Synthesis of inulin derivatives with quaternary phosphonium salts and their antifungal activity

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A B S T R A C T

Inulin is a kind of renewable and biodegradable carbohydrate with good water solubility and numerous physiological functions. For further utilization of inulin, chemical modification can be applied to improve its bioactivities. In this paper, five novel inulin derivatives were synthesized via chemical modification with quaternary phosphonium salt. Their antifungal activity against three kinds of plant pathogens including Colletotrichum lagenarium, Phomopsis asparagi, and Fusarium oxysporum was assessed with radial growth assay in vitro. Results revealed that all the inulin derivatives exhibited improved antifungal activity compared with inulin. Particularly, inulin modified with triphenylphosphine (TPHAIL) exhibited the best antifungal activity with inhibitory indices of 80.0%, 78.8%, and 87.4% against Colletotrichum lagenarium, Phomopsis asparagi, and Fusarium oxysporum at 1.0 mg/mL respectively. The results clearly showed that chemical modification of inulin with quaternary phosphonium salt could efficiently improve derivatives’ antifungal activity. Further analysis of results indicated that the antifungal activity was influenced by alkyl chain length or electron-withdrawing ability of the grafted quaternary phosphonium salts. Longer alkyl chain lengths or the stronger electron-withdrawing groups would lead to enhanced antifungal efficacy.

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1. Introduction

In the last few decades, a number of natural polysaccharides have been developed as biomaterials to meet an urgent need of sustainable development on account of their renewable and biodegradable properties [1–3]. Polysaccharides such as chitosan and starch are natural, non-toxic, cheap, quite abundant, and economically important biomaterials. They are amenable to be modified on purpose which makes them especially attractive biomaterials to produce value-added materials in such fields as drug delivery, tissue engineering scaffolds, biomedical devices, and waste water treatment [4–11]. Like these polysaccharides, inulin, being a kind of carbohydrate storage reserve for plants, is widely distributed in >36,000 plant species. Commercially it is extracted primarily from chicory, dahlia, and Jerusalem artichoke [12]. It is a type of fructose polymer linked by β(2→1) linkages of α-fructosyl fructose and commonly terminated by a glucose residue [13]. This fantastic structure can prevent inulin from being digested like a typical carbohydrate in the upper gastrointestinal tract [14]. Therefore, inulin generates low caloric value and is generally used as a fat or sugar replacer in food industry [15,16]. Furthermore, inulin shows a prebiotic effect as it stimulates the proliferation of bifidobacteria in the colon [17–19]. Inulin also exerts favorable properties in decreasing the risk of many diseases of the intestinal tract, particularly irritable bowel diseases (IBD) and colon cancer [19]. Based on these properties, inulin is widely utilized as an additive in many products such as functional foods, health care products, and some natural medicine [20–23]. However, its weak antimicrobial activity limits its further utilization. Because biological activities of polysaccharides are related with their molecular structure, we may improve inulin antimicrobial activity by chemical modification, which can introduce wide range of functional groups to its framework.

Among all the polysaccharide derivatives, cationic carbohydrate polymers such as quaternary ammonium salts and quaternary phosphonium salts have been the most popular and most efficient target molecules in medical applications [24] and water treatment [25]. Recently, quaternary phosphonium salts were reported to be the better antimicrobial agents compared with quaternary ammonium salts with the same structure except cationic part [26,27].

Quaternary phosphonium salt is a new generation of efficient, broad-spectrum antiseptic. It has been extensively studied as an active group for preparing antibacterial agent [28–30]. However, these small molecule antiseptics usually suffer from toxicity and short effective...
duration. Meanwhile, the rising incidence of drug resistant pathogens emphasizes the urgent need for new approaches of antifungal agent. Antifungal polymers represent a novel direction for the development of novel antifungal agent with advantages of high efficiency, low toxicity, and slow-release. Qiu et al. immobilized quaternary phosphonium salt onto chlorinated natural rubber (CNR) to prepare polymeric quaternary phosphonium salt bactericides and all the products exhibited excellent antibacterial activity against both Escherichia coli and Staphylococcus aureus [31]. Song et al. grafted triphenyl phosphonium onto starch and the starch derivative also exhibited high antibacterial activity [32]. Moreover, quaternary phosphonium salt has been grafted onto chitosan as a strategy to improve water solubility of chitosan as well as antibacterial ability [33–36]. However, quaternary phosphonium salt grafted onto inulin is infrequent in literature, until now. It is reasonable to assume that the modification of inulin by quaternary phosphonium salts can help to enhance biological activity and application value of inulin.

As a cheap, environmentally benign, biodegradable, and biocompatible renewable bioresources, the utilization of inulin was insufficient. One of the drawbacks may be a lack of active groups such as amino, carboxyl and sulfate ester. Chemical modification could be a powerful way to introduce new functional groups onto inulin. This study aimed to investigate the structure-activity relationships of inulin derivatives modified with various trialkylphosphonium salts and triphenylphosphonium salts. In this paper, we reported the synthesis of a series of inulin derivatives with quaternary phosphonium salt as substituent including 2-(P, P-trimethyl-phosphine) acetyl inulin chloride (TMPAIL), 2-(P,P,P-tripropyl-phosphine) acetyl inulin chloride (TPPAIL), 2-(P,P,P,tributyl-phosphine) acetyl inulin chloride (TBPAIL), 2-(P,P,P-tricyclohexyl-phosphine) acetyl inulin chloride (TCHPAIL), and 2-(P,P,P-triphenyl-phosphine) acetyl inulin chloride (TPPhAIL) (Scheme 1). Firstly we synthesized the chloroacetyl inulin (CAIL) according to acylation reaction between the C-6 hydroxyl of inulin and chloroacetyl chloride. We have reported that CAIL is an excellent intermediate of the reaction as the chlorine of CAIL can be easily attacked by some nucleophilic reagent [37]. Subsequently, trimethylphosphine, tripropylphosphine, tributylphosphine, tricyclohexylphosphine, or triphenylphosphine was grafted onto inulin respectively through the reaction mentioned above. The synthesized inulin derivatives were selected by virtue of antifungal activity. The inulin derivatives modified in this way were expected to possess advantages such as high antifungal activity and good water solubility. The chemical structures of the derivatives were characterized by FTIR, 1H NMR, and 13C NMR spectra. The antifungal activity of inulin and all the synthesized inulin derivatives were evaluated by mycelium growth rate method in vitro. Furthermore, the relationship between the structure and their antifungal activity of inulin and its derivatives were studied.

2. Experimental

2.1. Material

Inulin was purchased from Xi’an Haoyuan Biological Technology Co., Ltd.. Trimethylphosphine, tripropylphosphine, tributylphosphine, tricyclohexylphosphine, and triphenylphosphine were purchased from the Aladdin Industrial Corporation. The other reagents are all analytical grade and used without further purification.

FTIR spectra were recorded on a Jasco-4100 Fourier Transform Infrared Spectrometer (Japan, provided by JASCO Co., Ltd Shanghai, China) at 25 °C with KBr disks.

Nuclear magnetic resonance spectra (1H NMR and 13C NMR) were measured with a Bruker AVIII-500 Spectrometer (Switzerland, provided by Bruker Tech. and Serv. Co., Ltd. Beijing, China) at 25 °C with tetramethylsilane (TMS) as internal standard on ppm scale (δ).

2.2. Synthesis

2.2.1. Synthesis of chloroacetyl inulin (CAIL) [37]

CAIL was synthesized as follows: 1.62 g (10 mmol) of inulin was dissolved in 100 mL of H2O at room temperature, after that 30 mmol of chloroacetyl chloride was added dropwise. After stirring for 12 h at room temperature, the solution was concentrated under reduced pressure. When the system was cooled to room temperature, excess diethyl ether was added to the flask to precipitate the concentrated solution, and the precipitate was filtered. The products were washed with diethyl-ether for three times and freeze dried overnight in vacuum, yield: 92% (Scheme 1).

2.2.2. Synthesis of inulin derivatives (TMPAIL)

A solution of CAIL (2 mmol) and trimethylphosphine (6 mmol) in 20 mL of N,N-dimethylformamide (DMF) was refluxed at 60 °C for 24 h. The solutions were precipitated with excess diethyl-ether, filtered, washed carefully with diethyl-ether. The product was freeze dried overnight in vacuum, yield: 46% (Scheme 1).

2.2.3. Synthesis of inulin derivatives (TPPAIL, TBPAIL, TCHPAIL, and TPPhAIL)

A solution of CAIL (2 mmol) and tripropylphosphine (6 mmol), tributylphosphine (6 mmol), tricyclohexylphosphine (6 mmol), or triphenylphosphine (6 mmol) in 20 mL of DMF was stirred at 60°C for 24 h. The solutions were precipitated with excess acetone and filtered, washed carefully with acetone and diethyl-ether. The product was obtained by freeze drying, yield: 54%–62% (Scheme 1).
2.3. Antifungal ability assay

Antifungal ability was evaluated against three plants-threatening fungi including *Colletotrichum lagenarium* (Pass) Ell.et halst (*C. lagenarium*), *Phomopsis asparagi* (Sacc.) Bubak (*P. asparagi*), and *Fusarium oxysporum f.sp.niveum* (*F. oxysporum*), which were provided by Qingdao Academy of Agricultural Sciences. Antifungal assay was carried out according to Guo's methods [37]. Briefly, the compounds were dissolved in distilled water respectively at a concentration of 10 mg/mL. Then, each sample (inulin and inulin derivatives) solution was added to sterilized fungi agar medium to give a final concentration at 0.1, 0.5, and 1.0 mg/mL. After the mixture was cooled and solidification, 5.0 mm diameter of fungi was transferred to the test plate and incubated at 27 °C for 2–3 days. Blank control plate was prepared using identical volume of distilled water substituted samples. When the mycelium of fungi reached the edges on control plate, the antifungal index was calculated as follows:

\[
\text{Antifungal index} (\%) = \frac{1 - D_a}{D_b} \times 100
\]

where \(D_a\) is the diameter (mm) of the growth zone in the test plates and \(D_b\) is the diameter (mm) of the growth zone in the blank control plate. Each experiment was performed three times, and the data were reported with mean ± SD. Results with \(P < 0.05\) were considered statistically significant [38].

3. Results and discussion

3.1. Structure characterization

The synthesized inulin derivatives were characterized by FTIR (Fig. 1), \(^1^H\) NMR (Fig. 2), and \(^1^3^C\) NMR (Fig. 3).

3.1.1. FTIR spectroscopy

As shown in Fig. 1, the FTIR spectra of raw material inulin show peaks of saccharide at 848.53 cm\(^{-1}\), 1025.94 cm\(^{-1}\), and 3401.82 cm\(^{-1}\) [39]. The peak at 1025.94 cm\(^{-1}\) was considered as C–O stretching of...
CH$_2$—OH. The peak at 1149.37 cm$^{-1}$ should be the stretching vibrations of the C—O—C stretch in inulin [40]. After the reaction of chloroacetyl chloride and inulin, a new peak appears at 1747.19 cm$^{-1}$ apparently, which can be attributed to the characteristic peak of carbonyl (C=O) vibration deformation. Another new peak at 790.67 cm$^{-1}$ was assigned to the CCl group which indicated that CAIL was synthesized successfully [37]. After reacting with trimethylphosphine, the peak of CCl at 790.67 cm$^{-1}$ disappears. Meanwhile, a new peak appears at 1423.21 cm$^{-1}$, which can be assigned to the absorbance of P—C vibration in the spectrum of TMPAIL [31,41]. As for the spectra of TPPAIL, TBPAIL, TCHPAIL, and TPhPAIL, the absorbance of P—CH$_2$ vibration appears at 1440 cm$^{-1}$–1457 cm$^{-1}$. Moreover, the peak of carbonyl (C=O) vibration makes a red shift to 1735.62 cm$^{-1}$.

3.1.2. NMR spectroscopy

To confirm the successful modification of inulin, the nuclear magnetic resonance spectra ($^1$H NMR and $^{13}$C NMR) of the samples were further recorded. For example, in Fig. 2, the signals between 3.0 ppm and 5.4 ppm were assigned to the protons of inulin [42]. After reacting with chloroacetyl chloride, new signals appear at 4.3 ppm in CAIL, which are related to the proton of —CH$_2$Cl. For inulin derivatives, signal at 4.3 ppm is greatly weakened. Meanwhile, new signals appear at about 0.92–2.62 ppm which are related to the protons of —CH$_2$ or —CH$_3$. As to TPhPAIL, the signal between 7.67 and 7.86 ppm belongs to the protons from benzene rings of triphenyl phosphonium [41], which should be attributed to the conjugate structure. And those are further proved by the $^{13}$C NMR spectra of inulin and the synthesized inulin derivatives (Fig. 3). In Fig. 3, the chemical shifts of inulin are all above 60.1 ppm, which is consistent with the Ren's report [42]. In $^{13}$C NMR spectrum of CAIL, new signals appear at about 169.79 ppm (—COO—) and 41.5 ppm (—CH$_2$Cl). After conjugating with trimethylphosphine, tripropylphosphine, tributylphosphine, or tricyclohexylphosphine, new chemical shifts at 12.64–31.45 ppm of —CH$_2$ or —CH$_3$ carbons appear [43]. Meanwhile, the signals of ester group (—COO—) move to 165.0–166.2 ppm and the chemical shifts of pyranose rings are still at 60.1–105.9 ppm. For TPhPAIL, new chemical shifts appear at 130.3–135.6 ppm, which can be attributed to the benzene ring carbons. All of these spectra prove the successful modification of inulin.

3.2. Degree of substitution

The degree of substitution (DS) for inulin derivatives (As shown in Table 1.) was evaluated on the basis of the integral values in the $^{13}$C NMR spectrum [44–47]. The formula to determine DS of inulin derivatives is shown below:

$$DS = \frac{A}{B}$$

where A represents the integration areas of protons in ester group (—COO—) of inulin derivatives, B represents the integration areas of protons at C-2 ($\delta = 98.7$ to 101.1 ppm) of furanose rings.

| Compound | Degree of substitution |
|----------|-----------------------|
| CAIL     | 1.10                  |
| TMPAIL   | 0.85                  |
| TPPAIL   | 0.87                  |
| TBPAIL   | 0.81                  |
| TCHPAIL  | 0.78                  |
| TPhPAIL  | 0.70                  |
3.3. Antifungal activity

The antifungal activity of inulin, CAIL and the target products against C. lagenarium, P. asparagi, and F. oxysporum was determined by mycelium growth rate test according to the literatures [37,42].

As shown in Fig. 4, all the inulin derivatives show inhibitory effect against C. lagenarium and their antifungal indices have a positive correlation with the concentration. The inhibitory index of TPhPAIL reaches 80.0% at 1.0 mg/mL. The inhibitory indices of inulin derivatives against C. lagenarium show an order as follows: TPhPAIL > TBPAIL > TPPAIL > TCHPAIL > TMPAIL > CAIL > inulin. It is valid to find that when taking DS into account, the antifungal activity in inulin is in accord with the alkyl chain length of graft quaternary phosphonium salt. And it would be reasonable to presume that phenyl in quaternary phosphonium salt contributes more to the antifungal activity than alkyl since phenyl has better electron-withdrawing ability. For CAIL, the inhibitory index at 1.0 mg/mL is 10.5%. This is possible due to the introduction of chloroacetyl group, and it has been proved that chlorine grafted to the synthesized inulin derivatives could improve the antifungal activity of them [37].

Figs. 5 and 6 show the antifungal activity of inulin and all the derivatives against P. asparagi and F. oxysporum. All the compounds show antifungal activity against P. asparagi and F. oxysporum except inulin, and the inhibitory indices of them mount up with increasing concentration. The inhibitory indices of CAIL, TMPAIL, TPPAIL, TBPAIL, TCHPAIL, and TPhPAIL against P. asparagi are 16.4%, 9.3%, 18.4%, 35.1%, 48.2%, and 78.8% at 1.0 mg/mL respectively. And the inhibitory indices of CAIL, TMPAIL, TPPAIL, TBPAIL, TCHPAIL, and TPhPAIL against F. oxysporum are 17.7%, 10.1%, 47.5%, 48.6%, 65.1%, and 87.4% at 1.0 mg/mL respectively. Similar to the antifungal activity against C. lagenarium, five novel inulin derivatives exhibited much stronger antifungal activity than unmodified inulin. The results further confirmed that (1) quaternary phosphonium salt groups grafted onto the synthesized inulin derivatives contributed a lot to the antifungal action and consequently increased derivatives’ antifungal activity; (2) chlorine grafted to the synthesized inulin derivative could improve derivative antifungal activity; (3) antifungal activity of the synthesized inulin derivatives increased with an increase in the chain length of alkyl substituent; (4) the antifungal activity should be affected by electron-withdrawing ability of the alkyl substituent.

4. Conclusion

In summary, five novel inulin derivatives modified with quaternary phosphonium salt were designed and synthesized. Their structures were confirmed by FTIR, 1H NMR, and 13C NMR spectra and their antifungal activity against three kinds of phytopathogens were estimated by hypha measurement in vitro. All the inulin derivatives exhibited higher antifungal indices than inulin. These data demonstrated that quaternary phosphonium salt-functionalized inulin derivative could be used as antimicrobial biomaterial potentially. In addition, the antifungal activity of the synthesized inulin derivatives increased with the chain length of alkyl substituent, and the antifungal activity was enhanced by stronger electron-withdrawing ability of the synthesized inulin derivatives.

This paper provides a practical strategy to develop new inulin derivatives bearing quaternary phosphonium salts with high antifungal activity. The products described in this paper may serve as new leading structures for further design of antifungal agents and have the potential to prepare novel antifungal biomaterials.

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