Bull. Korean Fish. Soc. 25(6), 501~510, 1992 韓水誌, 25(6), 501~510, 1992

Characterization of Lipid Prooxidants in Sardine Skin

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Lipid prooxidants in sardine skin was characterized. Prooxidants in the sardine skin extract with 0.05*M* phosphate buffer was purified by successive chromatography on Sephadex G-200, DEAE-Sephadex A-50 and CM-Sephadex A-50. Prooxidants of sardine skin exist mainly in the intermediate molecular weight fractions. Observations of the thermounstability and optimum pH(pH 7.0) suggest that the major prooxidants are enzymes and hemoproteins. They can oxidize well both free and esterified linoleic acid and form conjugated hydroperoxides. From these results, the major prooxidants in sardine skin are assumed to be lipoxygenase-like enzymes.

Introduction

Lipid oxidation in marine foods is much more serious than in any other food, because fish contains specifically abundant of polyunsaturated fatty acids such as eicosapentaenoate(EPA) and docosahexaenoate(DHA). Oxidation of polyunsaturated fatty acids can proceed autocatalytically, but the reaction may also be catalyzed by transit metals and hemoproteins. Hydrolytic enzymes like lipase and phospholipase can also accelerate oxidation, because free fatty acids are known to be more labile to oxidation than esterified ones. Lipid oxidation in marine foods containing these catalytic compounds is suggested to be initiated by these enzymatic and nonenzymatic reactions(Hardy, 1980).

We have previously suggested the occurrence of strong prooxidants in sardine skin, because the oxidation of ethyl EPA proceeded most rapidly in skin in dry model systems(Cho *et al.*, 1989a, 1989 b). Furthermore, prooxidants in sardine skin might be some enzymes, because they were labile to cook. The preferential oxidation of skin lipids in both lean and fatty fish has been recognized by many investigators(Toyomizu *et al.*, 1980; Ke and Ackman, 1977; Yamaguchi *et al.*, 1984). Reports by Tsukuda and co-workers(Tsukuda and Amano, 19 67, 1968; Tsukuda, 1970a, 1970b) provided a definitive evidence for the involvement of enzyme(s) in the oxidative deterioration of fish skin lipids by showing that fading or discoloration of carotenoid pigments in the skin of red fishes could be delayed by introducing a variety of enzyme inhibitors to skin extracts. Also, the involvement of lipoxygenase in the initiation of lipid oxidation in fish skin tissue extracts containing high heme concentrations has been recently reported(Yamaguchi and Toyomizu, 1984; German and Kinsella, 1985, 1986). However, all these papers suggesting the involvement of some enzymes, tentatively lipoxygenase, in lipid oxidation of fish skin, used a crude extracts from fish skin as the enzyme preparation. Therefore, so far it is still obscure whether lipoxygenase really exists in fish skin or not.

In the present studies, to characterize the prooxidants existing in sardine skin, it was extracted with several buffers with different ionic strength, and the prooxidant activity of each extract and fraction by various column chromatography were evaluated.

Materials and Methods

Preparation of Acetone Powder

Skin, ordinary and dark muscles were excised from fresh sardine *Sardinops melanosticta* purchased from a fish market of Sendai, Japan. Each tissues was homogenized with an equivalent volume of deionized water and then centrifuged at 0℃ by 10,000 rpm for 15 min. The upper lipid layer was successively extracted with 3 times volume of cold acetone and ethyl ether. The resulting precipitate was airblast-dried at 5℃.

Preparation of Crude Enzyme Extracts from Skin of Sardine

Skin was removed from fresh sardine and homogenized with 3 times volume of deionized water at 5℃. The homogenate was centrifuged at 0℃, by 10,000 rpm for 15 min. The supernatant was filtered under a reduced pressure through a Toyo No. 2 filter paper and used for the subsequent experiments. On the other hand, the precipitate was further extracted stepwise with 0.05, 0.2 and 0.5*M* sodium phosphate buffer at pH 7.0, and then each extract was treated similarly with the supernatant of deionized water-extraction as described above. All steps of preparation was performed at 5℃ except where otherwise noted.

Gel Filteration and Ion Exchange Column Chromatography of Sardine Skin Extracts

The extract from sardine skin was fractionated by gel exclusion chromatography on a Sephadex column. An aliquots of the extract was loaded on a Sephadex G-200 column(2.5×90*cm*) and eluted with 0.05*M* phosphate buffer(pH 7.0) at 17*ml/h*. Fractions of 5*ml* were collected with the aid of a fraction collector. Alternative fractions were assayed for prooxidant activity, protein and heme contents. The prooxidant fractions were poured into cellophane tubes and concentrated with polyethylene glycol.

The concentrated Sephadex G-200 pooled fraction was loaded on a DEAE-Sephadex A-50 column (1.2×20*cm*) previously equilibrated with 0.01*M* sodium phosphate buffer(pH 7.0) and eluted with the same buffer(90*ml*). Then, the column was eluted with a linear gradient solution consisting of 0.01*M* phosphate buffer(pH 7.0) and 1*M* NaCl containing 0.01*M* phosphate buffer(pH 7.0). Elutions were achieved by gravitational flow at a rate of 20*ml/h* and fractions of 3*ml* each were collected. Three prooxidant fractions corresponding to three separate peaks were pooled, concentrated and dialyzed against 0.01*M* phosphate buffer(pH 7.0). The major fraction(G-II-1) was applied to a cation-exchange CM-Sephadex A-50 column(1.2×20*cm*) previously equilibrated with 0.01*M* sodium phosphate buffer (pH 6.5). The column was eluted with 90*ml* of the same buffer. Then the column was eluted with an increasing linear gradient solution consisting of 0.01 *M* phosphate buffer(pH 6.5) and 1*M* NaCl containing 0.01*M* phosphate buffer(pH 6.5). The elution method was similar to that for the DEAE-Sephadex A-50 column chromatography. All three columns chromatography were performed at 5℃.

Determination of Prooxidant Activity

Linoleic(Lo) and eicosapentaenoic(EPA) acids were used as the substrate. The substrate solution with a concentration of 50mM Lo or 2.5～10mM EPA was prepared by the method of Surrey(1964) using 1.2% (w/v) Tween 20 as an emulsifier. Each reaction mixture consisted of 0.5*ml* of substrate solution, 0.5*ml* of enzyme preparation and 4.0*ml* of 0.2*M* sodium phosphate buffer(pH 6/7). The reaction mixture was incubated at 25℃ with constant shaking. The prooxidant activity was determined by oxygen absorption method or measurement of conjugated diene formation rate. The oxygen absorption was determined by gas chromatography with a thermoconductivity detector as described previously(Cho *et al.*, 1987), and the activity was expressed as oxygen content consumed per min at 25℃($nmol \cdot min^{-1} \cdot g^{-1}$ or *nmol/ml*). On the other hand, the conjugated diene formation rate was measured as follows: After incubation for an appropriate time(20～60min), 0.5*ml* of the reaction mixture was withdrawn and diluted with 4.5*ml* of 60% ethanol. Absorbance at 234*nm* of the resultant clear alcoholic solution was read against the control prepared at zero time of incubation. The activity was

expressed as conjugated diene content formed for the first 20min of the incubation at 25℃.

Determination of Protein and Heme Concentration

Protein concentration was determined by the method of Lowry *et al.*(1951) or from the absorbance at 280*nm*. Heme content was determined from Soret absorbance at 420*nm*(Parkin and Hultin, 1987).

Inhibitory Effect of Various Reagents on Prooxidant Activity

Various antioxidants or enzyme inhibitors(0.5~2 *mM*) were added to crude enzyme extracts, and incubated with 0.25*mM* of EPA at 25℃ for 1*h*. After incubation, the residual activity was determined by oxygen absorption rate of the mixture as described above.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed on the prooxidant fractions according to the method of Laemmli(1970) using a 7.5% acrylamide gel at 5℃ with a voltage of 200V.

Results and Discussion

Lipid Proxidant Activity of Sardine Skin

The prooxidant activity in emulsion system(pH 6, 25℃) of skin, dark and ordinary muscles of sardine is presented in Table 1. The activity was determined as oxygen absorption rate of the reaction mixture containing 1*mM* of EPA as the substrate. It was confirmed in this emulsion system where enzymes are more active than in dry systems that the skin among various parts of sardine has the strongest prooxidant activity similar to that of in dry model systems reported previously(Cho *et al.*, 1989 a, 1989b).

To isolate the prooxidant matters from sardine skin, the sardine skin was extracted gradually with 0.05, 0.2 and 0.5*M* sodium phosphate buffer at pH 7.0. The effect of pH on the oxidation(oxygen absorption rate of the mixture with 0.3*mM* of EPA) of the sardine skin extracts at 25℃ for 30min is presented in Fig. 1. The extract with 0.05*M* phosphate buffer showed the most prominent prooxidant activity among the other extracts of sardine skin. The optimum pH was found to be approximately 4. Whereas, the fractions extracted with buffers with higher ionic strength showed relatively a low activity which is nonspecific to pH. From these experiments, the major prooxidants in sardine skin are suggested to be extractable with 0.05*M* phosphate buffer.

Table 1. Prooxidant activity* of skin, dark and ordinary muscles of sardine at 25℃, pH 6

($nmol \cdot min^{-1} \cdot$dry matter g^{-1})

Ordinary muscle	0.01
Dark muscle	0.22
Skin	0.66

* Oxygen absorption rate of the mixture with 1 *mM* of EPA.

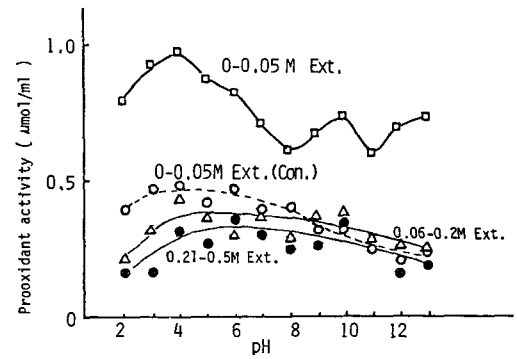

Fig. 1. The pH dependance of prooxidant activity (oxygen absorption rate of the mixture with 0.3*mM* of EPA) of sardine skin extracts at 25℃ for 30min.

Effect of Enzyme Inhibitors and Heating on the Lipid Prooxidants in Sardine Skin

Table 2 shows the inhibitory effects of various reagents and heating(100℃, 5*min*) on the prooxidant activity of the skin extract with 0.05*M* phosphate buffer. The reagents were preincubated with the skin extracts at 25℃ for 1*h*. Heating at 100℃

for 5min inhibited 89% of the original activity. This result indicates that the major prooxidant catalyst (s) in sardine skin are heat-labile and may be enzyme(s). Addition of KCN inhibited oxidation almost completely(93%) at pH 4. O'Brien(1969) reported the complete inhibition of hemoprotein-catalyzed hydroperoxide decomposition by cyanide. The strong inhibition of linoleate oxidation by KCN suggests the involvement of hemoproteins or any other type of transit metal-containing protein in prooxidant activity(Decker and Schanus, 1986). Furthermore, the addition of aspirin or allopurinol, which are inhibitors of cyclooxygenase or xanthineoxidase, respectively, also blocked oxidation about 30%.

Although we used the oxygen absorption method for prooxidant activity determination, it was suggested that this method may give exaggerated values in case of crude preparations; because many oxidation metabolisms, such as respiration, can proceed druing incubation(Fig. 1). Therefore, alternative conjugated diene method was also applied to determine the prooxidant activity of sardine skin. The time course of conjugated diene formation in 5*mM* linoleic acid(Lo) by incubation with the sardine skin extract(0.05*M* phosphate buffer) at 25℃ is shown in Fig. 2. The conjugated diene formation in the sardine skin extract incubated without a lipid substrate(Control) was negligible during 1*h* of incubation at 25℃, while in the sardine skin extract incubated with 5*mM* Lo; it increased linearly for 30 min at pH 4 and 7. These results showed that the prooxidant activity of sardine skin extract was confirmed by the conjugated diene method as well as by the oxygen absorption method.

Table 2. Inhibitory effects of various reagents and heating(100℃, 5*min*) on prooxidant activity of the skin extract(0~0.05*M* fraction) at 25℃ for 1*h**

	Concentration	Inhibition(%)	
	(*mM*)	pH 4	pH 6
Heating		89	-
BHA	0.5	40	-
KCN	2	93	41
Aspirin	2	-	32
Allopurinol	2	-	34

* Oxygen absorption rate of the mixture with 0.25*mM* of EPA.

** -: not determined.

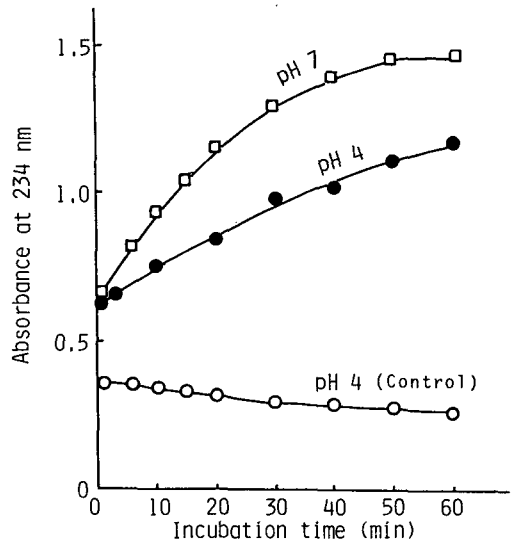

Fig. 2. Time course of conjugated diene formation by incubation of sardine skin extract(0~0.05*M* fraction) with 5*mM* linoleic acid at 25℃.

Isolation of the Lipid Prooxidants

Sardine skin extract by 0.05*M* phosphate buffer was resolved into four proteinic fractions by Sephadex G-200 chromatography as shown in Fig. 3. The prooxidant activity on linoleate was localized mainly in the 2nd and 3rd fractions. Plottings of the prooxidant activity gave one small and two major peaks, which were incompatible with the heme content estimated by Soret absorbance(Fig. 3). Heating of the each prooxidant fraction at 100℃ for 5min inhibited the activity as follows: G-I, 93%, G-II, 70% and G-III, 15%. The high thermostability of G-III fraction suggests that the catalyst in G-III fraction may not be enzymatic. Hence, the peak G-III was found to coincide in the position with that of hemoprotein. As the nonenzymatic prooxidant activity of hemoprotein such as hemoglobin and myoglobin are known to be not retarded but accelerated by heat treatment(Eriksson, 1987), the activity of G-III are suggested to nonenzymatic.

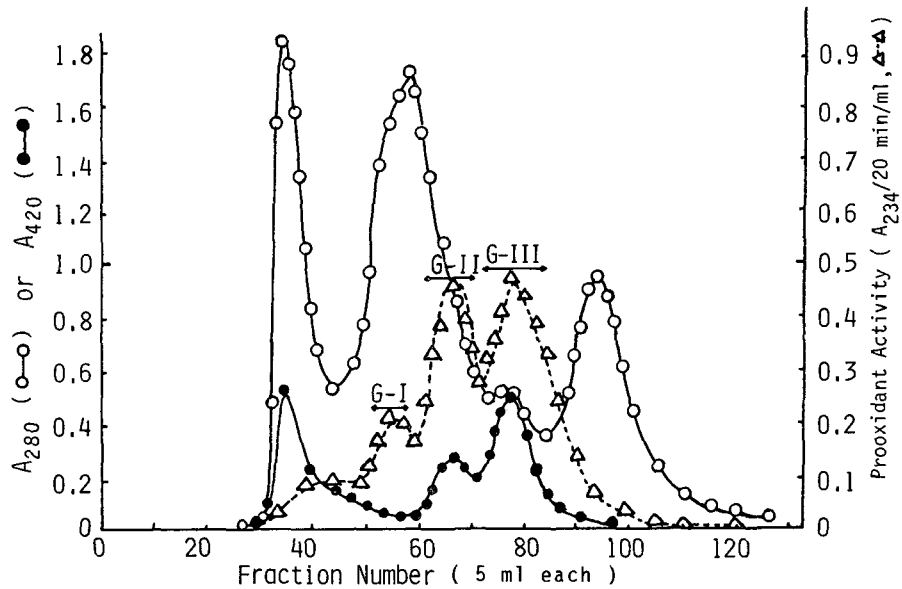

Fig. 3. Elution profile of Sephadex G-200 gel filtration (2.5×95*cm*) of crude sardine skin extract.

However, G-II fraction is suggested to contain some prooxidant enzymes judging from the heat inactivation. Though G-I was also suggested to contain some lipid-oxidizing enzyme(s), this fraction was not examined further because this is a minor constituent. The G-II fraction was further resolved with DEAE-Sephadex A-50 anion-exchange resin. Three proteinic fractions, G-II-1~3, were obtained as shown in Fig. 4. Fraction G-II-1 showed the highest prooxidant activity in the three fractions. Because this fraction was eluted without being adsorbed to the anion-exchange resin, the fraction G-II-1 was applied successively to CM-Sephadex A-50 cation-exchange chromatography (Fig. 5). Three proteinic fractions were obtained as shown in Fig. 5. Among three proteinic fractions the fraction CM-1 showed the most prominent prooxidant activity. Inhibitory effects of heat treatment of these ion-exchange chromatographic fractions at 100℃ for 5min on the prooxidant activity are as follows; G-II-1, 70%; G-II-2, 89%; G-II-3, 94%; CM-1, 80% and CM-2, 94%. Almost complete heat inactivation of these prooxidant fractions suggests that the prooxidants in these fractions may be enzymes.

To observe the protein composition of prooxidant fractions of chromatography on DEAE 50 and CM 50 columns, they were subjected to polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue. The results are summarized in Fig. 6. The relative mobility of fraction G-II-2(Fig. 4) was similar to that of catalase with about 240,000 Dalton of molecular weight. Moreover, the relative mobilities of the high prooxidant fractions, G-II-2 (Fig. 4) and CM-1(Fig. 5) clearly differed from those of myoglobin and hemoglobin.

The pH Dependence on Activity

The effect of pH on the prooxidant activity of the crude enzyme preparation(G-II, Fig. 3) is presented in Fig. 7. The optimal pH was approximately 7.0. Will(1965) reported that an increase in non-heme iron catalyzed oxidation of linoleate with decreasing pH, and Eriksson *et al.*(1971) reported similar results with ferrous and cupric ion catalyzed oxidation. Ben-Aziz *et al.*(1970) reported that optimal pH values for hemoproteins ranged from 4 to 5. From these published data, because the unsaturated fatty acid oxidation non-enzymatically catalyzed by transition metals or hemoprotein proceeds more rapidly in the acidic condition than in the neutral, the catalytic mechanism in sardine skin is

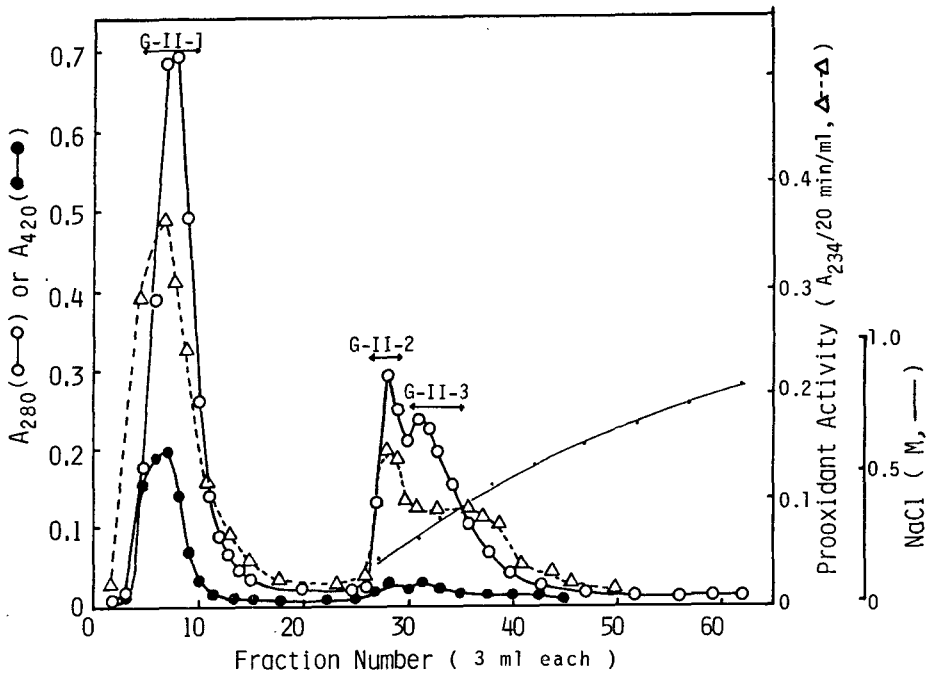

Fig. 4. DEAE-Sephadex A-50 gradient anion-exchange column(1.2×15*cm*, pH 7, 0.01*M* sodium phosphate buffer, 10*ml/h*) chromatography of G-200 intermediate molecular weight fraction (G-II) from the crude sardine skin extract.

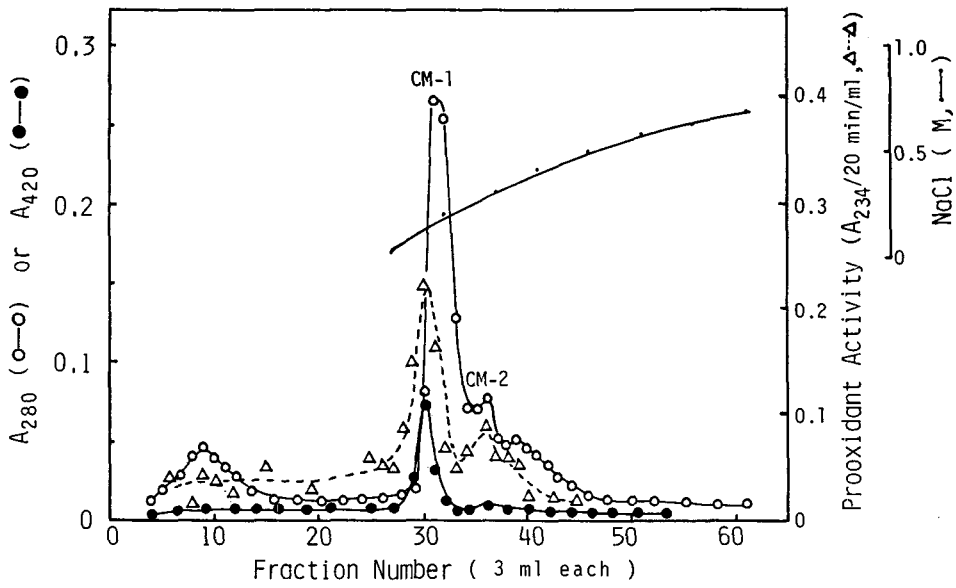

Fig. 5. CM-Sephadex A-50 gradient cation-exchange column(1.2×20*cm*, pH 7, 0.01*M* sodium phosphate buffer, 10*ml/h*) chromatography of crude sardine skin extract, previously chromatographed in DEAE-Sephadex A-50 column.

suggested to be different from the non-enzymatic one due to transition metals or hemoproteins. McDonald *et al.*(1979) reported an optimum pH of 6.5 for microsomal lipid oxidation of red hake muscles. Although no enzyme responsible for lipid oxidation could be isolated from fish skin, Tsukuda(1972) reported the occurrence of an enzyme which promotes the oxidative discoloration of a red pigment in skin of rockfish and also Yamaguchi and Toyomizu(1984) found a "lipoxygenase-like" enzyme in sardine skin. The optimum pH of these enzymes were reported to be 7 and 13, respectively. A pH optimum of 7.0(Fig. 7) also suggests that the prooxidant activity of fraction G-II(Fig. 3) may be at least partially enzymatic in nature as well as its heat-unstability.

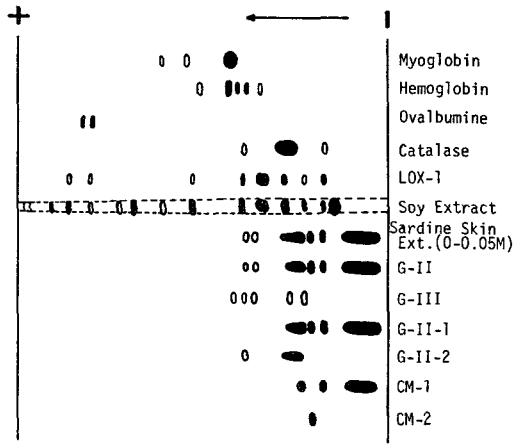

Fig. 6. Polyacrylamide gel electrophoresis of prooxidant enzymes obtained from various purification steps of the crude sardine skin extract.

Substrate Specificity

A variety of lipoxygenases have been examined for their substrate specificity and found to show different activities on esterified fatty acids. For example, among three isoenzymes of soybean lipoxygenase, L-2 can oxidize glycerol esters, while L-1 and -3 can oxidize only free fatty acids(Gardner, 1980). On the other hand, animal lipoxygenases which localize in leucocytes and involve in the leucotriene formation are regarded to be specific to free fatty acids(Hansson *et al.*, 1983). Cytochrome c, which is also responsible for lipid peroxidation in biological systems, stimulates the autoxidation of free fatty acids but unable to catalyze the peroxidation of methyl esters(Grossman *et al.*, 1972). As shown in Table 3, the sardine skin extract(Fr. CM-1) is highly active on methyl Lo. This fact suggests that the sardine skin prooxidant enzyme(s) are di-

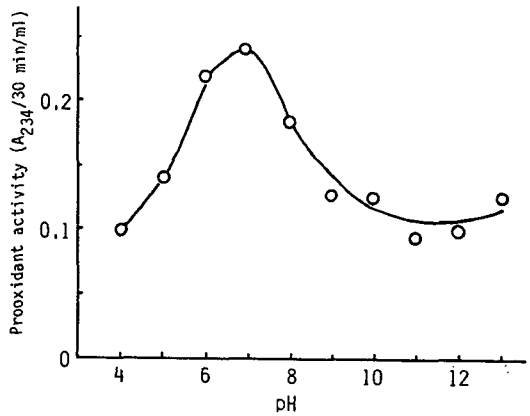

Fig. 7. Effect of pH on prooxidant activity of G-200 intermediate molecular weight fraction(G-II) from the crude sardine skin extract.

Table 3. Substrate specificity of partially purified prooxidant enzyme in sardine skin

Substrate	Relative activity			
	Sardine skin prooxidant enzyme (CM-1 fraction: pH 7)	Horse heart[a] cytochrome c (pH 11)	Eggplant[b] lipoxygenase (pH 6.5)	Fusarium[b] lipoxygenase (pH 13)
Linoleate	100	100	100	100
Methyl linoleate	73	0	21	49

a) The data are quoted from *report of Grossman et al.*(1972).
b) The data are quoted from report of *Matsuda et al.*(1976).

stinctly different from cytochrome c and leucocyte lipoxygenase(Hansson *et al.*, 1983; Grossman *et al.*, 1972). Furthermore, this sardine skin prooxidant enzyme are suggested to contain heme(Fig. 3~6). This property is quite different from general lipoxygenases containing non-heme iron(Gardner, 1980) as an active center. Only one lipoxygenase has been known to contain heme iron so far. That enzyme was isolated from a fungi *Fusarium oxysporum* (Matsuda *et al.*, 1976).

From these results of the experiments, the occurrence of highly prooxidant and thermounstable substances were demonstrated in sardine skin. The prooxidants are resonably assumed to be enzymes and can oxidized linoleate to conjugated diene-hydroperoxides(Fig. 2). Therefore, the prooxidants in sardine skin are suggested to be lipoxygenase or lipoxygenase-like enzymes. Further studies, especially the stereospecific abstraction of proton and successive oxygenation should be proved to identify these enzymes as lipoxygenases together with the specificity on cis, cis-1, 4-pentadiene structure. The occurrence of lipid prooxidants in fish tissues has been noticed since long but the activity has been attributed to the non-enzymatic catalysis by hemoprotein such as hemoglobin and myoglobin. Although the enzymes responsible for linoleate oxidation found in the present studies contain heme, these are apparently different from either hemoglobin or myoglobin. The characterization of prooxidants in fish tissues are believed to be beneficial and even essential to prevent the lipid oxidation in fish products.

Acknowledgment

This research was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

References

Ben-Aziz, A., S. Grossman, I. Ascarelli and P. Budowski. 1970. Linoleate oxidation induced by lipoxygenase and heme proteins; A direct spectrophotometric assay. *Analytical Biochemistry,* 34, 88~100.

Cho, S. Y., K. Miyashita, T. Miyazawa, K. Fujimoto and T. Kaneda. 1987. Autoxidation of ethyl eicosapentaenoate and docosahexaenoate under light irradiation. *Nippon Suisan Gakkaishi,* 55, 541~544.

Cho, S. Y., Y. Endo, K. Fujimoto and T. Kaneda. 1989a. Oxidative deterioration of lipids in salted and dried sardine during storage at 5℃. *Nippon Suisan Gakkasihi,* 55, 541~544.

Cho, S. Y., Y. Endo, K. Fujimoto and T. Kaneda. 1989b. Autoxidation of ethyl eicosapentaenoate in a defatted fish dry model system. *Nippon Suisan Gakkasihi,* 55, 545~552.

Decker, E. A. and E. G. Schanus. 1986. Catalysis of Linoleate oxidation by soluble chicken muscle proteins. *J. Am. Oil Chem. Soc.,* 63, 101~104.

Eriksson, C. E. 1987. In "Autoxidation of unsaturated lipids"(ed. by H. W. -S. Chan), Academic Press, London, pp. 207~231.

Eriksson, C. E., P. A. Olsson and S. G. Svensson. 1971. Denatured hemoproteins as catalysts in lipid oxidation. *J. Am. Oil Chem. Soc.,* 48, 442~447.

Gardner, H. W. 1980. In "Autoxidation in food and biological systems"(ed. by M. G. Simic and M. Karel), Plenum Press, New York, pp. 447~504.

German, J. B. and J. E. Kinsella. 1985. Lipid oxidation in fish tissue; Enzymatic initiation via lipoxygenase. *J. Agric. Food Chem.,* 33, 680~683.

German, J. B. and J. E. Kinsella. 1986. Lipoxygenase in trout gill tissue affecting on arachidonic, eicosapentaenoic, and docosahexaenoic acids. *Biochim. Biophys. Acta.,* 875, 12~20.

Grossman, S., M. Trop, R. Avtalion and A. Pinsky. 1972. Egg plant lipoxygenase; Isolation and partial characterization. *Lipids,* 7, 467~475.

Hansson, G., C. Malmstein and O. Radmark. 1983. In "Prostaglandins and ralated substances"(ed. by Pace-Asciak and E. Granstorm), Elsevier, Amsterdam, pp. 127~158.

Hardy, R. 1980. In "Advances in fish science and

technology"(ed. by J. J. Connell), Fishing News Books Press, Farnham, England, pp. 103~111.

Ke, P. J. and R. G. Ackman. 1977. Metal-catalyzed oxidation in mackerel skin meat lipids. *J. Am. Oil Chem. Soc.*, 54, 417~420.

Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T_4. *Nature*(London), 227, 680~685.

Lowry, O. H., A. L. Farr, N. J. Rosenbrough and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, 193, 265~275.

Matsuda, Y., T. Sato, M. Takashio, K. Sato, T. Beppu and K. Arima. 1976. Purification and properties of Co^{2+} requiring heme protein having lipoxygenase activity. *Agric. Biol. Chem.*, 40, 963~976.

McDonald, R. E., S. D. Kellefer and H. O. Hultin. 1979. Membrane lipid oxidation in a microsomal fraction from red hake muscle. *J. Food Biochemistry*, 3, 125~134.

O'Brien, P. J. 1969. Intracellular mechanism for the decomposition of a lipid peroxide. I. Decomposition of a lipid peroxide by metal ions, heme compounds, and nucleophiles. *Can. J. Biochem.*, 47, 485~490.

Parkin, K. L. and H. O. Hultin. 1987. Spectrophotometric evidence for a hemoprotein in fish muscle microsomes: Possible involvement in trimethylamine N-oxide(TMAO) demethylase activity. *J. Agri. Food Chem.*, 35, 34~41.

Toyomizu, M., K. Hanaoka and T. Nakamura. 1980. Lipid oxidation in the skin during storage of fish in the round at -5℃ and the susceptibility predictable for lipid oxidation. *Nippon Suisan Gakkaishi*, 46, 1011~1018.

Tsukuda, N. 1970a. Studies on the discoloration of red fishes—VI. Partial purification and specificity of the lipoxidase like enzyme responsible for carotenoid discoloration in fish skin. *Nippon Suisan Gakkaishi*, 36, 725~733.

Tsukuda, N. 1970b. Studies on the discoloration of red fishes—VII. Effect of chemicals on enzymatic discoloration. *Nippon Suisan Gakkaishi*, 36, 806~811.

Tsukuda, N. 1972. Studies on the discoloration of red fishes. *Bull. Tokai. Reg. Fish. Res. Lab.*, 70, 108~174.

Tsukuda, N. and K. Amano. 1967. Studies on the discoloration of red fishes—IV. Discoloration of astaxanthin, tunaxanthin, and β-carotene by the tissue homogenates of fishes. *Nippon Suisan Gakkaishi*, 33, 962~969.

Tsukuda, N. and K. Amano. 1968. Discoloration of red fishes. II. An enzyme involved in the discoloration of carotenoid pigments in fish skin tissues. *Nippon Suisan Gakkaishi*, 34, 633~639.

Yamaguchi, K. and M. Toyomizu. 1984. Acceleration of lipid oxidation in the extract from the skin of fish. *Nippon Suisan Gakkaishi*, 50, 2049~2054.

Yamaguchi, K., T. Nakamura and M. Toyomizu. 1984. Preferential lipid oxidation in the skin of round fish. *Nippon Suisan Gakkaishi*, 50, 869~874.

Wills, E. D. 1965. Mechanisms of lipid peroxide formation in tissues; Role of metals and haematin proteins in the catalysis of the oxidation of unsaturated fatty acids. *Biochem. J.*, 98, 238~251.

Received October 2, 1992
Accepted November 6, 1992

정어리표피중에 존재하는 지방산화촉진물질의 검색확인 및 그 특성구명

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정어리표피 중에 존재하는 지방산화촉진물질의 검색확인 및 그 특성구명을 시도하였다. 정어리표피중의 산화촉진물질은 0.05*M* 인산염 완충액으로 거의 모두 추출되었고, 이 조추출액에 대해 Sephadex G-200, DEAE-Sephadex A-50 및 CM-Sephadex A-50 등으로 연속적인 분리를 행하였다. 정어리표피 중의 주 산화촉진물질은 열에 극히 불안정하고, 지방산화촉진의 최적 작용 pH가 7이며, 특징적인 Sohret흡수를 나타내는 점 등으로부터 햄을 가진 효소임을 알 수 있었다. 특히 이들은 기질인 유리 및 에스테르화 리놀산에 모두 잘 작용하여 빠른 속도로 공역과산화물을 형성하였다. 이상의 결과로부터 정어리표피 중의 주 지방산화촉진물의 본태는 새로운 형태의 리폭시지네이스유사 효소인 것으로 추정되었다.