M_w$ 6068 Da 12.47% | $M_w$ 6781 Da 22.3% |
| Total yield[c] | 61.58% | 74.8% | 81.4% | 86.5% |

[a] Method 1 meant that dextransucrase and dextranase added into sucrose solution simultaneously. Method 2 referred to dextranase beginning degrading at 12 h. Method 3 was dextranase beginning degrading at 18 h. Method 4 was dextranase beginning degrading after dextransucrase finishing polymerization.
[b] All of the data was the average value of three parallel experimental results.
[c] The total yield of dextran products was calculated according to the following formula: [weight of all kinds of dry dextrans]/[0.5 × sucrose content] × 100%.

### 3.4. Structure and qualitative analysis of dextran

#### 3.4.1. FTIR spectroscopy

The infrared spectroscopy mainly identified some functional groups of specific compounds. Fig. 6A shows that all four kinds of dextrans had similar functional groups. First, the peak at 842 $cm^{-1}$ demonstrated that dextrans belonged to α-glycosidic bond, and the peak at 1015 $cm^{-1}$ was the characteristic absorption peak of α-(1,6) linkages (Shingel, 2002). The peaks at 915 and 760 $cm^{-1}$ were the D-glucopyranosyl C—O—C skeleton asymmetric and symmetric stretching vibrations, respectively. Second, the O—H stretching vibration peak of hydrogen bonds formed between the dextran molecules was confirmed by the peak at 3397 $cm^{-1}$, and the peak at 1648 $cm^{-1}$ belonged to bound water. The saturated C—H stretching vibration caused the band in 2927 $cm^{-1}$ region (Mukhopadhyay et al., 2014). Furthermore, the band in 1110 $cm^{-1}$ region was due to the alcoholic hydroxyl deformation vibration. The C—O absorption peak in the exocyclic D-glucopyranosyl ring was located at 1156 $cm^{-1}$ (Nikonenko, Buslov, Sushko, & Zhbankov, 2000). In addition, the bands between 1178 and 1138 $cm^{-1}$ (not shown) can be interpreted as the performance of the glycosidic linkages (Jockusch et al., 2004).

We synthesized 12 dextrans with $M_w$ ranging from 5241 Da to 22,521 Da according to previously described methods. Fig. 6B shows that these 12 dextrans exhibited high purities and low distribution indexes, indicating evenly distributed $M_w$, particularly dextrans with a $M_w$ of 6 kDa. The higher division of alcohol precipitation grade resulted in a more uniform $M_w$ distribution.

Fig. 6C shows the $^1$H NMR spectra of 6587 and 22,521 Da dextran (synthesized in this study) and 24,514 Da dextran (industrial

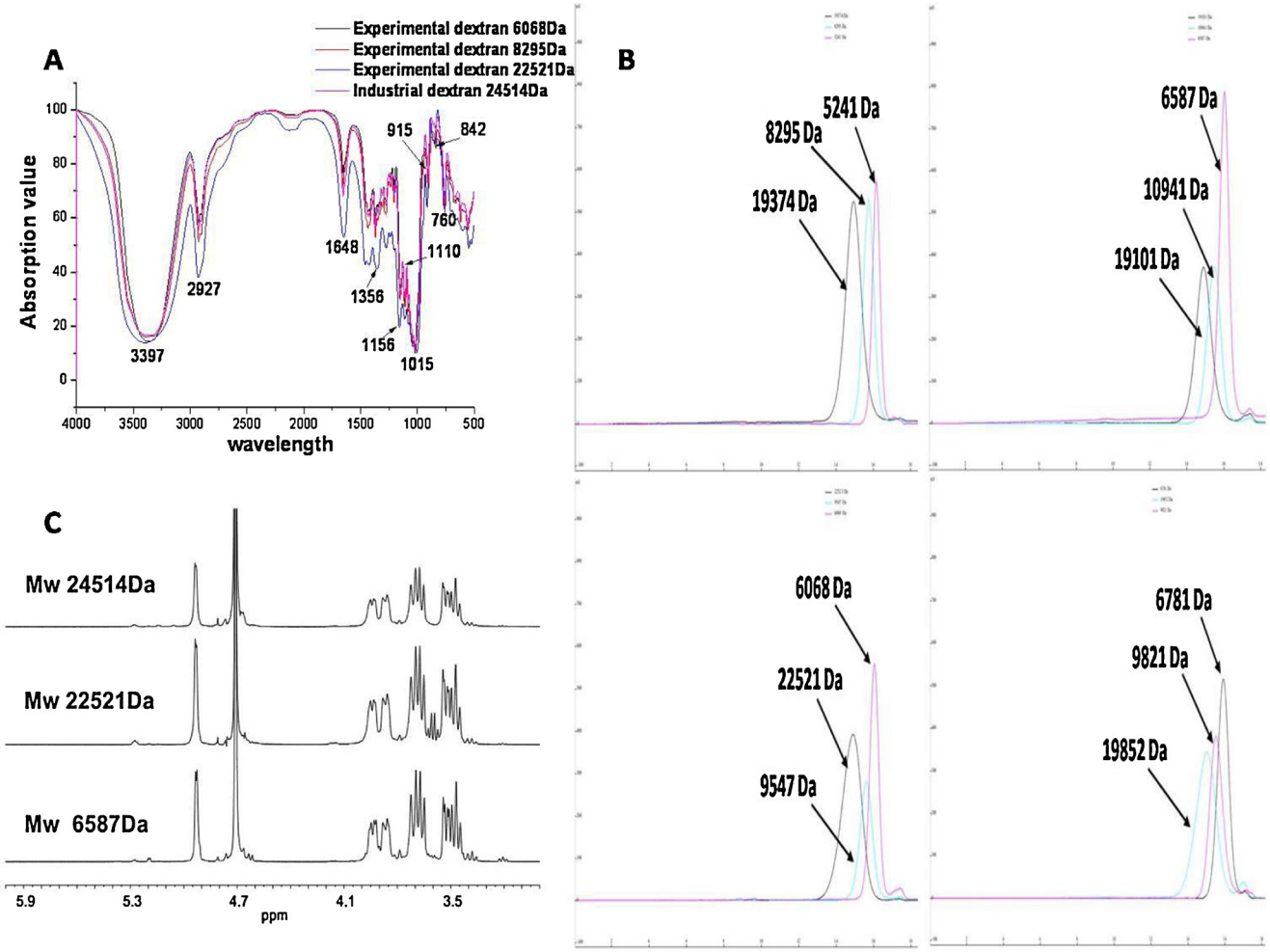


**Fig. 6.** The FTIR (A), HPLC (B) and $^1$H NMR (C) spectra of dextrans.

**Table 4**
$^1$H NMR chemical shifts of experimental and industrial oligodextrans.

| | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | H-6′ |
|---|---|---|---|---|---|---|---|
| Experimental 6587 Da | 4.91 | 3.50 | 3.61 | 3.44 | 3.84 | 3.92 | 3.67 |
| Experimental 22,521 Da | 4.93 | 3.54 | 3.66 | 3.45 | 3.86 | 3.95 | 3.70 |
| Industrial 24,514 Da | 4.94 | 3.61 | 3.68 | 3.47 | 3.86 | 3.94 | 3.71 |

dextran 20 kDa). Three kinds of dextrans shared similar NMR spectra in spite of differences in some details. The experimental spectra exhibited again that the synthesized dextrans belonged to α-glycosidic bonds. Signals at 4.708 ppm exhibited the deuterated solvent ($D_2O$) peak. Generally, the end proton signals were located at 4.3 ppm to 6.0 ppm and other protons signals were between 3.2 ppm and 4.2 ppm (Patel, Pendrill, Mallajosyula, Widmalm, & Mackerell, 2014; Probert, Whittaker, Crispin, Mitchell, & Dixon, 2013). The results corresponded with the experimental spectra (Table 4). The α-1,6-glycosidic linkage in the main chains was

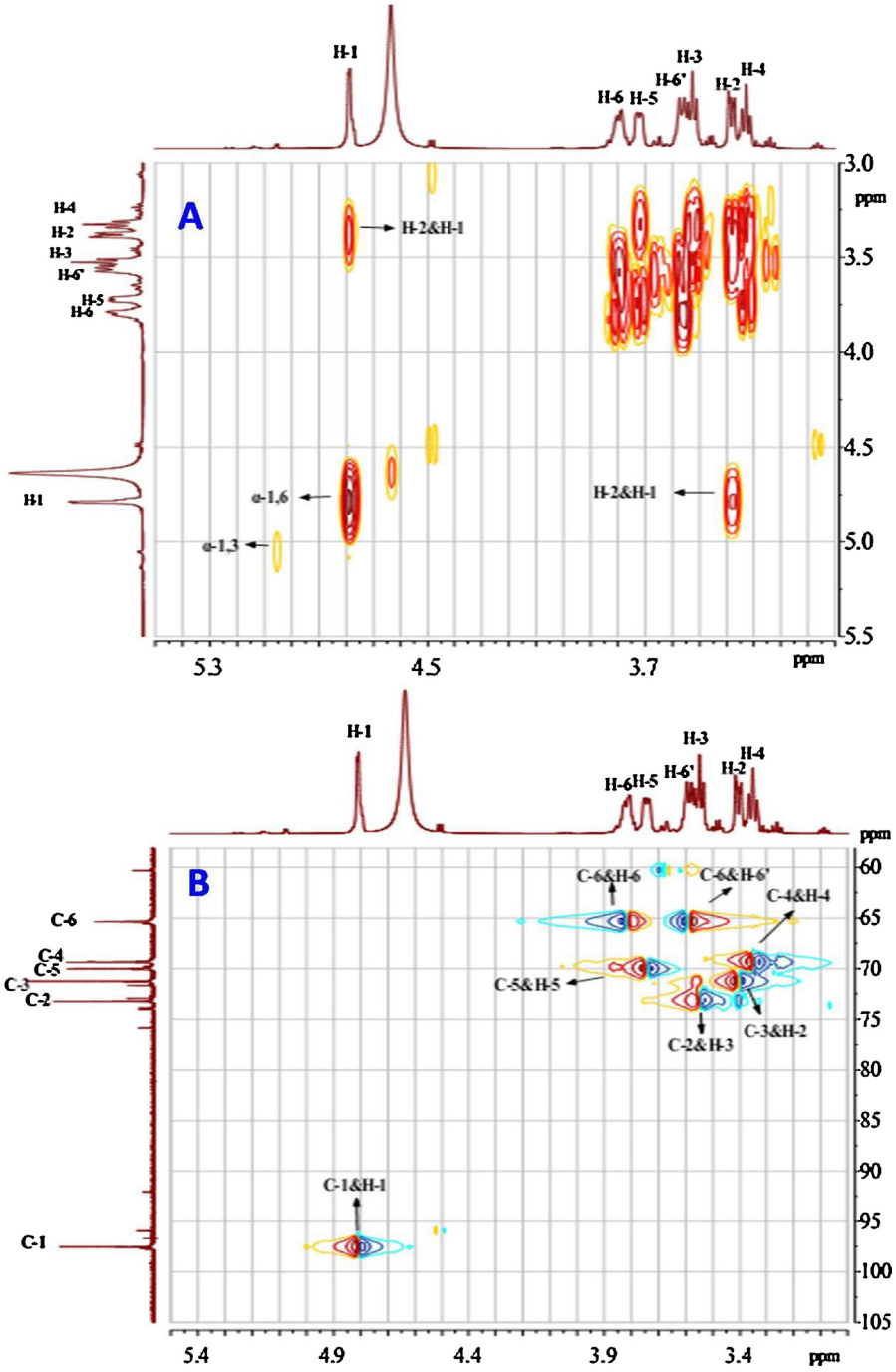


**Fig. 7.** COSY (A) and HSQC (B) spectra of the synthesized oligodextran 6587 Da using $D_2O$ as the deuterated reagents.

**Table 5**
Percentage contents of glycosidic linkages in oligodextrans.

| | Dextran 6587 Da[a] (%) | Dextran 22,521 Da[b] (%) | Dextran 24,514 Da[c] (%) |
|---|---|---|---|
| α-1,6-Glycosidic linkage | 96.82 | 96.03 | 95.5 |
| α-1,3-Glycosidic linkage | 3.18 | 3.97 | 4.5 |

[a] Dextran synthesized in method 2 as described in Table 4.
[b] Dextran synthesized in method 3.
[c] The industrial dextran 20 kDa obtained from Lu'an Huayuan Pharmaceutical Co, Ltd.

demonstrated at a peak of 4.93 ppm, whereas α-1,3-glycosidic linkage in the branch is located at approximately 5.20 ppm (Dong, Zhang, Hao, Tang, & Wang, 2013).

COSY and HSQC spectra (Fig. 7) provided better understanding on the internal H—H and H—C correlations of oligodextran. Three major correlations were observed on the H—H COSY spectrum (Bashari et al., 2013). First, the strong correlation of an anomeric proton H-1 and a C-2 proton (H-2) appeared at 4.79/3.38 (Leonard, 1975) (Fig. 7A). In addition, the H-3/H-2, H-3/H-4, H-4/H-5, and H-5/H-6 correlations are within the contour line of 3.5 ppm (Wondraczek, Elschner, & Heinze, 2011). Second, a distinctive correlation was observed of the anomeric H—H of α-1,6-glycosidic linkages located at 4.79/4.79 (Falconer, Mukerjea, & Robyt, 2011). H-1/H-2 correlation also appeared in this contour as 4.79/3.38. Lastly, a weak visible correlation appeared at ~5.20 ppm, which was α-1,3-glycosidic linkages (Wondraczek et al., 2011). On the HSQC spectrum, seven C—H correlations were observed (Fig. 7B). The corresponding carbon and proton chemical shifts (Bashari et al., 2013) were 97.62 and 4.82 (C-1 and H-1), 71.22 and 3.45 (C-3 and H-2), 73.15 and 3.68 (C-2 and H-3), 69.36 and 3.37 (C-4 and H-4), 69.96 and 3.76 (C-5 and H-5), 66.32 and 3.84 (C-6 and H-6), and 66.34 and 3.67 (C-6 and H-6′).

The peak area ratio of C-1 protons represented the proportions of different glycosidic linkages. Table 5 shows the percentage contents of glycosidic linkages in the three dextrans according to the integration calculation. The synthesized dextrans had relatively high percentage of α-1,6-glycosidic linkage in main chains, indicating a predominantly linear structure. For example, the degree of polymerization (DPn) in 6587 Da could be calculated according to the following formula: $DPn = (M_w n - 18)/162$ (Falconer et al., 2011). In this study, $M_w n$ was the number average $M_w$ of dextran. Therefore, 6587 Da dextran contained approximately $(6587/1.12 - 18)/162 = 36$ glucose units, including $36 \times 96.82\% = 35$ α-1,6-glycosidic units and only one α-1,3-linked glycosyl residue. Similarly, the 22,521 Da dextran contained $(22{,}521/1.31 - 18)/162 = 106$ glucose units, including $106 \times 96.03\% = 101$ α-1,6-glycosidic units and five α-1,3-linked glycosyl residues may be variously distributed in the main chains, including disorderly linked to the main chains, regularly distributed on every glucose units, or branched with different chain lengths (Vettori, Franchetti, & Contiero, 2012). This result should be further investigated.

## 4. Conclusions

Products of dextransucrase could be fast used by dextranase in a dual enzyme system. In this study, we used four different combinations of dextransucrase and dextranase to understand their synergistic catalysis manner by tracking the change of dextran products. Moreover, a double-substrate experiment and measurement of different $K_m$ values demonstrated that *P. aculeatum* dextranase preferentially degraded dextran with higher $M_w$. With these research, 12 kinds of oligodextrans were synthesized (19,374, 19,101, 22,521, 19,852, 8295, 10,941, 9547, 9821, 5241, 6587, 6068, and 6781 Da) with high yields. FTIR and 2D NMR confirmed the functional groups and internal H—H/C—H correlations of oligodextrans. $^1$H NMR showed that the structure of 22,521 Da dextran contained five α-1,3-glycosidic branches per 106 α-1,6-linked glycosyl units which showed great potential clinical values. Moreover, 6587 Da dextran was theoretically composed of 35 α-1,6-glycosidic units and only one α-1,3-linked glycosyl residue, this kind of dextran could be used to prepare derivatives.

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