Lipids
DOI 10.1007/s11745-015-4112-y

ORIGINAL ARTICLE

Analysis of Plasmalogen Species in Foodstuffs

Shinji Yamashita[1,2] · Susumu Kanno[2] · Ayako Honjo[2] · Yurika Otoki[2] · Kiyotaka Nakagawa[2] · Mikio Kinoshita[1] · Teruo Miyazawa[2,3,4]

Received: 20 July 2015 / Accepted: 4 December 2015

Abstract Ethanolamine plasmalogen (PlsEtn), which is present at high levels in brains, is believed to be involved in neuronal protection. The present study was performed to search for PlsEtn resources in foodstuffs. The foodstuffs examined showed a wide range of PlsEtn contents from 5 to 549 μmol/100 g wet wt. The marine invertebrates, blue mussel, and ascidian had high PlsEtn contents (over 200 μmol/100 g wet wt). Profiling of the molecular species showed that the predominant fatty acids of PlsEtn species were 20:5 (EPA) and 22:6 (DHA) at the *sn*-2 position of the glycerol moiety in marine foodstuffs, whereas major PlsEtn species in land foodstuffs were 20:4. Following quantitative analysis by multiple reaction monitoring, the ascidian viscera were shown to contain the highest levels of 18:0/20:5-PlsEtn and 18:0/22:6-PlsEtn (86 and 68 μmol/100 g wet wt, respectively). In order to evaluate a neuronal antiapoptotic effect of these PlsEtn species, human neuroblastoma SH-SY5Y cells were treated with ethanolamine glycerophospholipid (EtnGpl), purified from the ascidian viscera, under serum starvation conditions. Extrinsic EtnGpl from ascidian viscera showed stronger suppression of cell death induced by serum starvation than with bovine brain EtnGpl. The EtnGpl from ascidian viscera strongly suppressed the activation of caspase 3. These results suggest that PlsEtn, especially that containing EPA and DHA, from marine foodstuffs is potentially useful for a therapeutic dietary supplement preventing neurodegenerative diseases, such as Alzheimer's disease (AD).

Keywords Plasmalogen · DHA · EPA · Ascidian · Antiapoptosis · Neuronal cells · Alzheimer's disease

Abbreviations

AD	Alzheimer's disease
Aβ	Amyloid-β
DHA	Docosahexaenoic acid (22:6)
DMEM	Dulbecco's modified Eagle's medium
EPA	Eicosapentaenoic acid (20:5)
EtnGpl	Ethanolamine glycerophospholipid
FBS	Fetal bovine serum
HPLC	High-performance liquid chromatography
PakEtn	1-*O*-Alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine
PlsEtn	1-*O*-Alkenyl-2-acyl-*sn*-glycero-3-phosphoethanolamine or ethanolamine plasmalogen
PtdEtn	1,2-Diacyl-*sn*-glycero-3-phosphoethanolamine
PUFA	Polyunsaturated fatty acids
TLC	Thin-layer chromatography

Introduction

Ethanolamine glycerophospholipids (EtnGpl) are a major class of glycerophospholipids found in biological membranes. Moreover, EtnGpl exist in three forms with alkyl,

✉ Teruo Miyazawa
miyazawa@m.tohoku.ac.jp

[1] Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan

[2] Food and Biodynamic Chemistry Laboratory, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

[3] Food and Biotechnology Innovation Project, New Industry Creation Hatchery Center (NICHe), Tohoku University, Sendai 980-8579, Japan

[4] Food and Health Science Research Unit, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

Published online: 05 January 2016

alkenyl, or acyl linkages at the *sn*-1 position of the glycerol moiety (1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine, PakEtn; 1-*O*-alkenyl-2-acyl-*sn*-glycero-3-phosphoethanolamine, PlsEtn; and 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine, PtdEtn, respectively). The alkenylacyl form is called plasmalogen [1]. The aliphatic moiety at the *sn*-1 position consists of C16:0, C18:0, or C18:1 carbon chains, whereas the *sn*-2 position mainly consists of polyunsaturated fatty acids (PUFA). PUFA released from PlsEtn can be metabolized to eicosanoids and docosanoids, showing various bioactivities [2, 3]. While PlsEtn is restricted in most tissues and cells of animals [4], the nervous system has high PlsEtn contents. Owing to its propensity for hexagonal phase formation, PlsEtn is involved in membrane fusion, which occurs during synaptic transmission [5]. PlsEtn can also protect neuronal cells by scavenging reactive oxygen species such as singlet oxygen ($^1O_2$) and superoxide (O_2^-) at its alkenyl (vinyl ether) linkages [6–8].

Alzheimer's disease (AD) presents with brain atrophy caused by neuronal loss as a prominent pathological feature. This neuronal loss in AD was shown to occur through apoptosis [9–11]. Therefore, suppression of neuronal apoptosis is an essential part of AD treatment. On the other hand, PlsEtn levels were reported to be specifically decreased in postmortem brains from patients with AD [12–14]. In our previous study, we showed that PlsEtn from bovine brain suppresses neuronal cell death [15]. Moreover, PlsEtn with 22:6 (DHA) showed the strongest suppression of neuronal apoptosis [16]. These observations suggest that PlsEtn is involved in neurodegenerative diseases, such as AD, and extrinsic PlsEtn, especially that containing DHA, may prevent the pathogenesis and progression of AD via suppression of neuronal apoptosis.

Although bovine brain had mainly been used as a source of PlsEtn, its use became difficult with the outbreaks of bovine spongiform encephalopathy. On the other hand, most EtnGpl exists as PlsEtn in some marine invertebrates [17]. Furthermore, PlsEtn from marine invertebrates are expected to effectively suppress neuronal apoptosis because they are abundant in DHA [18].

The present study was performed to search for PlsEtn resources, especially containing DHA, in foodstuffs and to confirm their neuroprotective effects.

Materials and methods

Materials

The materials used were prepared from the sources shown: foodstuffs examined (Table 1) were purchased from local supermarkets in Sendai, Japan. The muscles of cattle, pig, and chicken were leg parts (round, ham, and thigh, respectively); 18:0/22:6-PlsEtn, 18:0/20:4-PlsEtn, and 18:0/18:1-PlsEtn were purchased from Avanti Polar Lipids (Alabaster, AL); fetal bovine serum (FBS) was purchased from Biowest (Nuaillé, France); EtnGpl (90 % PlsEtn) from bovine brain was purchased from Doosan Serdary Research Laboratories (Toronto, ON, Canada); SH-SY5Y (human neuroblastoma-derived cells) was purchased from Ds Parma Biomedical Co., Ltd. (Osaka, Japan); Dulbecco's modified Eagle's medium (DMEM), Ham's F12 medium, and L-glutamine were purchased from Nissui (Tokyo, Japan); penicillin–streptomycin and trypsin–EDTA were purchased from Gibco (Grand Island, NY); cell counting kits were purchased from Dojindo (Kumamoto, Japan); caspase colorimetric protease assay kits were purchased from MBL Co., Ltd. (Nagoya, Japan); 18:0/20:5-PlsEtn was purified according to the methods reported previously [18].

Lipid Extraction and Assay

Samples were weighed immediately after purchasing and stored at −80 °C. Samples frozen were freeze-dried, weighted, crushed, and kept at a temperature below −30 °C for further processing. The total lipids were prepared according to the method of Folch *et al*. [19]. Phospholipid

Table 1 Taxonomic classification of the foodstuffs examined

Kingdom	Phylum	Class	Scientific name	Common name
Chromalveolata	Heterokontophyta	Phaeophyoeae	*Laminariaceae Bory*	Brown algae
Animalia	Mollusca	Bivalvia	*Mytilus galloprovincialis*	Blue mussel
			Patinopecten yessoensis	Scallop
		Cephalopoda	*Todarodes pacificus*	Cuttlefish
	Protochordata	Ascidiacea	*Halocynthia roretzi*	Ascidian
	Chordata	Actinopterygii	*Oncorhynchus kisutsh*	Salmon
			Seriola quinqueradiata	Amberjack
		Mammalia	*Bos taurus*	Cattle
			Sus scrofa domesticus	Pig
		Aves	*Gallus gallus domesticus*	Chicken

content was determined according to the method described by Rouser *et al*. [20]. EtnGpl content was analyzed by high-performance liquid chromatography (HPLC) with evaporative light-scattering detection (ELSD) [21]. PlsEtn content was analyzed by two-dimensional reaction thin-layer chromatography (TLC) [22]. Following color formation by ninhydrin, EtnGpl and lyso-EtnGpl on TLC were analyzed using Scion Image Beta 4.0.4 for Windows (Scion Corp.).

MS/MS Analysis

Lipid extracts from foodstuffs were pretreated with Sep-Pak Plus silica cartridges (Waters, Milford, MA). Following conditioning of the cartridge with 20 mL of chloroform, 1 mg of extract as total lipid was loaded onto the cartridge with the following solvent systems: 12 mL of chloroform, 6 mL of chloroform–methanol (85:15, v/v), and 12 mL of chloroform–methanol (1:1, v/v). The final fraction was analyzed by HPLC with a 4000 QTRAP quadrupole/linear ion-trap tandem mass spectrometer (AB Sciex) [18]. EtnGpl species were analyzed using a silica column (Inertsil SIL-100A, 2.1 × 100 mm, φ 3 μm; GL Sciences, Tokyo, Japan) with a binary gradient consisting of solvent A [acetonitrile–methanol–1 M aqueous ammonium formate (pH 6.0) (78:20:2, by vol)] and solvent B [acetonitrile–methanol–1 M aqueous ammonium formate (pH 6.0) (49:49:2, by vol)]. The gradient profile was as follows: 0–1.0 min, 70 % B; 1.0–1.1 min, 70–100 % B linear gradient; 1.1–5.5 min, 100 % B. The flow rate was 0.2 mL/min, and the column temperature was 40 °C. Electrospray ionization was performed in negative and positive ion modes. The negative ion mode was used as an ion source with collision energy of −50 eV, transition dwell time of 100 ms, turbo gas temperature of 600 °C, and a spray voltage of −4500 V. Nitrogen pressure values for turbo, nebulizer, and curtain gases were set at 40, 70, and 40 pounds per square inch, respectively. Negative ion spectra were collected in the *m/z* range of 100–900. The positive ion mode was used as an ion source with collision energy of 20 eV and a spray voltage of 4800 V. The other conditions conformed to the negative ion mode.

Precursor ion scanning was performed for profiling EtnGpl molecular species. In negative mode, EtnGpl generated a fragment of 196 Da, with two carbon chains and one H_2O removed from EtnGpl, and a fragment from fatty acid at the *sn*-2 position (e.g., 281, 303, 301, and 327 Da are 18:1, 20:4, 20:5, and 22:6, respectively). Moreover, precursor ion scanning in positive ion mode was used to identify PlsEtn species. PlsEtn at *sn*-1 with 16:0, 18:0, 18:1, and 20:1 were detected by scanning parent ions that yielded fragment ions of 364, 392, 390, and 418 Da, respectively.

To quantify PlsEtn species, multiple reaction monitoring of the transition of parent ions to product ions was performed. Quantification of PlsEtn species in foodstuffs was performed for four molecular species (18:0/18:1-PlsEtn, 18:0/20:4-PlsEtn, 18:0/20:5-PlsEtn, and 18:0/22:6-PlsEtn).

Purification of EtnGpl from Ascidian Viscera

PlsEtn was prepared by a modification of our previous method [18]. Briefly, neutral lipid was removed from freeze-dried ascidian viscera with acetone. After the residue was prepared according to the method described by Folch *et al*. [19], neutral lipid and sphingolipid were removed with acetone and diethyl ether. The crude glycerophospholipid was subjected to silica gel column chromatography with the following solvent systems: chloroform–methanol (95:5, v/v), chloroform–methanol (4:1, v/v), and chloroform–methanol (3:2, v/v). Subsequently, EtnGpl fraction was subjected to silica gel column chromatography twice with the following solvent systems: chloroform–methanol–30 % ammonium hydroxide (70:24:3, by vol), chloroform–methanol–30 % ammonium hydroxide (65:25:3, by vol), and chloroform–methanol–30 % ammonium hydroxide (65:25:3.5, by vol).

Cell Culture

Cells were cultured in DMEM and F12 mixture supplemented with 15 % heat-inactivated FBS, 1 % penicillin–streptomycin, 2 mM L-glutamine, and 1 % sodium bicarbonate. Cells were kept in a 5 % CO_2 incubator under humid conditions at 37 °C. Cells were passaged at 2.0×10^5 cells/mL before reaching 50 % confluence. In the experiments, after incubation to confluence, cells were cultured in medium without FBS. EtnGpl was dissolved in ethanol to prepare a stock solution of 100 mM and diluted by adding medium without FBS at the time of use (final ethanol concentration, 0.1 %).

Cell Proliferation Assays

Cell viability was measured using a colorimetric assay for 96-well plates with a cell counting kit, containing 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (water-soluble tetrazolium salt) reagent [23]. Cell viability was measured at 450 nm (reference 655 nm) in a model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA).

Caspase Activity Assay

Caspase activities were measured using caspase colorimetric protease assay kits [24, 25]. The proteins containing caspases were extracted in cell lysis buffer (25 mM HEPES, 2.5 mM EDTA, 0.25 M sucrose, 0.05 % SDS, 0.5 mM DTT, 0.2 mM PMSF, 0.1 μM aprotinin, 0.1 μM

pepstatin A, phosphatase inhibitor cocktail). Cell lysates were centrifuged at 10,000×*g* for 5 min at 4 °C. The supernatant was incubated with each caspase substrate and measured at 405 nm.

Statistical Analysis

The data are expressed as mean ± SD and were tested by one-way ANOVA, followed by Scheffe's *F* test. Differences were considered significant at $P < 0.05$.

Results

Lipid Contents in Foodstuffs

The lipid contents of foodstuffs are indicated at Table 2. The total lipid contents were 0.5–19.8 % (wet wt). In particular, the viscera of cuttlefish and scallop had high lipid contents. On the other hand, the parts of brown algae had low lipid contents as a result of the high moisture content. The phospholipid levels of total lipid were very high in blue mussel and cuttlefish muscle. In the sporophyll of brown algae and the viscera of invertebrates examined, total lipid consisted mostly of non-phospholipid.

EtnGpl Contents in Foodstuffs

As shown in Table 3, EtnGpl contents of phospholipid were as follows: at least 30 mol% in the parts of ascidian; 20–30 mol% in blue mussel, amberjack muscle, and muscle + mantle of scallop. Blue mussel also contained very high levels of EtnGpl per wet wt. PlsEtn contents of phospholipid were as follows: greater than 20 mol% in blue mussel and the parts of ascidian; 10–20 mol% in cattle and chicken muscle. The ratios of PlsEtn to EtnGpl (as mol%) were very high in blue mussel, the parts of ascidian, and cattle muscle. In contrast, PlsEtn levels were low in fishes and the parts of cuttlefish.

EtnGpl Species Profile in Foodstuffs

Precursor ion scanning of EtnGpl species in foodstuffs is shown in Figs. 1 and 2. The respective peaks were specified by precursor ion scanning relevant to alkenyl and acyl chains. Minor fatty acids were confirmed by analyzing the product ions (Fig. 3).

In blue mussel, EtnGpl species consisted mostly of PlsEtn. The prominent species were 38:5-PlsEtn (18:0/20:5-PlsEtn and 18:1/20:4-PlsEtn) and 38:2-PlsEtn (18:0/20:2-PlsEtn). The fatty acid at *sn*-2 was composed of 18:1, 20:1, 20:2, 20:3, 20:4, 20:5, 22:2, and 22:6.

In the muscle + mantle of scallop, the predominant EtnGpl species were 38:5-PlsEtn (18:0/20:5-PlsEtn and 18:1/20:4-PlsEtn) and 40:6-PlsEtn (18:0/22:6-PlsEtn). The prominent PtdEtn was 36:5-PtdEtn (16:0/20:5-PtdEtn and 16:1/20:4-PtdEtn). The fatty acid at *sn*-2 was composed of 20:4, 20:5, 22:2, and 22:6. In scallop viscera, the predominant EtnGpl species were 38:5-PlsEtn (18:0/20:5-PlsEtn and 18:1/20:4-PlsEtn) and 40:6-PlsEtn (18:0/22:6-PlsEtn and 20:1/20:5-PlsEtn) (Fig. 1a). The component fatty acids at *sn*-2 were the same as in the muscle + mantle.

Table 2 Lipid contents in the foodstuffs examined

	Part	Total lipids		Phospholipids		Non-phospholipids	
		Wet wt%	Dry wt%	Wet wt%	Dry wt%	Wet wt%	Dry wt%
Brown algae	Sporophyll	0.5 ± 0.0	9.1 ± 0.3	0.0 ± 0.0	0.8 ± 0.1	0.5	8.3
	Leaf	1.1 ± 0.1	2.8 ± 0.2	0.2 ± 0.0	0.6 ± 0.1	0.9	2.2
	Caulome	0.5 ± 0.0	1.5 ± 0.1	0.3 ± 0.0	0.8 ± 0.0	0.3	0.8
Blue mussel	Muscle	4.6 ± 0.1	14.3 ± 0.2	2.0 ± 0.1	6.4 ± 0.3	2.5	7.8
Scallop	Muscle + mantle	0.7 ± 0.0	4.0 ± 0.0	0.5 ± 0.0	2.8 ± 0.2	0.2	1.2
	Viscera	11.5 ± 0.2	49.8 ± 0.8	0.5 ± 0.0	2.3 ± 0.1	10.9	47.5
Cuttlefish	Muscle	1.8 ± 0.1	9.3 ± 0.4	1.4 ± 0.1	7.4 ± 0.5	0.4	1.9
	Viscera	19.8 ± 0.2	50.7 ± 0.6	0.5 ± 0.0	1.2 ± 0.1	19.3	49.5
Ascidian	Muscle	2.1 ± 0.1	10.3 ± 0.5	0.7 ± 0.0	3.6 ± 0.2	1.4	6.8
	Viscera	4.1 ± 0.0	20.7 ± 0.1	0.6 ± 0.0	3.1 ± 0.1	3.5	17.6
Salmon	Muscle	3.8 ± 1.2	11.3 ± 3.5	0.9 ± 0.3	2.5 ± 0.8	3.0	8.8
Amberjack	Muscle	1.4 ± 0.3	4.8 ± 1.1	0.8 ± 0.0	2.7 ± 0.1	0.6	2.1
Cattle	Leg muscle	4.7 ± 0.5	15.2 ± 1.5	1.1 ± 0.2	3.5 ± 0.6	3.6	11.7
Pig	Leg muscle	13.0 ± 0.5	38.3 ± 1.4	1.6 ± 0.3	4.7 ± 0.9	11.4	33.6
Chicken	Leg muscle	4.8 ± 0.4	16.1 ± 1.4	1.2 ± 0.1	4.0 ± 0.3	3.6	12.1

Values are means ± standard deviation (n = 3–5). Non-phospholipids were determined as follows: (total lipids − phospholipids)

Table 3 Amounts of EtnGpl and PlsEtn in the foodstuffs examined

	Part	EtnGpl		PlsEtn		PlsEtn to EtnGpl mol% ratio
		Phospholpid mol%	μmol/100 g wet wt	Phospholpid mol%	μmol/100 g wet wt	
Brown algae	Sporophyll	Trace		–		–
	Leaf	Trace		–		–
	Caulome	Trace		–		–
Blue mussel	Muscle	24.4 ± 2.5	645 ± 67	20.8 ± 2.2	549 ± 58	85.2
Scallop	Muscle + mantle	22.3 ± 4.0	137 ± 24	9.0 ± 1.4	55 ± 9	40.3
	Viscera	12.3 ± 3.1	84 ± 21	7.4 ± 1.8	51 ± 12	60.7
Cuttlefish	Muscle	18.5 ± 1.9	336 ± 35	3.7 ± 0.4	67 ± 7	19.9
	Viscera	3.4 ± 0.8	20 ± 5	0.8 ± 0.0	5 ± 0	25.0
Ascidian	Muscle	30.0 ± 3.1	278 ± 29	24.4 ± 2.5	226 ± 23	81.3
	Viscera	33.2 ± 0.3	265 ± 3	27.0 ± 2.6	216 ± 21	81.4
Salmon	Muscle	13.7 ± 2.0	150 ± 22	2.4 ± 0.4	27 ± 4	17.6
Amberjack	Muscle	25.8 ± 1.9	260 ± 19	6.3 ± 0.4	64 ± 4	24.6
Cattle	Leg muscle	12.4 ± 2.0	168 ± 27	10.9 ± 1.7	148 ± 23	88.4
Pig	Leg muscle	4.1 ± 2.8	77 ± 52	2.8 ± 1.9	52 ± 36	68.4
Chicken	Leg muscle	18.9 ± 8.3	332 ± 146	10.1 ± 4.5	177 ± 80	53.3

Values are means ± standard deviation ($n = 5$). 769 was used as average molecular weight of EtnGpl and PlsEtn. Trace indicates less than 0.05 mol%

EtnGpl ethanolamine glycerophospholipid, *PlsEtn* ethanolamine plasmalogen

In cuttlefish muscle, PtdEtn species accounted for more than half of the EtnGpl species (Fig. 1b). The prominent EtnGpl species were 36:5-PtdEtn (16:0/20:5-PtdEtn), 38:6-PtdEtn (18:1/20:5-PtdEtn), 38:5-PtdEtn (18:0/20:5-PtdEtn), and 40:6-PtdEtn (18:0/22:6-PtdEtn and 20:1/20:5-PtdEtn). In cuttlefish viscera, the predominant EtnGpl species were 38:5-PtdEtn (18:0/20:5-PtdEtn), 38:6-PtdEtn (18:1/20:5-PtdEtn), and 38:6-PlsEtn (16:0/22:6-PlsEtn and 18:1/22:5-PlsEtn). In the muscle and viscera, the component fatty acids at *sn*-2 were 20:4, 20:5, and 22:6. The predominant fatty acid at *sn*-2 was 20:5.

In ascidian muscle, the predominant EtnGpl species was 38:5-PlsEtn (18:0/20:5-PlsEtn). In ascidian viscera, the predominant EtnGpl species was 38:5-PlsEtn (18:0/20:5-PlsEtn), and 40:6-PlsEtn (18:0/22:6-PlsEtn). In the muscle and viscera, the predominant fatty acids were 20:5 and 22:6. The fatty acid 17:1 was found only in the viscera.

In salmon muscle, the predominant EtnGpl species were 38:6-PtdEtn (16:0/22:6-PtdEtn) and 38:6-PlsEtn (16:0/22:6-PlsEtn) (Fig. 1c). Typically the alkenylacyl chains at *sn*-1 were not 18:0. The predominant fatty acid at *sn*-2 was 22:6.

In amberjack muscle, 40:6-PtdEtn (18:0/22:6-PtdEtn) accounted for most of the EtnGpl species.

In cattle muscle, EtnGpl species consisted mostly of PlsEtn. The most prominent species was 38:4-PlsEtn (18:0/20:4-PlsEtn and 18:1/20:3-PlsEtn) (Fig. 2a). The fatty acid at *sn*-2 was composed of 18:2, 20:3, 20:4, and 22:5.

In pig muscle, the predominant species were 38:4-PlsEtn (18:0/20:4-PlsEtn) and 36:4-PlsEtn (16:0/20:4-PlsEtn) (Fig. 2b). The fatty acid at *sn*-2 was composed of 18:2, 20:3, 20:4, and 22:5.

In chicken muscle, the predominant species were 38:4-PtdEtn (18:0/20:4-PtdEtn) and 38:4-PlsEtn (16:0/22:4-PlsEtn and 18:0/20:4-PlsEtn) (Fig. 2c). The fatty acid at *sn*-2 was composed of 18:1, 18:2, 20:4, 22:4, and 22:5.

Quantification of PlsEtn Species in Foodstuffs

The quantification of PlsEtn species in foodstuffs was performed for four molecular species (18:0/18:1-PlsEtn, 18:0/20:4-PlsEtn, 18:0/20:5-PlsEtn, and 18:0/22:6-PlsEtn) (Table 4). Of the foodstuffs examined, ascidian viscera contained the highest levels of 18:0/20:5-PlsEtn and 18:0/22:6-PlsEtn in EtnGpl. Ascidian viscera also contained the highest levels of these species per wet weight. Blue mussel contained high levels of 18:0/20:5-PlsEtn per wet weight, compared with the ascidian viscera. Cattle and pig muscle contained high levels of 18:0/20:4-PlsEtn in EtnGpl. Blue mussel and cattle muscle contained high levels of 18:0/20:4-PlsEtn per wet weight.

Purification of EtnGpl from Ascidian Viscera

The EtnGpl fraction from ascidian viscera contained 80 % EtnGpl (64.2 % as PlsEtn). The prominent fatty

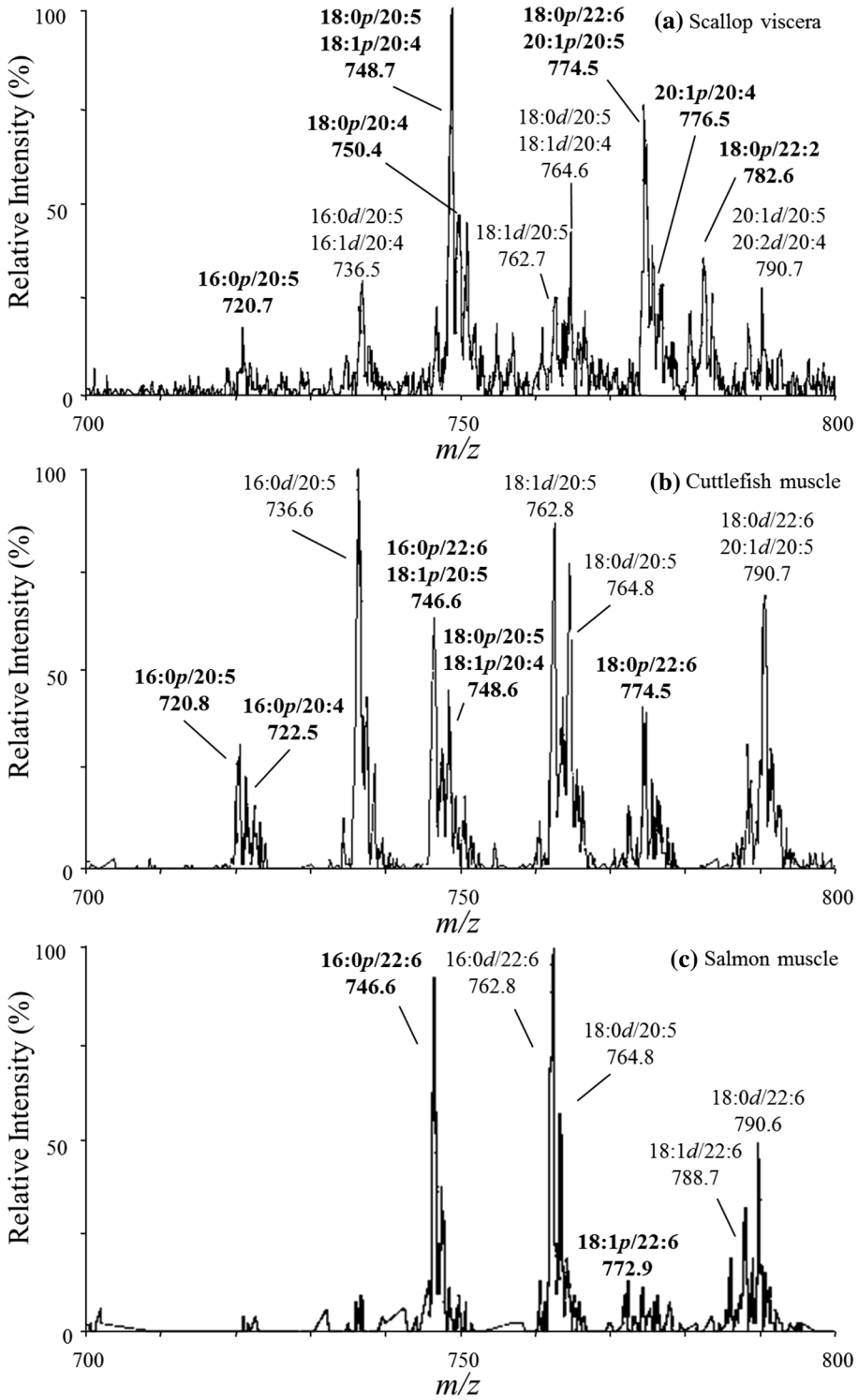

Fig. 1 Precursor ion scanning of EtnGpl species in the marine foodstuffs examined: **a** scallop viscera, **b** cuttlefish muscle, **c** salmon muscle. Spectra were obtained by precursor ion scanning for *m/z* 196 in negative ion mode. 16:0, 18:0, 18:1, and 20:1 at the *sn*-1 position of PlsEtn were determined by precursor ion scanning for *m/z* 364, 392, 390, and 418 in positive ion mode, respectively. Fatty acids at the *sn*-2 position of EtnGpl were determined by precursor ion scanning for *m/z* of each fatty acid in negative ion mode. *p* at *sn*-1 position and *bold characters* in spectra refer to PlsEtn, whereas *d* and *standard characters* refer to PtdEtn. *EtnGpl* ethanolamine glycerophospholipid, *PlsEtn* ethanolamine plasmalogen, *PtdEtn* phosphatidylethanolamine

acids were 20:5 (33.2 mol%), 22:6 (27.4 mol%), 18:0 (13.1 mol%), 18:1 (11.2 mol%), and 20:4 (6.1 mol%). The aldehyde from alkenyl chains at the *sn*-1 position consisted of 18:0 (89.3 mol%), 16:0 (5.4 mol%), and 18:1 (5.0 mol%). On the other hand, EtnGpl from bovine brain contained 90 % PlsEtn. The predominant fatty acids were 18:1 (35.1 mol%), 22:5 (17.3 mol%), 18:0 (15.8 mol%), 22:6 (11.9 mol%), and 20:4 (9.9 mol%). The aldehyde from alkenyl chains consisted of 18:0 (45.7 mol%), 16:0 (28.8 mol%), and 18:1 (25.5 mol%) [16].

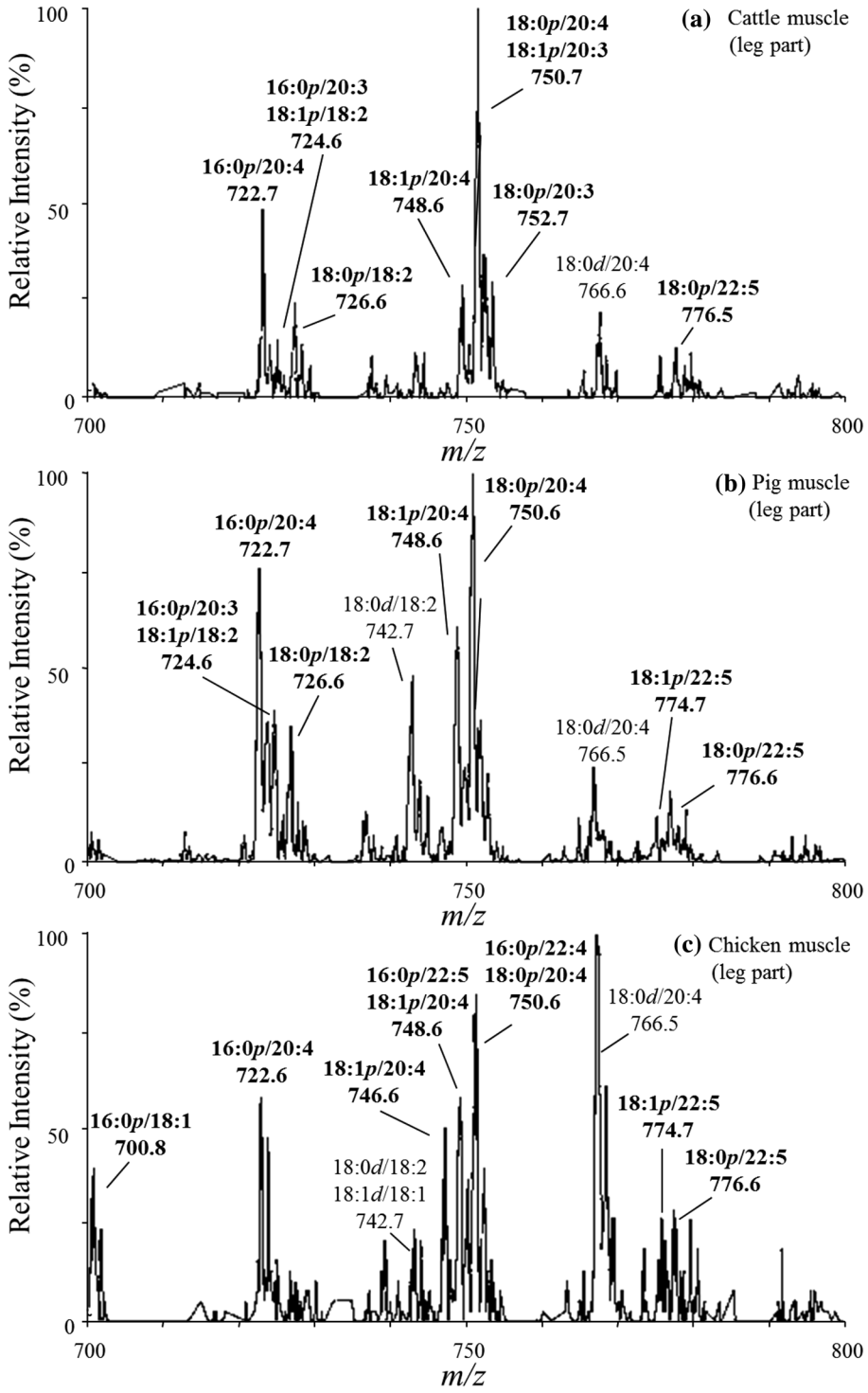

Fig. 2 Precursor ion scanning of EtnGpl species in the land foodstuffs examined: **a** cattle muscle, **b** pig muscle, **c** chicken muscle. Spectra were obtained by precursor ion scanning for *m/z* 196 in negative ion mode. *p* at *sn*-1 position and *bold characters* in spectra refer PlsEtn, whereas *d* and *standard characters* refer PtdEtn. *EtnGpl* ethanolamine glycerophospholipid, *PlsEtn* ethanolamine plasmalogen, *PtdEtn* phosphatidylethanolamine

Effects of EtnGpl from Ascidian Viscera on Suppression of Neuronal Death

To clarify the potential utility of EtnGpl from ascidian viscera, which is rich in 18:0/22:6-PlsEtn, its effects on cell viability and caspase activity were examined. In comparison to bovine brain EtnGpl, which was used in previous studies [26–28], EtnGpl from ascidian viscera attenuated the reduction in viability of SH-SY5Y cells induced by serum starvation (Fig. 4). The extrinsic EtnGpl suppressed the increase in caspase activity (i.e., 3, 8, and 9). For caspases 3 and 9, extrinsic EtnGpl from ascidian viscera showed stronger suppression compared to EtnGpl from bovine brain.

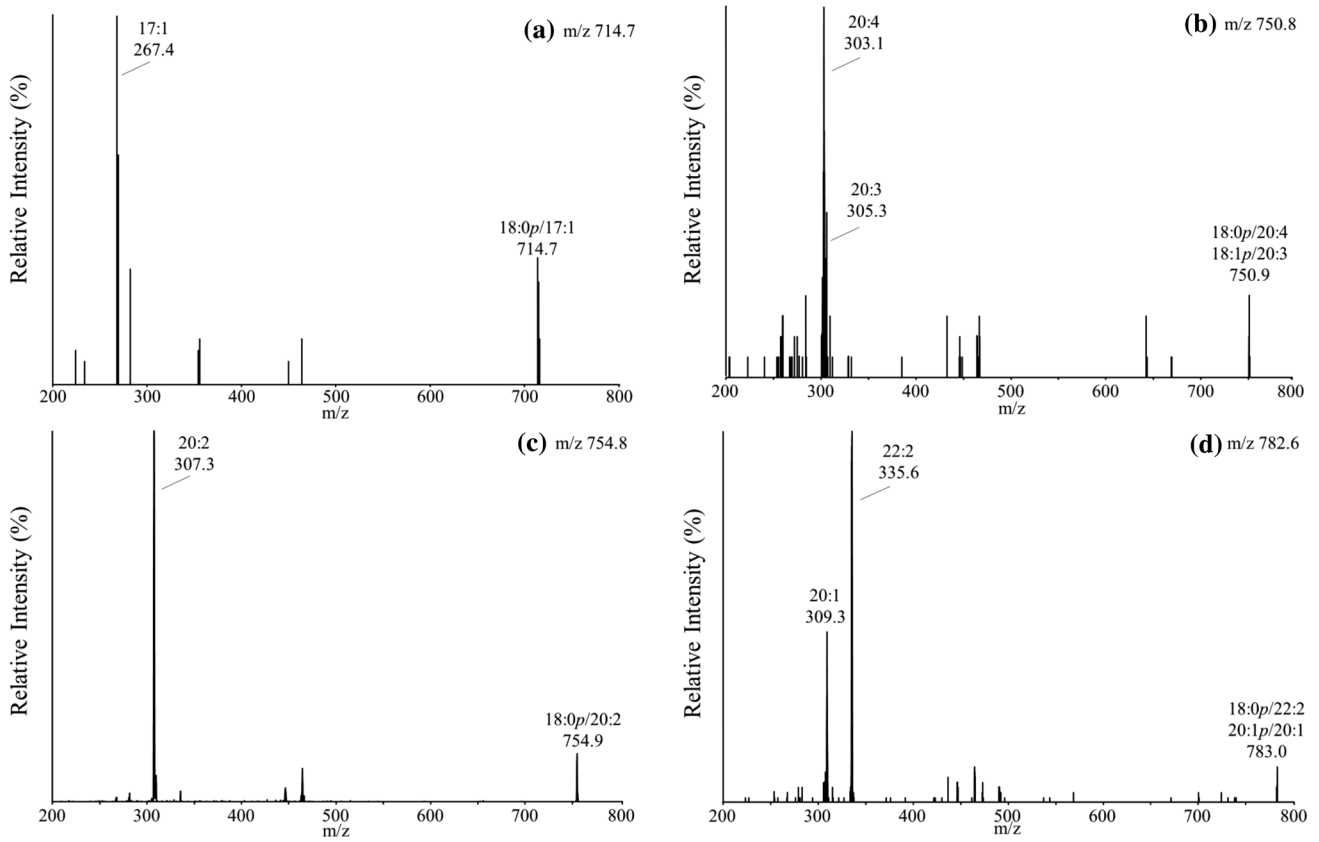

Fig. 3 Product ion scanning of PlsEtn species in the foodstuffs examined. Minor fatty acids at the *sn*-2 position of PlsEtn were confirmed by product ion scanning in negative ion mode. **a** Product ion of *m/z* 714.7 in ascidian viscera. **b**–**d** are product ions of *m/z* 750.8, 754.8, and 782.6 in blue mussel, respectively. *PlsEtn* ethanolamine plasmalogen

Discussion

This study was performed to search for PlsEtn resources, especially containing DHA, in foodstuffs, and confirmed the neuronal antiapoptotic effects by PlsEtn from foodstuffs.

Of the foodstuffs examined, ascidian, blue mussel, and cattle contained high levels of PlsEtn (greater than 80 mol% of EtnGpl). Kraffe *et al*. reported that PlsEtn in some bivalves accounted for 75.8–85.0 mol% of EtnGpl [29]. We also previously reported that ascidian and bivalves except scallop contained high levels of PlsEtn (greater than 80 mol% of EtnGpl) by a method using phospholipase C [17]. Thus, PlsEtn levels of ascidian and blue mussel examined in this study were nearly the same as those reported previously. Scott *et al*. reported that 65 mol% of EtnGpl was PlsEtn in skeletal muscles of ruminants, including cattle [30]. Although the PlsEtn level of cattle muscle examined in this study was higher than that reported previously, the difference was thought to be due to the part of muscle and aging: the previous study analyzed longissimus dorsi muscles immediately after slaughter, whereas we used leg muscle after aging for eating. Figure 2a also shows that the cattle muscle examined in this study contained high levels of PlsEtn.

The muscles of salmon and amberjack had low levels of PlsEtn (17.6 and 24.6 mol% of EtnGpl, respectively). Previous studies in fishes indicated that PlsEtn levels of the muscles of bonito and tuna species were 17.5–32.3 mol% [31] and 38.9–62.1 mol% [32] of EtnGpl, respectively. Therefore, not all fishes contain low levels of PlsEtn.

Comprehensive analysis using LC–MS/MS clarified the profiling of EtnGpl species in foodstuffs. Figures 1 and 2 show EtnGpl species detected by precursor ion scanning of 196 Da in negative ion mode. Although neutral loss scanning of 141 Da in positive ion mode can also be used to profile EtnGpl species, the intensities of PtdEtn differ from those of PlsEtn [33]. It was reported that spectra obtained by precursor ion scanning of 196 Da reflected the composition of PlsEtn and fatty acid, relatively [18]. PlsEtn containing DHA was detected as 18:0/22:6-PlsEtn, 16:0/22:6-PlsEtn, and 18:1/22:6-PlsEtn. The predominant fatty acids of PlsEtn species were EPA and DHA at the *sn*-2 position of the

Table 4 Comparison of amounts of PlsEtn species in the foodstuffs examined

	Part	18.0/18:1-PlsEtn		18:0/20:4-PlsEtn		18:0/20:5-PlsEtn		18:0/22:6-PlsEtn	
		EtnGpl mol%	μmol/100 g wet wt	EtnGpl mol%	μmol/100 g wet wt	EtnGpl mol%	μmol/100 g wet wt	EtnGpl mol%	μmol/100 g wet wt
Blue mussel	Muscle	1.1 ± 0.1	7.1 ± 0.9	3.7 ± 0.4	23.6 ± 2.7	12.6 ± 1.3	81.3 ± 8.5	3.9 ± 0.6	25.5 ± 4.2
Scallop	Muscle + mantle	Trace		Trace		0.5 ± 0.2	0.7 ± 0.2	Trace	
	Viscera	Trace		3.2 ± 0.4	2.7 ± 0.4	9.5 ± 0.1	8.0 ± 0.1	5.6 ± 0.7	4.7 ± 0.6
Cuttlefish	Muscle	Trace		0.5 ± 0.0	1.7 ± 0.0	2.3 ± 0.3	7.8 ± 1.0	2.9 ± 0.3	9.8 ± 1.1
	Viscera	Trace		Trace		Trace		Trace	
Ascidian	Muscle	1.2 ± 0.2	3.5 ± 0.6	1.7 ± 0.2	4.9 ± 0.5	16.0 ± 1.3	44.6 ± 3.5	7.2 ± 0.6	19.9 ± 1.6
	Viscera	5.2 ± 0.4	13.7 ± 1.2	5.9 ± 0.5	15.5 ± 1.4	32.5 ± 3.3	86.3 ± 8.7	25.5 ± 2.5	67.7 ± 6.7
Salmon	Muscle	Trace		Trace		Trace		0.9 ± 0.0	1.3 ± 0.0
Amberjack	Muscle	Trace		Trace		Trace		1.0 ± 0.2	2.5 ± 0.5
Cattle	Leg muscle	1.4 ± 0.4	2.4 ± 0.7	12.3 ± 1.7	21.3 ± 2.9	0.4 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	0.6 ± 0.0
Pig	Leg muscle	1.3 ± 0.5	1.1 ± 0.4	15.9 ± 1.2	13.4 ± 1.0	0.1 ± 0.0	0.1 ± 0.0	0.8 ± 0.0	0.6 ± 0.0
Chicken	Leg muscle	1.0 ± 0.0	2.8 ± 0.1	4.0 ± 0.3	11.8 ± 0.9	Trace		1.4 ± 0.3	4.2 ± 0.8

Values are means ± standard deviation ($n = 5$). EtnGpl was analyzed by HPLC-ELSD after solid-phase extraction and 769 was used as average molecular weight. Trace indicates less than 0.05 mol%

EtnGpl ethanolamine glycerophospholipid, *PlsEtn* ethanolamine plasmalogen

glycerol moiety in marine foodstuffs, whereas the PlsEtn species in land foodstuffs were 20:4. Moreover, land foodstuffs were characterized by the presence of PlsEtn containing 22:5, and marine foodstuffs had PlsEtn containing minor fatty acids as 17:1, 20:2, 20:3, and 22:2. In previous studies, it was also reported that PlsEtn from chicken skin, sheep muscle, and cattle muscle contain 20:4 (47.6, 27.1, and 19.9 mol%, respectively) as the main fatty acid and have 22:5 [30, 34]. On the other hand, PlsEtn from ascidian muscle, the viscera, and different bivalves contain a large amount of EPA (64.5, 51.7, and 12.8–36.7 mol%, respectively) and DHA (14.1, 18.1, and 4.7–26.9 mol%, respectively) and have minor fatty acids as described above [35, 36]. It is thought that the lipids of marine animals contain EPA, DHA, and minor fatty acids because the animals' diets consist of marine bacteria and algae which synthesize their fatty acids [37].

On quantitative analysis, the ascidian viscera were found to contain the highest levels of 18:0/20:5-PlsEtn and 18:0/22:6-PlsEtn in the foodstuffs examined. Although 16:0/22:6-PlsEtn and 18:1/22:6-PlsEtn were not quantified because no standards were available, these species were thought to be present at low levels in foodstuffs on the basis of the results of precursor ion scanning.

In this study, extrinsic EtnGpl from ascidian viscera suppressed the neuronal cell death compared with EtnGpl from bovine brain. The extrinsic EtnGpl suppressed activities of caspase family members, and EtnGpl from ascidian viscera strongly suppressed caspases 3 and 9. Caspases 9 and 8 are known to be associated with the mitochondrial pathway [38] and death receptor pathway [39], respectively. Caspase 3 induces apoptosis as the effector of these pathways. Therefore, the antiapoptotic effects of EtnGpl from ascidian viscera were thought to be largely dependent on suppression of the mitochondrial pathway. It was reported that the levels of mitochondrial pathway-associated protein (i.e., P53 and cytochrome c) expression in neuroblastoma Neuro2A cells were increased by serum starvation, and these increased expression levels were suppressed by extrinsic EtnGpl from bovine brain and chicken skin [16, 40]. On the other hand, although death receptor pathway-associated protein (i.e., activated ASK 1) expression was not increased by serum starvation, the expression was suppressed by extrinsic EtnGpl from bovine brain. Hence, regardless of the resource of PlsEtn, PlsEtn can suppress neuronal apoptosis via the mitochondrial pathway. Moreover, PlsEtn may also prevent AD progression by suppression of apoptosis via the death receptor pathway because tumor necrosis factor as the ligand is closely related to the injury in AD brain [41].

Our previous study indicated that the antiapoptotic effect of PlsEtn containing DHA was stronger than that of other PlsEtn species, and other phospholipids containing DHA did not show the effect [16]. In the present study, 18:0/22:6-PlsEtn in EtnGpl from bovine brain and ascidian viscera accounted for 10.3 and 25.5 mol% of EtnGpl (11.9, and 29.4 mol% DHA of fatty acids), respectively. Although 50 μM EtnGpl from bovine brain and 25 μM

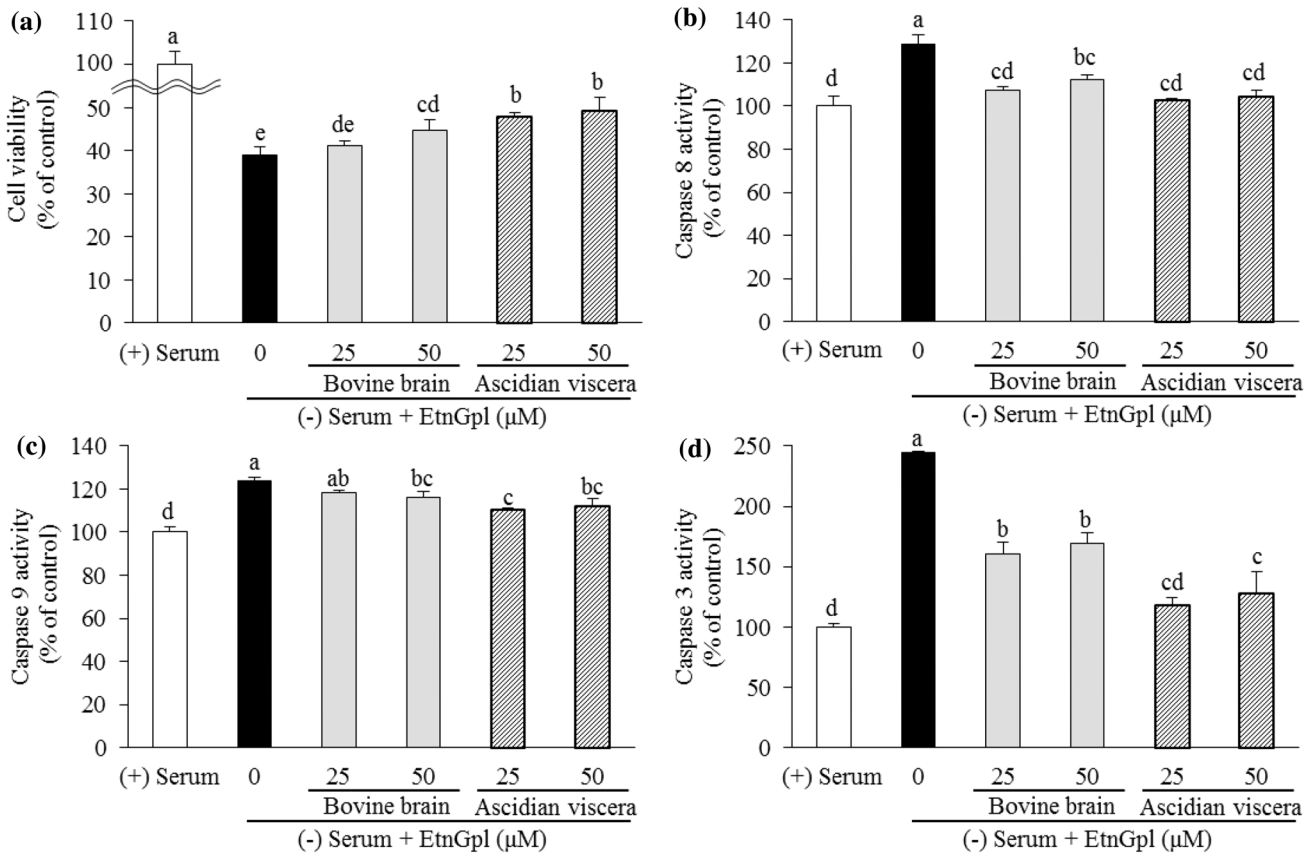

Fig. 4 Effects of EtnGpl containing high levels of PlsEtn on proliferation and caspase activities of neuroblastoma SH-SY5Y cells. **a** SH-SY5Y cells were cultured with PlsEtn from bovine brain or sea squirt in the absence of FBS for 36 h. Viable cells were estimated using the water-soluble tetrazolium salt assay. **b**, **c** Following incubation with each caspase substrate for 8 h, caspase activity was measured by the colorimetric method at 405 nm. **d** Following incubation with the caspase substrate for 24 h, caspase 3 activity was measured at 405 nm. Values in control cells in the presence of FBS were taken as 100 %. Values are mean ± SD, **a** $n = 4$, **b**, **c** $n = 3$, **d** $n = 6$. Means without a common letter are significantly different ($P < 0.05$). *EtnGpl* ethanolamine glycerophospholipid, *PlsEtn* ethanolamine plasmalogen

EtnGpl from ascidian viscera had nearly the same level of 18:0/22:6-PlsEtn, the latter showed stronger suppressive effects on neuronal apoptosis. Therefore, the antiapoptotic effects may also be related to PlsEtn containing EPA, which was abundant in ascidian viscera.

Although dietary PlsEtn have a low absorption factor [42], PlsEtn levels in the blood and liver could be markedly increased by ingestion of PlsEtn over a fixed term [28]. It is not clear whether PlsEtn administration could increase PlsEtn levels in the brain. On the other hand, amyloid-β (Aβ) is deposited in the form of plaques in patients with AD, inducing oxidative injury in the brain and progressing AD pathologies [43, 44]. Recently, it was reported that Aβ production in the liver has a connection with Aβ accumulation in the brain [45, 46]. PlsEtn administration suppressed Aβ accumulation in the brain induced by intraperitoneal injection of lipopolysaccharide [47]. Moreover, the level of PlsEtn with DHA decreases in the blood of patients with AD and has a negative correlation with plasma Aβ level [48]. It is also reported that PlsEtn suppresses Aβ production and aggregation [48, 49]. Therefore, an increase in PlsEtn levels in the blood and liver might also suppress AD progression.

In conclusion, we searched for resources of PlsEtn, especially containing DHA, in foodstuffs, and determined that PlsEtn containing DHA and EPA are abundant in ascidian viscera. Extrinsic EtnGpl from ascidian viscera suppressed neuronal apoptosis via the mitochondrial pathway. These results suggest that PlsEtn, especially that containing EPA and DHA, from marine foodstuffs is potentially useful for a therapeutic dietary supplement preventing neurodegenerative diseases, such as AD.

References

1. Dembitsky VM (1988) Quantification of plasmalogen, alkylacyl and diacyl glycerophospholipids by micro-thin-layer chromatography. J Chromatogr 436:467–473

2. Yang HC, Farooqui AA, Horrocks LA (1996) Plasmalogen-selective phospholipase A2 and its role in signal transduction. J Lipid Mediator Cell Signal 14:9–13
3. Farooqui AA, Horrocks LA (2006) Phospholipase A2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist 12:245–260
4. Hack MH, Helmy FM (1977) Thin-layer chromatographic resolution of molecular species of ethanolamine plasmalogen quantitatively unique to myelin. J Chromatogr 135:229–234
5. Glaser PE, Gross RW (1994) Plasmenylethanolamine facilitates rapid membrane fusion: a stopped-flow kinetic investigation correlating the propensity of a major plasma membrane constituent to adopt an HII phase with its ability to promote membrane fusion. Biochemistry 33:5805–5812
6. Wallner S, Schimtz G (2011) Plasmalogens the neglected regulatory and scavenging lipid species. Chem Phys Lipids 164:573–589
7. Broniec A, Klosinski R, Pawlak A, Wrona-Krol M, Thompson D, Sarna T (2011) Interactions of plasmalogens and their diacyl analogs with singlet oxygen in selected model systems. Free Radical Biol Med 50:892–898
8. Luoma AM, Kuo F, Cakici O, Inouye H, Crowther MN, Denninger AR, Avila RL, Brites P, Kirschner DA (2015) Plasmalogen phospholipids protect internal myelin form oxidative damage. Free Radical Biol Med 84:296–310
9. Busciglio J, Yankner BA (1995) Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons *in vitro*. Nature 378:776–779
10. Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267:1456–1462
11. Yamatsuji T, Matsui T, Okamoto T, Komatsuzaki K, Takeda S, Fukumoto H, Iwatsubo T, Suzuki N, Asami-Odaka A, Ireland S, Kinane TB, Giambarella U, Nishimoto I (1996) G protein-mediated neuronal DNA fragmentation induced by familial Alzheimer's disease-associated mutants of APP. Science 272:1349–1352
12. Ginsberg L, Rafique S, Xuereb JH, Rapoport SI, Gershfeld NL (1995) Disease and anatomic specificity of ethanolamine plasmalogen deficiency in Alzheimer's disease brain. Brain Res 698:223–226
13. Wells K, Farooqui AA, Liss L, Horrocks LA (1995) Neural membrane phospholipids in Alzheimer disease. Neurochem Res 20:1329–1333
14. Guan Z, Wang Y, Cairns NJ, Lantos PL, Dallner G, Sindelar PJ (1999) Decrease and structural modifications of phosphatidylethanolamine plasmalogen in the brain with Alzheimer disease. J Neuropathol Exp Neurol 58:740–747
15. Miyazawa T, Kanno S, Eitsuka T, Nakagawa K (2006) Plasmalogen: a short review and newly-discovered functions. In: Knapp HR, Huang YS, Yanagita Y (eds) Dietary Fats and Risk of Chronic Disease. AOCS, Urbana
16. Yamashita S, Kanno S, Nakagawa K, Kinoshita M, Miyazawa T (2015) Extrinsic plasmalogen suppresses neuronal apoptosis in mouse neuroblastoma Neuro-2A cells: importance of plasmalogen molecular species. RSC Adv 5:61012–61020
17. Yamashita S, Abe A, Nakagawa K, Kinoshita M, Miyazawa T (2014) Separation and detection of plasmalogen in marine invertebrates by high-performance liquid chromatography with evaporative light-scattering detection. Lipids 49:1261–1273
18. Yamashita S, Honjo A, Aruga M, Nakagawa K, Miyazawa T (2014) Preparation of marine plasmalogen and selective identification of molecular species by LC-MS/MS. J Oleo Sci 63:423–430
19. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509
20. Rouser G, Kritchevsky G, Simon G, Nelson GJ (1967) Quantitative analysis of brain and spinach leaf lipids employing silicic acid column chromatography and acetone for elution of glycolipids. Lipids 2:37–40
21. Higuchi O, Nakagawa K, Tsuzuki T, Suzuki T, Oikawa S, Miyazawa T (2006) Aminophospholipid glycation and its inhibitor screening system: a new role of pyridoxal 5′-phosphate as the inhibitor. J Lipid Res 47:964–974
22. Viswanathan CV, Phillips F, Lundberg WO (1968) Two-dimensional reaction thin-layer chromatography in the analysis of phosphatide plasmalogens. J Chromatogr 35:66–71
23. Ishiyama M, Tominaga H, Shiga M, Sasamoto K, Ohkura Y, Ueno K (1996) A combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. Biol Pharm Bull 19:1518–1520
24. Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP, Chapman KT, Nicholson DW (1997) A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. J Biol Chem 272:17907–17911
25. Wolf BB, Schuler M, Echeverri F, Green DR (1999) Caspase-3 is the primary activator of apoptotic DNA fragmentation via DNA fragmentation factor-45/inhibitor of caspase-activated DNase inactivation. J Biol Chem 274:30651–30656
26. Maeba R, Ueta N (2003) Ethanolamine plasmalogens prevent the oxidation of cholesterol by reducing the oxidizability of cholesterol in phospholipid bilayers. J Lipid Res 44:164–171
27. Maeba R, Ueta N (2003) Ethanolamine plasmalogen and cholesterol reduce the total membrane oxidizability measured by the oxygen uptake method. Biochem Biophys Res Commun 302:265–270
28. Nishimukai M, Wakisaka T, Hara H (2003) Ingestion of plasmalogen markedly increased plasmalogen levels of blood plasma in rats. Lipids 38:1227–1235
29. Kraffe E, Soudant P, Marty Y (2004) Fatty acids of serine, ethanolamine, and choline plasmalogens in some marine bivalves. Lipids 39:59–66
30. Scott TW, Ashes JR, Fleck E, Gulati SK (1993) Effect of fish oil supplementation on the composition of molecular species of choline and ethanolamine glycerophospholipids in ruminant muscle. J Lipid Res 34:827–835
31. Ohshima T, Wada S, Koizumi C (1989) 1-O-Alk-1′-enyl-2-acyl and 1-O-alkyl-2-acyl glycerophospholipids in white muscle of bonito *Euthynnus pelamis* (Linnaeus). Lipids 24:363–370
32. Medina I, Aubourg SP, Martin RP (1995) Composition of phospholipids of white muscle of 6 tuna species. Lipids 30:1127–1135
33. Berry KAZ, Murphy RC (2004) Electrospray ionization tandem mass spectrometry of glycerophosphoethanolamine plasmalogen phospholipids. J Am Soc Mass Spectrom 15:1499–1508
34. Yunoki K, Kukino O, Nadachi Y, Fujino T, Ohnishi M (2008) Separation and determination of functional complex lipids from chicken skin. J Am Oil Chem Soc 85:427–433
35. Jeong BY, Ohshima T, Koizumi C (1996) Hydrocarbon chain distribution of ether phospholipids of the ascidian *Halocynthia roretzi* and the sea urchin *Strongylocentrotus intermedius*. Lipids 31:9–18
36. Hanuša LO, Dmitri OL, Shkrobc I, Dembitsky VM (2009) Plasmalogens, fatty acids and alkyl glyceryl ethers of marine and freshwater clams and mussels. Food Chem 116:491–498
37. Tomaz ACD, de Miranda GEC, de Souza MDV, de Cunha EVL (2012) Analysis and characterization of methyl esters of fatty acids of some *Gracilaria* species. Biochem Syst Ecol 44:303–306

38. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X (1999) Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 15:269–290
39. Ashkenazi A, Dixit VM (1998) Death receptors: signaling and modulation. Science 281:1305–1308
40. Hossain MS, Ifuku M, Take S, Kawamura J, Miake K, Katafuchi T (2013) Plasmalogens rescue neuronal cell death through an activation of AKT and ERK survival signaling. PLoS One 8:e83508
41. Mattson MP, Barger SW, Furukawa K, Bruce AJ, Wyss-Coray T, Mark RJ, Mucke L (1997) Cellular signaling roles of TGF beta, TNF alpha and beta APP in brain injury responss disease. Brain Res Rev J 23:47–61
42. Hara H, Wakisaka T, Aoyama Y (2003) Lymphatic absorption of plasmalogen in rats. Br J Nutr 90:29–32
43. Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M, Morser J, Migheli A, Nawroth P, Stern D, Schmidt AM (1996) RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. Nature 382:685–691
44. Pratico D, Delanty N (2000) Oxidative injury in diseases of the central nervous system: focus on Alzheimer's disease. Am J Med 109:577–585
45. Sutcliffe JG, Hedlund PB, Thomas EA, Bloom FE, Hilbush BS (2011) Peripheral reduction of β-amyloid is sufficient to reduce brain β-amyloid: implications for Alzheimer's disease. J Neurosci Res 89:808–814
46. Skerrett R, Pellegrino MP, Casali BT, Taraboanta L, Landreth GE (2015) Combined liver X receptor/peroxisome proliferator-activated receptor γ agonist treatment reduces amyloid β levels and improves behavior in amyloid precursor protein/presenilin 1 mice. J Biol Chem 290:21591–21602
47. Ifuku M, Katafuchi T, Mawatari S, Noda M, Miake K, Sugiyama M, Fujino T (2012) Anti-inflammatory/anti-amyloidogenic effects of plasmalogens in lipopolysaccharide-induced neuroinflammation in adult mice. J Neuroinflammation 9:197
48. Yamashita S, Kiko T, Fujiwara H, Hashimoto M, Nakagawa K, Kinoshita M, Furukawa K, Arai H, Miyazawa T (2015) Alterations in the levels of amyloid-β, phospholipid hydroperoxide, and plasmalogen in the blood of patients with Alzheimer's disease: Possible interactions between amyloid-β and these lipids. J Alzheimers Dis. doi:10.3233/JAD-150640
49. Onodera T, Futai E, Kan E, Abe N, Uchida T, Kamio Y, Kaneko J (2015) Phosphatidylethanolamine plasmalogen enhances the inhibiting effect of phosphatidylethanolamine on γ-secretase activity. J Biochem 157:301–309