International Journal of Biological Macromolecules 108 (2018) 412–418

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

A novel low molecular weight *Enteromorpha* polysaccharide-iron (III) complex and its effect on rats with iron deficiency anemia (IDA)

Jiefen Cui [a], Yinping Li [a], Peng Yu [a], Qiping Zhan [a], Jingfeng Wang [a], Yongzhou Chi [a], Peng Wang [a,b,*]

[a] College of Food Science and Engineering, Ocean University of China, Qingdao 266003, PR China
[b] State Key Laboratory of Bioactive Seaweed Substances, Qingdao 266400, PR China

ARTICLE INFO

Article history:
Received 1 June 2017
Received in revised form
26 November 2017
Accepted 5 December 2017
Available online 6 December 2017

Keywords:
Iron deficiency anemia
Enteromorpha prolifera
Polysaccharide-iron (III) complex
Iron supplementation

ABSTRACT

Iron deficiency anemia (IDA) is a common nutritional disease that affects normal erythropoiesis. Traditional iron supplements usually cause gastrointestinal irritation. In this study, a novel low-molecular-weight polysaccharide from *Enteromorpha prolifera* (LPE) was prepared by oxidation degradation, and LPE-iron (III) complex was synthesized and characterized. The molecular weight of LPE-iron (III) complex was 21.25 kDa, and iron content was 25%. The therapeutic effects of LPE-iron (III) complex on IDA were investigated in rats. The hematological indices and organ coefficients of the rats were analyzed. Results showed a dose-dependent relationship, and a prior intragastric administration of LPE-iron (III) complex (2 mg Fe/kg body weight) exhibited considerable effect when compared with the positive control. Therefore, LPE-iron (III) complex could be exploited as a new iron fortifier.

1. Introduction

Iron is an essential cofactor and one of the most important trace minerals in the human body [1]. According to the World Health Organization (WHO), iron-deficiency anemia (IDA) is a global concern [2]. IDA is a common nutritional disease and may result from an inadequate iron intake, iron malabsorption, and excessive iron loss [3]. Therefore, oral iron supplements are required for the treatment and prevention of IDA [4]. However, currently available oral iron supplements, such as ferrous sulfate, ferrous fumarate, and ferrous gluconate could result in epigastric pain, diarrhea, and constipation [5]. Consequently, it is crucial to develop new iron supplements with little or no side effects [6].

In recent years, polysaccharides are extensively studied by many biochemical and nutritional researchers, due to their various biological functions [7]. Polysaccharide could be a kind of ligand while polysaccharide-iron (III) complex is stable and water-soluble [8]. In addition, polysaccharide-iron (III) complex could be absorbed by the human body, and their biocompatibilities are similar to ferrous sulfate [9]. In physiological conditions, polysaccharide-iron (III) complex is nontoxic, and therefore, it could be used to treat IDA [10]. Recently, various polysaccharides including tea polysaccharide [11] and *Angelica sinensis* [12] polysaccharide have been used to prepare polysaccharide-iron (III) complex. However, limited studies have been conducted on algal polysaccharide-iron (III) complex.

Enteromorpha prolifera, one of the most common green algae, is widely distributed along the intertidal zone. [13]. It is regarded as an abundant source of carbohydrates, minerals, crude fiber, vitamins, and fats [14,15]. Polysaccharides from *E. prolifera* (PE) possess antitumor, biosorption, antioxidant, and anticoagulant properties [16,17]. Compared with polysaccharides, oligosaccharides and their derivatives possess appreciable aqueous solubility and bioactivity, and thus, have been diversely applied in pharmaceuticals, functional foods, and cosmetics [18].

In this study, a low-molecular-weight PE (LPE) was prepared and used to synthesize LPE-iron (III) complex. The physicochemical properties and the effects of LPE-iron (III) complex on IDA upon iron supplementation were investigated in rats. This study would provide an experimental evidence for the treatment of IDA.

* Corresponding author at: College of Food Science and Engineering, Ocean University of China, 5 Yushan Road, Qingdao 266003, PR China.
E-mail address: pengwang@ouc.edu.cn (P. Wang).

https://doi.org/10.1016/j.ijbiomac.2017.12.033
0141-8130/

2. Materials and methods

2.1. Materials

E. prolifera was collected from the coasts of Qingdao, China. Dialysis membrane (molecular weight cut-off 7000) was purchased from Beijing Solarbio Science & Technology Co. The PL Aquagel-OH 30 column (0.75 cm × 30 cm) was purchased from Agilent (USA). Dextran standards (3.65, 5, 12, and 25 kDa), were purchased from Sigma Co. (USA). Niferex (capsule containing 150 mg iron) was manufactured by Schwarz Pharma, Inc. America. All other chemicals and reagents were of analytical grade.

2.2. Preparation of LPE

The PE was extracted following an improved method described by Li et al. [19]. Degradation of PE was achieved in the presence of H_2O_2 and Vc following Zhang's method [20]. Briefly, Vc (10 mM) and H_2O_2 (10 mM) were added to PE solution and stirred for 2 h. Then the solution was precipitated with 95% ethanol (1:5, V/V) and centrifuged to obtain the degraded product, LPE.

2.3. Synthesis of LPE-iron (III) complex

The LPE-iron (III) complex was prepared according to the method described by Tang et al. [21]. Briefly, 1 mL of 2 M $FeCl_3{\cdot}6H_2O$ was added dropwise under continuous stirring to 3% (w/w) LPE and 0.15% (w/w) sodium citrate aqueous solution. During the process, 1 M NaOH was used to maintain the pH around 7–8. The solution was heated at 50 °C for 4 h and centrifuged at 4000 r/min for 15 min. The supernatant was concentrated and then dialyzed in distilled water to remove unbound Fe^{3+}. Finally, the dialysate was concentrated and lyophilized to obtain LPE-iron (III) complex.

2.4. Characterization of LPE-iron (III) complex

The molecular weight of LPE-iron (III) complex was determined by Agilent 1260 HPLC system (Wilmington, USA) equipped with PL Aquagel-OH 30 column (0.75 cm × 30 cm) and a refractive index detector (RID). The column was eluted with 0.2 M $NaNO_3$ and 0.01 M NaH_2PO_4 at a flow rate of 0.8 mL/min. The molecular weight of LPE-iron (III) complex was estimated by using calibration curve made from dextran standards as a reference. Sulfate ester content was estimated according to the method described by Therho and Hartiala [22]. The monosaccharide composition was analyzed by using reversed-phase HPLC after pre-column derivatization [23]. The vibrational spectra of different atomic and polar bonds were studied by Fourier transform infrared spectroscopy (FTIR). Two milligrams of LPE and LPE-iron (III) complex were mixed with 200 mg KBr, respectively. The FTIR spectra of LPE and LPE-iron (III) complex were recorded in the wavelength range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using MAGNA-IR 560 E.S.P (Nicolet, USA).

2.5. In vivo experiment

Early weaned male SD rats (4-week-old, SPF) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animals were housed under controlled conditions (24 ± 1 °C, 50% humidity). For the control group, ten rats were selected randomly and were fed on a regular diet (iron content 228.2 mg/kg). Alternatively, remaining rats were fed on a low iron diet (iron content 3.2 mg/kg) for 4 weeks. This was combined with once a week bloodletting via caudal vein to build an IDA model. Hemoglobin (Hb) levels were tested weekly, and IDA was defined as Hb less than 100 g/L [24]. Forty rats with IDA were randomly selected and assigned into 4 experimental groups (according to the Hb level and body weight); model group, positive control group (Niferex, 2 mg Fe/kg body weight), low LPE-iron (III) complex group (0.7 mg Fe/kg body weight), and high LPE-iron (III) complex group (2 mg Fe/kg body weight). The intragastric administration was performed daily at 8:00 am for 28 d. The body weight of all the rats was measured every three days throughout the experimental period. After the last intragastric administration, all the rats were fasted for 12 h and anesthetized with ether. Blood was collected from the abdominal aorta in different tubes to determine various indices. Serum was obtained from blood samples after centrifugation (7500 r/min for 30 min) and stored at −80 °C for future analysis.

2.6. Hematological indices

The levels of Hb, red blood cells (RBC), and hematocrit (HCT) were measured with a Sysmex XT-1800i hematology analyzer (Kobe, Japan). Serum iron (SI), total iron-binding capacity (TIBC), and erythropoietin (EPO) reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute.

2.7. Organ coefficients of rats

In this study, all the rats were sacrificed, and their liver and spleen were excised and weighed. The organ coefficient was calculated as follows:

$$\text{Organcoefficient(g/100 g)} = \text{organweight/ratbodyweight} \times 100$$

2.8. Statistical analysis

All the data was expressed as a mean ± standard deviation. Statistical analyses were performed with SPSS version 12.0. Comparisons between the groups were performed using one-way analysis of variance (ANOVA) and least significant difference (LSD) test. A difference of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Degradation of PE

In recent years, acid hydrolysis, enzymatic hydrolysis, and oxidative degradation have been employed to prepare polysaccharides with low molecular weight. Acid hydrolysis could violently change structure of the sugar units and break bioactivity groups [25]. Enzymatic hydrolysis is extremely specific for cleaving glycosidic bonds [26]; however, it is still unavailable for commercial manufacturing. In fact, hydroxyl radical is a powerful reactant and can react with the hydrogen atoms of polysaccharides on plant cell, leading to the glycosidic bond cleavage. When heated, H_2O_2 decomposes into H_2O and O_2. Therefore, oxidation degradation (H_2O_2/Vc) is a convenient and an environmentally friendly method to obtain low molecular weight polysaccharides [20,27].

3.2. Characterization of LPE-iron (III) complex

LPE-iron (III) complex was synthesized under optimum conditions. It is a reddish brown powder with good solubility. As shown in Table 1, the molecular weight of LPE-iron (III) complex is 21.25 kDa. Corroborating with Tang et al.'s polysaccharide (103.51 kDa) from *E. prolifera*, which could be absorbed and utilized by hyperlipidemic rats [28]; LPE-iron (III) complex could also be absorbed by rats with IDA. The LPE-iron (III) complex consists of four monosaccharides, including rhamnose (52.42%), glucose (12.53%), xylose (14.72%),