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

Yunxia Hu[a,b], Jun Zhang[c], Chunwei Yu[c], Qing Li[a], Fang Dong[a], Gang Wang[a], Zhanyong Guo[a,*]

[a] Key Laboratory of Coastal Biology and Bioresource Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Laboratory of Environmental Monitoring, School of Tropical and Laboratory Medicine, Hainan Medical College, Haikou 571199, China

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ABSTRACT

A series of novel inulin derivatives were synthesized *via* reaction of chloracetyl inulin (**CAIL**) with amino-pyridines, including 2-(2-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 (**2,3DAPAIL**), and 2-(3,4-diamino-pyridyl)acetyl inulin (**3,4DAPAIL**). The antioxidant property of the products and 2-pyridylacetyl inulin chloride (**PAIL**) against hydroxyl radicals (•OH), superoxide radicals (O_2•), and DPPH radicals (DPPH•) were evaluated *in vitro*, respectively. Results showed that **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. Besides, the scavenging activity of **2,3DAPAIL** to O_2• 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.

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

* Corresponding author. Tel.: +86 535 2109171; fax: +86 535 2109000.
E-mail address: zhanyongguo@hotmail.com (Z. Guo).

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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-pyridines 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*-alkypyridinium 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, ^{13}C NMR, and ^{1}H 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 fructosyl 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. ^{13}C Nuclear magnetic resonance (^{13}C NMR) and ^{1}H Nuclear magnetic resonance (^{1}H 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 H_2O 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 acetone 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 acetone 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-Fe^{2+} (220 μM), safranine O (0.23 μM), and H_2O_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. 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 H_2O_2).

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 mothosulfate (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:

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