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Lipid Fractions with Aggregatory and Antiaggregatory Activity toward Platelets in Fresh and Fried Cod (*Gadus morhua*): Correlation with Platelet-Activating Factor and Atherogenesis

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Cod (*Gadus morhua*) is a popular part of the diet in many countries on both sides of the North Atlantic; in most cases it is consumed fried. In this study, total lipids of cod muscle were separated into neutral and polar lipids, which were further fractionated by HPLC. The lipid fractions were tested in vitro, against washed rabbit platelets, for the probable existence of lipid compounds that either exhibit an action similar to that of platelet-activating factor (PAF) or inhibit the action of PAF. The platelet bioassay was used to evaluate total lipids, total polar lipids, and total neutral lipids, before any further separation. Detection of these compounds in fresh and fried cod could be used to evaluate the nutritional value of this important fish. The in vitro biological study of lipids showed that in fresh cod lipid fractions, ranges of PAF-like and anti-PAF-like activities were present, whereas in fried cod lipid fractions, both neutral and polar, anti-PAF activities were mainly observed. Because it has already been reported that PAF is involved in atheromatosis generation, the existence of PAF inhibitors in cod may contribute to the possible protective role of fish, in this case cod, against atherosclerosis.

Keywords: *Cod (Gadus morhua); platelet-activating factor (PAF); PAF inhibitors, lipids; frying*

INTRODUCTION

Over the past decade, there has been increasing concern about the rising number of various types of coronary heart disease. The inflammation mediator (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is known to be a platelet-activating factor (PAF) that may be involved in atherogenesis (Demopoulos et al., 1979; Koltai and Braquet, 1992; Liapikos et al., 1994). PAF is thought to play a major role in the development of atheromatic plaque in the arteries (Antonopoulou and Demopoulos, 1997). PAF is also a very potent aggregating agent that is involved in the inflammatory development of plaque and the blockage of the blood vessels, resulting in the development of coronary heart disease. Recently, studies have been carried out on various foodstuffs to determine whether compounds with PAF-like or anti-PAF-like activities are present (Koussissis et al., 1994a,b; Rementzis et al., 1996; Antonopoulou et al., 1996a). The presence of the latter is very important in terms of nutritional value because compounds with anti-PAF-like activities could be potent agents not only against aggregation in the arteries but also in protection of atheromatosis generation. Consumption of food with a high anti-PAF content would thus minimize the probability of developing coronary heart disease.

The occurrence of lipids with PAF-like and anti-PAF-like activities has been studied in cow's milk and yogurt (Antonopoulou et al., 1996b). In the latter study, yogurt lipids were found to be stronger inhibitors of PAF than lipids of raw milk; this result was linked to the fact that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* biosynthesize important quantities of PAF inhibitors. In an analogous study in the fish mackerel (*Scomber scombrus*), gangliosides with a PAF-like activity were identified (Rementzis et al., 1997). These gangliosides have also been associated with scombroid food poisoning, either through a direct effect on cells or through stimulating the secretion of other inflammatory mediators such as histamine.

Cod (*Gadus morhua*) is one of the most widely consumed types of fish on both sides of the northern Atlantic Ocean and probably the most popular fish in the United Kingdom (Kurlansky, 1997). Health supplements such as cod liver oil are widely used, hence making a study on the biological role of cod lipids essential. Fish can be classified into "lean" fish that store their reserve fats as triacylglycerols in the liver (e.g., cod) and "fatty" fish that store their whose triacylglycerols in the flesh (e.g., mackerel, herring). Cod is considered to be a lean fish, and therefore an abundance of lipids in the flesh is not a feature (Kurlansky, 1997).

Cod is widely consumed fried. During frying, the fat content of the fish increases due to the oil absorption by the flesh, but the nutritional significance of this is difficult to determine as it varies according to the type and thermal history of the oil used and the amount entrained in the food (Fellows, 1997). It is thus important to study on an individual basis the effect of frying on the composition of lipids and any possible change on the biological activity of these lipids. Therefore, in this

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study, lipids of fresh and fried cod have been tested for PAF- and anti-PAF-like activities using the platelet bioassay.

MATERIALS AND METHODS

Apparatus. HPLC was performed on a Waters model series 600E supplied with a 100 μL loop Rheodyne injector. A Waters model series 996 photodiode array detector was used. The spectrophotometer was connected to a Hewlett-Packard (Avondale, PA) model HP-3396A integrator−plotter. The flow rate was 1 mL/min. For the analysis the following conditions were used: a reverse phase column Bondclone C_{18}, 25 cm × 4.6 mm, particle size = 10 μm from Phenomenex (Macclesfield, U.K.), for neutral lipids, and a cation exchange column SS Partisil 25 cm × 4.6 mm, particle size = 10 μm, PXS 10/25 SCX from Whatman (Clifton, NJ), for polar lipids. The PAF-induced aggregation was measured in a Chrono-Log (Havertown, PA) aggregometer coupled to an Omniscribe recorder (Houston, TX). NMR analysis was carried out using a Bruker DRX 500 MHz NMR spectrophotometer with $CDCl_3$ containing 0.03% TMS as solvent. GC-MS analysis was carried out using a Carlo Erba 4200GC coupled to a Kratos MS80 RFA mass spectrometer with a source temperature of 180 °C. A BP20 capillary column (25 mm × 0.32 mm × 0.5 μm film thickness; SGE) was held at 40 °C for 2 min and programmed from 40 to 220 °C at 5 °C min^{-1} using helium as a carrier gas at 2 mL min^{-1}. Injection of the sample (0.2 μL) was made on column. Mass spectra were recorded with an ion source energy of 70 eV.

Reagents. All reagents were of analytical grade and solvents were of HPLC grade, supplied by Sigma (Gillingham, U.K.). Fresh cod (flesh only) was purchased from a local market and used in all experiments immediately after the purchase, withour any prior storage or freezing. Semisynthetic PAF (80% C-16PAF and 20% C-18PAF) was synthesized in our laboratory as previously described (Demopoulos et al., 1979).

Procedure. Each cod fillet was cut into three similar pieces. These pieces were either chopped prior to homogenization and used as fresh samples or fried in a Kenwood deep fryer with sunflower oil at 170 °C for 7 min. The fried pieces were allowed to cool and dried on paper. The skin of the fillets was then removed, and these samples were composited and chopped prior to homogenization. For each run, three pieces from three different fillets were pooled and analyzed. Total lipids were extracted according to the Bligh−Dyer method (Bligh and Dyer, 1959). Total lipids were separated into neutral and polar lipids by countercurrent distribution (Galanos and Kapoulas, 1962). Neutral lipids were further fractionated on HPLC (Antonopoulou et al., 1994). Briefly, neutral lipids were separated on a reverse phase HPLC column with a gradient elution system that consisted of (A) methanol/water (4:1, v/v), (B) acetonitrile/methanol (3:2, v/v), (C) acetonitrile/tetrahydrofuran (99.5:0.5, v/v), (D) 2-propanol/acetonitrile (99:1, v/v), and (E) cyclohexane. The gradient elution system used was a linear gradient from 100% A to 100% B in 10 min, 100% B for 5 min, a linear gradient from 100% B to 100% C in 10 min, 100% C for 5 min, a linear gradient from 100% C to 100% D in 10 min, 100% D for 5 min, a linear gradient from 100% D to 100% E in 10 min, and 100% E for 20 min. Polar lipids were further fractionated on HPLC (Demopoulos et al., 1994). Briefly, polar lipids were separated on a cation exchange HPLC column, 10 μm, using an isocratic elution system consisting of acetonitrile 60% and methanol/water 4:1 (v/v) 40%. The purified fractions of neutral lipids and phospholipids from the above separations were separately collected and tested for biological activity as described recently (Fragopoulou et al., 2000). Briefly, PAF and the examined samples were dissolved in 2.5 mg of bovine serum albumin (BSA)/mL of saline. Thrombin was dissolved in saline. Various concentrations of the examined sample were added into the aggregometer cuvette, and the aggregation induced by the sample was studied in a Chronolog aggregometer (Demopoulos et al., 1979). Experiments with specific inhibitors, 0.7 mM creatine phosphate (CP)/creatine phosphate kinase (CPK), 13 units/mL of saline, 10 μM indomethacin (10% ethanol in water), and 0.1 mM BN 52021 (0.3% DMSO in water), were also performed. These inhibitors were added to washed rabbit platelets 1 min prior to the addition of the examined sample into the aggregometer cuvette. This experiment was carried out according to the method of Lazanas et al. (1988). In desensitization and cross-desensitization experiments, platelets were desensitized by the addition of the test lipid to the platelet suspension at a concentration that caused reversible aggregation. A second stimulation was performed immediately after complete disaggregation. The platelet aggregation induced by PAF (1.25×10^{-10} M, final concentration in the cuvette) was measured as PAF-induced aggregation, in washed rabbit platelets before (considered as 0% inhibition) and after the addition of various concentrations of the examined sample (Demopoulos et al., 1979). Consequently, the plot of percent inhibition (ranging from 0 to 100%) versus different concentrations of the sample is linear. From this curve, we calculated the concentration of the sample that inhibited 50% PAF-induced aggregation. This value is defined as IC_{50}, namely, 50% inhibitory concentration. The IC_{50} values are expressed as percent of total fraction required for 50% inhibition against PAF. This experiment was also performed with thrombin (0.125 unit/cuvette) to assess the inhibition of thrombin-induced aggregation.

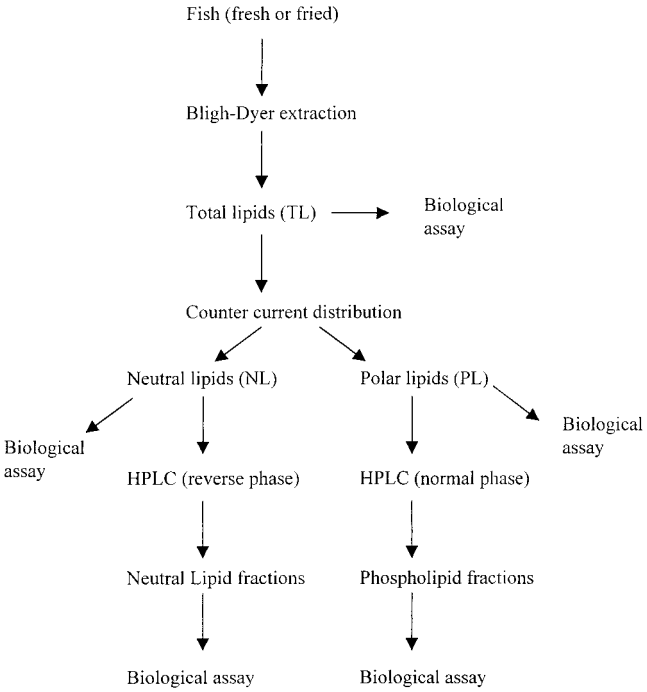

Figure 1. Schematic diagram of the extraction and purification procedure.

RESULTS AND DISCUSSION

A schematic diagram of the extraction and purification procedures used is shown in Figure 1. The biological activities of total lipid, neutral, and polar fractions of fresh and fried cod are shown in Table 1.

Total Lipids from Fresh Cod. Total lipids as well as total neutral lipids and total polar lipids were tested for their biological activity using the platelet bioassay. An amount of 0.0845 and 0.0422 mg of total lipids, added each time in the cuvette, induced rabbit platelet aggregation identical to that caused by 0.73×10^{-10} and 0.95×10^{-10} M PAF (final concentration), respectively, which indicates the presence of agonists as well as PAF inhibitors. Total neutral lipids did not appear to have any biological activity. Total polar lipids in an amount of 0.113 and 0.00565 mg induced rabbit platelet ag-

Table 1. Biological Activity of Total Lipid Fractions, as well as Total Neutral and Total Polar Lipid Fractions, of Fresh and Fried Cod

cod sample	lipid fraction	amount of lipids, mg	biological activity against PAF
fresh	total	0.0845	aggregation equivalent to 0.73×10^{-10} M PAF
		0.0422	aggregation equivalent to 0.95×10^{-10} M PAF
fresh	total neutral	0.0141	none
fresh	total polar	0.113	aggregation equivalent to 0.38×10^{-10} M PAF
		0.0056	aggregation equivalent to 1.0×10^{-10} M PAF
fried	total	0.169	5% inhibition of PAF
		0.0845	20% inhibition of PAF
fried	total neutral	0.0705	34% inhibition of PAF
		0.141	22% inhibition of PAF
fried	total polar	0.0565	aggregation equivalent to 0.85×10^{-10} M PAF
		0.0282	aggregation equivalent to 0.94×10^{-10} M PAF

gregation equivalent to that caused by 0.38×10^{-10} and 1.0×10^{-10} M PAF, respectively (Table 1), which indicates the presence of agonists as well as PAF inhibitors. We have examined the biological activity in two series of independent experiments using a different quantity of lipids each time in order to study whether the aggregation was dose-dependent or not. All experiments using the platelet bioassay were carried out once due to cost and time restrictions. The lipids are expessed in weight because their structures are not yet known.

When the sample caused an aggregation, the PAF concentration that gave equal aggregation is given. Thus, the calculation of the aggregation activity of each sample is based on PAF.

Total Lipids from Fried Cod. Total lipids as well as total neutral lipids and total polar lipids were tested for their biological activity using the platelet bioassay. To study whether the biological activity is dose-dependent, two different amounts of lipids were assayed: amounts of 0.169 and 0.0845 mg of total lipids caused 5 and 20% inhibition in PAF-induced aggregation, respectively. This result indicates the presence of agonists as well as PAF inhibitors.Total neutral lipids in amounts of 0.0705 and 0.141 mg caused 34 and 22% inhibition in PAF-induced aggregation, respectively, which indicates the presence of agonists as well as PAF inhibitors. Total polar lipids in amounts of 0.0565 and 0.0282 mg induced rabbit platelet aggregation equivalent to that caused by 0.85×10^{-10} and 0.94×10^{-10} M PAF (final concentration in the cuvette), respectively, which indicates the presence of agonists as well as PAF inhibitors (Table 1).

The amount of total lipids from either fresh or fried cod was separated into neutral and polar lipids by countercurrent distribution (Galanos and Kapoulas, 1962). Neutral and polar lipids were fractionated on HPLC C_{18} and cation exchange columns, respectively, with the elution systems described under Materials and Methods. Due to limitations of instrumentation and the nonmiscible nature of solvents A and E that were used in the HPLC separation of neutral lipids, the HPLC chromatograms of neutral lipids in fresh and fried cod are presented in two parts; the first part is for the retention time of 0−55 min, whereas the second part is for the retention time of 55−75 min.

Neutral Lipids of Fresh Cod. A typical profile of the neutral lipid separation of fresh cod on an HPLC C_{18} column, along with the elution times of standard lipids, is shown in Figure 2. Ten neutral lipid fractions were collected and tested for their biological activity. The results suggest that neutral lipid fractions that have PAF activity as well as lipid fractions that have anti-PAF activity are present in fresh cod. The results for the neutral lipid fractions of Figure 2 are shown in Table 2. The IC_{50} value was expressed as percent of total fraction required for 50% inhibition against PAF, and the platelet aggregation was induced by 1.25×10^{-10} M PAF (final concentration). The results of Table 2 suggest that lipids with strong anti-PAF activity are present in fresh cod. The lipids that are of biological importance for humans are those that exhibit anti-PAF activity. However, molecules that can compete with PAF for specific receptors are also very important because, as they are much less potent than PAF, in effect they act as PAF inhibitors, thus minimizing and preventing the undesirable actions of PAF. Neutral fraction 6 appears to be the most potent aggregating agent. The most important neutral fractions are 3, 6, and 10 with PAF-activity and fractions 2, 4, and 5 that exhibited anti-PAF activity. Current research is directed toward the structural elucidation of these compounds.

Four of the fractions (2, 4, 5, and 8) inhibited in a dose-dependent manner PAF-induced aggregation with IC_{50} values of 2.0, 5.25, 5.75, and 7.25%, respectively. Six of the 10 fractions (1, 3, 6, 7, 9, and 11 of Figure 2) induced rabbit platelet aggregation. It seems that fractions 1 and 9 act through PAF, ADP, and arachidonic acid methods (i.e., the three different ways of platelet aggregation) of aggregation for the following reasons: (1) Platelets desensitized to PAF were not aggregated by these lipids and vice versa; (2) PAF receptor specific inhibitor BN 52021 does inhibit the aggregation induced by these fractions; and (3) the enzymatic system CP/CPK and indomethacin, which specifically inhibit ADP and arachidonic acid-induced platelet aggregation, respectively, inhibit the aggregation induced by these fractions. Fraction 7 acts through PAF and ADP receptors because (1) PAF receptor specific inhibitor BN 52021 does inhibit the aggregation induced by these fractions, (2) indomethacin had no inhibitory effect on the aggregation induced by this fraction, and (3) the enzymatic system CP/CPK inhibited its aggregation. Fractions 3, 6, and 10 act through PAF because (1) platelets desensitized to PAF were not aggregated by these lipids and vice versa, (2) the enzymatic system CP/CPK and indomethacin which specifically inhibit ADP and arachidonic acid-induced platelet aggregation, respectively, have no inhibitory effect on the aggregation induced by these fractions, and (3) in contrast, PAF receptor specific inhibitor BN 52021 does inhibit the aggregation induced by these fractions.

Further NMR analysis of fractions 4 and 6 was carried out. Fraction 4 was tentatively identified as a free fatty acid, most probably octanoic acid, whereas fraction 6 was tentatively identified as an asymmetrical diglyceride. Preliminary GC-MS analysis of this diglyceride suggests that both fatty acids are of short carbon chain (C_2). These experimental results are in good agreement with work previously published in which oleic acid was reported to have anti-PAF-like activity (Nunez et al., 1990) and glycerides with short carbon chains were reported to have PAF-like biological activity (Demopoulos, 1986). Research is now being directed to

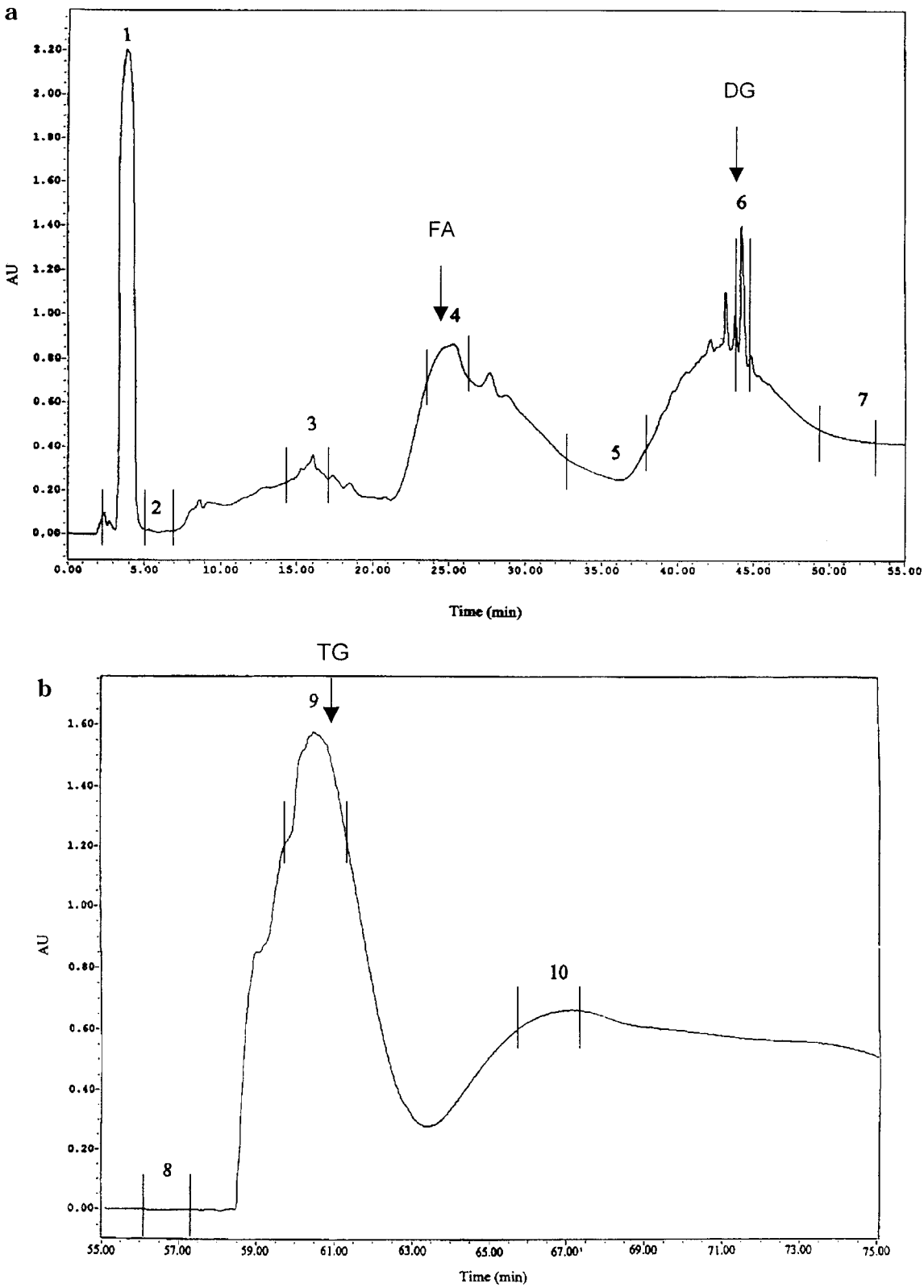

Figure 2. HPLC separation of neutral lipids from fresh cod on a C_{18} column (a, 0−55 min; b, 55−75 min) with a gradient elution system described under Materials and Methods. FA, fatty acids; DG, diglycerides; TG, triglycerides.

the complete structural elucidation of these two very important compounds.

Polar Lipids of Fresh Cod. A typical profile of the polar lipid separation of fresh cod on an HPLC cation exchange column, along with the elution times of standard lipids, is shown in Figure 3. Six polar lipid fractions were collected and tested for their biological activity. The biological activity of each fraction was assayed as described above, and the results are summarized in Table 2.

Two of these fractions (fractions 1 and 6 of Figure 3) inhibited in a dose-dependent manner PAF-induced aggregation with IC_{50} values of 0.46 and 0.42%, respectively. Amounts of 0.5 and 0.6% of total fractions 1 and 6, respectively, inhibited 100% thrombin-induced aggregation. Four of them (fractions 2−5 of Figure 3)

Table 2. Biological Activity of Each Lipid Fraction from the Neutral Lipids (Left) and Polar Lipids (Right) in Fresh Cod, after HPLC Separation

neutral lipids		polar lipids	
fraction	biological action	fraction	biological action
1	aggregation is exhibited; when present at 12.5%, the aggregation corresponds to that exhibited by a PAF concentration of 0.54×10^{-10} M	1	anti-PAF activity; $IC_{50} = 0.46\%$
2	anti-PAF activity; $IC_{50} = 2.0\%$	2	both PAF and anti-PAF activities
3	PAF activity; when present at 1.75%, aggregation caused equivalent to 0.94×10^{-10} M PAF	3	PAF activity; when present at 0.25%, aggregation caused equivalent to 0.77×10^{-10} M PAF
4	anti-PAF activity; $IC_{50} = 5.25\%$	4	PAF activity; when present at 0.25%, aggregation caused equivalent to 0.77×10^{-10} M PAF
5	anti-PAF activity; $IC_{50} = 5.75\%$	5	PAF activity; when present at 0.025%, aggregation caused equivalent to 0.58×10^{-10} M PAF
6	PAF activity; when present at 2.5%, aggregation caused equivalent to 0.95×10^{-10} M PAF	6	anti-PAF activity; $IC_{50} = 0.42\%$
7	PAF activity; when present at 1.25%, aggregation caused equivalent to 0.68×10^{-10} M PAF		
8	anti-PAF activity; $IC_{50} = 7.25\%$		
9	PAF activity; when present at 12.5%, aggregation caused equivalent to 0.54×10^{-10} M PAF		
10	PAF activity; when present at 10%, aggregation caused equivalent to 0.89×10^{-10} M PAF		

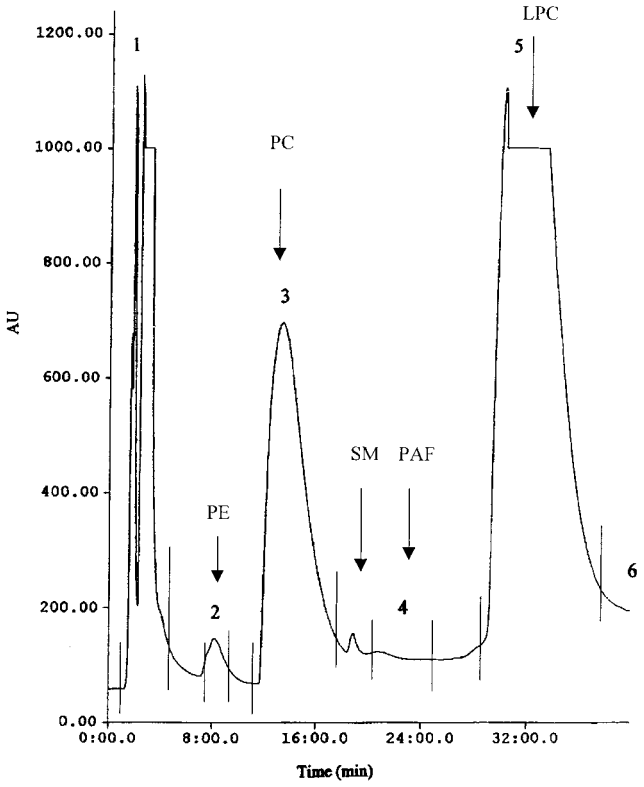

Figure 3. HPLC separation of polar lipids from fresh cod on a cation exchange column with acetonitrile 60% and methanol/water 4:1 (v/v) 40% (v/v). PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; PAF, platelet activating factor; LPC, lysophosphatidylcholine.

induced rabbit platelet aggregation, with a dose-dependent aggregation pattern, with the exception of fraction 2, in which an increase of aggregation was observed at lower concentrations. Fractions 2−4 act through PAF and arachidonic acid methods of aggregation, for the following reasons: (1) platelets desensitized to PAF were not aggregated by these lipids and vice versa; (2) platelets desensitized to these lipids were not aggregated by thrombin; (3) the enzymatic system CP/CPK had no inhibitory effect on the aggregation; and (4) PAF receptor specific inhibitor BN 52021 does inhibit the aggregation induced by these fractions. Fraction 5

Table 3. IC_{50} Values (Expressed as Percentage of the Total Fraction) of Neutral Lipids (Left) and Polar Lipids (Right) of Fried Cod against 1.25×10^{-10} M PAF (Final Concentration)

neutral lipids		polar lipids	
fraction	IC_{50} value	fraction	IC_{50} value
1	26.5	1	4.35
2	28.2	2	1.06
3	29.5	3	0.536
4	18.4	4	1.12
5	19.8	5	5.17
6	2.89	6	1.72
7	0.477	8	0.946
8	13.0		
9	11.8		
11	13.4		
12	2.48		
13	7.14		
15	0.906		
16	0.46		

seems to act through PAF and ADP receptors as follows: (1) platelets desensitized to PAF were not aggregated by this lipid and vice versa; (2) platelets desensitized to this lipid were aggregated by thrombin; and (3) the enzymatic system CP/CPK has partial (41%) inhibitory effect on the aggregation.

Neutral Lipids of Fried Cod. A typical profile of the neutral lipid separation of fried cod on an HPLC C_{18} column, along with the elution times of standard lipids, is shown in Figure 4. Sixteen neutral lipid fractions were collected and tested for their biological activity. Almost all of these have caused inhibition of platelet aggregation caused by PAF (anti-PAF activity). The IC_{50} was expressed as percent of total fraction required for 50% inhibition against PAF, and the platelet aggregation was induced by 1.25×10^{-10} M PAF (final concentration). The IC_{50} values for the fractions with anti-PAF activity were calculated and are shown in Table 3.

The IC_{50} value of fraction 10 has not been calculated because it had not been isolated in pure condition. However, fractions 9 and 10 together exhibited inhibition with an IC_{50} value of 12.5%, a weaker inhibition than fraction 9 alone. This result suggests that fraction 10 either is a very weak aggregation factor or is a weaker PAF inhibitor than fraction 9. Fraction 14 demonstrated a rather peculiar biological activity be-

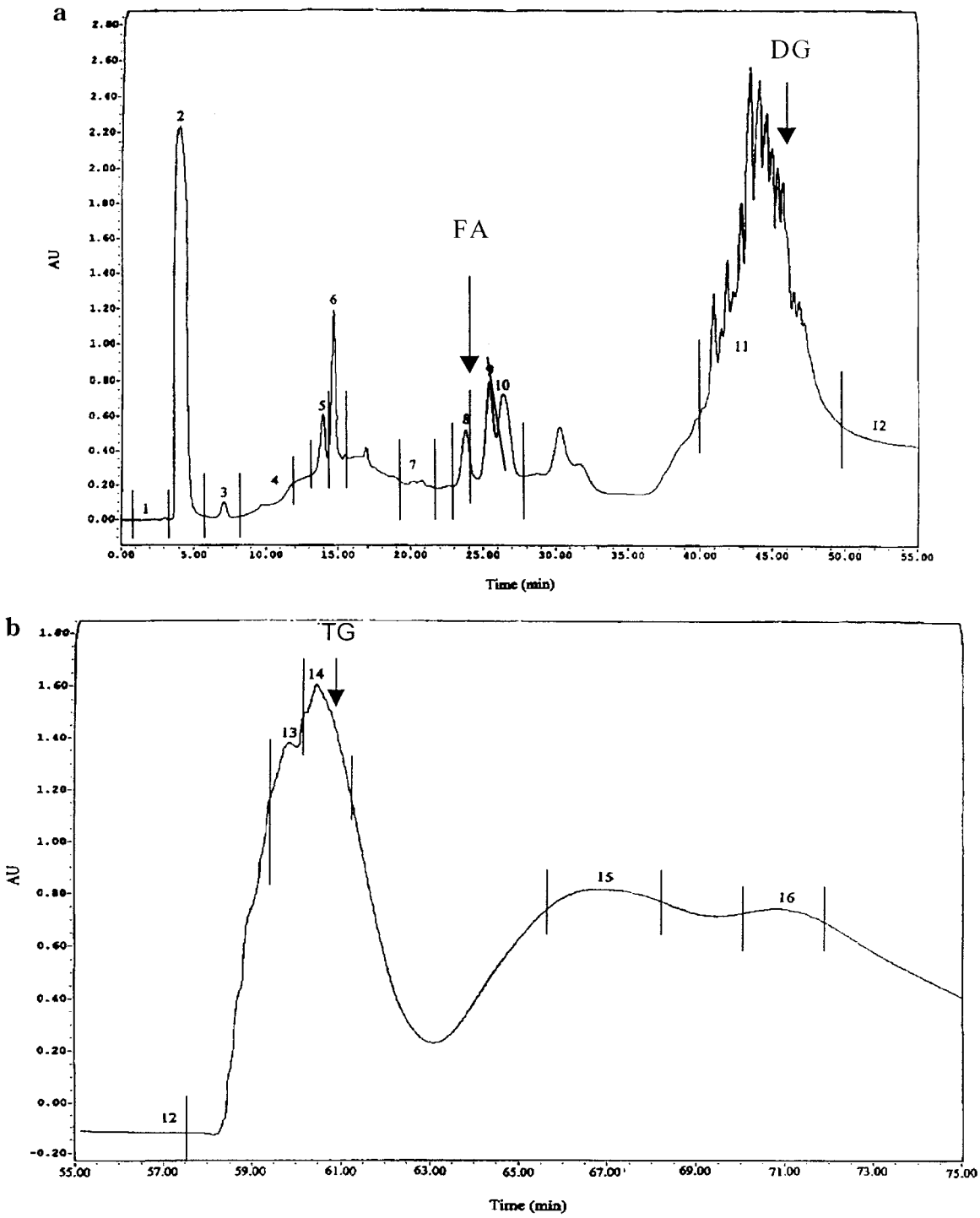

Figure 4. HPLC separation of neutral lipids from fried cod on a C_{18} column (a, 0−55 min; b, 55−75 min) with a gradient elution system system described under Materials and Methods. FA, fatty acids; DG, diglycerides; TG, triglycerides.

cause larger amounts of this fraction caused less inhibition of PAF activity. This behavior could be explained on the basis that this fraction contains compounds with weak platelet aggregation behavior that have rather similar molecular structures and cannot thus be separated by HPLC.

The different results obtained from the biological assay of the neutral lipids in fresh cod, in which lipid compounds exhibiting PAF-like activity have been found, are probably due to the different lipid composition of fried cod caused by oil absorption that takes place during frying. In fact, from the data obtained during the extraction of total lipids from fried cod, we have calculated that the total fat content of fried cod is 5.93%. This figure is much higher than the reported figure (0.4%) for the total fat content of fresh cod (Jay, 1992). In addition, changes in lipid compounds due to high-temperature application have also taken place because compounds in fresh and fried neutral lipid fractions with similar chromatographic behavior exhibit different biological activity.

We have shown (Demopoulos et al., 1999) that inhibitors of PAF occur in vegetable oils and olive oil. In that paper, only unprocessed oils were studied. In all of the types of oils, the fractions of total lipids, total neutral lipids, and total polar lipids were studied after having

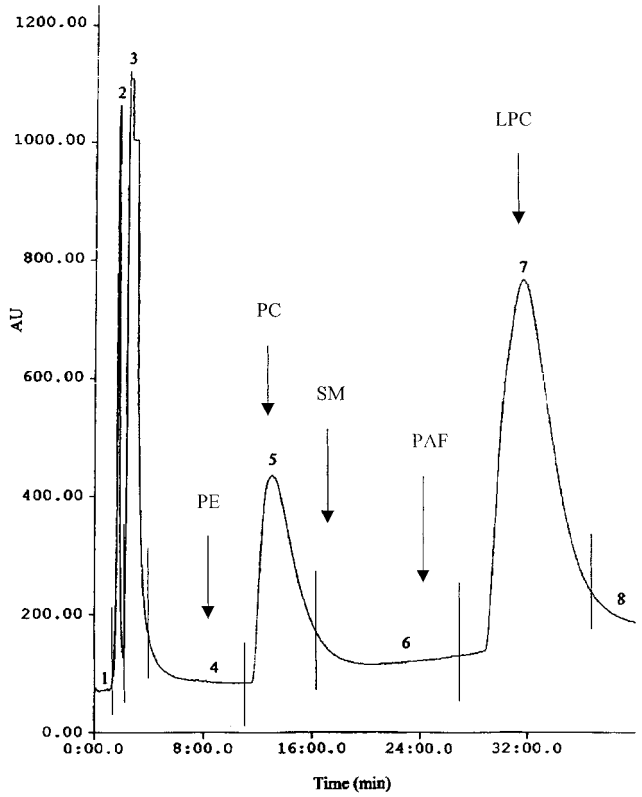

Figure 5. HPLC separation of polar lipids from fried cod on a cation exchange column with acetonitrile 60% and methanol/water 4:1 (v/v) 40% (v/v). PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin; PAF, platelet-activating factor; LPC, lysophosphatidylcholine.

been fractionated in a way identical to that in the work reported here. It was found that in the sunflower oil, all three total lipid fractions have some inhibitors of PAF. However, the lipid fractions of olive oil had greater biological activity. We therefore suggest that these lipid compounds play an important role in the protective effect of oils, especially olive oil, in atheromatosis generation.

Polar Lipids of Fried Cod. A typical profile of the polar lipid separation of fried cod on an HPLC cation exchange column, along with the elution times of standard lipids, is shown in Figure 5. Eight polar lipid fractions were collected and tested for their biological activity. All of these fractions, apart from fraction 7, showed an anti-PAF activity. The IC_{50} values for the fractions with anti-PAF activity were calculated and are shown in Table 3. An amount of 0.37% of total fraction 7 caused platelet aggregation identical to that caused by a PAF concentration of 1.05×10^{-10} M, whereas 0.12% of total fraction caused platelet aggregation identical to that caused by a PAF concentration of 0.85×10^{-10} M, suggesting that lipid compounds with strong PAF-like activity are present in this fraction, acting in a dose-dependent manner. Fraction 7 acts through PAF receptors because (1) platelets desensitized to PAF were not aggregated by these lipids and vice versa, (2) PAF receptor specific inhibitor BN 52021 does inhibit the aggregation induced by this fraction, (3) the enzymatic system CP/CPK has no inhibitory effect on the aggregation, and (4) indomethacine has no inhibitory effect on the aggregation.

From the results of Table 3, it could be suggested that the polar lipid fractions 3 and 8 exhibit rather strong anti-PAF-like activity. However, the strong PAF-like activity demonstrated by fraction 7 overbalanced the anti-PAF-like activity of the other fractions, and thus the whole fried cod polar lipid fraction caused aggregation of platelets.

The inhibition of PAF activity exhibited by these fractions is probably due to sunflower oil absorption that took place during frying. We have experimental results that show that many lipid fractions from vegetable oils have anti-PAF-like activity (unpublished data). In conclusion, the biological activities of lipids present in fresh cod against PAF as well as the effect of frying on these activities are reported here. It could be suggested that frying has a double effect on the nutritional value of fresh cod. First, the absorption of oil by the fish flesh results in a food much richer in fat and calories. This could be regarded as the negative effect of frying on the nutritional value of fish. However, the absorbed oil has changed the dietary value of lipids present in fried cod as all lipids (either neutral or polar) in fried fish were found to have anti-PAF-like activities (Table 3). This beneficial effect could be explained by the absorption of oil lipids with anti-PAF-like activities by the fish flesh during frying. This paper is the first report of a study on the dietary value of cod lipids with respect to platelet aggregation and atherosclerosis. It demonstrates that compounds with strong anti-PAF-like activity are present in both fresh and fried cod. However, compounds with PAF-like activity could also be beneficial as they act as weak PAF agonists and compete with PAF for common binding sites during the formation of atheromatotic plaque in blood arteries, protecting them from atheromatosis generation because they mainly act as PAF inhibitors. Research is being carried out to fully elucidate the structure of compounds with PAF-like and anti-PAF-like activities in fresh and fried cod.

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