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Study on the effect of eicosapentaenoic acid on phospholipids composition in membrane microdomains of tight junctions of epithelial cells by liquid chromatography/electrospray mass spectrometry

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Abstract

Tight junctions of epithelial cells determine epithelial membrane integrity and play an important role in selective paracellular permeability to ions and macromolecules. In this work, we investigated the effect of one of n-3 series polyunsaturated fatty acids, eicosapentaenoic acid (EPA) on the phospholipid composition of membrane microdomains of tight junctions. After treated by EPA, membrane microdomains of tight junctions were isolated by discontinuous sucrose density gradient ultracentrifugation, and raft phospholipids were extracted. The PE, PI, PS, PC and SM were separated and determined by high-performance liquid chromatography/quadrupole-linear ion trap mass spectrometry (HPLC Qtrap-MS), and were further identified by HPLC–MS/MS. It was found that EPA altered the fatty acyl substitution of phospholipids that constituted membrane microdomains of tight junctions by enriching the unsaturated fatty acyl chains of the phospholipids. It provides a new visual angle to explaining the intracellular mechanism involved in n-3 polyunsaturated fatty acids (PUFAs) modulation of intestinal tight junction barrier.

Keywords: Eicosapentaenoic acid; Tight junction; Phospholipids; HPLC–MS

1. Introduction

The structural foundations of intestinal mucosa barrier are intact enteric epithelia and the junctions between adjacent intestinal epithelia. Tight junctions are the primary ones between intestinal epithelia, and serve as the maintenance of epithelial cell polarity and the rate-limiting barrier to passive movement of hydrophilic solutes across intestinal epithelia [1]. It also plays an important role in maintaining integral intestinal epithelia and protecting intestinal barrier, as well as preventing bacterial endotoxin and other toxins into body. Tight junctions are specialized lipid microdomains. The increasing evidences show that tight junctions act a multifunctional platform for trafficking and signaling protein complexes [2,3]. Recently, tight junctions are considered as raft-like membrane compartments and represent structural and functional distinct membrane microdomains, which are fundamental in spatial organization of tight junctions and in regulation of paracellular permeability in epithelial cells [1].

Dietary lipids are essential components of each living cell, they are not only especially important for the integrity of bilipid structures of cell membranes [4], but also important sources of energy and precursors for numerous biologically active compounds. Humans can synthesize all lipids needed for various biological activities except the n-3 and n-6 polyunsaturated fatty acids (PUFAs). n-3 PUFAs have received increasing attention in many fields, such as anti-cancer effects [5], and modulability of immune responses [6], etc. In recent years, eicosapentaenoic

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acid (EPA) and docosahexaenoic acid (DHA) are considered to have important roles as "immunonutrients" and exert beneficial effects on inflammatory bowel diseases and autoimmune disease [4,6]. Moreover, the patients with an inflammatory bowel disease, such as Crohn's disease (CD), manifest a defect in tight junction barrier and an increase in intestinal permeability [7–9]; therefore the effect of n-3 PUFAs on tight junctions is an increasing research hotspot.

In recent years, a few groups have investigated the effects of PUFAs on tight junctions of both epithelial cells [10–12] and endothelial cells [13,14]. It was reported that EPA or DHA reduced the paracellular permeability of large molecules [13,14] or enhanced permeability of ipophobic small molecules [11,12]. Some researches have been done to explore the essential cause [10–14], such as up-regulating the expression of occludin or occludin messenger RNA in tight junctions. However, to date, there is little information on the effect of n-3 PUFAs on the fatty acyl chains of phospholipids of membrane microdomains of tight junctions, and the intracellular mechanism involved in n-3 PUFAs modulation of intestinal tight junction barrier remains unclear.

In this work, we suppose that supplementation of epithelial cells with PUFAs, such as EPA, possibly alters the fatty acyl substitution of phospholipids that constitute membrane microdomains of tight junctions. After treated by EPA, the membrane microdomains of tight junctions of T84 epithelial cells were isolated, and raft phospholipids were separated and determined by HPLC Qtrap-MS, and were further identified by HPLC–MS/MS. The results show that EPA altered the phospholipid composition of membrane raft in tight junctions, and this work provides a new angle to explaining the mechanism involved in n-3 PUFAs modulation of intestinal tight junction barrier.

2. Experimental

2.1. Materials

T84 epithelial cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Dulbecco's modified Eagle's medium (DMEM), F-12 nutrient mixture (Ham) and serum-free Iscove's modified Dulbecco's medium were from Invitrogen Inc. (Grand Island, NY), and fetal bovine serum from GibcoBRL (GibcoBRL, Grand Island, NY, USA). Bovine serum albumin (fraction V) was obtained from Roche Diagnostic Inc. (Indianapolis, IN). Complete protease inhibitor tablets were purchased from Boehringer Mannheim (Indianapolis, IN). *Cis*-5, 8, 11, 14, 17-eicosapentaenoic acid (EPA (20:5, n-3)) and stearic acid (18:0) were purchased from Sigma–Aldrich Inc. (St. Louis, MO). 1, 2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine (C14:0/C14:0 PE) and 1, 2-dimyristoyl-*sn*-glycero-3-phosphocholine (C14:0/C14:0 PC) were from Avanti Polar Lipids (Alabaster, AL). Other phospholipids standards were from Avanti Polar Lipids or Sigma (St. Louis, MO). 2, 6-Di-*tert*-butyl-4-methylphenol was from Aldrich-Chemie (Steinheim, Germany). Formic acid and all the solvents were HPLC grade (TEDIA); ammonia (25%) was analytical grade from Lian-Bang (Shenyang, China).

2.2. Epithelial cell culture and fatty acid treatment

T84 epithelial cells were incubated on 75 cm^2 flasks (Corning Costar, Acton, MA) at 37 °C with 5% CO_2 in a medium composing of 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium with 5% heat-inactivated fetal bovine serum, 14 mM $NaHCO_3$, 15 mM HEPES buffer, at a pH of 7.3 and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). For subcultures, cells were digested with 0.25% trypsin and 1 mM EDTA in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline. In the experiments the cells were cultured for 2 days in serum-free Iscove's modified Dulbecco's medium, supplemented with 0.4% (w/v) bovine serum albumin (fraction V), with addition of 50 μM of stearic acid (18:0) or EPA (20:5 n-3).

2.3. Isolation of membrane lipid raft in tight junctions

Lipid rafts were isolated from T84 epithelial cells as described [15] by discontinuous sucrose density gradient ultracentrifugation. The cell monolayers were washed three times with ice-cold PBS and then scraped. Cells were collected by centrifugation and resuspended in 1 ml extraction buffer (50 mM Tris, 25 mM KCl, 5 mM $MgCl_2{\cdot}6H_2O$, 1 mM EDTA, 40 mM NaF, 4 mM Na_3VO_4, pH 7.4) containing 1% TritonX-100 and protease inhibitor mixture, and sonated with 4 × 10-s bursts using an ultrasonic disrupter (Ultrasonic disrupter Virsonic 100, Virtis Co., Gardiner, NY). The sucrose concentration of the cell lysate was adjusted to 40%, placed at the bottom of an ultracentrifuge tube, and overlaid with sucrose gradients of 30, 25, 20 and 5% in the extraction buffer. The samples were centrifuged at 250,000 × *g* for 18 h at 4 °C in an Optima L-80XP ultracentrifuge (Beckman Coulter Inc. Fullerton, CA). Fractions of 1 ml were collected from the top of the gradients one by one and stored at −80 °C until analysis.

2.4. Extraction of phospholipids in the lipid raft

Polar lipids in detergent-resistant fractions from EPA-treated and controlled T84 epithelial cells were extracted using the method of Uran et al. [16] with some modifications. Briefly, appropriate amount of internal standards (C14:0/C14:0 PE, C14:0/C14:0 PC) were added to the samples and mixed, then 1 ml of methanol containing 0.01% (w/v) 2, 6-Di-*tert*-butyl-4-methylphenol was added and whirlmixed. After that, 4 ml of chloroform was added and stirred with the addition of 1 ml of methanol. After 250 μl of 200 mM KCl was added and vortexed, the sample was centrifuged at 4000 rpm for 10 min, the lower organic phase was transferred. The extraction procedure was repeated twice and the organic phase was collected, filtered by 0.45 μm organic filters, and dried using a rotary evaporator at 38 °C. Prior to analysis, the extracted samples were redissolved in 300 μl of chloroform/methanol (2:1, v/v) and diluted 3 times with hexane/1-propanol (3:2, v/v).

2.5. HPLC–MS for membrane raft phospholipids in tight junctions

An HP 1100 series HPLC system (Agilent Technologies, Palo Alto, CA), coupled to a QTrap tandem mass spectrometer (Applied Biosystems Instrument Corporation, USA) equipped with an electrospray ion source (ESI) was employed according to the procedure described in literature [17].

The analysis was performed on a diol column (Nucleosil, 100-5 OH), 250 mm × 3.0 mm (i.d.) × 5.0 μm (particle size), at a flow rate of 0.4 ml/min, and the column temperature was kept at 35 °C. Solvent mixture A was hexane/1-propanol/formic acid/ammonia (79:20:0.6:0.07, v/v), solvent mixture B was 1-propanol/water/formic acid/ammonia (88:10:0.6:0.07, v/v). The column was eluted with a linear gradient of 32–80% B over 0–20 min, the composition was held at 80% B for 13 min, and then returned to 32% B in 5 min.

The detection of phospholipids eluted from the chromatographic column was performed in the "enhanced MS" (EMS), and the structure of phospholipids was elucidated by the "enhanced" product ion (EPI) scan mode. The mass spectrometer was operated in negative-ion mode. The ion sprayer voltage was set at −4200 V, and the source temperature was set at 375 °C. The ion source and ion optic parameters were optimized with respect to the negative molecular related ions of the phospholipid standards. The curtain gas was set at 30 psi, with a nebulization gas pressure of 45 psi and an auxiliary heat gas pressure of 40 psi. The declustering potential was set at −80 V. The other parameters were as follows: EMS was employed as survey scan to scan from 417 to 917 *m/z* with 20 ms LIT fill time, and EPI was used as dependent scan with 150 ms LIT fill time and a collision energy of −40 eV. Q0 trapping was always on.

2.6. Data collection and normalization

Considering negative-ion mode ESI-MS gives more information-rich data than positive-ion ESI, negative-ion LC/MS chromatogram (Fig. 1) was inspected for profiling the phospholipid species in raft fraction. Due to the limit of the extraction method, we only detected the familiar phospholipids in cell membrane (e.g. PE, PS, PI, PC and SM), whereas other phospholipids in cell membrane (e.g. phosphatidic acids) were not determined. Masses corresponding to $[M-H]^-$ (for PE, PS, and PI species) or $[M-15]^-$ (for PC, and SM species) for each phospholipid species were plotted against elution time. After phospholipids' peaks were matched based on the mass-to-charge ratio and retention time of each class of phospholipid, the peak intensities in the extracted ion chromatography were normalized by that of internal standard with homemade software.

The total phosphorus content of the raft fraction was determined as previously described [18]. After calibrated by internal standard, the above data were transformed into internal standard equivalent/phosphorus content of the raft fraction.

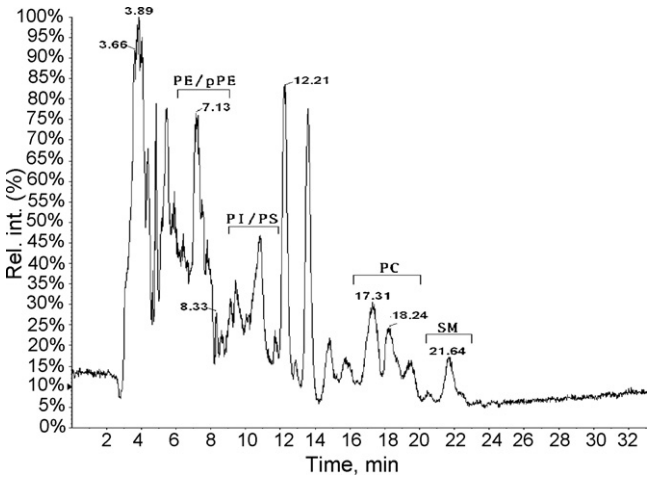

Fig. 1. Gradient negative-ion LC/ESI-MS of phospholipid mixture obtained from membrane raft phospholipids in tight junctions of epithelial cells showing the total ion current mass chromatogram. The experimental conditions of LC/ESI-MS are described in the text. PE, phosphatidylethanolamine; pPE, plasmalogen phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin.

2.7. Statistical analysis

Data were expressed as mean ± S.E.M. Significant differences between groups were evaluated using analysis of variance (ANOVA) in the SPSS 13.0 for windows. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. HPLC/MS analysis

The full scan of phospholipid species in the raft fraction of T84 epithelial cells was performed in negative-ion mode to obtain the relatively high sensitivity for most of phospholipids. Under our separation conditions, PE molecules were firstly eluted, and PS (PI), PC and SM molecules were eluted in turns. Phospholipid molecules of a class have the same polar head, thus their retention times (tr) in normal-phase HPLC are very adjacent. The tr difference in different phospholipids in a class is much smaller than that in two different classes, this fact could be used to match and identify phospholipid species in the extraction ion chromatography (XIC).

In the negative-ion mode, masses corresponding to the quasi-molecular anions of PE, PS and PI species mainly were $[M-H]^-$ ion, and those of PC and SM species were $[M-CH_3]^-$ and $[M+HCOO^-]^-$ ions. The selection of C14:0/C14:0 PE, C14:0/14:0 PC as internal standards is based on their solubility and their very low content in various cells (≪1%), which was also confirmed through acquiring a mass spectrum without internal standards. The quasi-quantifications were achieved by comparison of the ion peak intensity of phospholipids with that of a corresponding internal standard. PI/PS and SM quasi-quantification was based on PE and PC internal standards (C14:0/C14:0 PE, C14:0/14:0 PC) respectively, owing to the lack of commercial internal standards, and their

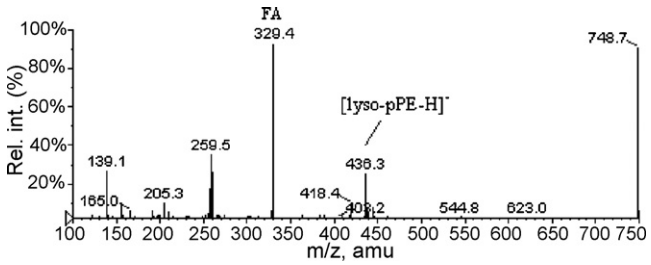

Fig. 2. Enhanced product ion (EPI) spectrum of *m/z* 748.7 representing the $[M-H]^-$ ion of pPE. FA: fatty acid residues, lyso-pPE-H: pPE losing one of the two fatty acid residues.

peaks being close to those of PE and PC species respectively as shown in Fig. 1. Identification of the phospholipid structures was achieved by HPLC-ESI-MS/MS [19–21]. For instance, the negative enhanced product ion spectrum of *m/z* 748.7 representing the $[M-H]^-$ ion of pPE was shown in Fig. 2. The fragment ion at *m/z* 436.3 corresponded to C16:0 lyso-pPE ion, the fragment ion detected at *m/z* 329.4 corresponded to C22:5 fatty acid residue (carboxylate anion fragment). The position of the acyl chains in the glycerol backbone of a phospholipid molecule is obviously important for their degree of dissociation. In this paper, we adopt the idea that the phospholipids isolated from animals most often contain a saturated fatty acid in *sn*-1 position and an unsaturated fatty acid in *sn*-2 position [22].

3.2. EPA treatment alters fatty acyl substitution of phospholipids in raft membrane microdomains in tight junctions of T84 epithelial cells

As shown in Table 1, in raft membrane microdomains of tight junctions from EPA-treated cells, PE species substituted with 34:2 (16:1/18:1, 16:0/18:2) decreased significantly, whereas 36:5 (p16:0/20:5), 38:6 (p16:0/22:6), 38:5 (p16:0/22:5), 38:4 (p18:0/20:4, p16:0/22:4), 38:6 (18:1/20:5), 40:5 (p18:0/22:5), 40:4 (p20:0/20:4), 40:7 (18:1/22:6), 40:6 (18:0/22:6) and 40:5 (18:0/22:5, 20:1/20:4, 20:0/20:5) fatty acyl substitutions markedly increased comparing with controls. EPA treatment observably enhanced concentration of PE species substituted with 20:5, 22:5 and 22:6 acyl chains in rafts. In PI species 34:1 (16:0/18:1), 36:4 (16:0/20:4), 38:5 (18:1/20:4), 38:4 (18:0/20:4), 40:6 (18:0/22:6) and 40:5 (18:0/22:5) acyl chains were obviously more abundant in raft fractions from EPA-treated cells than that in controls. Substitutions of PS with 34:1 (16:0/18:1), 36:1 (18:0/18:1), 38:5 (16:0/22:5), 40:6 (18:0/22:6), 40:5 (18:0/22:5) and 40:4 (18:0/22:4) acyl chains were evidently increased in raft membrane microdomains of tight junctions from EPA-treated cells comparing with control cells. The concentrations of 32:1(16:0/16:1), 32:0 (16:0/16:0), 36:5 (16:0/20:5), 38:6 (18:1/20:5, 16:0/22:6), 38:5 (18:0/20:5, 16:0/22:5), 38:4 (18:0/20:4, 18:1/20:3) and 40:6 (18:1/22:5, 20:3/20:3, 20:1/20:5) acyl chains in PC species were enriched in raft fractions of EPA-treated cells. In addition, there were no significant differences for acyl chain substitution of SM species.

In order to legibly compare the change between the phospholipid composition in-raft membrane microdomains of tight junctions treated by 18:0 fatty acid and EPA respectively, the data in Table 1 were summed up and showed in Fig. 3. It was observed that after treated by EPA, PE, PC, PI and PS with fatty acyl substitutions of PUFAs in raft membrane microdomains of tight junctions increased, and the degree of the change is PE > PC > PI > PS (Fig. 3a). Phospholipids with fatty acyl chains of n-3 PUFAs also obtained the similar enrichment in detergent-resistant membrane microdomains of tight junctions, especially the increase of PE species was the most marked, and the rest were PC, PI, PS in turn (Fig. 3b). Nevertheless, the alteration of phospholipids with fatty acyl substitutions of n-6 PUFAs is dissimilar, the PE species still gained the most notable increase, PI and PS species got smaller rise than PE species. However, the PC species did not get a significant change compared with controls (Fig. 3c).

3.3. The relative content alteration of PE species with different fatty acyl substitutions after treated by EPA

Negative-ion HPLC/EMS/MS of PE species in raft membrane microdomains of tight junctions from T84 epithelial cells

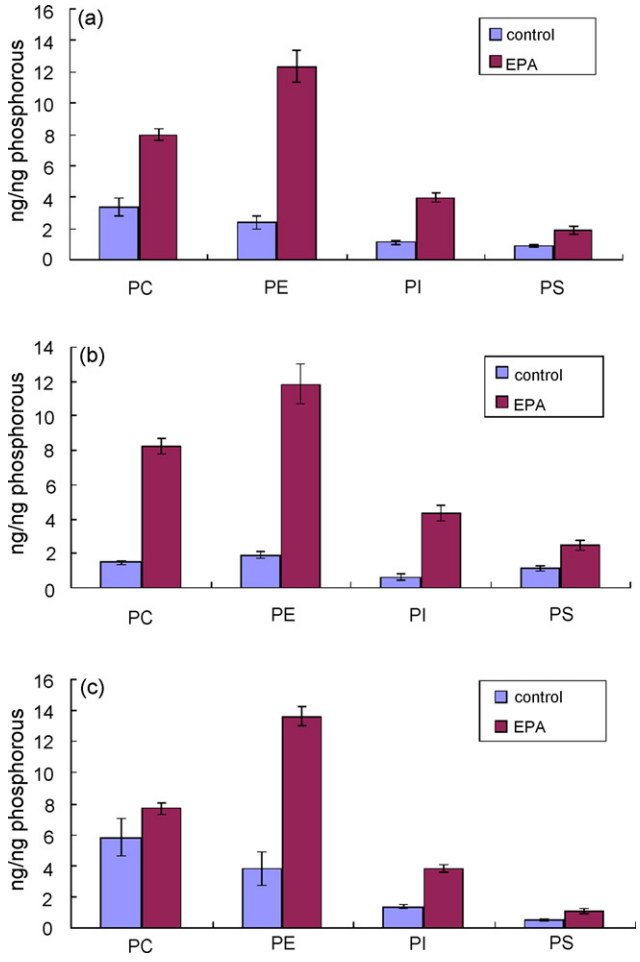

Fig. 3. Effect of EPA on different phospholipid classes with (a) fatty acyls of PUFAs; (b) fatty acyls of n-3 PUFAs; (c) fatty acyls of n-6 PUFAs from membrane microdomains of tight junctions. Cells treated with stearic acid (18:0) and 20:5 fatty acid were as control and EPA, respectively.

Table 1
Fatty acid composition of PE, pPE, PI, PS PC and SM in membrane microdomains of tight junctions from EPA-treated cells and the control

m/z	Lipid	Control	EPA treatment
PE and pPE			
714.6	34:2(16:1/18:1, 16:0/18:2)	8.62 ± 1.30	4.94 ± 0.15*
716.5	34:1(16:0/18:1)	19.55 ± 2.19	18.56 ± 1.60
720.5	36:5(p16:0/20:5)	0.62 ± 0.02	18.93 ± 2.60**
722.6	36:4(p16:0/20:4)	6.39 ± 1.72	8.76 ± 0.94
726.5	36:2(p18:1/18:1, p18:0/18:2)	6.21 ± 1.97	5.37 ± 0.64
728.6	36:1(p18:0/18:1)	3.94 ± 1.04	4.43 ± 0.44
742.5	36:2(18:1/18:1,18:0/18:2)	13.11 ± 1.32	9.97 ± 1.38
744.6	36:1(16:0/20:1,18:0/18:1)	10.81 ± 1.38	8.31 ± 0.99
746.6	38:6(p16:0/22:6)	3.13 ± 0.56	8.20 ± 0.82**
748.5	38:5(p16:0/22:5)	3.76 ± 1.03	35.20 ± 3.42***
750.6	38:4(p18:0/20:4,p16:0/22:4)	4.58 ± 1.42	9.93 ± 0.90*
762.5	38:6(18:1/20:5)	0.48 ± 0.06	9.78 ± 0.40***
776.5	40:5(p18:0/22:5)	1.34 ± 0.11	10.54 ± 1.33**
778.5	40:4(p20:0/20:4)	0.48 ± 0.07	22.19 ± 0.42***
788.6	40:7(18:1/22:6)	3.08 ± 0.23	5.38 ± 0.42**
790.5	40:6(18:0/22:6)	2.01 ± 0.05	3.29 ± 0.22**
792.6	40:5(18:0/22:5,20:1/20:4,20:0/20:5)	0.60 ± 0.09	3.49 ± 0.32***
PI			
835.6	34:1(16:0/18:1)	2.79 ± 0.29	4.58 ± 0.12**
857.7	36:4(16:0/20:4)	0.53 ± 0.11	1.98 ± 0.13***
861.6	36:2(18:0/18:2,16:0/20:2)	2.71 ± 0.37	1.77 ± 0.20
863.7	36:1(18:0/18:1)	6.06 ± 0.77	8.13 ± 1.11
883.7	38:5(18:1/20:4)	0.47 ± 0.08	4.57 ± 0.58**
885.8	38:4(18:0/20:4)	1.72 ± 0.29	6.82 ± 0.55***
909.6	40:6(18:0/22:6)	0.52 ± 0.04	0.98 ± 0.08**
911.7	40:5(18:0/22:5)	0.73 ± 0.30	7.73 ± 0.82***
PS			
760.6	34:1(16:0/18:1)	3.69 ± 0.78	6.85 ± 0.81*
786.7	36:2(18:1/18:1,18:0/18:2)	1.63 ± 0.34	2.61 ± 0.39
788.8	36:1(18:0/18:1)	2.71 ± 0.39	5.97 ± 0.88*
808.7	38:5(16:0/22:5)	0.70 ± 0.15	2.47 ± 0.29**
810.6	38:4(18:0/20:4)	0.81 ± 0.14	1.48 ± 0.21
834.8	40:6(18:0/22:6)	0.79 ± 0.16	1.42 ± 0.15*
836.7	40:5(18:0/22:5)	1.83 ± 0.24	3.59 ± 0.51*
838.8	40:4(18:0/22:4)	0.22 ± 0.03	0.63 ± 0.11*
PC			
716.6	32:1(16:0/16:1)	16.13 ± 2.04	27.92 ± 1.85*
718.7	32:0(16:0/16:0)	4.83 ± 0.48	13.12 ± 1.39**
742.6	34:2(16:0/18:2)	10.76 ± 2.07	7.93 ± 0.36
744.7	34:1(16:0/18:1)	33.00 ± 7.03	32.95 ± 1.11
764.7	36:5(16:0/20:5)	0.62 ± 0.08	18.29 ± 1.02***
766.8	36:4(16:0/20:4,18:2/18:2)	4.17 ± 0.70	3.48 ± 0.33
790.6	38:6(18:1/20:5,16:0/22:6)	2.07 ± 0.42	3.83 ± 0.28*
792.7	38:5(18:0/20:5,16:0/22:5)	2.51 ± 0.64	7.28 ± 0.24**
794.8	38:4(18:0/20:4,18:1/20:3)	2.57 ± 0.96	11.64 ± 0.62***
818.7	40:6(18:1/22:5,20:3/20:3,20:1/20:5)	0.70 ± 0.08	3.55 ± 0.36**
SM			
685.7	34:2	1.08 ± 0.19	1.17 ± 0.06
687.8	34:1(d18:1/16:0)	23.66 ± 0.63	22.24 ± 4.78

Fatty acid composition of phospholipids in membrane microdomains of tight junctions was analyzed by LC–MS and expressed in nanograms of C14:0/C14:0 PE or C14:0/C14:0 PC equivalents per nanogram of phosphorus (mean ± S.E.M.). Data presented here were from three experiments. Significant differences between raft fractions of EPA-treated cells and control were indicated by asterisks ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

with 18:0 fatty acid (control) or 20:5 fatty acid (EPA) treatment was shown in Fig. 4, respectively. Obviously, there was a considerable alteration of the relative abundance of PE molecules in detergent-resistant microdomains of tight junctions from EPA-treated cells compared with controls. PE molecules with *m/z* of 748.5 (p16:0/22:5), 778.5 (p20:0/20:4), 720.5 (p16:0/20:5) were more abundant than others (Fig. 4b). Whereas, PE molecules with *m/z* of 716.5 (16:0/18:1), 742.5 (18:1/18:1, 18:0/18:2), 744.5 (16:0/20:1, 18:0/18:1) revealed higher ion intensity in controls as shown in Fig. 4a.

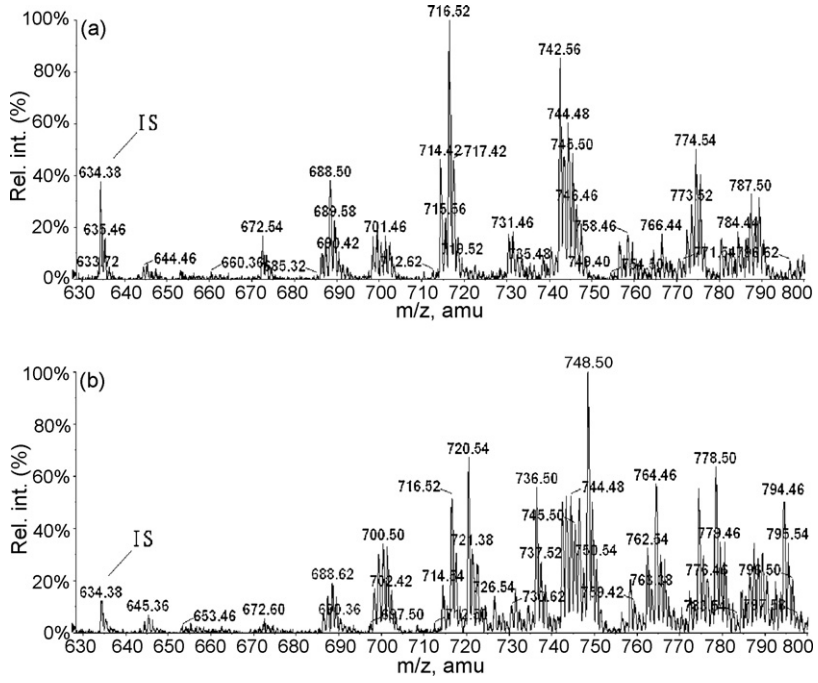

Fig. 4. Negative-ion HPLC/EMS/MS of PE species in raft membrane microdomains of tight junctions from T84 epithelial cells with (a) 18:0 fatty acid (control) treatment and (b) 20:5 fatty acid (EPA) treatment.

In Fig. 5, the above-mentioned alterations were displayed more visually. Here PE molecule of 728.6 (p36:1(p18:0/18:1)) was used as a reference to determine the corresponding PE content index. The PE molecules with *m/z* 714, 716, 726, 728, 742, 744 all had fatty acyl substitutions of monounsaturated fatty acids (MUFAs) attached to the *sn*-2 position, and got inconspicuous decrease (Fig. 5A) after treated by EPA. While there were different degree increases (Fig. 5B and C) of PE molecules with *m/z* 722, 750, 778 and 720, 746, 748, 762, 776, 788, 790, 792 which had fatty acyl chains of n-6 PUFAs and n-3 PUFAs attached to the *sn*-2 position, respectively. Thereinto, the relative content of PE molecules with 748.5 (p16:0/22:5), 778.5 (p20:0/20:4), 720.5 (p16:0/20:5), 762.5 (18:1/20:5), 776.5 (p18:0/22:5) obtained greater increase.

4. Discussion

Intestinal epithelia tight junctions are dynamic permeability barriers, and possess diplex function, which includes not only preventing the passage from lumen into the systemic circulation of larger potentially toxic compounds or pathogens, but also permitting the absorption of nutrient, electrolytes, and water.

Tight junctions are specialized lipid microdomains. Lately, it was reported that the tight junction is composed of cholesterol-rich, detergent-insoluble membrane microdomains [1,23]. It was reported that n-3 PUFAs were incorporated into lipids of the membrane leaflet in raft and could change the lipid composition, which would potentially affect the function of lipid rafts [6]. Therefore, we supposed that n-3 PUFAs may change the

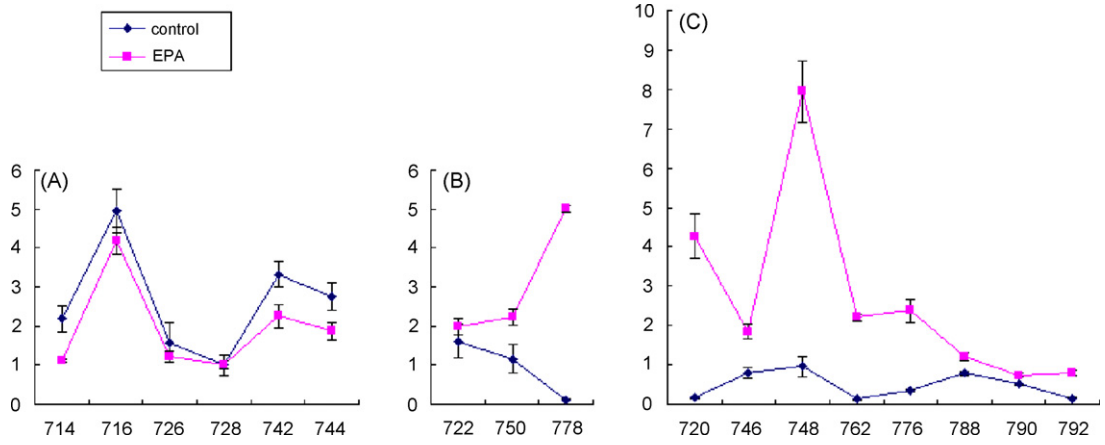

Fig. 5. Alteration in the relative abundance of PE species with different fatty acyl substitutions in membrane microdomains of tight junctions from T84 epithelial cells with 18:0 fatty acid (control) and 20:5 fatty acid (EPA) treatment. (A) fatty acyls of MUFAs, (B) fatty acyls of n-6 PUFAs, (C) fatty acyls of n-3 PUFAs. PE molecule of 728.6 (p36:1(p18:0/18:1)) was used as a reference to determine the corresponding PE content index.

lipid composition and fatty acyl substitution of phospholipids in membrane microdomains in tight junctions, which adjusted tight junctions function through redistributing tight junction proteins in membrane microdomains. To our knowledge there is no previous information concerning the effect of n-3 PUFAs on the substitution of phospholipids of membrane microdomains of tight junctions by HPLC–MS. This study described EPA, one of n-3 series of PUFAs, exerted an evident effect on the membrane raft phospholipids in tight junctions, by enriching the unsaturated fatty acyl substitution of phospholipids in tight junctions.

It is well-known that PC and SM are located at the outer leaflet of the plasma membrane, whereas most of PE, PI and PS are localized to the inner cytoplasmic leaflet of the membrane [24]. Previous studies have clarified that tight junctions contain numerous raft membrane microdomains that constitute the sealing elements of tight junctions [25]. Whether the fatty acyl substitutions of phospholipids in raft is related to the specialized functions of tight junction membranes remains to be explored. In this study, as shown in Table 1, the alteration of fatty acyl chains of membrane in the cytoplasmic leaflet lipids (PE, PI and PS) was shown by enriching quite a number of PUFAs. The exoplasmic lipid (PC) of membrane microdomains was apt to be affected by unsaturated fatty acyl. There was no significant alteration in fatty acyl substitutions of SM by the EPA treatment. In other words, the considerable changes in fatty acyl composition both in cytoplasmic and exoplasmic lipid leaflets were observed in membrane microdomains of tight junctions from EPA-treated cells.

A study reported that EPA enriched the unsaturation of fatty acids in the membrance and increased the membrane fluidity in epithelial cells [26]. In our study, EPA obviously increased the unsaturation of the fatty acyl substitution of phospholipids that constituted membrane microdomains of tight junctions. It was also illuminated that PUFAs were incorporated into cellular phospholipids and greatly influence membrane fluidity and the activation of intracellular signaling pathway [4]. Recently, the research of Hossain et al. [27] demonstrated that the function of tight junctions in epithelial cells gained similar changes after treated by EPA-enriched PC compared with that treated by EPA. Thus, it is very likely that the increase of membrane fluidity was at least partly induced by the increased unsaturation of fatty acyl chains of phospholipids in tight junction membrane microdomains. In other words, the unsaturation degree of the fatty acyl composition of phospholipids could likewise influence the membrane fluidity. Under the same condition, a well fluidity is beneficial for the integrality and function of the membrane, as the defendable protein could arrive at the injured place, safeguard the membrane and tight junction barrier integrity. Proteins, locating at the tight junctions of epithelia cells, are important for the function of tight junctions. The changes in the organization of tight junction proteins in the epithelial lateral membrane will influence tight junction function consequentially [28]. It was reported that n-3 PUFAs displace the proteins from membrane rafts of T cells by altering raft lipid composition [29]. Tight junctions are specialized raft membrane microdomains of plasma membrane. Thus, maybe the function of tight junction could be altered by EPA through changing the organization of tight junction proteins, and further the signaling of some of them. In addition, it was suggested that the saturation degree of phospholipids correlated with plasma membrane fluidity and endocytosis [30]. Tight junction complexes may contain machinery required for vesicular trafficking in endocytosis [31]. Thus, the alteration in phospholipids in membrane microdomains of tight junction may influence endocytosis. So the effect of EPA on the fatty acyl chains of phospholipids in membrane microdomains of tight junctions is probably one of the modes that EPA affects tight junctions. Furthermore, this conclusion suggests a new angle to explore the reason for the helpful effect of PUFAs on Crohn's disease (CD), as the defect in tight junction barrier is an important etiologic factor of CD [8].

5. Conclusions

To sum up, we have presented evidence that EPA changed the phospholipid composition of membrane microdomains of tight junctions by enriching the unsaturated fatty acyl substitution of phospholipids. This provides an angle to explaining the mechanism involved in n-3 PUFAs modulation of intestinal tight junction barrier. Nevertheless, this experiment is an *in vitro* research; a further *in vivo* study is required to gain a more significant result.

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