Carbohydrate Polymers 85 (2011) 268–271

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Synthesis and hydroxyl radicals scavenging activity of *N*-(aminoethyl)inulin

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ARTICLE INFO

Article history:
Received 17 December 2010
Received in revised form 11 January 2011
Accepted 20 January 2011
Available online 28 January 2011

Keywords:
N-(Aminoethyl)inulin
Reactive precursor
Antioxidant activity

ABSTRACT

Inulin, a kind of abundant polymeric material, is mainly extracted from such low-requirement crops as Jerusalem artichoke, chicory, and yacon. The objective of this study was to develop a reactive precursor for chemical manipulation of inulin to encourage the employment of this currently underutilized biodegradable and environmentally benign polysaccharide. *N*-(Aminoethyl)inulin was prepared with a 1.0 degree of substitution at C-6. Additionally, its potential hydroxyl radicals scavenging activity was evaluated with chitosan and vitamin C as positive comparison. Better than inulin, chitosan and vitamin C, *N*-(aminoethyl)inulin possesses scavenging activity of 62% at 2.4 mg/mL. The synthesized *N*-(aminoethyl)inulin was characterized by FT-IR spectra, ^{13}C NMR.

1. Introduction

Inulin consists primarily of β-fructosyl fructose units – always presented in furanose form – usually with a glucopyranose unit reducing end (GFn) (Rogge & Stevens, 2004). This polysaccharide has exhibited many interesting properties like beneficial nutritional attributes for human health, moderate average degree of polymerization and readiness of being obtained (Beylot, 2006; Causey, Feirtag, Gallaher, Tungland, & Slavin, 2000; Wei et al., 2007). These interesting characteristics suggest the wonderful potential that inulin could be widely employed in various aspects of function food and other ways (Rogge, Stevens, Colpaert, Levecke, & Booten, 2007). What is more, the demand for biodegradable and environment benign polymeric material is continuously increasing and this abundant resource may be well suited for some applications. Recent years, great deals of efforts are devoted to the employment of this extraordinarily renewable polymeric material. In a review, Stevens provided a relatively integral overview of the chemical modification of inulin and their probable industrialized application (Stevens, Meriggi, & Booten, 2001). However, comparing with the well development of other polysaccharide, the utilization of inulin was insufficient, especially when the total biomass and the wonderful biological activities of inulin are taken into account. One of the drawbacks of inulin may be its limited (polyol) functionality. It is safe to propose that a reactive precursor would steer the chemical manipulation of this polysaccharide toward a good direction of facility.

Chemical modifications are a powerful tool for enhancing employments of natural resources. To synthesize a reactive precursor, amino groups would be ideal candidates to be grafted on the backbone of the limited (polyol) functional polysaccharide. Not only because of the important part amino groups could play during chemical reactions, for instance through amino groups Roman synthesized fluorescently labeled cellulose nanocrystals (Dong & Roman, 2007), but excellent bioactivities aminoglycosides always exhibit. Given these characteristics of inulin and the projects, we started to synthesize *N*-(aminoethyl)inulin. Moreover, evidence has proved that aminated derivatives of saccharide were more potent than natural saccharide as a scavenger of hydroxyl radicals (Xie, Xu, & Liu, 2001). So, we have tested the potential scavenging ability of *N*-(aminoethyl)inulin with another bioactive polysaccharide chitosan and currently widely used antioxidant agent: vitamin C as positive comparison.

For above reasons the domain of this work mainly includes followings: (1) total toslyation of inulin at C-6 without using protecting groups; (2) quantitive and specific nucleophilic substitution of the leaving groups at C-6 with ethylenediamine; (3) complete removing of few tosyl groups at C-4 to give *N*-(aminoethyl)inulin. In addition, useful information was reported to increase the possibility of employing *N*-(aminoethyl)inulin as antioxidant agents.

2. Materials and methods

2.1. Materials

Inulin was purchased from E. Merck (Darmstadt, Germany). Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China) and its degree of deacetylation was 97%. p-Toluenesulfonyl

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doi:10.1016/j.carbpol.2011.01.041

chloride, lithium chloride and ethylenediamine (Et_3N) were from the Sigma–Aldrich Chemical Co. The other reagents were analytical grades and were purified and dried by standard procedures. FT-IR spectrometers were recorded on a Jasco-4100 (Tokyo, Japan, provided by JASCO China (Shanghai) Co. Ltd., Shanghai, China) and the ^{13}C NMR was recorded on a Bruker AVIII-500 spectrometer (Fällanden, Switzerland, provided by Bruker BioSpin CN/Bruker (Beijing) Tech. and Serv. Co. Ltd., Beijing, China).

2.2. Preparation of N-(aminoethyl)inulin

In a typical preparation: 1.62 g inulin (10 mmol of fructose equivalents) and 0.63 g lithium chloride (dried at 100 °C overnight in vacuum) were dissolved in N,N-dimethylformamide (DMF) at 70 °C with stirring under a nitrogen atmosphere until homogenous. The solution was cooled to 0 °C, and a 1.5-fold unit of Et_3N was added. Then a solution of p-toluenesulfonyl chloride (2.85 g) in DMF was added dropwise. After stirred at 0 °C for 36 h under nitrogen atmosphere, the reaction mixture was poured into 450 mL acetone and the tosylated inulin crystallized easily, which was filtered off and washed carefully with acetone. After dialyzed against deionized water for 3 days, it was freeze dried.

The tosylated inulin (3.8 g) was reacted with ethylenediamine (20 mL) at 70 °C for 6 h. After the reaction mixture was cooled to 40 °C, 10% sodium hydroxide solution (3 mL) was added and the resulting solution was stirred at 40 °C for 24 h. The product was recovered by precipitation into ethyl ether–water (1:3, 300 mL), filtered off, washed carefully with ethanol, and after dialyzed for 3 days, it was dried.

2.3. Hydroxyl-radical scavenging ability assay

The scavenging activity of •OH was assessed according to Smirnoff and Cumbes (1989). Briefly, the reaction mixture, a total volume 4.5 mL, containing the samples, was incubated with EDTA–Fe^{2+} (220 μM), safranine O (0.23 μM), 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. Hydroxyl radicals bleached the safranine O, so increased absorbance of the reaction mixture indicated decreased hydroxyl radicals scavenging ability and the scavenging effect of the samples was computed using the following equation:

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

where $A_{\text{blank }520\,\text{nm}}$ was the absorbance of the blank (distilled water instead of the samples) and $A_{\text{control }520\,\text{nm}}$ was the absorbance of the control (distilled water instead of the H_2O_2).

All data are expressed as means ± SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the means were separated by Duncan's multiple range test. The results were processed by the computer programs: Excel and SPSS.

3. Results and discussion

3.1. Chemical syntheses

To complete and regioselective introduction of N-(aminoethyl) into C-6 of inulin full tosylating inulin at C-6 is a prerequisite for the subsequently total nucleophilic substitution by ethylenediamine, although this might result in partially tosylating at C-4 (Liu & Baumann, 2002). Then as long as the reaction temperature was no higher than 70 °C, a quantitive and specific nucleophilic substitution took place at C-6 with ethylenediamine. After the N-(aminoethyl) groups were selectively introduced into C-6 of inulin, 10% sodium hydroxide could completely remove the few tosyl groups at C-4 (Scheme 1).

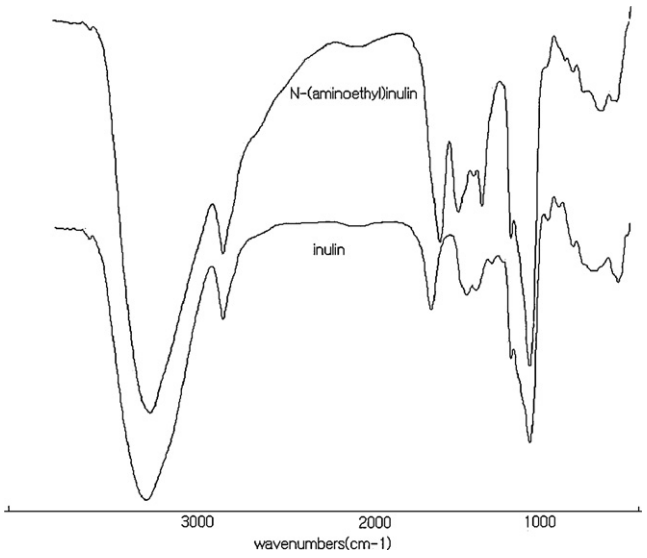
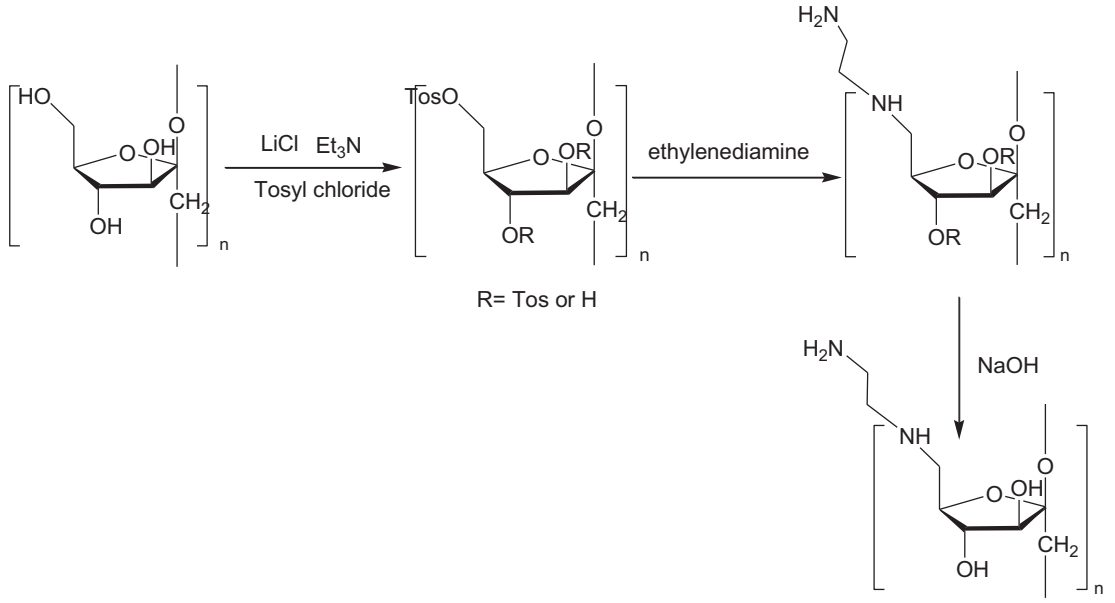


Fig. 1. FT-IR spectra of inulin and *N*-(aminoethyl)inulin.

The FT-IR spectra of inulin and *N*-(aminoethyl)inulin are shown in Fig. 1, and the ^{13}C NMR in Fig. 2. The FT-IR spectrum of inulin shows peaks of saccharide at 852 cm^{-1}, 1029 cm^{-1} and 3041 cm^{-1}. Characteristic peak of amine vibration (N–H) of *N*-(aminoethyl)inulin appear at 1589 cm^{-1} and another new peak at 1473 cm^{-1} is assigned to the methylene adjacent to amine groups (Guo, Xing, Liu, Zhong, & Li, 2008). Fig. 2 reveals that the chemical shifts of ^{13}C NMR of inulin were all above 60.1 (Rogge & Stevens, 2004). Comparing with the ^{13}C NMR of inulin, that of the product have no shift at 60.1 ppm (signal for C-6-OH of inulin) but new signals appear at 42.3 ppm, 49.7 ppm and 56.4 ppm (signals for $-CH_2-NH_2$, C-6–NH–, –NH–CH_2– respectively) (Liu & Baumann, 2002). Moreover, signals at 73.5 ppm of C-4–OH groups have no change between the two ^{13}C NMR. So all C-6–OH groups were quantitatively transformed as $-NH-CH_2-CH_2-NH_2$ groups and the C-4–OH groups of inulin were retained (Rogge et al., 2007). The results mentioned above evidently substantiated the obtainment of *N*-(aminoethyl)inulin.

3.2. Hydroxyl radical scavenging activity

Under normal condition, direct addition of Fe^{2+} to a reaction mixture containing phosphate buffer generates hydroxyl radicals (Halliwell & Gutteridge, 1990), which were scavenged by the tested samples. Fig. 3 reveals the •OH scavenging ability of the samples. We could see that all the scavenging effects of samples have positive correlation with concentration. Of all the samples, *N*-(aminoethyl)inulin exhibits the best scavenging ability against •OH which approaching 62% at 2.4 mg/mL. After grafting amido groups on inulin, *N*-(aminoethyl)inulin possesses obvious better activity than inulin and somewhat better than chitosan and vitamin C at test concentrations. IC_{50} values of *N*-(aminoethyl)inulin, chitosan and vitamin C are 0.87, 0.92 and 2.06 mg/mL respectively. IC_{50}, a good parameter to evaluate the scavenging activity, means the concentration of test sample to reduce the radical by 50%. It is reasonable to propose that the enhanced scavenging capability of *N*-(aminoethyl)inulin benefits from NH_2 and –NH–. These groups can form ammonium groups NH_3^+ by absorbing hydrion and then react with •OH. What's more, the active amino groups may also react straightly with •OH to form stable macromolecular radicals (Xie et al., 2001).



Scheme 1. Synthetic pathway for *N*-(aminoethyl)inulin.

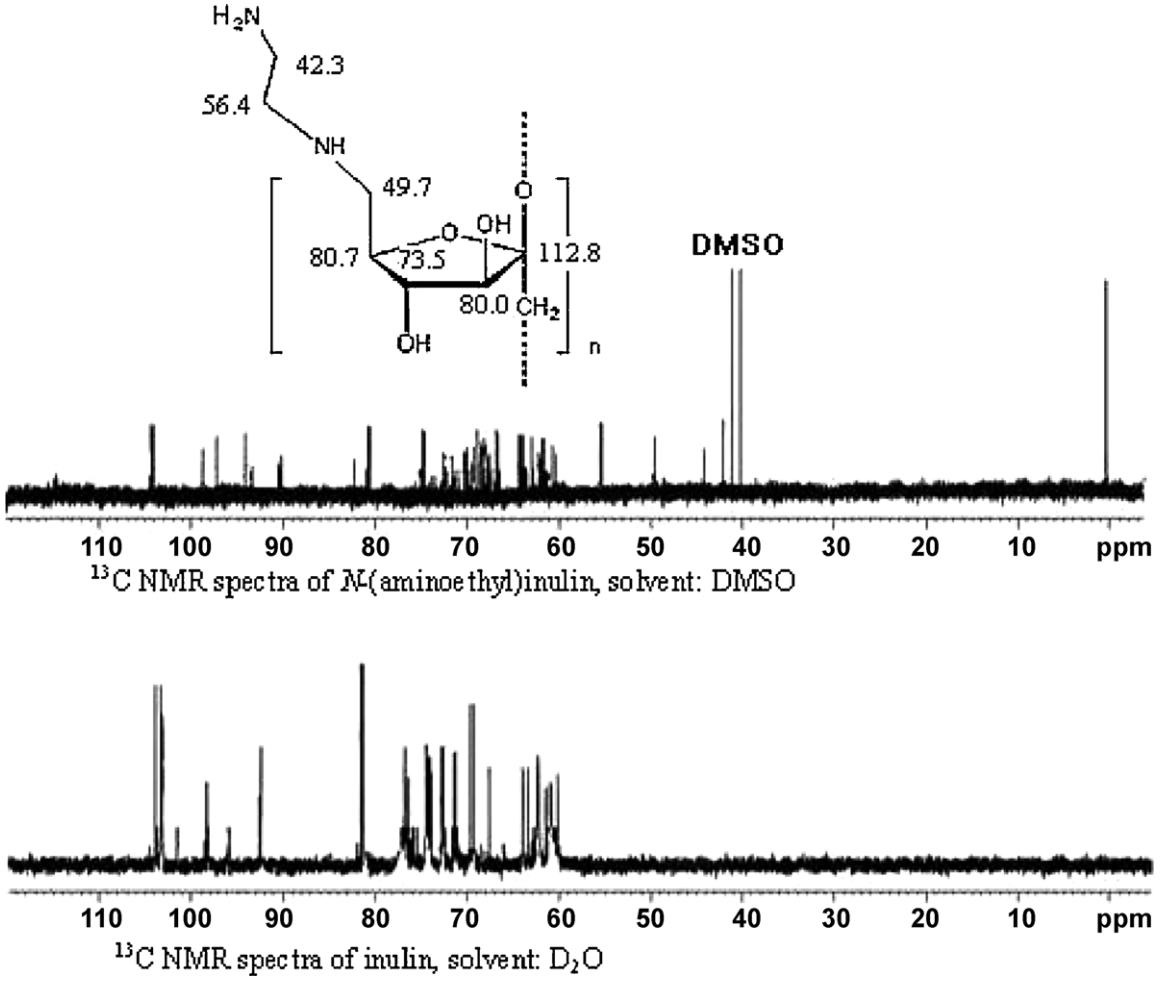


Fig. 2. ^{13}C NMR spectra data of *N*-(aminoethyl)inulin and original inulin.

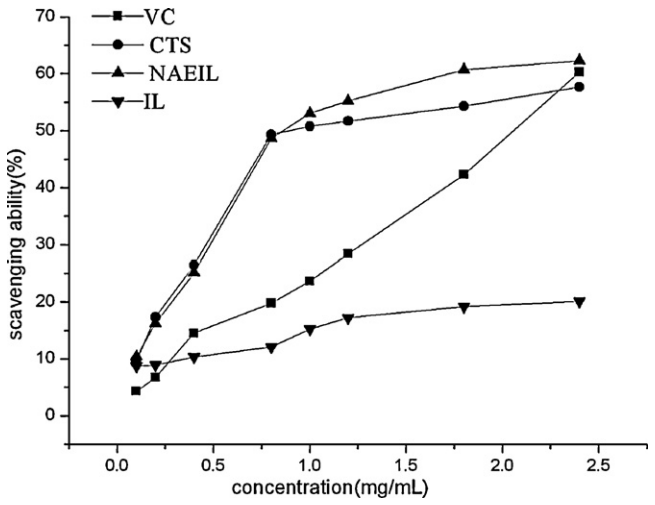


Fig. 3. Hydroxyl radical scavenging ability of N-(aminoethyl)inulin (NAEIL), inulin (IL), chitosan (CTS), and vitamin C (VC).

4. Conclusion

In conclusion, an effective preparation of *N*-(aminoethyl)inulin with 1.0 degree of substitution (DS) at C-6 via easy-going chemical reactions has been established and the investigation of its potential antioxidant ability against hydroxyl radicals at different concentrations has also been described. For synthesis of *N*-(aminoethyl)inulin, we have thoroughly tosylated inulin at C-6–OH which may as well somewhat occur at C-4. After having selectively synthesized C-6-*N*-(aminoethyl)inulin through controlling reaction temperature, we totally released C-4–OH of inulin with 10% sodium hydroxide. The product is a suitable precursor for facile chemical manipulation of inulin. For the investigation of scavenging ability against hydroxyl radicals, the data obtained in vitro models clearly suggested the antioxidant potency of the substances. The mechanism of the enhanced activities of N-(aminoethyl)inulin was also discussed in this paper. This may be probably strengthened by the grafted NH_2 groups in the N-(aminoethyl)inulin, which were more active groups when react with super-radicals.

Acknowledgements

This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (kzcx2-yw-225) and project of National Science & Technology Pillar Program (2011 BAC02B00), which are gratefully acknowledged.

References

Beylot, M. (2006). Effects of inulin-type fructans on lipid metabolism in man and in animal models. *British Journal of Nutrition*, *93*(Suppl. 1), S163–S168.
Causey, J. L., Feirtag, J. M., Gallaher, D. D., Tungland, B. C., & Slavin, J. L. (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal, environment in hypercholesterolemic men. *Nutrition Research*, *20*(2), 191–201.
Dong, S., & Roman, M. (2007). Fluorescently labeled cellulose nanocrystals for bioimaging applications. *Journal of the American Chemical Society*, *129*(45), 13810–13811.
Guo, Z., Xing, R., Liu, S., Zhong, Z., & Li, P. (2008). Synthesis and hydroxyl radicals scavenging activity of quaternized carboxymethyl chitosan. *Carbohydrate Polymers*, *73*(1), 173–177.
Halliwell, B., & Gutteridge, J. M. C. (1990). Role of free-radicals and catalytic metal-ions in human-disease—An overview. *Methods in Enzymology*, *186*, 1–85.
Liu, C., & Baumann, H. (2002). Exclusive and complete introduction of amino groups and their N-sulfo and N-carboxymethyl groups into the 6-position of cellulose without the use of protecting groups. *Carbohydrate Research*, *337*(14), 1297–1307.
Rogge, T. M., & Stevens, C. V. (2004). Facilitated synthesis of inulin esters by transesterification. *Biomacromolecules*, *5*(5), 1799–1803.
Rogge, T. M., Stevens, C. V., Colpaert, A., Levecke, B., & Booten, K. (2007). Use of acyl phosphonates for the synthesis of inulin esters and their use as emulsion stabilizing agents. *Biomacromolecules*, *8*(2), 485–489.
Smirnoff, N., & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, *28*(4), 1057–1060.
Stevens, C. V., Meriggi, A., & Booten, K. (2001). Chemical modification of inulin, a valuable renewable resource, and its industrial applications. *Biomacromolecules*, *2*(1), 1–16.
Wei, L. Y., Wang, J. H., Zheng, X. D., Teng, D., Yang, Y. L., Cai, C. G., et al. (2007). Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers. *Journal of Food Engineering*, *79*(3), 1087–1093.
Xie, W. M., Xu, P. X., & Liu, Q. (2001). Antioxidant activity of water-soluble chitosan derivatives. *Bioorganic & Medicinal Chemistry Letters*, *11*(13), 1699–1701.