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In vivo anti-radiation activities of the *Ulva pertusa* polysaccharides and polysaccharide–iron(III) complex

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ABSTRACT

Polysaccharides with different molecular weights were extracted from *Ulva pertusa* and fractionated by ultrafiltration. Iron(III) complex of the low molecular-weight *U. pertusa* polysaccharides were synthesized. Atomic absorption spectrum showed that the iron content of iron(III)–polysaccharide complex was 27.4%. The comparison between *U. pertusa* polysaccharides and their iron(III) complex showed that iron chelating altered the structural characteristics of the polysaccharides. The bioactivity analysis showed that polysaccharide with low molecular weight was more effective than polysaccharide with high molecular weight in protecting mice from radiation induced damages on bone marrow cells and immune system. Results also proved that the anti-radiation and anti-oxidative activity of iron(III) complex of low molecular-weight polysaccharides were not less than that of low molecular-weight polysaccharides.

1. Introduction

Radiation source is a kind of physical stresses that human risk in the events of nuclear pollutions, medical diagnosis [1], cancer therapy [2] and space flights [3]. Radiation exposure increases the oxidative pressure and induces further damages such as DNA lesions, cell death, cancer and other diseases. In the past decades, there has been a growing interest in exploring radioprotective medicines and foods to improve human health condition. Some chemicals and biological agents have been proved to have radioprotective activities [4]. But in recent years, people prefer nature medicine and health food to keep health and treat illness. Recent researches proved that one of the functional compounds of the nature products used as anti-radiation substances such as herbal drugs was polysaccharide [5].

Polysaccharides from functional plants are known to have beneficial bioactivities including radioprotection, antioxidant and immunomodulatory [6,7] activities. Recently, polysaccharides from marine organisms have garnered attentions because they have great potential to be used as ingredients of new medicines and functional foods [8–11]. Green seaweed is one of the most abundant resources of the marine organisms. Polysaccharides extracted from edible green seaweed, known as ulvan, have also been found to possess varieties of pharmacological activities such as immunomodulatory, antitumor, anti-inflammatory, anticoagulant and antiviral activities [12–15]. However, little attention has been given to the anti-radiation activities of the ulvan polysaccharides.

U. pertusa is familiar edible green seaweed which has great potential to be used as functional seafood due to its various bioactivities. Ulvans extracted from *U. pertusa* also exhibited antioxidative and antihyperlipidemia activities [16]. All of the previous findings prompted us to detect whether ulvans from *U. pertusa* could be a rich source of radioprotective medicines and foods. The biological activities of the polysaccharides have been reported to be related with their molecular weights [17]. In this study, the polysaccharides were extracted from *U. pertusa* and fractioned according to the molecular weight. The *in vivo* anti-radiation activities of those compounds were detected in mice.

Nowadays, iron deficiency has been a worldwide problem. So, it is important to develop oral iron supplements for treatment and prevention of iron deficiency in humans. Previous researches proved that polysaccharide–iron(III) complexes (PIC) were not inferior to ferrous sulfate, a popular iron supplement, on their absorbability and biocompatibilities. Moreover, the component of polysaccharide in PIC could exhibit multiple bioactivities to improve human health [18]. In our work, iron(III) complexes of polysaccharides from *U. pertusa* were prepared. The structural characteristics and anti-radiation properties of the

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polysaccharides–iron(III) complexes were investigated and compared with the polysaccharides.

2. Materials and methods

2.1. Materials and reagents

Green seaweed *U. pertusa* was obtained from the coast of Dalian, Liaoning Province, China. After washed with distilled water, the seaweed was air-dried, milled and stored at −80 °C. All chemicals and reagents used in this work were analytical grade.

2.2. Isolation and purification of low-molecular-weight ulva polysaccharide

2.2.1. Extraction of crude polysaccharides

Total of 200 g *U. pertusa* was milled and pretreated with 85% ethanol for 12 h at room temperature [7]. The residual part was separated by centrifugation at 5000 rpm for 10 min. After dried at room temperature, the material was extracted with distilled water at 65 °C for 3 h. After centrifuged at 5000 rpm for 10 min, the supernatants were concentrated by evaporation under reduced pressure at 60 °C for 2 h. The solution of polysaccharides was precipitated again with three times volume of 95% ethanol and placed at 4 °C overnight [8,19]. The polysaccharide was obtained by the filtration of the solution with a 0.45 μm membrane and washed three times with 95% ethanol, followed by acetone, and then dried at room temperature.

2.2.2. Fractionation of the polysaccharides

Ultrafiltration was used to obtain different molecular-weight polysaccharides from crude ulva polysaccharides. The membrane used in this study was 100 kDa and 30 kDa. Prior to use for experiments, the membranes were soaked overnight in distilled water and then washed impurities or additives used for stabilization. After used, membrane was filled with 0.1 M sodium hydroxide and subsequently washed with plenty of water till they returned normal filtrate flux. The filtrate flux was measured as a function of TMP, temperature, pH or ionic strength using timed collection. The required pH values were adapted by dropwise addition of 0.1 M HCl or 0.1 M NaOH. The ionic strength adjustment was prepared by adding proper solid NaCl or KCl in solutions.

2.3. Determination of carbohydrate content and protein content

Total carbohydrate content was determined by the phenol–sulfuric acid method, using D-glucose as the standard [20]. Protein contents were measured by the Bradford method using bovine serum albumin as standard [21].

2.4. The polysaccharide–iron complex

60 mL solution containing polysaccharide (2.0 g) and sodium citrate (0.5 g) was heated and keep at 70 °C. The solution was neutralized slowly with 20% NaOH. $FeCl_3$ (2 mol/L) was dropped slowly into the polysaccharide solution until brown powder appeared. During this procedure, 20% NaOH was used to keep pH at 8–9. The solution was heated at 70 °C for at least 2 h, desalted and concentrated by flow dialysis, and spray dried. The polysaccharide–iron complex was obtained as a brown powder and soluble in water.

2.5. Electron microscopy

Samples for analysis were mounted directly onto formvar-coated copper grids. These were rinsed with deionized water to prevent salt precipitation, air-dried, and coated with carbon. Samples were examined on S-360 scanning electron microscope operated at an accelerating voltage of 20.0 kV, equipped with a Tracor Northem 524 energy dispersive X-ray energy spectrometer.

2.6. IR spectroscopy

The structural characteristics of polysaccharides and the iron(III) complex were determined by Fourier transform IR spectrophotometer (Nicolet 6700). Dried homogeneous polysaccharide and KBr were mixed and then pressed into pellets for transform IR spectral measurement in the frequency range of 400–4000 cm^{-1}.

2.7. Atomic absorption spectrometry

Atomic absorption spectrometer SpectrAA 220FS (Varian) with air-acetylene flame atomization (acetylene flow 1.5 L min^{-1}, air flow 6.5 L min^{-1}), equipped with an HCL lamp for the determinations of iron (wavelength 248.3 nm, slit 0.5 nm, lamp current 5.0 mA) was used to detect the iron content of the polysaccharides–iron(III) complex.

2.8. In vivo anti-radiation activity

2.8.1. Grouping, irradiation and administration of polysaccharides and the iron(III) complexes

A total of 60 BALB/c mice (30 male and 30 female) of 6–8 weeks old, weighing 20.0 ± 2 g, were obtained from experimental animal center of Heilongjiang University of Chinese Medicine (Harbin, China), and maintained under conditions of controlled temperature (23 ± 2 °C) and humidity (50 ± 5%), and a 12 h light/dark cycle. 60 mice were randomly divided into ten groups (n = 6 per group, 3 male and 3 female). The polysaccharides were dissolved in normal saline (NS). Mice were exposed daily to aqueous solutions of polysaccharides at dosing levels of 4 mg and 8 mg for 21 consecutive days. The mice of control groups were given NS daily on the base of equal volume. All mice, except those from the normal control group, were irradiated by medical electron linear accelerator at dose of 4.0 Gy and dose rate of 1.0 Gy/min. Mouse was placed in a separate plastic container (20 cm × 20 cm × 100 cm), and exposed to whole body irradiation. Mice of each group were killed by cervical dislocation at 4 days after irradiation. The procedures of the experiment were strictly performed according to the generally accepted international rules and regulations.

2.8.2. Liver, spleen and thymus indices

The spleen index, thymus index and liver index were measured as previously described [22]. 4 days after irradiation, the mice were sacrificed; the spleen and liver were removed, and weighed for calculation of the immune organ index. Body weight (BW) was measured at the same time points. Liver index equals to liver weight/BW × 1000; spleen index equals to spleen weight/BW × 1000.

2.8.3. Micronuclei of bone marrow cells in mice

6 mice from each group were sacrificed for bone marrow micronucleus test. The micronuclei assay was based on Lu's method [23]. Briefly, the bone marrow from the right femur of mice was flushed out and re-suspended in a few drops of neonatal calf serum, and collected after centrifugation at 1500 rpm for 10 min at 4 °C. A cell pellet was re-suspended in a drop of fetal calf serum and bone marrow smears were prepared. Slides were air dried, and fixed in methanol for 5 min and then stained with Giemsa's stain for 15 min. A total of 6000 polychromatic erythrocytes from six mice of each group were scored and the frequency of micronuclei was calculated.