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Article

Screening of Korean Marine Plants Extracts for Inhibitory Activity on Protein Tyrosine Phosphatase 1B

Hee Jung Lee[2], You Ah Kim[1], Jung Im Lee[1], Burm-Jong Lee[3] and Youngwan Seo[1,*]

[1]*Division of Marine Environment and Bioscience, Korea Maritime University, Youngdo-Gu, Busan 606-791, Korea*

[2]*Busan Regional Food & Drug Administration, Test & Analysis Center, Busan 608-829, Korea*

[3]*Department of Chemistry, Inje University, Gimhae 621-749, Korea*

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Crude extracts of 69 marine organisms (27 salt marsh plants and 42 seaweeds) were screened for the inhibitory activity against the protein tyrosine phosphatase 1B (PTP1) *in vitro*. The most active extracts were methanol extracts from *Derbesia marina* (80.6% in inhibitory activity) and *Symphycladia latiscula* (85.6%) at the concentration of 15 μg/mL. Methanol extracts of *Codium adhaerens* and *Hisikia fuziformis* were moderately inhibitory with 71.2 and 69.1% inhibition, respectively. It was peculiar that only the extracts from seaweeds show inhibitory activity where those from salt marsh plants do not show any significant effect.

Key words: *diabetes mellitus, protein tyrosine phosphatase 1B, salt marsh plant, seaweed*

Diabetes mellitus is classified into type 1 and type 2. Type 1 is caused by absolute deficiency of insulin by the destruction of insulin-producing β-cells, while type 2 is caused by insulin resistance of target tissues and decrease of insulin production. Since the receptor of insulin is a tyrosine kinase, it has been considered that protein tyrosine phosphatase (PTPase) negatively regulates insulin signaling via receptor dephosphorylation. The phosphorylation of protein on tyrosine is controlled both by the concerted action of protein tyrosine kinases (PTKases) that transfer the terminal phosphate from nucleoside triphosphate to tyrosine on the protein and protein tyrosine phosphatases (PTPases) that remove phosphate from phosphotyrosine-containing proteins. Tyrosine phosphorylation levels are regulated by the balance of the activities of the PTKase and PTPase acting on the individual protein. Therefore, PTPase is a possible target for type 2 diabetes with insulin resistance [Hong *et al.*, 2004; Kenndy, 1999; Montalibet and Kennedy, 2005; Kennedy and Ramachandran, 2000; Umezawa *et al.*, 2003; Cheon *et al.*, 2004]. Several PTPases have been identified in major insulin-sensitive tissues, such as skeletal muscle, liver and adipose tissues. In fact, PTP1B is known to be a negative regulator of insulin receptor associated with signal transduction [Kenner *et al.*, 1996]. PTP1B deficient mice showed increased insulin sensitivity in muscle and liver and resistance to obesity [Elchebly *et al.*, 1999]. Overexpression of PTP1B inhibits proximal and distal insulin signaling events. Therefore, PTPases are considered to be involved in the etiology of diabetes mellitus.

In our continuous search for biologically active metabolite from marine resources [Lee *et al.*, 2004a,b; Seo *et al.*, 2004; Lee and Seo, 2006; Jung *et al.*, 2004; Seo *et al.*, 2005; Lee *et al.*, 2006], we have attempted to screen inhibitory metabolites from Korean salt marsh plants (27 species) and seaweeds (42 species) against the protein tyrosine phosphatase 1B.

Materials and Methods

Sample collection, extraction, and fractionation. The species of seaweeds were collected by hand in March 2004, along with the shores of Cheju Island and Busan, Korea and identified by Dr. J. S. Yoo, at the Research Institute of Marine Science and Technology, Korea Maritime University, Korea. The salt marsh plants were collected at Daebudo, Yangpori, Pohang and Geojedo, Korea. The specimen was identified by Prof. S. G. Moon, Department of Biology, Kyungsung University, Korea.

Shade-dried seaweeds were extracted with a volumetrically equal mixture of acetone and dichloromethane for 24 hours at room temperature and then with methanol. Each

*Corresponding author
Phone: +82-51-410-4328; Fax: +82-51-404-3988
E-mail: ywseo@hhu.ac.kr

step was repeated twice. The shade-dried salt marsh plants were chopped into small pieces and repeatedly extracted for 2 days with CH_2Cl_2 and then methanol according to the same procedure. The extracted solutions with the same solvent for each sample were combined and then evaporated under vacuum, yielding a dark and sticky material. Each of the crude extracts was used as experimental material. The prepared samples were then stored in a refrigerator at −20°C, for later study. Each of the combined solvent extracts of *Derbesia marina*, *Symphyocladia latiscula*, *Codium adhaerens* and *Hisikia fuziformis* was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 fraction was further partitioned with *n*-hexane and 85% aq. MeOH and the H_2O fraction was successively fractionated with *n*-BuOH and H_2O.

Chemicals. PTP1B (human, recombinant) and p-nitrophenylphosphate were purchased from BIOMOL International LP (USA) and Sigma-Aldrich Chemical Company (St. Louis, MO, USA), respectively.

Table 1. Inhibition of PTP1B by the crude extracts of salt marsh plants

Species (15 μg/mL)	% inhibition	
	CH_2Cl_2 ext.	MeOH ext.
Rosa rugosa Thunberg	3.2	37.5
Messerschmidia sibirica	11.2	-
Lathyrus japonicus willdenow	-	-
Carex scabrifolia	-	-
Sonchus brachyotus	-	-
Limonjm tetragonum	-	-
Salsola komarvii lljin	-	-
Suaeda asparagoides	-	-
Suaeda japonica	-	-
Erigron annuus	-	-
Ixeris tamagawaensis kitamura	-	-
Imperata cylindrica	-	-
Aster tripolium	-	-
Calystegia soldanella	-	-
Glehnia littoralis	-	-
Artemisia capillaris Thungerg	-	-
Tetragonia tetragonoides	-	-
03H-4	-	-
Vitex rotundifolia	-	-
Corydalis heterocarpa	-	-
Carex kobomugi	-	-
Polygonum bellardi	-	-
Hypochoeris radicata	-	-
Suaeda asparagoides	-	-
Limonjm tetragonum	-	-
03U-3	-	-
Erigron annuus	3.9	-

Assay method of PTP1B inhibitory activity. For inhibition assay, test sample (3 μL in DMSO) was added to a reaction mixture containing enzyme (2 μL), reaction

Table 2. Inhibition of PTP1B by the crude extracts of seaweeds

Species (15 μg/mL)	% inhibition	
	Acetone/ CH_2Cl_2 ext.	MeOH ext.
Sargassum yezoense	54.4	-
Sargassum fluvellum	36.1	-
Sargassum hornerii	46.2	-
Sargassum sagamianum	21.4	-
Sargassum coreanum	27.5	-
Sargassum hemiphyllum	44.1	-
Sargassum siliquastrum	14.8	-
Sargassum confusum	-	-
Chondrus ocellathus	41.5	-
Chondrus crispus	27.6	-
Corallina spp	-	-
Corallina pilulifera	58.3	-
Padina crassa	26	-
Padina arborescens	54.1	54.1
Carpopeltis cornea	-	-
Gymnogongrus flabelliformis	38.6	-
Gracillaria textori	24.9	15.6
Pachydictyon coriaceum	-	-
Spatoglossum pacificum	-	-
Dictyota dichotoma	22.8	53.2
Ulva pertusa	25.8	48.1
Enteromorpha linza	42.1	35.4
Lomentaria hakodatensis	-	-
Carpopeltis affinis	1.1	-
Prionitis cornea	21	-
Laruencia okamurae	12.8	33.3
Chondria crassicaulis	-	-
Laurencia intermedia	43.4	-
Halymenia acuminata	30.4	-
Gratelopia turutura	33.1	61.7
Pachymeniopsis lanceolata	13.2	9.5
Grateloupia lanceolata	45.6	-
Porphyra suborbiculata	44.9	-
Colpomenia sinuosa	51.2	6.9
Colpomenia bullosa	53.9	59.5
Gelidium amansii	45.3	23.7
Hisikia fuziformis	26.5	69.1
Codium adhaerens	51.5	71.2
Polysiphonia sp.	14.6	-
Scytosiphon lomentaria	18.7	-
Derbesia marina	61.7	80.6
Symphyocladia latiscula	85.6 (Crude ext.)	

Table 3. Inhibition of PTP1B activity against *n*-hexane, 85% aq. MeOH, *n*-BuOH and H_2O fraction of 4 seaweeds

Seaweeds	% Inhibition			
	n-hexane	85% aq. MeOH	*n*-BuOH	H_2O
Symphyocladia latiscula	87.6	90.6	73	1.3
Hisikia fuziformis	99	91.3	77.8	16.6
Codium adhaerens	20.8	52.4	69.4	-
Derbesia marina	91.5	73	80.1	56.2

buffer [10 μL, 50 mM citrate, pH 6.0, 0.1 M NaCl, 1 mM EDTA, 1 mM dithiothreitol (DTT)], water (35 μL), and 50 μL of p-nitrophenylphosphate (p-NPP, 20 mM). The reaction mixture was placed in a 37°C incubator for 30 min and the reaction was terminated with 1 N NaOH. The amount of produced p-nitrophenol was estimated by measuring the increase in absorbance at 405 nm. Nonenzymatic hydrolysis of 20 mM p-NPP was corrected by measuring the increase in absorbance at 405 nm in the absence of PTP1B [Oh *et al.*, 2005].

Results and Discussion

Even though physiological function and role of PTP1B are not completely elucidated, a number of evidences accumulated from the various biochemical studies are now suggesting that PTP1B is a major negative regulator of insulin receptor signaling [Cheng *et al.*, 1999; Zhang *et al.*, 1999; Klaman *et al.*, 2000]. Accordingly, PTP1B is considered as an attractive target for the treatment of type 2 diabetes and related metabolic syndromes [Johnson *et al.*, 2002; Dadke and Chernoff, 2003; Tonks, 2003].

As shown in Table 1 and 2, the *in vitro* PTP1B inhibitory activities of 71 marine plant species from Korea showed that the extracts from *D. marina*, *S. latiscula*, *C. adhaerens*, and *H. fuziformis* were significant PTP1B inhibitors and those from *C. pilulifera*, *S. yezoense*, *P. arborescen*, *C. cornea*, *D. dichotoma*, *G. turutura*, *C. sinuosa*, *C. bullosa* were moderately active with inhibition rate ranging 50-62% against PTP1B in comparison to the known phosphatase inhibitor, RK-682 (68.3% inhibition) as a positive control in the assay [Hamaguchi *et al.*, 1995]. However, the crude extracts of 27 salt marsh plants didn't show any effect on PTP1B enzyme activity *in vitro*.

Interestingly, high PTP1B inhibitory activities were observed from the methanol extracts of *D. marinaand* and *S. latiscula* with 80.6 and 85.6% inhibition at the concentration of 15 μg/mL, respectively. The methanol extracts of *H. fuziformi* and *C. adhaerens* also exhibited comparatively high PTP1B inhibitory activities (69.1% and 71.2%).

Each crude extract of these four seaweeds was fractionated with *n*-hexane, 85% aq. MeOH, *n*-BuOH and H_2O successively and then each of the solvent fractions was evaluated for PTP1B inhibitory activity. Other fractions of *S. latiscula*, *H. fuziformi*, *D. marinaand*, except for the H_2O fraction showed good PTP1B inhibitory activities. Activity of four fractions from *H. fuziformi* and *D. marina* increased in the order of *n*-hexane > 85% aq.MeOH > *n*-BuOH > H_2O while that of *S. latiscula* increased in the order of 85% aq.MeOH > *n*-hexane > *n*-BuOH > H_2O, suggesting inhibitory activity is presumably attributed to relatively nonpolar constituents of seaweeds. In the case of *C. adhaerens*, the highest PTP1B inhibitory activity was observed from the *n*-BuOH fraction (69.4%), which is expected to include relatively polar constituents.

To our knowledge, this is the first report on PTP1B inhibitory effect of Korean seaweeds and salt marsh plants. From the above results, it is obvious that some seaweeds have PTP1B inhibitory effect, at least *in vitro*. This study suggests that several seaweed extracts have a potential as anti-diabetic agent.

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