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Decreasing mechanism of lipoxygenase-mediated peroxides by seaweed constituents

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Introduction

Lipoxygenases (EC 1.13.11.12) mediate peroxidation of lipids to produce hydroperoxylipids which are starting compounds of various mediators [1,2]. The products are converted to leukotrienes as inflammatory mediators. Lipoxygenase inhibitors are widely distributed in natural plant sources [1,2]. The inhibitors are expected to prevent inflammatory diseases. Various inhibitor candidates have obtained from seaweed extracts showing decreasing activity of hydroperoxide production mediated with soybean lipoxygenase, an n-6-lipoxygenase, in our study. They have been identified as fatty acid methyl ester mixture (FAMEM, **1**), carotenoid (fucoxanthin, FX, **2**), chlorophyll-related compound (pheophytin a, PPA, **3**) [3], and bromophenol (2,3-dibromo-4,5-dihydroxybenzyl methyl ether, BP, **4**) [4] (Fig. 1). However hydroperoxide-decreasing mechanisms of them are unclear. In the present study, we investigated that hydroperoxide-decreasing mechanisms of them, along with nordihydroguaiaretic acid (NDGA, **5**) as a positive control, using various lipoxygenase activity assays.

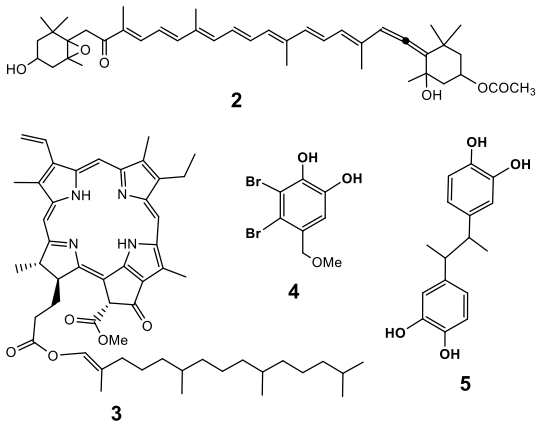

Fig. 1. Hydroperoxide-decreasing compounds.

Materials and methods

Fatty acid methyl ester mixture was isolated from the MeOH extract of *Chondrus yendoi*. Compounds **2**-**4** were previously obtained from *Sargassum horneri*, *Odonthalia corymbifera* and *Chondrus pinnulatus*, respectively. Compound **5** was purchased from Aldrich. Hydroperoxide production with lipoxygenase was assessed by indamine dye method converted from lipid peroxide using soybean lipoxygenase and linoleic acid as a substrate [3]. The hydroperoxide produced was converted with 3-methyl-2-benzothiazolinone hydrazone and 3-dimethylaminobenzoic acid to indamine dye. The assay consisted of lipoxygenase reaction (LR), indamine dye formation (IDF) and measurement of absorbance (MA) steps (Fig. 2). Test compounds at final concentration of 20 μg/mL were added before MA, LR and IDF steps in experiments A, B and C, respectively.

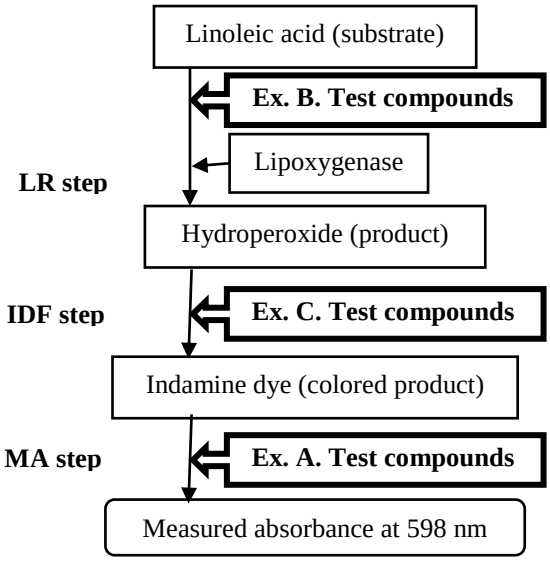

Fig. 2. Scheme of determination of hydroperoxide production mediated with lipoxygenase. Abbreviations of LR, IDF and MA steps are represented as lipoxygenase reaction, indamine dye formation and measurement of absorbance steps, respectively. Ex. A-C are represented as adding timing of teste compounds in experiments A-C.

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Results and discussion

*Effect of compounds **1**-**5** on produced indamine dye (experiment A)*

When compounds **1**-**5** were added before the AM step (experiment A), all the resulting absorbance values were same to the value without adding the test compound (Table 1). All the compounds examined did not affected the formed indamine dye.

Table 1. Relative absorbance by adding test compounds in the experiment 1

Compound	Relative absorbance[a]
1	1.02±0.02
2	0.98±0.02
3	1.02±0.02
4	0.98±0.02
5	0.98±0.02

[a]Relative absorbance to absorbance of the negative control without test compounds in the experiment 1. Data are mean±SEM (n=3).

*Effect of compounds **1**-**5** by adding before (experiment B) and after (experiment C) lipoxygenase reaction*

Decreasing ratios of product in experiments B and C (DRB and DRC) are employed for determination of mechanisms of hydroperoxide production decreasing. In the case of only lipoxygenase inhibition, while hydroperoxide production is decreased in experiment B, it would not be decreased in experiment C (DRC/DRB = 0). In the case of only hydroperoxide reduction, hydroperoxide production would be decreased on the same level in both experiments B and C (DRC/DRB = 1). In the case of both lipoxygenase inhibition and hydroperoxide reduction, decreasing ratio of hydroperoxide production in experiment B would be higher than experiment C (0 < DRC/DRB < 1). All the compounds **1**-**5** decreased hydroperoxide production monitored by absorbance of produced indamine dye in experiment B (Table 2). FAMEM (**1**), BP (**4**) and NDGA (**5**) did not decrease the production in experiment C (DRC/DRB = 0). Thus, compounds **1**, **4** and **5** were decided to be lipoxygenase inhibitors. Compound **1** may be a substrate mimic, and **4** and **5** may bind with protein. FX (**2**) decreased the production comparably in both experiments B and C (DRC/DRB = 1). These results suggested that compound **2** was a reductant to hydroperoxide, not a lipoxygenase inhibitor. Carotenoids are recognized to prevent various oxidative stress [5]. This function may come from chemical reducing potency. PPA (**3**) decreased hydroperoxide production in both experiments B and C, however decreasing ratio of hydroperoxide production in experiment B would be higher than experiment C (DRC/DRB = 0.41). Thus, compound **3** possesses both characteristics of an inhibitor and a reductant. Compound **3** consists of tetrapyrrole and phytol moieties. The former and the latter moieties may act as a reductant and a substrate mimic, respectively.

Table 2. Decreasing ratios of absorbance by adding test compounds in the experiments B and C

Compound	DRB[a] (%)	DRC[a] (%)	DRC/DRB	Mechanism[b]
1	43.6±1.3	0[c]	0	Inhibition
2	11.8±0.1	12.2±0.5	1.03	Reduction
3	24.4±1.5	9.9±0.3[c]	0.41	Both
4	21.7±3.1	0[c]	0	Inhibition
5	38.0±2.4	0[c]	0	Inhibition

[a] DRB and DRC were represented as decreasing ratios (%) of resulting absorbance to negative control in the experiments B and C, respectively. Data are mean±SEM (n=3).
[b]Decreasing mechanism of hydroperoxide: Inhibition, lipoxygenase inhibition; Reduction, reduction of hydroperoxide; Both, Both mechanisms of lipoxygenase inhibition and reduction of hydroperoxide.
[c]Significant difference vs DR2 ($p<0.05$).

Conclusions

Hydroperoxide-decreasing mechanisms of compounds **1**-**5**, derived from seaweeds and a well-known inhibitor, were investigated to determine lipoxygenase inhibitors for **1**, **4** and **5**, a reductant of hydroperoxide for **2**, and both an inhibitor and a reductant for **3**.

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