Journal of Applied Phycology (2006) 18: 409–412
DOI: 10.1007/s10811-006-9038-6

Formation of aldehyde flavor (*n*-hexanal, 3*Z*-nonenal and 2*E*-nonenal) in the brown alga, *Laminaria angustata*

Kangsadan Boonprab[1,*], Kenji Matsui[2], Yoshihiko Akakabe[2], Miyuki Yoshida[2], Norishige Yotsukura[3], Anong Chirapart[4] & Tadahiko Kajiwara[2]
[1]*Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok, 10900, Thailand;* [2]*Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, 753-8515, Japan;* [3]*Institute of Algological Research, Faculty of Science, Hokkaido University, Hokkaido, 051-0003, Japan;* [4]*Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok, 10900, Thailand*

[*]Author for correspondence: e-mail: ffisksb@ku.ac.th, bffisksb@yahoo.com; fax: +66(0)29428363

Key words: *Laminaria angustata*, lipoxygenase, fatty acid hydroperoxide lyase, *n*-hexanal, 3Z-nonenal, 2*E*-nonenal

Abstract

2*E*-Nonenal and *n*-hexanal are the major and minor flavor compounds in the edible brown alga, *Laminaria angustata*, respectively. They are believed to characterize the flavor of this alga. However the metabolism of the two compounds is not precisely known. The pathways were clarified by elucidation of the intermediate structure through purification of the intermediate compounds from an enzymatic reaction and identification using HPLC and GC-MS techniques. Formation of *n*-hexanal, 3*Z*-nonenal and 2*E*-nonenal are proposed to be *via* two cascades from unsaturated fatty acids. They are C18:2(*n*-6), linoleic acid cascade and C20:4(*n*-6), arachidonic acid cascade through their hydroperoxides as intermediates by the lipoxygenase/fatty acid hydroperoxide lyase pathway.

Introduction

Biogeneration of aldehydes in higher plants is generally known to be accomplished *via* one of the oxylipin pathways (lipoxygenase/fatty acid hydroperoxide lyase). C9 aldehydes like nonenal and nonadienal are formed from 9-hydroperoxides of unsaturated C18 fatty acids through a cleavage by fatty acid hydroperoxide lyase. On the other hand, in animals such as fish, these aldehydes could be formed *via* C20 or C22 fatty acid (Cadwallader, 2000). Marine algae have C20, C22 and C18 unsaturated fatty acids, and they can produce both plant (C18) and animal type (C20 and C22) fatty acid hydroperoxides. From this it has been postulated that nonenal may be formed from C18 and/or C20 unsaturated fatty acids *via* their hydroperoxides (Gerwick, 1994; Fujimura & Kawai, 2000). The pathway *via* the animal type hydroperoxides was then proposed in marine algae (Kajiwara, 1997). However, convincing evidence to support their enzymatic formation has not been obtained.

Not only nonenal is formed as a major component by the brown alga, *Laminaria angustata* (the well-known edible alga in Japan and other Asian countries) but *n*-hexanal (green, fresh flavor) is also formed and was reported as a minor component (Kajiwara et al., 1996). In higher plants, *n*-hexanal is formed *via* the lipoxygenase/fatty acid hydroperoxide lyase (LOX/HPL) system through 13-hydroperoxy linoleic acid (Blée, 1998). Thus, it was suggested that this pathway might exist also in the algae. Therefore, this study was aimed to clarify the metabolic pathway of the volatile aldehydes [C6 aldehyde (*n*-hexanal) and C9 aldehyde (3*Z*- and 2*E* – nonenal)] in *L. angustata*. We identified precursors for the aldehyde formation in homogenates of *L. angustata* as hydroperoxides of linoleic acid and arachidonic acid. Furthermore, two enzymatic pathways to generate C6 and C9 aldehydes through linoleic acid and

arachidonic acid were proposed. The finding is interesting because the brown alga can use different precursors for the production of short chain aldehydes, probably through different types of LOX/HPL systems. The experiments were performed and described in detail by the study of biogeneration of C6 and C9 aldehyde, biosynthesis of C6 aldehyde (*n*-hexanal) from linoleic acid and biosynthesis of C6 aldehyde (*n*-hexanal) and C9 aldehydes (*n*-hexanal, 3*Z*-nonenal and 2*E*-nonenal) from arachidonic acid, and the following results obtained.

Pathways of aldehyde formation

Biogeneration of C6 and C9 aldehydes

In this study, an enzymatic reaction was performed by the incubation of frond homogenate at 4 °C, for 80 min, to form volatile compounds that were explored by using simultaneous distillation extraction (SDE) and solid phase micro extraction (SPME) techniques. An increase in the compounds after incubation was observed, which suggested that they were formed by an enzymatic reaction, especially C6 aldehyde (*n*-hexanal) and C9 aldehyde (2*E*-nonenal). In the reaction with crude enzyme and unsaturated fatty acid as the substrate, C9 aldehydes (3*Z*-nonenal and 2*E*-nonenal) are mainly formed from arachidonic acid, while C6 aldehydes (*n*-hexanal) are formed from either C18 or C20 fatty acids (Boonprab et al, 2003b). This indicates that *Laminaria angustata* has at least two metabolic pathways to form short chain aldehydes.

Biosynthesis of C6 aldehyde (n-hexanal) from linoleic acid

The results from the above biogeneration study of C6 and C9 aldehydes indicate that *L. angustata* could form relatively high amounts of C6 and C9 aldehydes. When linoleic acid was added to a homogenate prepared from the fronds of this alga, formation of *n*-hexanal was observed. When glutathione peroxidase was added to the reaction mixture together with glutathione, the formation of *n*-hexanal from linoleic acid was inhibited, and oxygenated fatty acids accumulated. By chemical analyses, one of the major oxygenated fatty acids was shown to be (*S*)-13 hydroxyoctadecadienoic acid. Therefore, it is assumed that *n*-hexanal is formed from linoleic acid *via* a sequential action of LOX and HPL, by a similar pathway as the counterpart found in higher plants. HPL partially purified from the fronds has a rather strict substrate specificity, and only 13-hydroperoxide of linoleic acid, and 15-hydroperoxide of arachidonic acid are the essentially suitable substrates for the enzyme. (Boonprab et al., 2003a)

Biosynthesis of C6 aldehyde (n-hexanal) and C9 aldehydes (n-hexanal, 3Z-nonenal and 2E-nonenal) from arachidonic acid

In higher plants, C6 and C9 aldehydes are formed from C18 fatty acids, such as linoleic acid or linolenic acid, through the formation of 13- and 9-hydroperoxides, followed by their stereospecific cleavage by fatty acid hydroperoxide lyases. Some marine algae can also form C6 and C9 aldehydes, but the precise biosynthetic pathway has not been fully elucidated. According to the biogeneration of C6 and C9 aldehydes study, *L. angustata* could generate C6 and C9 aldehydes enzymatically. C9 aldehydes were formed exclusively from the C20 fatty acid, arachidonic acid, while C6 aldehydes are derived either from C18 or from C20 fatty acid. Thus experiments to identify the intermediates in the reaction were set up. The intermediates were trapped using a glutathione/glutathione peroxidase system, and subjected to structural analyses by co-injection with the standard hydroperoxide compounds, and by GC-MS for their mass spectrum. Formation of (*S*)-12-, and (*S*)-15-hydroperoxy arachidonic acids [12(*S*) hydroperoxyeicosatetraenoic acid and 15(*S*) hydroperoxyeicosatetraenoic acid] from arachidonic acid could be found and confirmed by chiral HPLC analyses (Boonprab et al., 2003b). This accounts respectively for the formation of C9 and C6 aldehydes. The fatty acid hydroperoxide lyase that catalyzes formation of C9 aldehydes from 12(*S*) hydroperoxyeicosatetraenoic acid seems highly specific for hydroperoxides of C20 fatty acids.

Conclusion

Based on these results it is proposed that there are at least two pathways to form volatile aldehydes in *L. angustata* as shown in Figure 1. The brown algae can use different precursors for the production of short chain aldehydes, probably through different types of LOX/HPL systems.

The marine algae are major components of the earth's biomass, responsible for significant carbon fixation, and occupy an extreme diversity of climatic

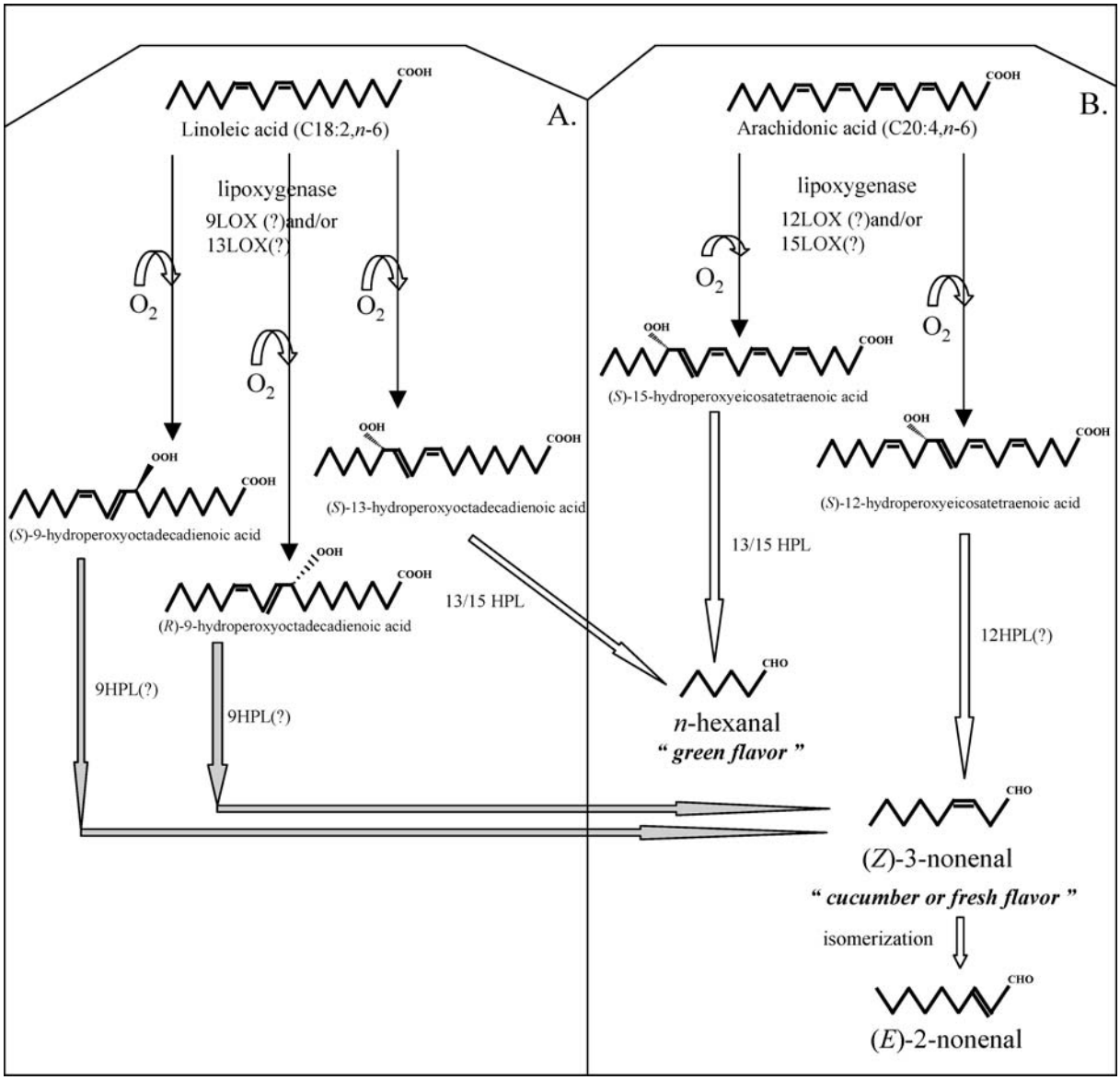

Figure 1. The proposed pathway for the metabolism of linoleic acid and arachidonic acid mediated C6 (*n*-hexanal) and C9 aldehydes [(*Z*)-3-nonenal and (*E*)-2-nonenal] branch of oxylipin pathway in the brown alga, *L. angustata*.
LOX: Lipoxygenase
HPL: Fatty acid hydroperoxide lyase
⇒Minor pathway
⇨Major pathway
A. was reported by Boonprab et al. (2003a)
B. was reported by Boonprab et al. (2003b)

niches. Further studies would provide insight into the physiology or regulation of these pathways, which may be involved in growth development, chemical defense, oxidative stress or other mtabolic functions in algae.

Acknowledgements

The support of the JSPS-NRCT Core University Program on "Development of thermotolerant microbial resources and their applications" under the cooperation

of Japanese and Thai scientists, in association with Kasetsart University (Thailand) and Yamaguchi University (Japan), is acknowledged.

References

Blée E (1998) Phytooxylipins and plant defense reactions. Prog. Lipid Res. 37: 33–72.

Boonprab K, Matsui K, Yoshida M, Akakabe Y, Chirapart A, Kajiwara T (2003a) C6-aldehyde formation by fatty acid hydroperoxide lyase in the brown alga *Laminaria angustata*. Z. Naturf. 58c: 207–214.

Boonprab K, Matsui K, Akakabe Y, Norishige Y, Kajiwara T (2003b) Hydroperoxy-arachidonic acid mediated *n*-hexanal and (*Z*)-3- and (*E*)-2-nonenal formation in *Laminaria angustata.* Phytochem. 63: 669–678.

Cadwallader KR (2000) Enzymes and Flavor Biogenesis in Fish. In Haard NF, Simpson BK (eds.), Seafood Enzymes. Utilization and Influence on Postharvest Seafood Quality, Marcel Dekker, Inc., New York: pp. 365–383.

Fujimura T, Kawai T (2000). Enzymes and seaweed flavor. In Haard N.F., Simpson BK (eds.), Seafood Enzymes. Utilization and Influence on Postharvest Seafood Quality, Marcel Dekker, Inc., New York, pp. 385–407.

Gerwick WH, Proteau PJ., Nagle DG, Wise ML, Jiang ZD, Bernart MW, Hamberg M (1993) Biologically active oxylipins from seaweeds. Hydrobiologia 260/261: 653–665.

Kajiwara T, Matsui K, Akakabe Y (1996) Biogeneration of volatile compounds via oxylipins in edible seaweeds. In Takeoka GR, Teranishi R, Williams PJ, Kobayashi A (eds.), Biotechnology for Improved Foods and Flavors, American Chemical Society Symposium Series 637, Washington, DC, pp. 146–166.

Kajiwara T (1997) Dynamic studies on bioflavor of seaweed. Koryo 196: 61–70. (in Japanese with English summary)