Carbohydrate Polymers 121 (2015) 86–91

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Novel vanadyl complexes of alginate saccharides: Synthesis, characterization, and biological activities

Shengyi Liu[a,b], Guangyang Liu[a,b], Yuetao Yi[a,*]

[a] Yantai Institute of Coastal Zone Research Chinese Academy of Sciences, Yantai 264003, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 13 June 2014
Received in revised form
26 November 2014
Accepted 29 November 2014
Available online 31 December 2014

Keywords:
Vanadyl alginate polysaccharides
Vanadyl alginate oligosaccharides
Antioxidant activity
Antiproliferative activity
Protein tyrosine phosphatase 1B
Cytocompatibility

Chemical compounds studied in this article:
Vanadium (PubChem CID: 23990)
Alginate (PubChem CID: 70678572)
Sodium alginate (PubChem CID: 6850754)
Vanadyl sulfate (PubChem CID: 34007)
Hydroxyl radical (PubChem CID: 961)
DPPH (PubChem CID: 2735032)
Sulforhodamine B (PubChem CID: 9916275)
p-Nitrophenol phosphate (PubChem CID: 255)

ABSTRACT

Vanadium compounds present many physiological functions. However, vanadium(IV) and (V) salts are difficult for gastrointestinal absorption and have strong side effects. Therefore organic oxovanadium compounds gain more attention. Vanadyl alginate polysaccharides (VAPS) and vanadyl alginate oligosaccharides (VAOS) were obtained from aqueous solutions of $VOSO_4$ at pH 12. They were characterized by infrared spectroscopy, UV–vis spectroscopy and inductively coupled plasma-mass spectrometry (ICP–MS). The antioxidant activity of oxovanadium(IV) complexes was investigated in hydroxyl and DPPH radical scavenging systems *in vitro*. The results reveal that activities of VAPS and VAOS in the two systems were stronger than those of alginate polysaccharides (APS) and alginate oligosaccharides (AOS), respectively. In addition, VAPS and VAOS promoted significantly the antiproliferation of ligands of human hepatoma cell line BEL-7402. Oxovanadium(IV) complexes were potent inhibitors of protein tyrosine phosphatase 1B (PTP1B) with IC_{50} values in the range of 6.4–18.7 μg/mL, indicated in biochemical assays. In addition, Vanadyl-alginate had no significant side effects on proliferation and viability of HL-7702 hepatic cells. In the future, they can be added to medicines and ease the growing threat that cancer and diabetes mellitus cause to human health.

1. Introduction

Vanadium(V), a transition metal element (Etcheverry, Williams, & Baran, 1997) is essential for physiological functions of human beings and animals. Vanadium ions and complexes are found with potent insulin-like effects (Evangelou, 2002), anti-diabetic activities (Baran, 2004), and anti-cancer activities (Ashiq et al., 2008). Moreover, several recent studies show that many vanadium compounds present significant antioxidant activities (Ashiq et al., 2008; Etcheverry et al., 2008; Mohammadi & Yazdanparast, 2010; Naso et al., 2011) *in vitro* and *in vivo*. However, vanadium(IV) and (V) salts are difficult for gastrointestinal absorption and have strong side effects (Li, Ding, Baruah, Crans, & Wang, 2008) indicated in biochemical assays. Therefore, synthesis of novel vanadium complexes with organic chelating ligands has become a scientific task and their biological and pharmacological properties have been evaluated and reported in animal and cell models (Thompson et al., 2004; Thompson & Orvig, 2006).

Alginate, a natural acidic linear polysaccharide (Gacesa, 1988), consists of α-L-guluronate and β-D-mannuronate with 1 → 4 glycosidic linkages. It occurs in the cell walls of seaweed and can be synthesized with some bacteria (Costerton, 1999). Due to their variable properties, alginate and its derivatives have been widely used in food, biotechnology, pharmaceutical and cosmetic industry (Iwamoto et al., 2005; Yamamoto, Kurachi, Yamaguchi, & Oda, 2007). However, its high molecular weight limits the application in many fields. Recent reports show that, low molecular weight polysaccharide and oligosaccharide prepared from alginate feature many biochemical activities, including antioxidant activity (Tusi, Khalaj, Ashabi, Kiaei, & Khodagholi, 2011; Wang

* Corresponding author. Tel.: +86 535 2109162; fax: +86 535 2109000.
E-mail addresses: yiyuetao@gmail.com, ytyi@yic.ac.cn (Y. Yi).

http://dx.doi.org/10.1016/j.carbpol.2014.11.069
0144-8617/

et al., 2007), anti-hypersensitive activity (Burana-osot et al., 2009), anti-allergy property (Uno, Hattori, & Yoshida, 2006), enhancing protection against infection with some pathogens (Tusi et al., 2011) and advanced glycation end-products (AGEs) inhibitory effect (Sattarahmady, Khodagholi, Moosavi-Movahedi, Heli, & Hakimelahi, 2007).

In this work, VAPS and VOAS were two new coordination compounds, therefore it was necessary to confirm their surface chemical structure. According to biological-pharmacological effects that vanadium compounds may have, the anti-proliferative activities of VAPS and VAOS onto human hepatoma cell line BEL-7402 and the ability against PTP1B were tested. Then they can contribute to ease the growing threat that cancer and diabetes mellitus cause to human health. Their antioxidant effects were tested using hydroxyl radical and DPPH radical models. To study the anti-proliferative activities of VAPS and VAOS onto human hepatoma cell line BEL-7402, tests were carried out by the sulforhodamine B (SRB) method. To understand their ability against PTP1B, the main negative regulatory factor in insulin signaling pathways, tests were carried out as per Ryszard et al. (Gryboś, Paciorek, Szklarzewicz, Matoga, Zabierowski, & Kazek, 2013). Besides, MTT assay was conducted to test their cytocompatibility.

2. Experimental sections

2.1. Materials

Alginate was purchased from Sinopharm Chemical Reagent Co Ltd (China). All other reagents were in analytical grade and used without further purification. IR spectra were measured on a Jasco-4100 FT-IR spectrometer with KBr disks. The content of vanadium was determined by ELAN DRC II ICP–MS. The average molecular weight of the alginate saccharides were determined by DNS methods (Costantino et al., 1999).

2.2. Preparation of APS and AOS

Alginate polysaccharides/alginate oligosaccharides were prepared by acid hydrolysis (Zhang et al., 2006)/oxidative degradation (Tian, Liu, Hu, & Zhao, 2004; Yang, Li, & Guan, 2004). Sodium alginate (10 g) was dissolved in 500 mL water, using sulfuric acid adjusting the concentration of hydrion to 0.22 mol/L under reflux at 90 °C for 24 h. After regulated reaction was completed, the resulting solution was adjusted to pH 7 with NaOH and filtered using a Buchner funnel under vacuum condition. Hydrolysis alginate solution was purified by hydrogen-SAC (strong acidic cation) exchange resins and anion exchange resins in hydroxylic form. The eluent was concentrated and then precipitated four times with absolute ethanol. APS were dried in a vacuum oven at 40 °C for 24 h.

Sodium alginate (10 g) was dissolved in 200 mL water, adding 20 mL 30% H_2O_2 under reflux at 100 °C for 50 min. After regulated reaction was completed, the resulting solution was adjusted to pH 7 with NaOH and filtered using a Buchner funnel under vacuum condition. The following steps were the same as what had been done for APS. Then AOS was gained.

2.3. Preparation of VAPS and VAOS

Aqueous solution (10 mL) of alginate saccharide (1 g) was prepared by adjusting the pH to 12 using 1 mol/L NaOH and stirring for 2 h at room temperature. Then, 0.25 g $VOSO_4{\cdot}5H_2O$ was dropped slowly into the saccharide solution with constant stirring, and pH of the solution was maintained at 12 during whole process. After incubatiom for 4 h, the solution was concentrated and precipitated four times with absolute ethanol. The obtained oxovanadium alginate saccharides were dried in a vacuum oven at 40 °C.

2.4. Hydroxyl radical scavenging activity

The hydroxyl-radical scavenging activities of alginate saccharides and oxovanadium alginate saccharides were measured according to the method described by Guo et al. (2005), Guo et al. (2007) with slight modification. The reaction solution (total volume 4.5 mL) containing 0.5 mL saccharide (2, 4, 6, 8, 10 mg/mL) was incubated with 1 mL EDTA–Fe^{2+} (0.945 mM), 1 mL safranine O (80 mg/L), 1 mL·H_2O_2 (0.03%) and 1 mL potassium phosphate buffer (0.15 mM, pH 7.4) for 30 min at 37 °C. Vitamin C was used as the positive control in the experiment. Finally, the absorbance of the reaction solution was measured at 520 nm. The effect of scavenging hydroxyl radicals was calculated using the following equation:

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

Where $A_{\text{blank 520 nm}}$ was the absorbance of the blank (distilled water instead of the samples), $A_{\text{control 520 nm}}$ was the absorbance of the control (distilled water instead of the sample and EDTA–Fe^{2+}).

2.5. DPPH radical scavenging activity

The DPPH radical scavenging activities of alginate saccharides and oxovanadium alginate saccharides were estimated in triplicate using a modified Yamaguchi Method (Yamaguchi, Takamura, Matoba, & Terao, 1998). An ethanolic solution of DPPH (2 mL, 0.2 mM) was added to 1 mL of the saccharide solutions (2, 4, 6, 8, 10 mg/mL) in 1 mL Tris–HCl buffer (0.1 M pH 7.4). The mixture was left in the darkness at room temperature for 30 min, and then the absorbance of the mixture was measured at 517 nm. Vitamin C was used as the positive control in the experiment. The effect of scavenging DPPH radicals was calculated using the following equation:

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

where A_{blank} was the absorbance of the blank (0.1 M Tris–HCl buffer instead of the samples).

2.6. Cell proliferation assay

Anti-proliferative activity was evaluated with human hepatoma cell line BEL-7402 by the SRB method (Iwasa, Moriyasu, Yamori, Turuo, Lee, & Wiegrebe, 2001). RPMI–1640 was used as the culture medium. An amount of 200 μL cell suspension was cultured in 96-cell plates in density of 2×10^5 cells/mL. Different doses of samples were added into each well for incubation 72 h and the final concentration of samples were 16, 63, 250, 1000, and 4000 μg/mL. Then, cells were fixed with trichloroacetic acid (12%) and the cell layer stained with 0.4% SRB. The absorbance of SRB solution was measured at 515 nm.

$$\text{Inhibition ratio} = \left(1 - \left(\frac{A_{\text{sample}}}{A_{\text{blank}}}\right)\right) \times 100\%$$

A_{sample} is the absorbance of the sample at 515 nm; A_{blank} is the absorbance of the blank at 515 nm.