Synthesis and antioxidant property of novel 1,2,3-triazole-linked starch derivatives via ‘click chemistry’

Wenqiang Tan, Qing Li, Wancong Li, Fang Dong, Zhanyong Guo

Key Laboratory of Coastal Biology and Bioresource Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

University of Chinese Academy of Sciences, Beijing 100049, China

1. Introduction

Reactive oxygen species (ROS), including hydroxyl radicals (·OH), hydrogen peroxide (H₂O₂), and superoxide anion (O₂⁻izione) [1–3], can induce damage to cellular constituents [4], which can cause neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases, cancer, hypertension, diabetes, and many other diseases associated with aging in biological systems [5–9]. The role of antioxidants has received increased attention during the past decades. However, the use of synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate, has potential health hazards [10]. Therefore, searching for natural antioxidants as alternatives to synthetic ones is of great interest among researchers.

Starch is the major energy reserve for a large variety of higher green plants, such as cereals, legumes, and tubers. Starch is mainly composed of α-D-glucopyranosyl unit [11–14]. Several interesting properties such as maximum energy supplement for human, moderated average degree of polymerization, and readiness of being obtained have been shown by starch. As an abundant, cheap, environmentally benign, biodegradable, and biocompatibility polysaccharide, starch has a certain range of applications involving food, pharmaceutical, beverages, papermaking, packaging, and textiles [15,16]. However, it is known that native starch is inherently unsuitable for further industrial applications. One valid solution is often tailored through chemically to enhance desirable functional properties [11]. Moreover, increasing attention has been attracted to the structure–activity relationship of polysaccharides, as biological activities of polysaccharide are related with its molecular structure [17].

The Cu(I) catalyzed azide-alkyne [3+2] cycloaddition (CuAAC) or ‘click chemistry’, initiated by Sharpless et al., has emerged as a powerful strategy for the design of sophisticated biomaterials with high levels of precision and control [18,19]. As reaction products of CuAAC, 1,2,3-triazoles are attractive constructs of target molecules due to their wide range of biological properties, such as antimicrobial [20], antitubercular [21], antimalarial [22], antibiotic [23], anticancer [24,25], cytotoxic agents [26,27], and antioxidant [28,29]. Recently, the application of ‘click chemistry’ in polysaccharide modification has aroused great interest. The modification...
of carbohydrate polymers by ‘click chemistry’ could overcome their disadvantages, such as low selectivity, various side reactions, and low yields [30]. Moreover, it will also improve certain biological properties of carbohydrate polymers via ‘click chemistry’. Dong et al. reported synthesis of amphiphilic aminated inulin by azide-alkyne click reaction and the obtained derivative exhibited improved antibacterial property against Staphylococcus aureus [30]. Qin et al. described the synthesis and potential antifungal applications of (1,2,3-triazol-4-yl)methyl nicotinate chitosan utilizing azide-alkyne click reaction [31]. However, there was very few research on synthesis and bioactivity (such as antioxidant) of starch derivatives with 1,2,3-triazole, which were very indispensable contents of starch researches.

In order to study the relationship of structure–antioxidant activity of starch derivatives with 1,2,3-triazole, we reported the synthesis and antioxidant property of a group of starch derivatives with 1,2,3-triazole as substituent including HMTST, HETST, HPTST, and HBTST via ‘click chemistry’ in this paper. The 6-bromo-6-deoxy starch was first selectively synthesized by reaction between the C6–OH of starch and N-bromosuccinimide (NBS) in N,N-dimethylformamide (DMF)/LiBr, which was been selected as the reaction medium because it could save both time and labor [32]. Then, the 6-azido-6-deoxy starch was obtained by reaction between the 6-bromo-6-deoxy starch and NaN3. Subsequently the alkylene components were introduced into 6-azido-6-deoxy starch through the Huisgen 1,3-dipolar cycloaddition reaction. The starch derivatives designed in this way were expected to have advantageous characteristics such as high antioxidant activity and good water solubility. The chemical structures of the derivatives were characterized by FT-IR, 1H NMR, and 13C NMR. Three common free radicals, including hydroxyl-radical, DPPH-radical, and superoxide-radical, were selected to evaluate the antioxidant property of starch and starch derivatives synthesized in vitro. The relationship between the structure and antioxidant activity of starch derivatives was discussed, simultaneously.

2. Experimental

2.1. Materials

Soluble starch from potato was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), 2-Propyn-1-ol, 3-butyln–1-ol, 4-pentyn-1-ol, and 5-hexyn–1-ol were obtained from Aladin Chemical Corp (Shanghai, China). The other reagents were all of analytical grade and used without further purification.

2.2. Analytical methods

Fourier transform infrared (FTIR) spectra were performed ranging from 4000 cm⁻¹ to 400 cm⁻¹ using a Jasco–4100 FT-IR spectrometer (Japan, provided by JASCO Co., Ltd. Shanghai, China) with KBr disks. 13C Nuclear magnetic resonance (13C NMR) and 1H Nuclear magnetic resonance (1H NMR) spectra were all measured with a Bruker AVIII–500 Spectrometer (500 MHz, Switzerland, provided by Bruker Tech. and Serv. Co., Ltd. Beijing, China), using (CD3)2SO (DMSO-d6) as solvents with tetramethysilane (TMS) as internal standard. Chemical shift values were given in δ (ppm). The UV–vis absorbencies of the tested mixtures were measured with a T6 New Century UV spectrometer (China, provided by P General Co., Ltd., Beijing, China). The elemental analyses (C, H, and N) were performed on a Vario EL III (Elementar, Germany). The Degrees of Substitution (DS) of starch derivatives were calculated on the basis of the percentages of carbon and nitrogen.

2.3. Synthesis

2.3.1. The dissolution of starch

Soluble starch (3.24 g, 20 mmol) was stirred in 80 mL anhydrous N,N-dimethylformamide (DMF), while the mixture was heated to 120 °C for 1 h. The slurry was then allowed to cool to 90 °C, at which point LiBr (3.47 g, 40 mmol) was added. The starch dissolved within 5 min to form a transparent solution. The contents of the flask were allowed to cool further to room temperature while stirring.

2.3.2. Synthesis of 6-bromo-6-deoxy starch

As shown in Scheme 1, when transparent solution above-mentioned was cooled to 0 °C, N-bromosuccinimide (NBS) (14.24 g, 80 mmol) and triphenylphosphine (Ph3P) (20.99 g, 80 mmol) were added. The reaction solution was heated to 80 °C for 3 h under an argon atmosphere. The product was isolated by adding the reaction mixture slowly to 400 mL of 95:5 (v/v) mixture of absolute ethanol and deionized water, followed by filtration. The unreacted NBS, Ph3P, and other outgrowth (succinimide, triphenylphosphine oxide (Ph3PO)), were extracted in a Soxhlet apparatus with ethanol and aceton for 48 h, respectively. The 6-bromo-6-deoxy starch was obtained by freeze-drying overnight in vacuum, yield: 89.3%.

2.3.3. Synthesis of 6-azido-6-deoxy starch

In a 100 mL three-necked round-bottom flask, 6-bromo-6-deoxy starch (2.25 g, 10 mmol) was weighed and dissolved in 40 mL of anhydrous dimethylsulfoxide (DMSO). Then, NaN3 (1.3 g, 20 mmol) was added to the flask and dissolved. The solution was heated to 80 °C and stirred for 24 h under an argon atmosphere. The product was isolated by pouring the reaction solution into 200 mL of absolute ethanol. The precipitate was collected by filtration, and washed with acetone. After being extracting in a Soxhlet apparatus with ethanol for 48 h and being dialyzed against deionized water for 2 days to remove the probable remained sodium azide, the 6-azido-6-deoxy starch was obtained by freeze-drying, yield: 71.1%.

2.3.4. Synthesis of amphiprotic starch derivatives (HMTST, HETST, HPTST, and HBTST)

6-Azido-6-deoxy starch (187 mg, 1 mmol) was dissolved in 20 mL DMSO, cuprous iodide (19 mg, 0.1 mmol), triethylamine (0.14 mL, 1 mmol), and terminal alkynie derivative (2-propyn-1-ol, 3-butyln-1-ol, 4-pentyn-1-ol, and 5-hexyn-1-ol) (3 mmol) were
were added, and the solution was stirred at 75 °C for 24 h under an argon atmosphere. The mixture was precipitated in acetone, and collected by filtration. The probable remained reagents were extracted in a Soxhlet apparatus with acetone for 2 days. After being dialyzed against deionized water for 2 days, the starch derivatives with 1,2,3-triazoles were obtained by lyophilization of their aqueous solutions, yield: 86.34–94.57%.

2.4. The investigation of the antioxidant ability

2.4.1. Hydroxyl-radical scavenging ability assay

The test of hydroxyl-radical scavenging ability was carried out according to Liu’s methods with minor modification [33]. The reaction mixture, a total volume 4.5 mL, containing the samples of starch or starch derivatives (HMTST, HETST, HPTST, and HBTST) (10 mg/mL, 0.045, 0.09, 0.18, 0.36, and 0.72 mL), were incubated with EDTA-Fe$^{2+}$ (220 μM), safranine O (0.23 μM), and H$_2$O$_2$ (60 μM) in potassium phosphate buffer (150 mM, pH 7.4) for 30 min at 37 °C. The absorbance of the mixture was measured at 520 nm. In the blank, samples were substituted with distilled water. Meanwhile, in the control, H$_2$O$_2$ was substituted with potassium phosphate buffer. Three replicates for each sample concentration were tested. The capability of scavenging hydroxyl radicals of the products was computed using the following equation:

Scavenging effect(%) = \[\left(\frac{A_{\text{sample} \, 520 \, \text{nm}} - A_{\text{blank} \, 520 \, \text{nm}}}{A_{\text{control} \, 520 \, \text{nm}} - A_{\text{blank} \, 520 \, \text{nm}}}\right) \times 100\]

where $A_{\text{blank} \, 520 \, \text{nm}}$ is the absorbance of the blank and $A_{\text{control} \, 520 \, \text{nm}}$ is the absorbance of the control.

2.4.2. DPPH-radical scavenging ability assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH$^\cdot$) scavenging property of the products was evaluated by following method [17]: testing samples (10 mg/mL, 0.03, 0.06, 0.12, 0.24, and 0.48 mL) and 2 mL ethanol solution of DPPH (180 μM) were incubated for 30 min at 25 °C. Then, the absorbance of the remained DPPH radical was measured at 517 nm against a blank. In the blank, samples were substituted with distilled water. Meanwhile, in the control, DPPH was substituted with ethanol. Three replicates for each sample concentration were tested and the scavenging effect was obtained according to the following equation:

Scavenging effect(%) = \[\left(1 - \frac{A_{\text{sample} \, 517 \, \text{nm}} - A_{\text{control} \, 517 \, \text{nm}}}{A_{\text{blank} \, 517 \, \text{nm}}}\right) \times 100\]

where $A_{\text{control} \, 517 \, \text{nm}}$ is the absorbance of the control and $A_{\text{blank} \, 517 \, \text{nm}}$ is the absorbance of the blank.

2.4.3. Superoxide-radical scavenging ability assay

The superoxide-radical scavenging ability was assessed following the model of Ren’s methods with minor modification [34]. The reaction mixture, a total volume 3 mL, involving testing samples of starch or starch derivatives (5 mg/mL, 0.06, 0.12, 0.24, 0.48, and 0.96 mL), phenazine mothsulfate (PMS, 30 μM), nicotinamide adenine dinucleotide reduced (NADH, 338 μM), and nitro blue tetrazolium (NBT, 72 μM) in Tris–HCl buffer (16 mM, pH 8.0), was incubated at 25 °C for 5 min. The absorbance was read at 560 nm against a blank. In the blank, samples were substituted with distilled water. Meanwhile, in the control, NADH was substituted with distilled water. Three replicates for each sample concentration were tested and the capability of scavenging superoxide-radical was calculated using the following equation:

Scavenging effect(%) = \[\left(1 - \frac{A_{\text{sample} \, 560 \, \text{nm}} - A_{\text{control} \, 560 \, \text{nm}}}{A_{\text{blank} \, 560 \, \text{nm}}}\right) \times 100\]

where $A_{\text{control} \, 560 \, \text{nm}}$ is the absorbance of the control and $A_{\text{blank} \, 560 \, \text{nm}}$ is the absorbance of the blank.

2.5. Statistical analysis

All data were expressed as means ± SD. Data were analyzed by an analysis of variance (P < 0.05) to guarantee statistical significance and the means were separated by Duncan’s multiple range test. The results were processed by the computer programs: Origin and Statistic software SPSS.

3. Results and discussion

3.1. Chemical synthesis and characterization

Each step of the synthesis was followed by FTIR, $^1$H NMR, and $^{13}$C NMR spectroscopy. The FTIR, $^1$H NMR, and $^{13}$C NMR spectra of starch, 6-bromo-6-deoxy starch, 6-azido-6-deoxy starch, HMTST, HETST, HPTST, and HBTST were shown in Figs. 1–3 respectively.

In Fig. 1, the FTIR spectrum of starch showed that saccharide contained the following characteristic bands: ν(O–H)
was synthesized at 991.23 ppm. The peaks appeared at approximately 3428.81 and 1427.07 cm\(^{-1}\), indicating the presence of the hydroxyl groups of starch. The bands at 2927.41 and 1650.77 cm\(^{-1}\) could be attributed to the C-H stretching vibration and associated with water bending vibration. The spectrum also showed C-H deformation at 1373.07 cm\(^{-1}\). In the fingerprint region (1200–990 cm\(^{-1}\)), three characteristic peaks appeared at 1160.94, 1087.66, and 991.23 cm\(^{-1}\), respectively. The bands at 1160.94 and 1087.66 cm\(^{-1}\) were attributed to the stretching vibrations of the anhydroglucose ring C-O stretch. The peak at 991.23 cm\(^{-1}\) was most likely attributed to C-H stretch of the C-O-C in starch and indicated the presence of the 1,1,6 linkage [11]. For 6-bromo-6-deoxy starch, the new peak at 543.83 cm\(^{-1}\) was assigned to C-Br group [32]. As far as the FTIR spectrum of 6-azido-6-deoxy starch, the reaction of NaN\(_3\) with 6-bromo-6-deoxy starch led to new strong peak at 2105.89 cm\(^{-1}\), which could be attributed to azide group [30,31]. This characteristic peak could indicate the 6-azido-6-deoxy starch was synthesized successfully. After the click reaction with terminal alkynes, the absorbance of azide group at 2105.89 cm\(^{-1}\) disappeared completely and new peaks appeared at 1554.34–1562.06 cm\(^{-1}\) [30], and 1430.92–1434.78 cm\(^{-1}\) which were assigned to the absorbance of C6-1,2,3-triazoles and methylenes in the spectra of HMTST, HETST, HPTST, and HBTST.

Fig. 2 presented the \(^1\)H NMR spectra of starch and the synthesized starch derivatives. It was known that all of the signals at 3.0–5.7 ppm were assigned to the protons of starch. New signals appeared at 7.6–7.8 ppm (proton of 1,2,3-triazole) [19], 3.85–3.95 ppm (proton of C6–CH\(_2\)–), and 2.09 ppm (proton of alcoholic hydroxyl group) in HMTST, HETST, HPTST, and HBTST. Besides, the new chemical shift at 4.3 ppm (in HMTST), 4.6, 2.7 ppm (in HETST), 4.4, 2.4, and 1.6 ppm (in HPTST), 4.4, 2.3, 1.47, and 1.40 ppm (in HBTST) were assigned to the rest of methylenes. Moreover, the structures of the synthesized products were further demonstrated by \(^13\)C NMR spectra (Fig. 3). The signals between 60 ppm and 100 ppm were assigned to the chemical shift of \(^13\)C NMR of starch. After reacting with NBS and Ph\(_3\)P, new signal appeared at about 34.8 ppm in highly regioselective 6-bromo-6-deoxy starch, which was related to the carbon of C6–Br [30]. Besides, small peaks at 20, 56, and 162 ppm indicated the presence of a low DS of acetate ester groups attached to C6–OH of starch, which could also be found clearly in the FTIR and \(^1\)H NMR of 6-bromo-6-deoxy starch. The reasonable interpretation was that during the SN\(_2\) reaction with starch alkoxyphosphonium salt intermediate, the acetate group was a product of the DMF solvent sometimes acting as a nucleophile instead of bromide [32]. Fortunately, these disturbances were disappeared absolutely after proceeding to next step. Moreover, a new characteristic peak at 51 ppm appeared comparing with the spectrum of starch, which was assigned to the carbon of C6–N\(_3\) [30,35]. After ‘click reaction’, new chemical shift at 140–148 and 123–130 ppm of carbons of 1,2,3-triazoles appeared [30,35]. In addition, new peaks of the rest of methylenes of HMTST, HETST, HPTST, and HBTST were appeared below 40 ppm, respectively. All of those spectra indicated the successful synthesis of starch derivatives.

3.2. Solubility and antioxidant activity

HMTST, HETST, HPTST, and HBTST had favorable water solubility under the tested concentration (0.1–1.6 mg/mL) at room temperature probably due to triazole as the hydrophilic moiety. The large dipole moment of 1,2,3-triazole could make it functionalized as a weak hydrogen bond donor. Moreover, N-2 and N-3 acted as good H-bond acceptors, which could favor in improving water solubility [29].

The elemental analyses, yields, and the degrees of substitution of starch derivatives were shown in Table 1. The DS of starch derivatives were virtually identical. Meanwhile, generated by the
reaction of Fe-EDTA complex with H₂O₂ in the phosphate buffer, hydroxyl radicals are harmful to body through reacting with such biological molecule as amino acid and DNA. Fig. 4 exhibits the hydroxyl-radical scavenging ability of starch and derivatives synthesized at various concentrations. According to the graph we could conclude the results as follows: firstly, the hydroxyl-radical scavenging effect of samples enhanced generally with increasing concentration. Secondly, all of the synthesized products had better ability of scavenging hydroxyl-radical compared with starch. This was possible due to the presence of hydroxyl group more exposed to the outside through a direct route on the triazole ring at the C4 position. Thirdly, of all the tested samples, the longer molecular chain contained hydroxyl group that products possessed, the stronger that their scavenging ability against hydroxyl-radical was. The scavenging property of starch and the aimed products against DPPH-radical was shown in Fig. 5. The results were similar to those of scavenging against hydroxyl-radicals. These products also possessed remarkable antioxidant activity, compared with starch. Meanwhile, the scavenging activity against DPPH-radical decreased in the order: HBTST > HPTST > HETST > HMTST > starch. The superoxide-radical scavenging ability of the obtained derivatives was shown in Fig. 6. They had conspicuous antioxidant activity compared with starch. Moreover, the scavenging effect of the obtained products against superoxide-radical attained 90% above at minimum concentration tested (0.1 mg/mL). Based on the results mentioned above, the scavenging effects of the products against hydroxyl-radical, DPPH-radical, and superoxide-radical were significant enhanced at tested concentrations compared with starch. Generally, the antioxidant activity decreased in

![Fig. 3. ¹³C NMR spectra of starch and starch derivatives.](image)

![Fig. 4. Hydroxyl-radical scavenging ability of starch and starch derivatives.](image)

Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yields (%)</th>
<th>Elemental analysis (%)</th>
<th>Degrees of substitution (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>N</td>
</tr>
<tr>
<td>HMTST</td>
<td>94.57</td>
<td>39.76</td>
<td>13.48</td>
</tr>
<tr>
<td>HETST</td>
<td>92.08</td>
<td>42.20</td>
<td>13.03</td>
</tr>
<tr>
<td>HPTST</td>
<td>89.56</td>
<td>43.27</td>
<td>12.12</td>
</tr>
<tr>
<td>HBTST</td>
<td>86.34</td>
<td>45.08</td>
<td>11.62</td>
</tr>
</tbody>
</table>

<sup>a</sup> Degrees of substitution refer to the C6-OH substitution degree of 1,2,3-triazole in starch derivatives.
reaction' would be beneficial to enhance the antioxidant activity of starch. Moreover, it would be reasonable to presume that the electron-donating ability of different substituted groups in the 1,2,3-triazole groups could influence the antioxidant property of these starch derivatives. The substituted groups with stronger electron supplying capacity enhanced the antioxidant activity of the starch-linked-1,2,3-triazole derivatives. Based on the results mentioned above, significant scavenging effects against hydroxyl-radical, DPPH-radical, and superoxide-radical are evident at tested concentration of the products, which suggest the potential of the products to be developed as antioxidants for human consumption. However, comprehensive studies need to be carried out to ascertain the safety of starch derivatives in experimental animal models.

Acknowledgements

We thank the Science and Technology Service Network Initiative (KJF-EW-STS-060), the National Natural Science Foundation of China (41206152), the Science and Technology Project of Wei hai (2012GN5004), and the Taishan Scholar Program of Shandong Province for financial support of this work.

References


