Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 45 (2007) 2390–2396
www.elsevier.com/locate/foodchemtox

Studies on the expression of liver detoxifying enzymes in rats fed seaweed (*Monostroma nitidum*)

Albert Linton Charles [a], Cheng-Kai Chang [b], Mei-Li Wu [b], Tzou-Chi Huang [b,*]

[a] *Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology (NPUST), Pingtung 91201, Taiwan, ROC*
[b] *Department of Food Science, NPUST, 912, Pingtung, Taiwan*

Received 28 October 2006; accepted 10 June 2007

Abstract

The expression level of phase I (CYP1A1 and CYP1A2) and phase II (GST, and UGT) enzyme-coded genes were measured in liver microsomes of 30 Sprague–Dawley rats fed sea weed (*Monostroma nitidum*). Quantitative and qualitative analysis of the detoxifying enzymes were investigated using reverse transcription polymerase reaction (RT-PCR) and real time polymerase reaction (Real-time PCR) techniques. The antioxidative properties of seaweed were screened and investigated for its hepatoprotective activity in rat. There was no significant induction of GSTYa1, GSTYa2, and CYP1A2. However, an *M. nitidum* diet was found to significantly increase UGT1A1 and UGT1A6 mRNA levels and to decrease CYP1A1 mRNA levels in rat liver. Structural studies confirmed the presence of sulfated polysaccharides in the seaweed samples. The results demonstrate the potential of seaweed as a natural source of sulfated polysaccharide substances with potential use in chemoprevention medicine.

Keywords: Seaweed; Sulfated polysaccharide; Phase II enzymes; Liver; Chemoprevention

1. Introduction

Seaweeds, traditionally used as food, are a rich source of dietary fiber, minerals, and proteins (Lahaye, 1991) and are considered to be a rich source of antioxidants with the ability to scavenge reaction oxygen species and free radicals (Lim et al., 2002). Studies revealed that seaweeds decreased serum total cholesterol in the seaweed-based diet groups compared with those of the control rat group (Wong et al., 1999) and various seaweed species, screened for their hepatoprotective activity using CCl4-induced liver injury in the rat as a model of chemical hepatitis (Wong et al., 2000), were reported to exhibit significant hepatoprotection by reducing the acute increase of glutamic pyruvic transaminase (GPT) and glutamic oxoaloacetic transaminase (GOT). Seaweeds administration enhanced dioxin excretion and reduced dioxin absorption in rats administered the dioxin mixture (Morita and Nakano, 2002).

Cell wells from marine algae contain sulfated polysaccharides which are not found in land plants (Kloareg and Quatrano, 1988). Functionalities of sulfated polysaccharides were found to be anticoagulant (Siddhanta et al., 1999), antiviral (Watson et al., 1999), antioxidative (Lin et al., 1999), immuno-modulating (Shan et al., 1999) and antitumor (Koyanagi et al., 2003). Similarly, active sulfated-polysaccharides, which are referred to as heparinoids, have been used as substitutes and are well known to have remarkable anticoagulant activity. However, it is unknown whether seaweed species such as *M. nitidum* could induce important detoxifying enzymes referred to as Phase I and II enzymes.

* Corresponding author. Tel.: +886 8 7703202; fax: +886 87740213.
E-mail address: tchuang@mail.npust.edu.tw (T.-C. Huang).

0278-6915/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2007.06.014

Phase I enzymes results in the activation of chemicals to a toxic or mutagenic product, including aflatoxins and polycyclic aromatic hydrocarbons. Another potentially damaging effect of phase I enzymes is the production of oxygen free radicals that occurs as a result of cytochrome P450 activity (Percival, 1997). The balance between the abilities of potential chemoprotective agents to inhibit P450s and induce phase II enzymes may be critical for their capacity to function as anticarcinogenic agents (Krajka-Kuzniak and Baer-Dubowska, 2003).

In this study, the characterization, chemical and viability or proliferative properties of the active polysaccharide of *M. nitidum* were carried out. RT PCR and quantitative real-time PCR techniques were used to investigate the induction and quantify the expression of Phase I and Phase II enzymes in liver tissues of rats fed *M. nitidum*.

2. Materials and methods

2.1. Extraction and chemical analysis of crude seaweed polysaccharides

Seaweed samples were obtained from Peng-Chu Fishery Ltd. Co. Air-dried samples were dipped into 10 vol of water at room temperature for 1 h then homogenized into a thick viscous gum in an Osterizer blender and refluxed for 2 h at 100 °C. The hot water extract was separated from the seaweed residues by centrifugation. The supernatants were concentrated under reduced pressure, dialyzed in cellulose membrane against distilled water for 3 successive days. The retention was recovered, concentrated under reduced pressure, and lyophilized to obtain hot-H_2O soluble crude polysaccharides. Fractions of crude seaweed polysaccharides (CSP) were roughly fractionated by anion exchange chromatography on Q Sepharose Fast Flow with elution with stepwise increments in ionic strengths of NaCl at 0.5, 1.0, 2.0, and 3.0 mL^{-1}. The fraction containing the most abundant carbohydrate was further purified by Sephacryl S-400/HR gel filtration chromatography. The major fractions were pooled, concentrated, desalted and freeze-dried. The purified sulfated polysaccharide (SP1) was obtained as a colorless powder.

2.2. Composition analysis

Polysaccharide (CSP) was first hydrolyzed using 1M H_2SO_4 at 100 °C for 2 h. Total sugar content was determined with the phenolsulfuric method (Dubois et al., 1956) using rhamnose as standard. Ash was quantified gravimetrically after 12 h at 550 °C and further 4 h at 900 °C. Protein content was determined as described by Bradford, 1976. The neutral sugars were determined, using gas chromatography, by converting them into acetylated aldononitrile derivatives (Mao et al., 2004). Sugar identification was done by comparison with reference sugars. FTIR assay was carried out according to Charles et al. (2006).

3. *In vivo* experiments

3.1. Animals and treatments

Thirty Sprague–Dawley rats (weighing 200–250 g) were obtained from the School of Veterinary Medicine, of the NPUST. Animals were housed in polycarbonate cages on hardwood chip bedding and during the experiment were allowed free access to food (Purina Lab Chow #5010, St. Louis, MO) and water *ad libitum* for 4 weeks (0%, control group). The animals were divided into two groups based on their adulterated diets, prepared by combining the appropriate amount (0.5% and 1.0%, w/v) of seaweed with a pre-weighed amount of a powdered diet in a V-blender (Table 1). To circumvent the possible problem of palatability of the diet, food intake was given with gavage dosing of the test substance.

Table 1
Composition of the commercial and adulterated diets of control and treated groups with seaweed

	Experimental diet (g/kg diet)[a]		
	Control	Group 1	Group 2
Purina Lab Chow 5010 (g)	950	945	940
Corn oil (g)	50	50	50
Seaweed (g)	–	5	10
Water	500	500	500

[a] Before use, the dry matter was ground and mixed with water (water/dry matter = 50/100, w/w).

At the end of treatment, each animal was starved on the previous day, then killed by CO_2 asphyxiation and the entire liver was carefully removed from each, trimmed free of extraneous tissue and immediately frozen in liquid nitrogen and kept at −70 °C.

3.2. Total RNA extraction

Total RNA was extracted from frozen liver specimens according to the manufacturer's instructions using Trizol reagent. Total RNA integrity was visualized by 1% agarose gel electrophoresis.

3.3. cDNA synthesis

First strand cDNA was synthesized from 5 μg total RNA using the BD PowerScript Reverse Transcriptase (Clontech) according to the manufacturer's instructions. A typical PCR reaction consisted of 2 μl of the reverse transcription reaction, 200 μM each of dNTPs, 100 nM gene-specific primers, 1× PCR buffer and 0.5 U Taq DNA polymerase (Takara) in a total volume of 30 μl. Primers used in the analysis included β-actin (F): 5-CTCTTCCAGCCTTCCTTCCTG-3, (R): 5-GTGGTGG-TACATGGG TCCGT-3; *Phase I enzyme*: CYP1A1 (F): 5-TGGGGAGGTTACTGGTTCTG-3, (R): 5-CAGAT AGG GCA GCTGAGGTC-3; CYP1A2 (F): 5-TCTTC-TGGAGCATTTTGC TA-3, (R): 5-CAC AAA GGGGT-CTTTCCACT-3; *Phase II enzymes*: UGT1A1 (F): 5-GGT GTG CCG GAG CTCATGTTCG-3, (R): 5-AGA-CAGCAGCATACT GGAGTCCC-3; UGT1A6 (F): 5-TTGCCTTCTTCCTGCTGC-3, (R): 5-TCTGAA GAG-GTAGATGGA AGGC-3; GSTYa1 (F): 5-ATGAGAAG-TTTATACAAACTCC-3, (R): 5-GATCTA AAATGCCT-TCGGTG-3. Primer express software (version 2.0 Applied Biosystems, Foster City, CA, USA) was used to determine an optimal primer pair anchored in the conserved regions