Synthesis, characterization, and antioxidant properties of novel inulin derivatives with amino-pyridine group

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Abstract

A series of novel inulin derivatives were synthesized via reaction of chloracetyl inulin (CAIL) with amino-pyridines, including 2-(3-amino-pyridyl)acetyl inulin chloride (2APAIL), 2-(3-amino-pyridyl)acetyl inulin chloride (3APAIL), 2-(4-amino-pyridyl)acetyl inulin chloride (4APAIL), 2-(2,3-diamino-pyridyl)acetyl inulin chloride (23DAPAIL), and 2-(3,4-diamino-pyridyl)acetyl inulin chloride (34DAPAIL). The antioxidant property of the products and 2-pyridylacetyl inulin chloride (PAIL) against hydroxyl radicals (•OH), superoxide radicals (O2•−), and DPPH radicals (DPPH*) were evaluated in vitro, respectively. Results showed that 4APAIL and 34DAPAIL exhibited remarkable improvement on scavenging •OH and DPPH*, which can scavenge the radical of •OH completely at 0.4 mg/mL. Besides, the scavenging activity of 23DAPAIL to O2•− was excellent among all of the tested samples, reaching 85% at 1.6 mg/mL. These data indicate that all of the inulin derivatives have better antioxidant activities than inulin, and the scavenging effect indices are affected by the number and position of the amino group on pyridine grafted to the inulin derivatives.

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1. Introduction

Oxidation, caused by reactive oxygen species (ROS), is a pervasive biological process in physiology and metabolism of many organisms [1]. ROS are normally generated in the human body and scavenged by antioxidant defenses system when ROS remains at physiological concentrations [2]. It is essential to preserve the endogenous antioxidant defense systems and normal cell functions when ROS remains at physiological concentrations. Therefore, the body can have the capacity to avoid many harmful damages [3]. However, these systems are insufficient to prevent the harm entirely [4]. It is reported that free radicals, including superoxide anion, hydroxyl radical, and hydrogen peroxide can cause pathological damages like cancer disease, diabetes, atherosclerosis, coronary heart disease, and many other diseases associated with aging to the organism, and lead to harmful alterations in foods and pharmaceutical industries [5–7]. Therefore, it is urgent to develop antioxidant supplements to help the human body reduce oxidative scratch.

Inulin is a natural, biodegradable, and plant-derived storage polysaccharide [8], isolated mainly from low requirement crops such as the tubers of Helianthus tuberosus (Jerusalem artichoke), Cichorium intybus (chicory), and Polymnia sonchifolia (yacon) [9]. The structural framework of this linear polysaccharide consists primarily, if not exclusively of β (2 → 1) fructosyl fructose units, usually with one glucopyranose unit at the reducing end [10]. Inulin exhibits some interesting properties such as beneficial nutritional attributes for human health, moderate average degree of polymerization, and readiness of being obtained [11,12]. These characteristics make it possible to be widely applied in food, feed, biofuel, water purification, and pharmaceutical industries, which indicate that inulin has become a promising candidate to satisfy the rising demand for renewable and environmental-friendly polymeric material. It is generally accepted that biological activities of polysaccharides related with its molecular structure including monosaccharide composition, glycosidic bond of the main chain, sugar component, and conformation of the main chains. Therefore, increasing attention has been attracted to structure–activity relationship of polysaccharides. In recent years, many studies concerning on the chemical modification of polysaccharides have been published [13,14]. Our previous work also reported the
antioxidant activities and antifungal activities of inulin derivatives, which proved the chemical modification could improve the bioactivity of inulin [15–17].

Pyridine and its derivatives are known to possess various pharmacological applications such as antibacterial, antitumor, antiparasite, and analgesic activity [18–21]. It is reported that most of the 3-amino conjugates containing pyridine groups had good activity in vitro [22]. Meanwhile, evidence has suggested that saccharide derivatives containing amino groups were more excellent than natural saccharide as a scavenger of hydroxyl radicals [23]. Moreover, many reports have proposed that appropriate macromolecular systems, namely antioxidant-polymer conjugates, could combine the merits of several components, which retains the admirable biological activities of antioxidant molecules, possessing a higher stability and a slower degradation rate than the antioxidant molecules [24–26].

In this paper, we reported the synthesis and antioxidant properties of a series of inulin derivatives with amino-pyridine as substituent including 2APAIL, 3APAIL, 4APAIL, 2,3DAPAIL, and 3,4DAPAIL (Scheme 1). The chloracetyl inulin (CAIL) was first synthesized by reaction between the C-6 hydroxyl of inulin and chloracetyl chloride. CAIL is an excellent intermediate of the project as the chlorine of CAIL can easily attack pyridine to give N-alkylpyridinium salts [15,27,28]. Subsequently the pyridine containing one or two amino groups was grafted into inulin through the reaction mentioned above. The inulin derivatives modified in this way were expected to have advantages such as high antioxidant activity and good water solubility. The chemical structures of the derivatives were characterized by FT-IR, 13C NMR, and 1H NMR. The antioxidant activities of inulin and the synthesized inulin derivatives were evaluated in vitro, and the relationship between the structure and the antioxidant activity of inulin was discussed.

2. Experimental

2.1. Materials

Inulin was purchased from E. Merck (Darmstadt, Germany). Its average degree of polymerization is around 20 fructose fructose units. 2-Aminopyridine, 3-aminopyridine, 4-aminopyridine, 2,3-diaminopyridine, and 3,4-diaminopyridine were purchased from the Sigma–Aldrich Chemical Corp. The other reagents are all of analytical grade and used without further purification.

2.2. Analytical methods

FT-IR spectra were measured on a Jasco-4100 Fourier Transform Infrared 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 (Switzerland, provided by Bruker Tech. and Serv. Co., Ltd Beijing, China). The UV–vis absorbance of the tested mixture was measured with a Puxi-TU1810 UV spectrometer (China, provided by P General Co., Ltd., Beijing, China).

2.3. Synthesis

2.3.1. Preparation of CAIL

CAIL was synthesized as follows [29]: 1.61 g (10 mmol) inulin was dissolved in 100 mL H2O at room temperature (r.t.), and 20 mmol chloracetyl chloride was added drop wise. After stirring for 12 h at r.t., the solution was concentrated under reduced pressure. The concentrated solution was precipitated by the addition of excess aceton and the precipitate was filtered. The products were washed with ether and dried at 60 °C for 24 h, yield: 54%.

2.3.2. Synthesis of inulin derivatives

A solution of CAIL (2 mmol) and pyridine or amino-pyridines (6 mmol) in 15 mL N,N-dimethylformamide was stirred for 24 h at 60 °C. The solutions were precipitated with excess acetic acid and filtered, washed carefully with acetone. The unreacted amino-pyridines and other byproducts were extracted in a Soxhlet apparatus with acetone for two days. The products were obtained by freeze drying [15], yield: 80–90%.

2.4. The investigation of the antioxidant ability

2.4.1. Hydroxyl-radical scavenging ability assay

The test of the hydroxyl-radical scavenging ability was carried out according to Ren’s methods with minor modification [30]. The reaction mixture, a total volume 4.5 mL, containing the samples of inulin or inulin derivatives (CAIL, PAIL, 2APAIL, 3APAIL, 4APAIL, 2,3DAPAIL, and 3,4DAPAIL), were incubated with EDTA-Fe2+ (220 μM), safranine O (0.23 μM), and H2O2 (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. Three replicates for each sample concentration were tested. The capability of scavenging hydroxyl radicals of the product was computed using the following equation:

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

where \( A_{\text{blank}520\text{nm}} \) is the absorbance of the blank (distilled water instead of the samples) and \( A_{\text{control}520\text{nm}} \) is the absorbance of the control (distilled water instead of the H2O2).

2.4.2. Superoxide-radical scavenging ability assay

The superoxide radical scavenging ability was assessed following the model of Xing’s methods with minor modification [31]. Involving testing samples of inulin or inulin derivatives (CAIL, PAIL, 2APAIL, 3APAIL, 4APAIL, 2,3DAPAIL, and 3,4DAPAIL), 30 μM phenazine methosulfate (PMS), 338 μM nicotinamide adenine dinucleotide reduced (NADH), and 72 μM nitro blue tetrazolium (NBT) in Tris–HCl buffer (16 mM, pH 8.0), the reaction mixture was incubated at 25 °C for 5 min. The absorbance was read at 560 nm against a blank. Three replicates for each sample concentration were tested and the capability of scavenging superoxide radical was calculated using the following equation:

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

where \( A_{\text{control}560\text{nm}} \) is the absorbance of the control (distilled water instead of NADH for each concentration) and \( A_{\text{blank}560\text{nm}} \) is the absorbance of the blank (distilled water instead of the samples).

2.4.3. DPPH-radical scavenging ability assay

The DPPH* scavenging properties of the products were evaluated by the following method: testing samples and 2 mL ethanol solution of DPPH (180 μmol/L) was incubated for 30 min at 25°C. Then, the absorbance of the remained DPPH radical was measured at 517 nm against a blank. Three replicates for each sample concentration were tested and the scavenging effect was obtained according to the following equation:

\[
\text{Scavenging effect} (\%) = \left[ 1 - \frac{A_{\text{sample}517\text{nm}} - A_{\text{control}517\text{nm}}}{A_{\text{blank}517\text{nm}}} \right] \times 100%
\]
where $A_{\text{control}517\text{nm}}$ is the absorbance of the control (ethanol instead of DPPH for each concentration) and $A_{\text{blank}517\text{nm}}$ is the absorbance of the blank (distilled water instead of the samples).

2.5. Statistical analysis

All data were expressed as means ± SD. The Scheffe method was used to evaluate the differences in antioxidant index in the antioxidant assays. Results with $P<0.05$ were considered statistically significant [32].

3. Results and discussion

3.1. Structure of the inulin derivatives

Each step of the synthesis was followed by FT-IR, $^{13}$C NMR, and $^1$H NMR spectroscopy. The FT-IR, $^{13}$C NMR, and $^1$H NMR spectra of inulin, CAIL, PAIL, 2APAIL, 3APAIL, 4APAIL, 2,3DAPAIL, and 3,4DAPAIL are shown in Figs. 1–3 respectively.

In the spectrum of unmodified inulin, peaks of saccharide is about at 852 cm$^{-1}$, 1029 cm$^{-1}$, and 3041 cm$^{-1}$ [17], and peak at 1029 cm$^{-1}$ is attributed to C–O stretching of –CH$_2$–OH. The peak at 1145 cm$^{-1}$ is the asymmetric bridge oxygen (C–O–C) stretching [33,34]. Peaks at 1361 cm$^{-1}$ and 1319 cm$^{-1}$ may be assigned to O–H deformation of –CH$_2$–OH and –CH–OH [33,34]. For CAIL, the reaction of chloracetyl chloride with inulin leads to a new peak at 1747 cm$^{-1}$, which can be attributed to the carbonyl (C=O) [15]. The characteristic peak of secondary hydroxyl groups (–CH–OH) is still at 1319 cm$^{-1}$, and the peak of O–H deformation of –CH$_2$–OH at 1361 cm$^{-1}$ almost disappears. It seems that the primary hydroxyl groups are involved in the substitution reaction instead of secondary hydroxyl groups. Meanwhile, another new peak at 782 cm$^{-1}$ is assigned to the C–Cl group [15]. All of these characteristic peaks indicate that CAIL is synthesized successfully. After the addition of pyridine, the absorbance of C–Cl at 782 cm$^{-1}$ disappeared and new peaks appear at 771, 1465, and 1542 cm$^{-1}$ which are assigned to the absorbance of pyridine in the spectrum of PAIL [29]. As for the spectra of 2APAIL, 3APAIL, 4APAIL, 2,3DAPAIL, and 3,4DAPAIL, new peak at about 1658 cm$^{-1}$ may be assigned to the carbonyl (C=O), and peaks at about 860, 1427, 1515 cm$^{-1}$ may be attributed to the pyridine ring of amino-pyridine, and another prominent peak in the range of 1577–1596 cm$^{-1}$ belongs to the amino group.

Fig. 2 presents the $^1$H NMR spectra of inulin and the synthesized inulin derivatives. It is known that all of the signals at 3.0–5.4 ppm are assigned to the protons of inulin [17], which makes the signal of the proton of amino groups in amino-pyridines been covered. After reacting with chloracetyl chloride, new signals appear at 1.1–1.3 ppm in CAIL, which are related to the proton of –CH$_2$–Cl [15,29]. New signals appear at 7.9–8.9 ppm (proton of pyridine) in PAIL, and corresponding signals of the proton of pyridine move to high field at about 6.8–8.5 ppm in 2APAIL, 3APAIL, 4APAIL, 2,3DAPAIL and 3,4DAPAIL, which should be attributed to the conjugate structure. And this was further proved by the $^{13}$C NMR spectra of inulin and the synthesized inulin derivatives (Fig. 3). The chemical shifts of $^{13}$C NMR of inulin are all above 60.1 ppm, which is consistent with the Ren's report [17]. In $^{13}$C NMR spectrum of CAIL, new signals appear at about 164.5 ppm (C=O of ester). After conjugating with pyridine or amino-pyridines, new chemical shifts at 127.4–145.8 ppm of pyridine ring carbons appear [27,35]. Meanwhile, the signals of C=O of ester still exist at 162.5–168.2 ppm and the chemical shifts of pyranose rings are still at 60.1–107.8 ppm [27]. All of those spectra indicate the successful synthesis of inulin derivatives.

![Fig. 1. FTIR spectra of inulin and inulin derivatives.](image)

![Scheme 1. Synthetic pathway of inulin derivatives.](image)
Fig. 2. $^1$H NMR spectra of inulin and inulin derivatives.

Fig. 3. $^{13}$C NMR spectra of inulin and inulin derivatives.
3.2. Antioxidant activities

Hydroxyl radicals, generated by reaction of Fe-EDTA complex with \( \text{H}_2\text{O}_2 \) in phosphate buffer, are harmful to the body through reacting with biological molecule such as amino acid or DNA. Fig. 4 reveals the *OH scavenging activity of inulin and the synthesized inulin derivatives at various concentrations. According to the data shown in Fig. 4, we can conclude as follows. Firstly, all the scavenging effects of samples have a positive correlation with tested concentrations. Secondly, all of the aimed products have an evident inhibitory against hydroxyl radicals compared with inulin, CAIL, and PAIL. This is possible due to the introducing of pyridine containing amino groups, which is in accord with the conclusion that aminated derivatives of saccharide are more potent than natural saccharide as a scavenger of hydroxyl radicals [23]. Thirdly, of all the tested samples, 4APAIL and 3,4DAPAIL exhibited remarkable improvement on scavenging *OH and DPPH*, which can scavenge the radical of *OH completely at 0.4 mg/mL. Finally, most of the derivatives containing two amino groups have a better scavenging effect than that of derivatives with one amino group. The scavenging property of inulin and aimed products against DPPH* radicals is shown in Fig. 5. The result is similar to that of scavenging against *OH radicals. These products also possess a remarkable antioxidant activity, compared with inulin, CAIL, and PAIL. Meanwhile, 4APAIL and 3,4DAPAIL have the best inhibitory activity against DPPH* radicals, and the scavenging indices are all higher than 80% at 0.4 mg/mL. It would be reasonable to presume that the amino group at the C-4 position of pyridine ring should be an important factor that influences the scavenging activity against hydroxyl radicals and DPPH radicals. Besides, the enhanced scavenging capability against hydroxyl radicals and DPPH radicals may be affected by the number of amino groups of pyridine ring. As shown in Fig. 6, the superoxide-radical scavenging ability of the obtained derivatives, differs from the scavenging properties against hydroxyl radicals and DPPH radicals (Fig. 5). It is 2,3DAPAIL that has the best enhanced scavenging activity, reaching 85% at 1.6 mg/mL. The results further confirm that the number of amino groups could influence the antioxidant activity of inulin derivatives. Based on the results mentioned above, significant scavenging effect against hydroxyl radicals and DPPH radicals are evident at tested concentrations of the products, which suggests the potential of the products to be developed as hydroxyl radicals and DPPH radicals scavenging reagents for human consumption.

4. Conclusion

In summary, a group of antioxidant-polymer products including six inulin derivatives were synthesized via a convenient reaction between CAIL and pyridine or amino-pyridines with high yield. Their antioxidant activities against three kinds of radicals were evaluated in vitro. All of the inulin derivatives have good solubility in water, and exhibit higher antioxidant indices than inulin. These data indicate that the chemical modification of inulin with amino-pyridines would be beneficial to enhance the antioxidant activity of inulin. Moreover, it would be reasonable to presume that the number and the position of amino group on pyridine could influence the antioxidant property of these inulin derivatives. Further comprehensive investigation to ascertain this hypothesis on antioxidant and structure–activity relationships will be carried out.

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