日本農芸化学会2018年度大会 SCIEX協賛ランチョンセミナー

過酸化脂質(脂質ヒドロペルオキシド)の質量分析

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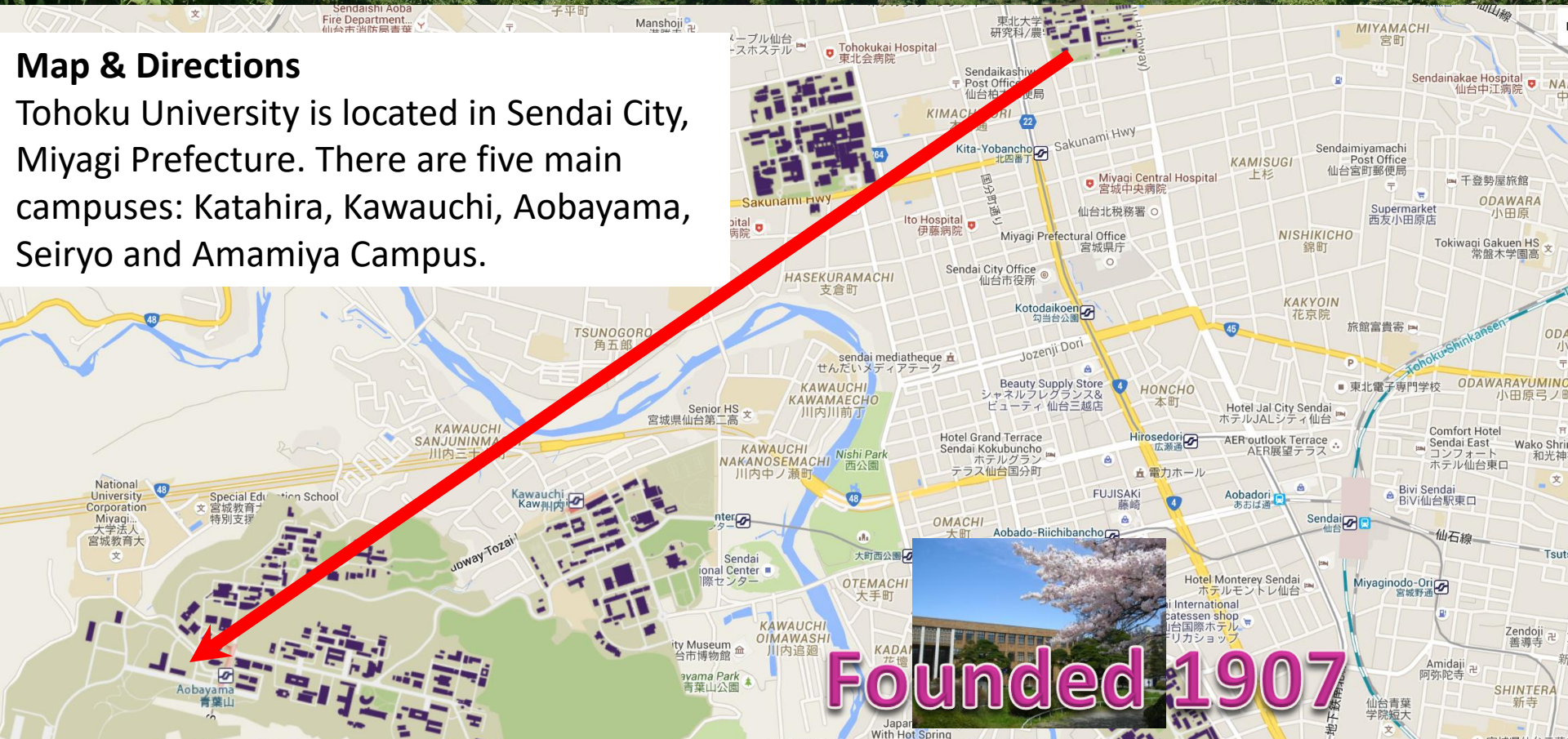
Sendai

Map & Directions

Tohoku University is located in Sendai City, Miyagi Prefecture. There are five main campuses: Katahira, Kawauchi, Aobayama, Seiryo and Amamiya Campus.



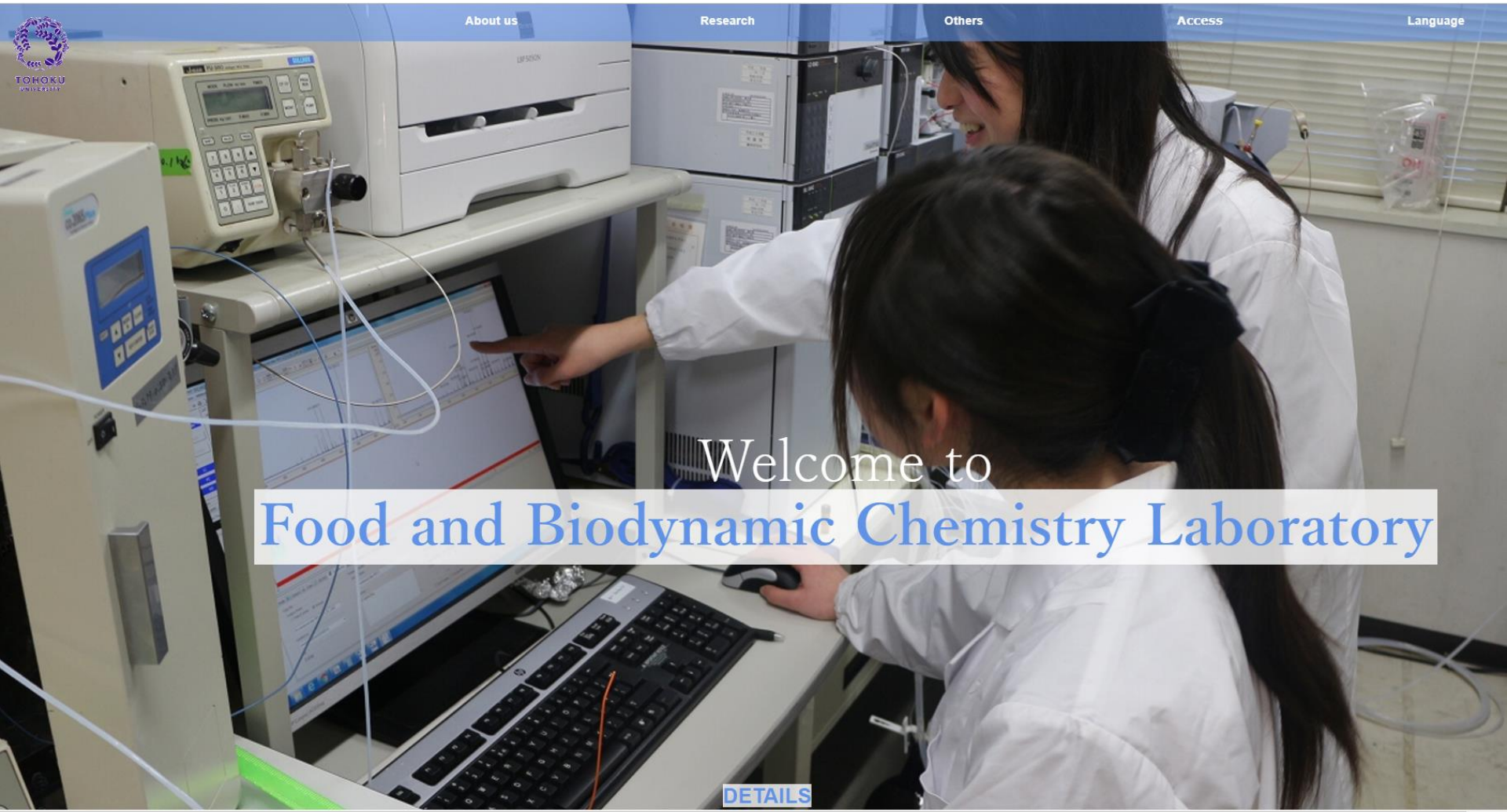
Founded 1907

Graduate School of Agricultural Science, Tohoku University

Founded 1947

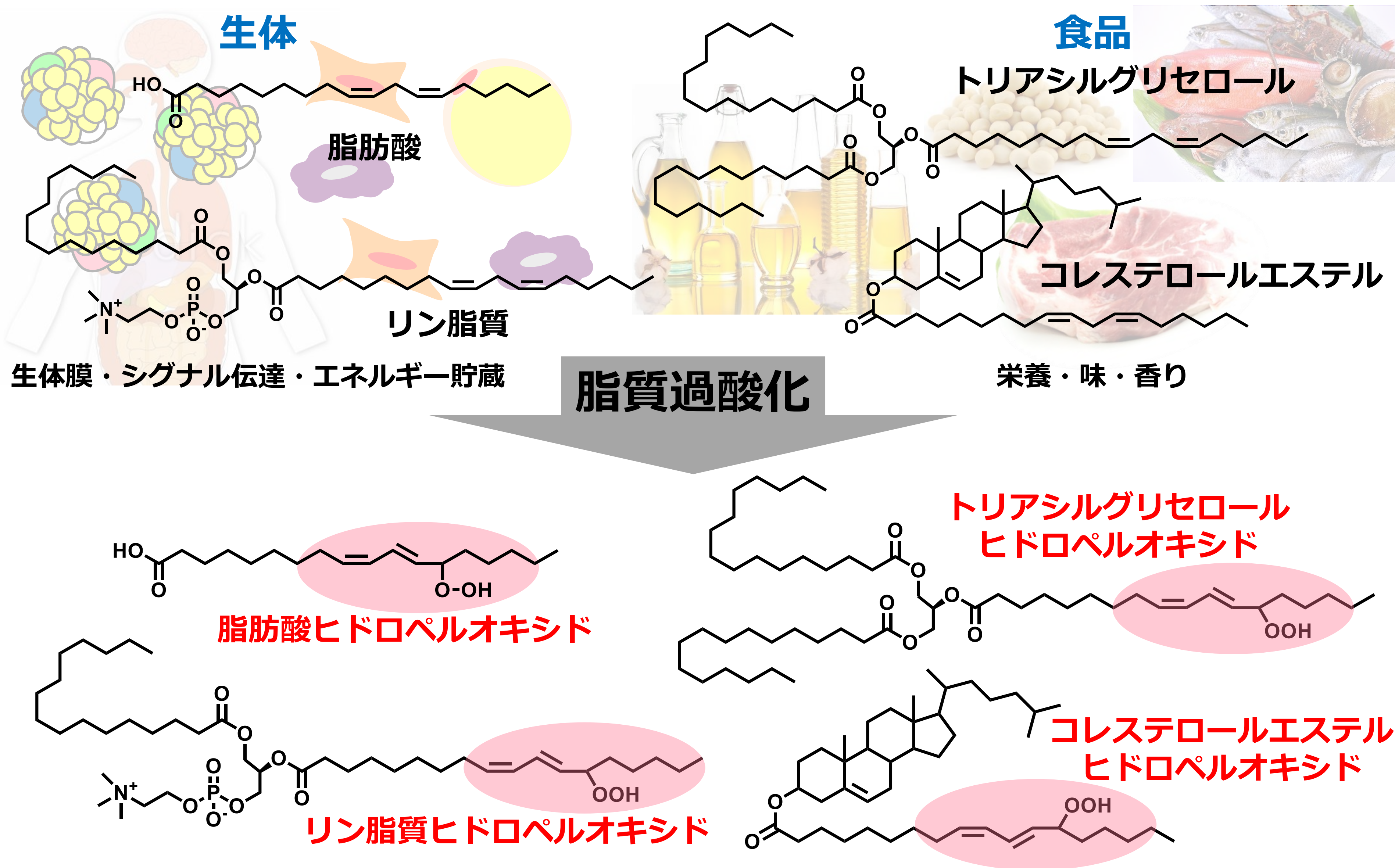
About 200 stuffs support the student life and perform the frontier researches in the aim for "Safe and Sustainable Food Production and Environmental Conservation for our Healthy Life".

Food & Biodynamic Chemistry Laboratory

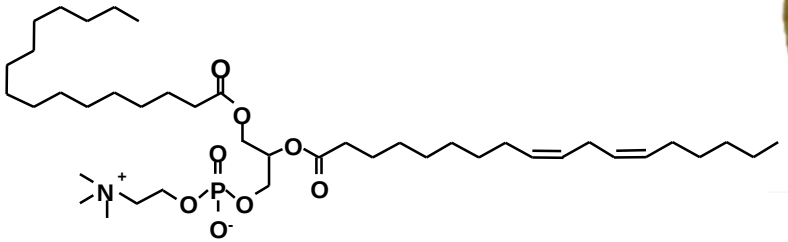


http://www.agri.tohoku.ac.jp/kinoubun

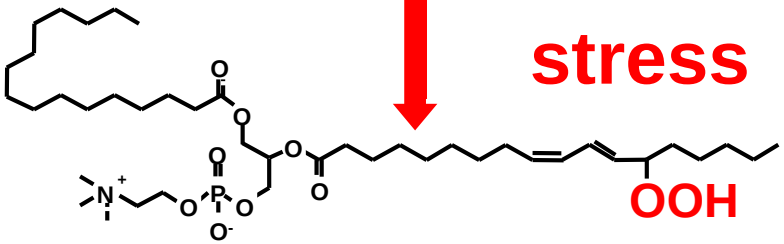
脂質過酸化は生体老化や食品劣化に大きく関与する



What's PCOOH?



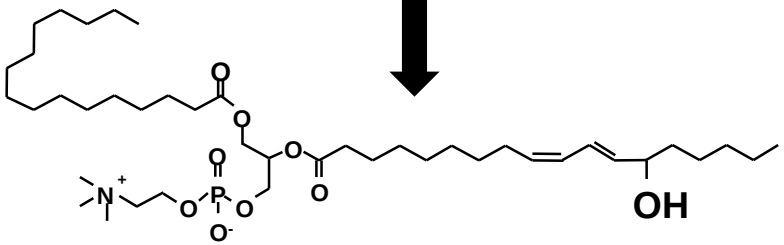
Phosphatidylcholine (PC)



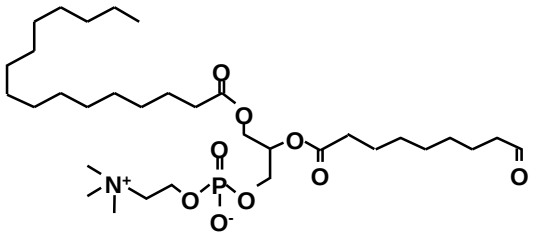
Phosphatidylcholine hydroperoxide (PCOOH)

Chemiluminescence detection (CL)-HPLC

T. Miyazawa et al. *J Lipid Res.* 1992

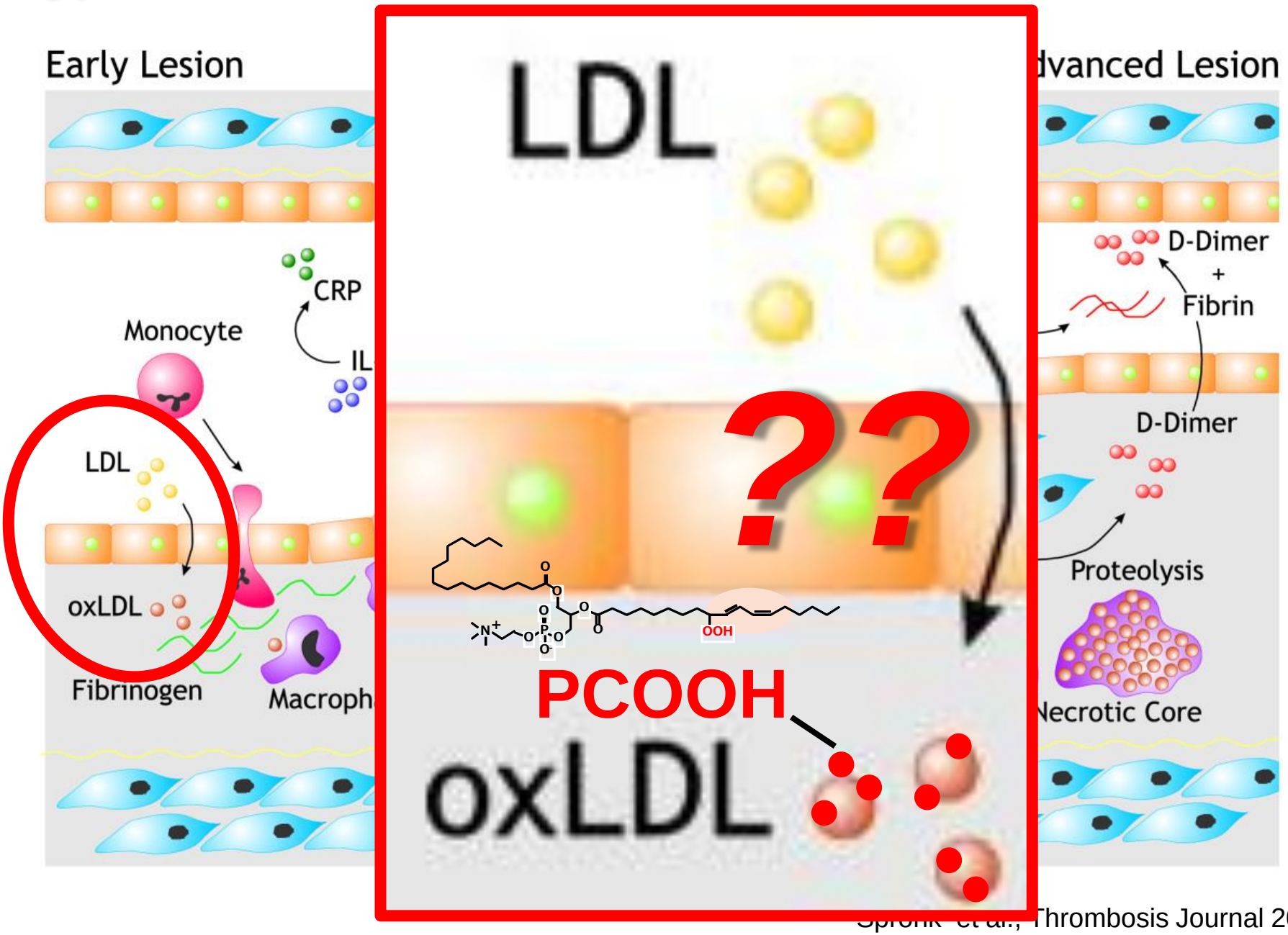


Phosphatidylcholine hydroxide (PCOH)



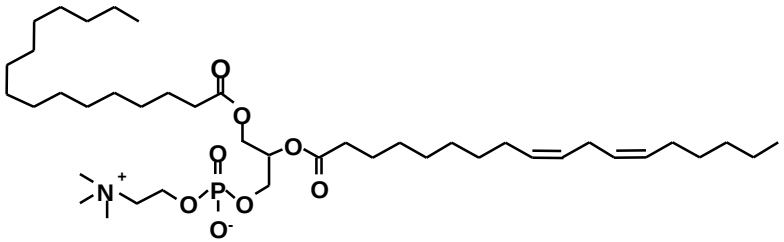
***sn2*-Truncated phosphatidylcholine**

Hypothesis

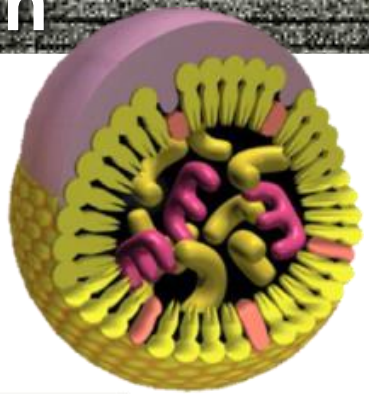


Sprink et al., Thrombosis Journal 2004

Phospholipid peroxidation

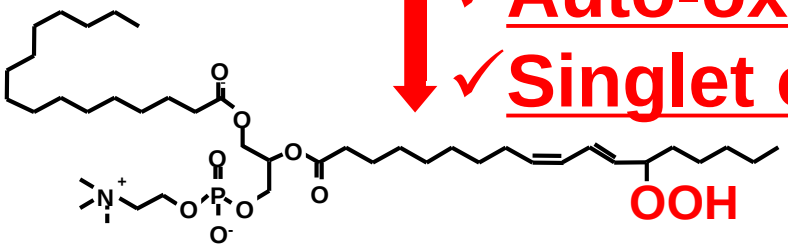


Phosphatidylcholine (PC)



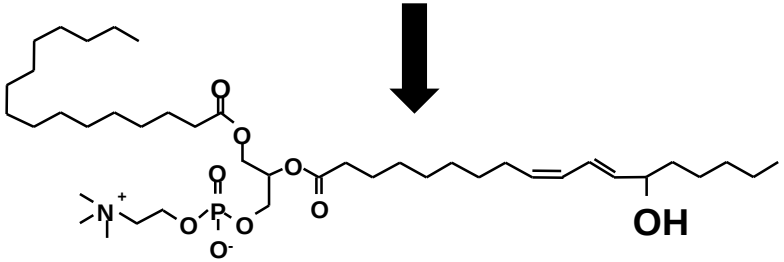
$[O_2]$

- ✓ **Enzymatic oxidation**
- ✓ **Auto-oxidation**
- ✓ **Singlet oxygen-induced oxidation**

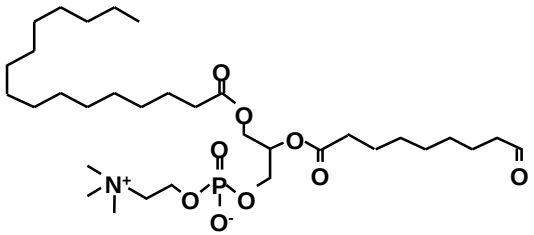


Phosphatidylcholine hydroperoxide (PCOOH)

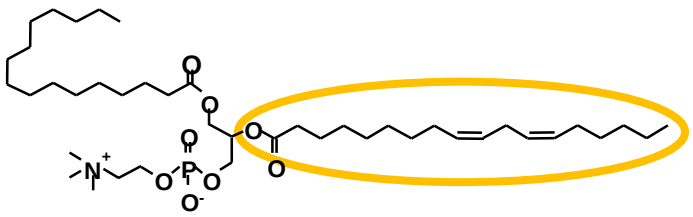
PC is oxidized by three distinct mechanisms.



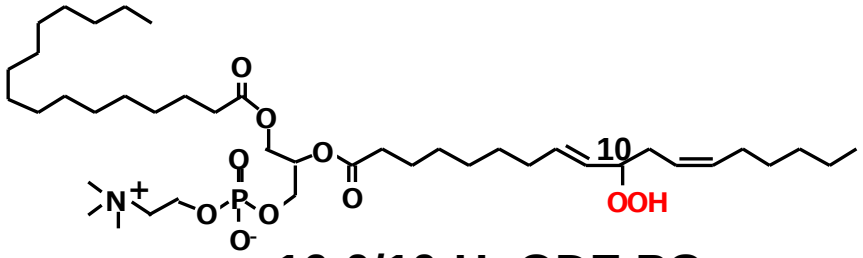
Phosphatidylcholine hydroxide (PCOH)



***sn2*-Truncated phosphatidylcholine**

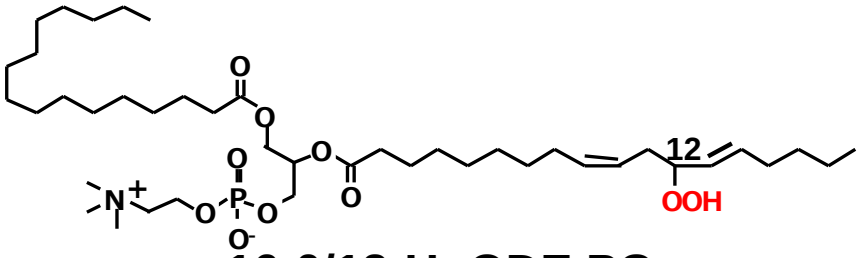


PC possessing linoleic acid at *sn*-2



16:0/10-HpODE PC

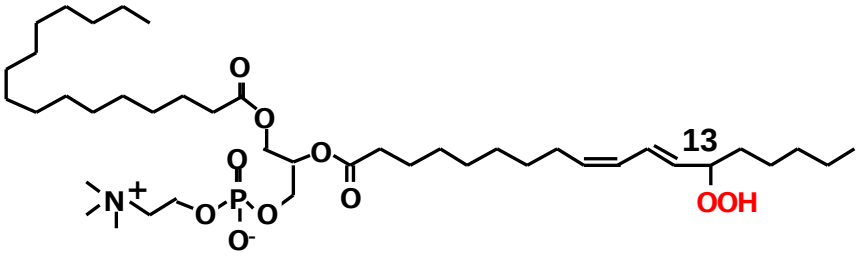
(Singlet oxygen-induced oxidation)



16:0/12-HpODE PC

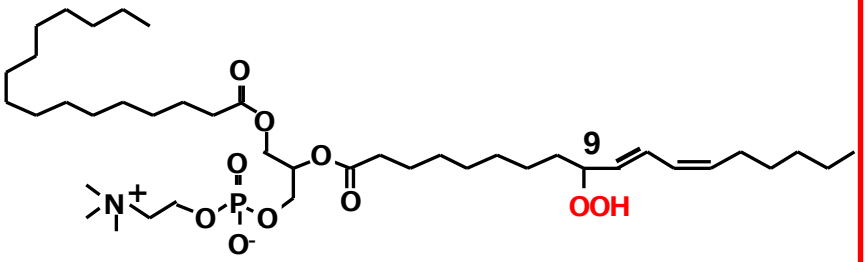
(Singlet oxygen-induced oxidation)

HpODE: Hydroperoxyoctadecadienoic



16:0/13-HpODE PC

(Singlet oxygen-induced oxidation
Auto-oxidation, Enzymatic oxidation)

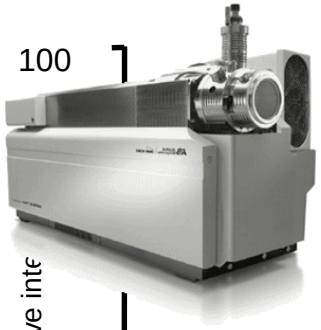


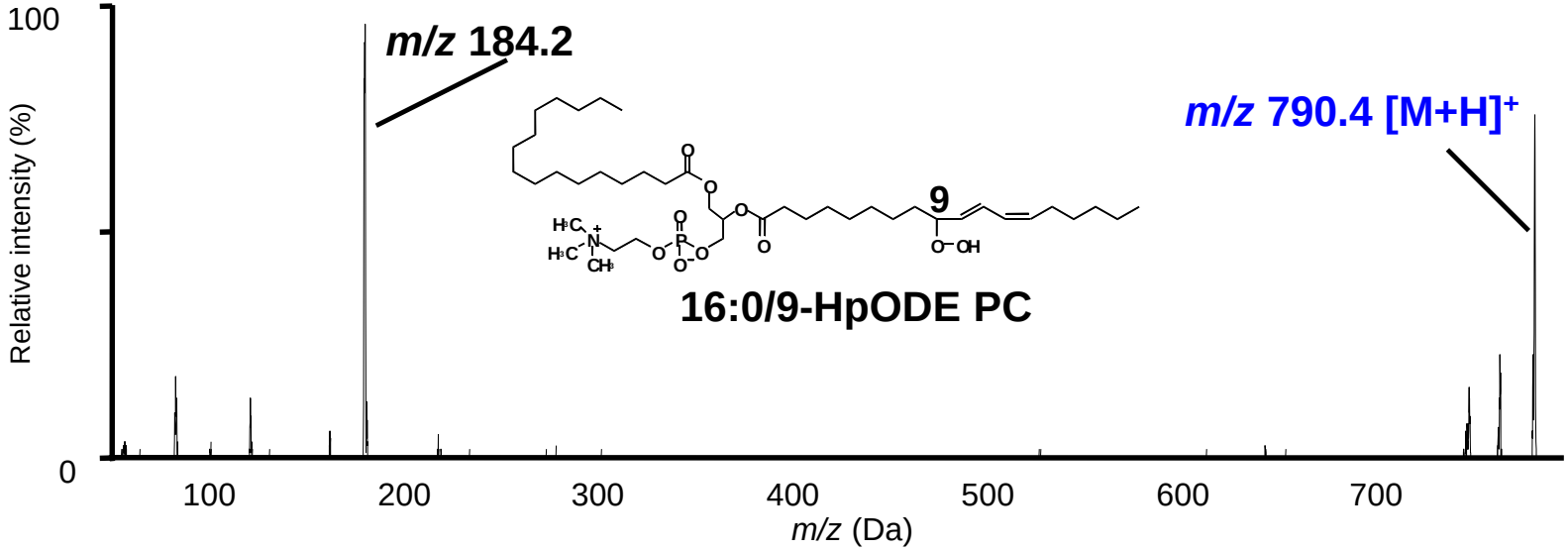
16:0/9-HpODE PC

(Singlet oxygen-induced oxidation,
Auto-oxidation, Enzymatic oxidation)

Product ion mass spectra (MS/MS) of 16:0/HpODE PC

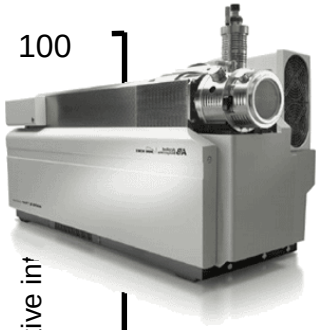
Absence of alkali metals

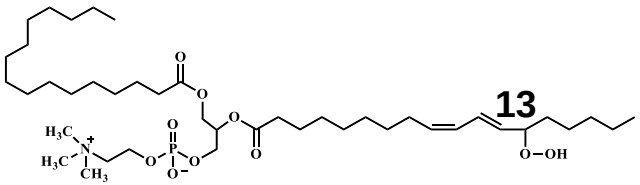




Product ion mass spectra (MS/MS) of 16:0/HpODE PC

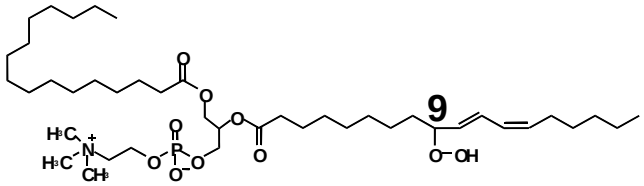
Presence of alkali metals (e.g., sodium)





16:0/13-HpODE PC

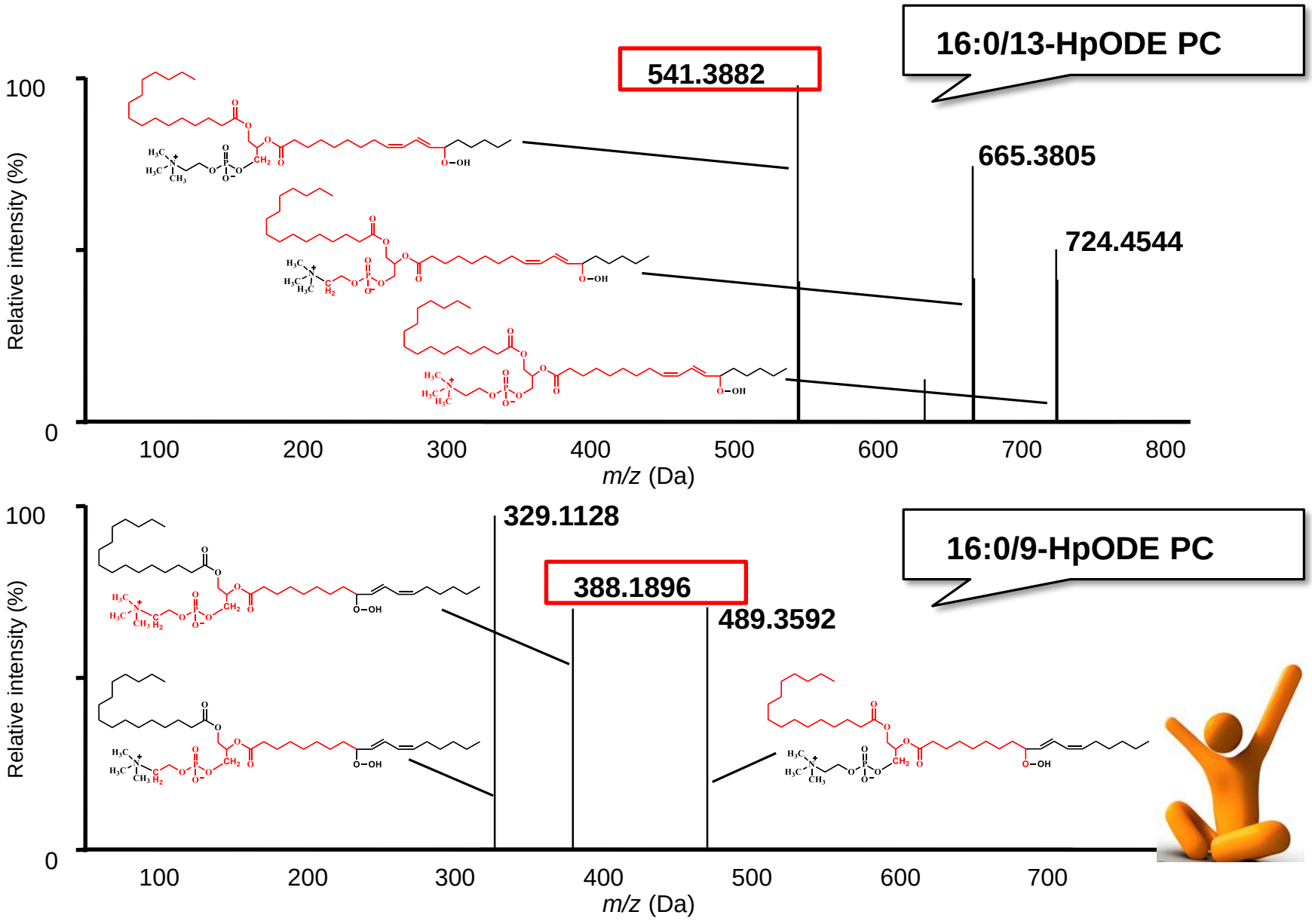
m/z 812.2 [M+Na]+



16:0/9-HpODE PC

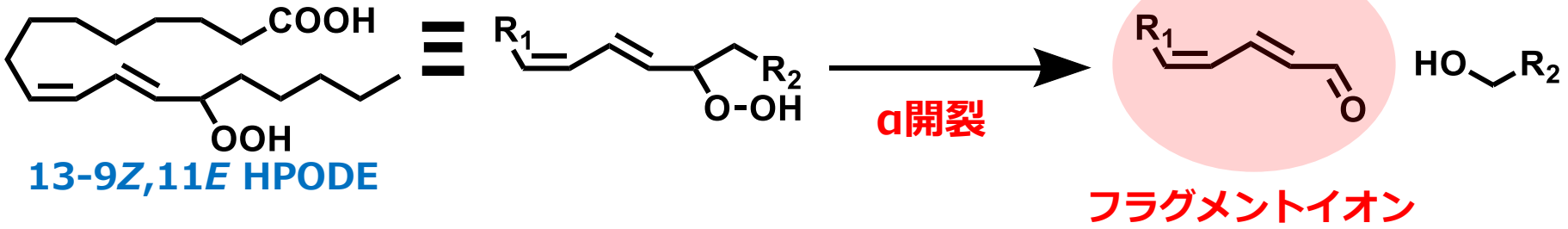
m/z 812.2 [M+Na]+

Differences of product ion intensities



α-開裂とHock転位反応

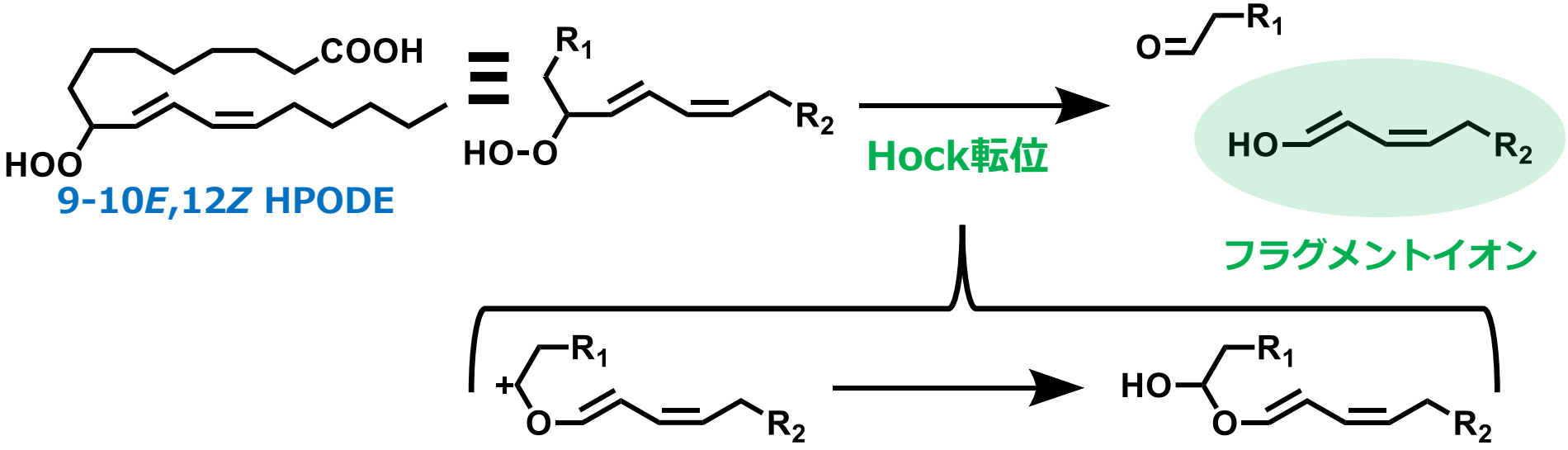
α開裂



13-9*Z*,11*E* HPODE

フラグメントイオン

Hock転位



9-10*E*,12*Z* HPODE

フラグメントイオン

SCIENTIFIC REPORTS

OPEN

A novel chiral stationary phase LC-MS/MS method to evaluate oxidation mechanisms of edible oils

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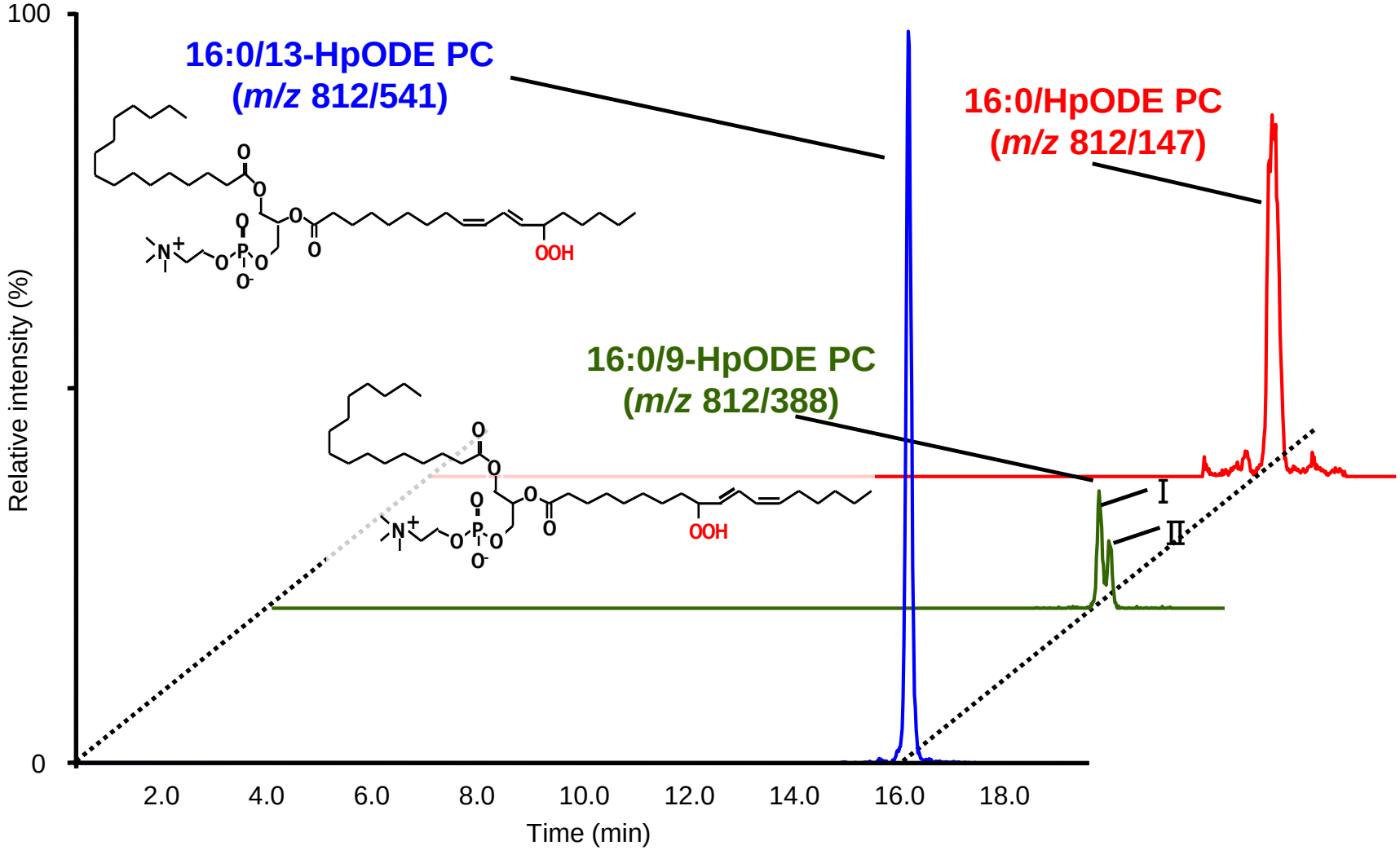
Junya Ito[1], Naoki Shimizu[1], Eri Kobayashi[1], Yasuhiko Hanzawa[1], Yurika Otoki[1], Shunji Kato[1,2], Takafumi Hirokawa[3], Shigefumi Kuwahara[3], Teruo Miyazawa[1,4] & Kiyotaka Nakagawa[1]

The elucidation of lipid oxidation mechanisms of food is vital. In certain lipids, characteristic lipid hydroperoxide isomers are formed by different oxidation mechanisms (*i.e.*, photo-oxidation or auto-oxidation). For example, linoleic acid is photo-oxidized to 13-9*Z*, 11*E*-hydroperoxyoctadecadienoic acid (HPODE), 12-9*Z*,13*E*-HPODE, 10-8*E*,12*Z*-HPODE and 9-10*E*,12*Z*-HPODE, whereas 13-9*Z*, 11*E*-HPODE, 13-9*E*,11*E*-HPODE, 9-10*E*,12*Z*-HPODE and 9-10*E*,12*E*-HPODE are formed by auto-oxidation. Therefore, we considered that oxidation mechanisms could be evaluated by analyzing these characteristic positional and *cis/trans* lipid hydroperoxide isomers. In this study, we developed a novel chiral stationary phase LC-MS/MS (CSP-LC-MS/MS) method to analyze the positional and *cis/trans* isomers of HPODE, with the use of a chiral column and sodium ion. Also, as an application of the method, either light-exposed or heated edible oils were treated with lipase to hydrolyze triacylglycerols. The resultant fatty acids including HPODE isomers were analyzed with the developed method. As a result, HPODE isomers characteristic to photo-oxidation were certainly detected in light-exposed edible oils. On the other hand, in heated edible oils, the HPODE isomers characteristic to auto-oxidation were largely increased. Thus, the combination of the developed CSP-LC-MS/MS method with lipase proves to be a powerful tool to evaluate the involvement and mechanisms of lipid oxidation in the process of food deterioration.

Measurement of PCOOH in clinical specimens

Plasma PCOOH in patients with hyperlipidemia



Ⅰ.16:0/9-hydroperxyoctadeca-10*E*, 12*Z*-dienoyl PC
Ⅱ.16:0/9-hydroperxyoctadeca-10*E*, 12*E*-dienoyl PC

HPLC-MS/MS with MRM

Contents lists available at ScienceDirect

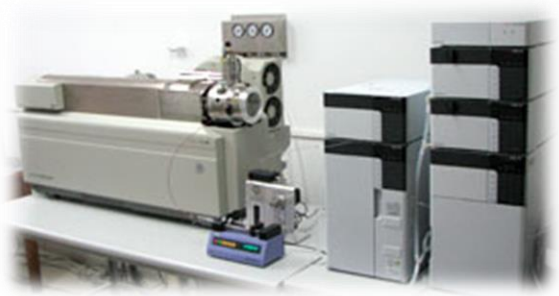
Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Liquid chromatography–tandem mass spectrometry determination of human plasma 1-palmitoyl-2-hydroperoxyoctadecadienoyl-phosphatidylcholine isomers via promotion of sodium adduct formation

Shunji Kato [a], Kiyotaka Nakagawa [a], Yuuri Suzuki [a], Akira Asai [b], Mototsugu Nagao [b], Kazuyuki Nagashima [c], Shinichi Oikawa [b], Teruo Miyazawa [a,*]

[a] Food and Biodynamic Chemistry Laboratory, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan
[b] Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo 113-8603, Japan
[c] Cardiovascular Institute, Tokyo 106-0031, Japan



analytical chemistry

Article

pubs.acs.org/ac

Tandem Mass Spectrometry Analysis of Linoleic and Arachidonic Acid Hydroperoxides via Promotion of Alkali Metal Adduct Formation

Junya Ito, Shunsuke Mizuochi, Kiyotaka Nakagawa,* Shunji Kato, and Teruo Miyazawa

Food and Biodynamic Chemistry Laboratory, Graduate School of Agricultural Science, Tohoku University, Sendai 980-8577, Japan

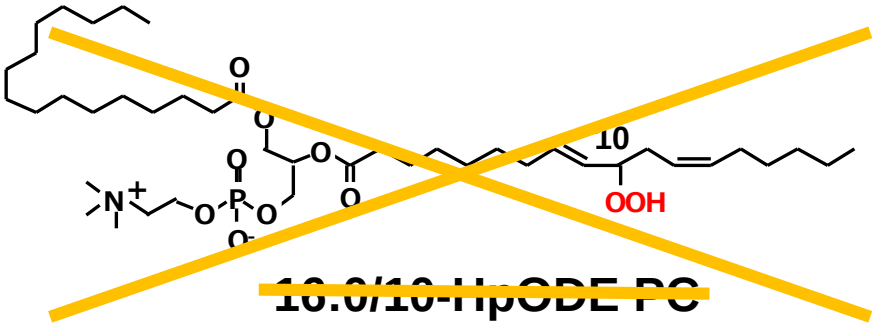
Measurement of PCOOH in clinical specimens

	16:0/13-HpODE PC	16:0/9-HpODE PC	16:0/HpODE PC*
		pmol/mL	
Healthy subjects n=8	25.4	22.6	49.8
	46.3	41.0	86.4
	50.5	46.0	103.4
	34.7	34.3	67.3
	31.9	30.9	66.1
	49.3	43.5	102.7
	19.0	16.6	38.2
	31.9	30.3	64.8
Patients with hyperlipidemia n=12	86.9	66.0	138.6
	32.9	32.2	64.4
	45.5	35.0	90.3
	39.0	36.0	77.8
	42.8	38.9	87.2
	112.5	84.3	194.6
	49.8	50.1	99.5
	67.4	69.4	132.5
	33.3	30.2	61.9
	43.6	34.9	80.6
	32.3	32.9	61.3
	42.9	32.8	79.1

16:0/13-HpODE PC + 16:0/9-HpODE PC = 16:0/HpODE PC

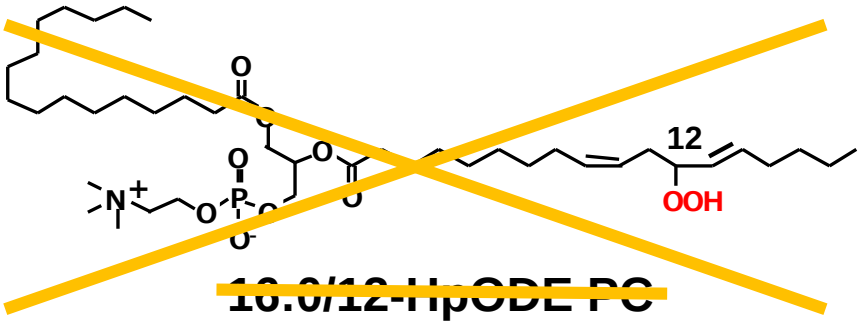
Discussion

16:0/13-HpODE PC + 16:0/9-HpODE PC = 16:0/HpODE PC



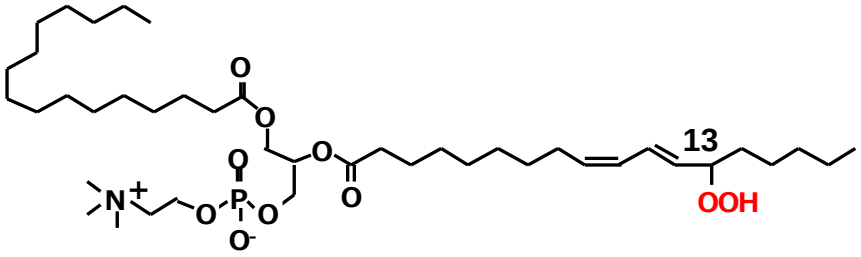
~~16:0/10-HpODE PC~~

(~~Singlet oxygen-induced oxidation~~)



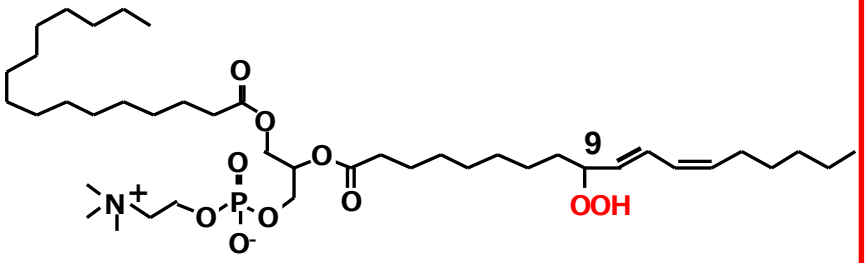
~~16:0/12-HpODE PC~~

(~~Singlet oxygen-induced oxidation~~)



16:0/13-HpODE PC

(Auto-oxidation, Enzymatic oxidation, ~~Singlet oxygen-induced oxidation~~)

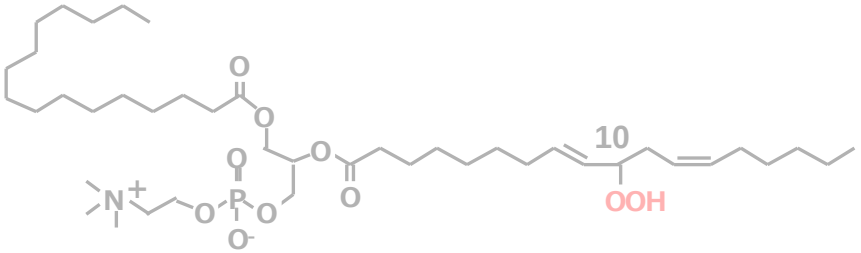


16:0/9-HpODE PC

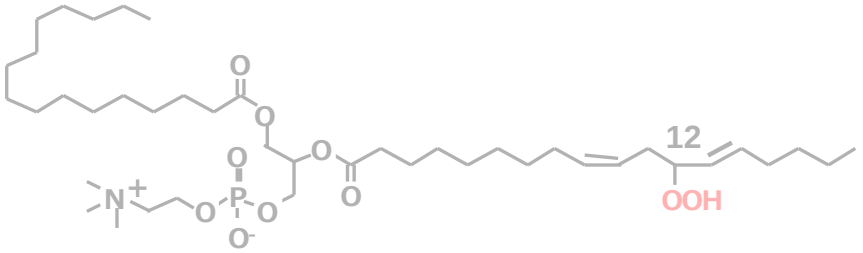
(Auto-oxidation, Enzymatic oxidation, ~~Singlet oxygen-induced oxidation~~)

Auto-oxidation and/or enzymatic oxidation, rather than singlet oxygen-induced oxidation, would cause PC peroxidation.

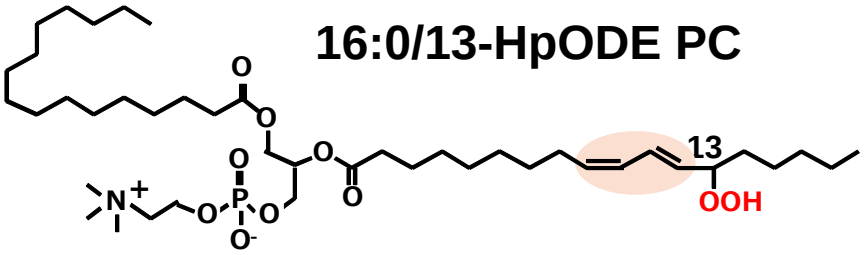
Discussion & on-going study



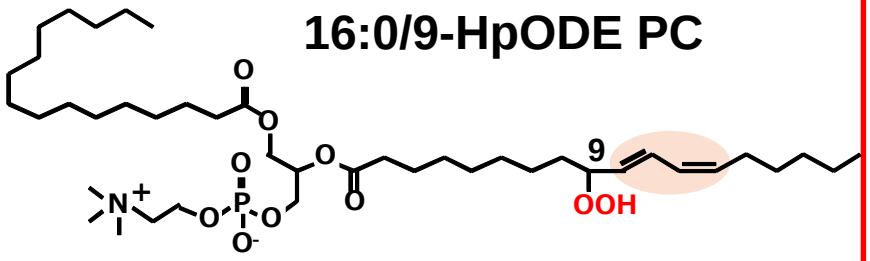
16:0/10-HpODE PC
(Singlet oxygen-induced oxidation)



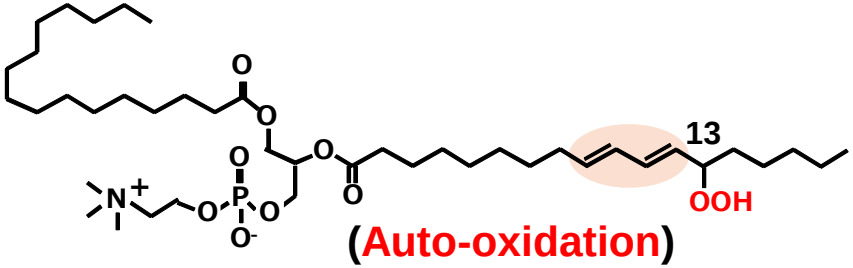
16:0/12-HpODE PC
(Singlet oxygen-induced oxidation)



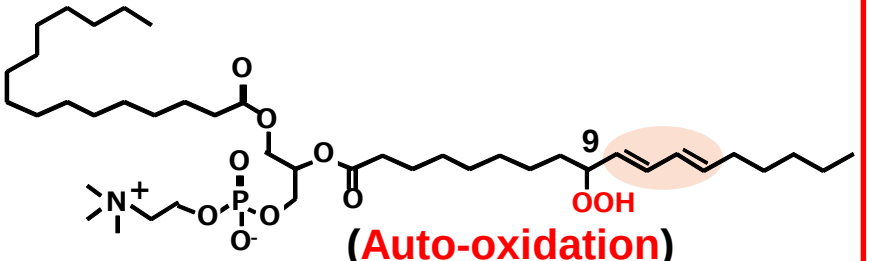
16:0/13-HpODE PC
(Auto-oxidation, Enzymatic oxidation, Singlet oxygen-induced oxidation)



16:0/9-HpODE PC
(Auto-oxidation, Enzymatic oxidation, Singlet oxygen-induced oxidation)



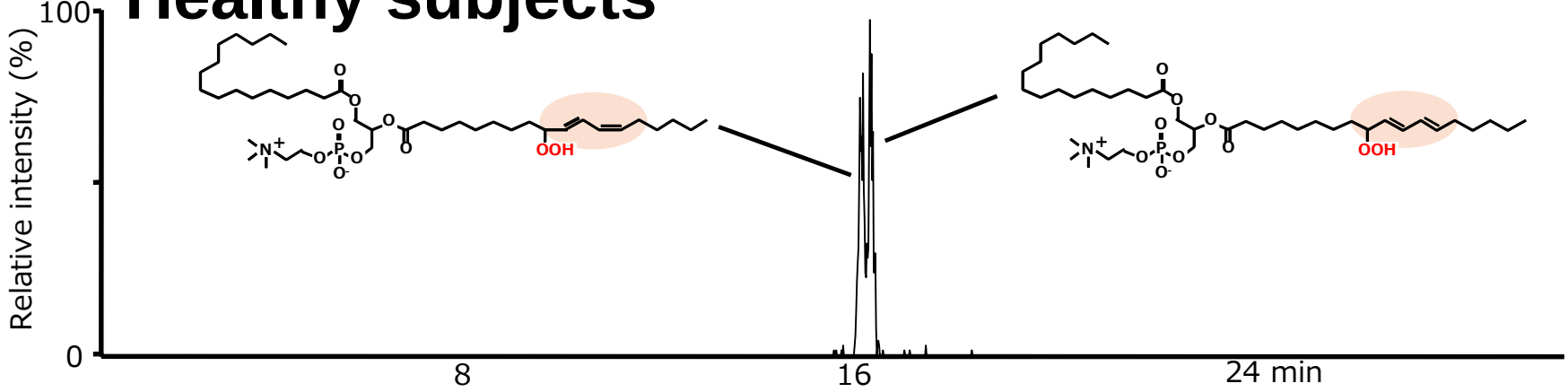
(Auto-oxidation)



(Auto-oxidation)

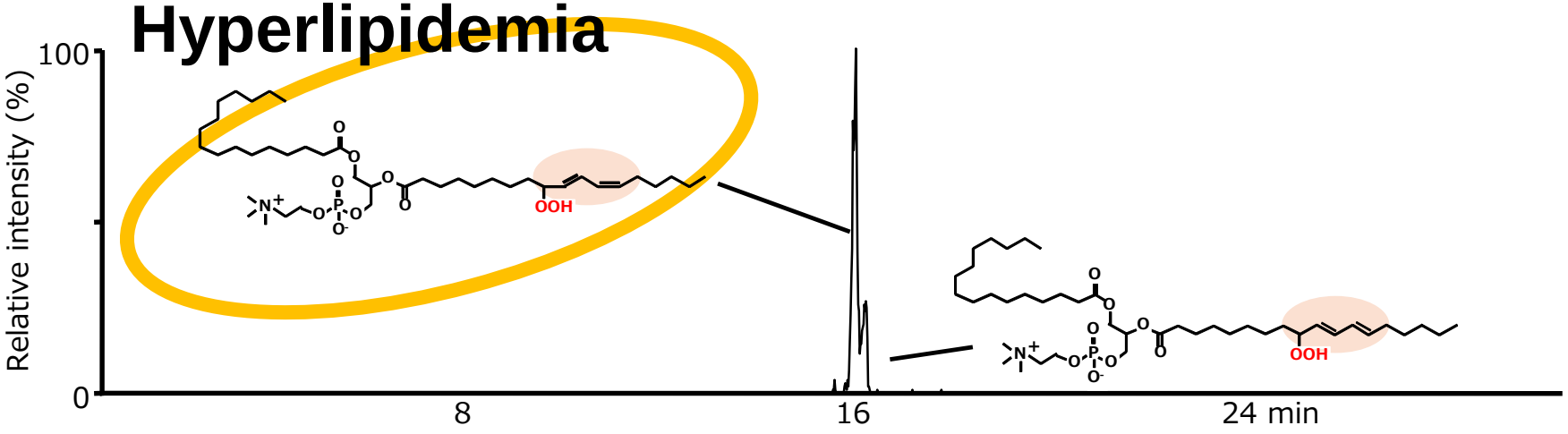
On-going study

Healthy subjects



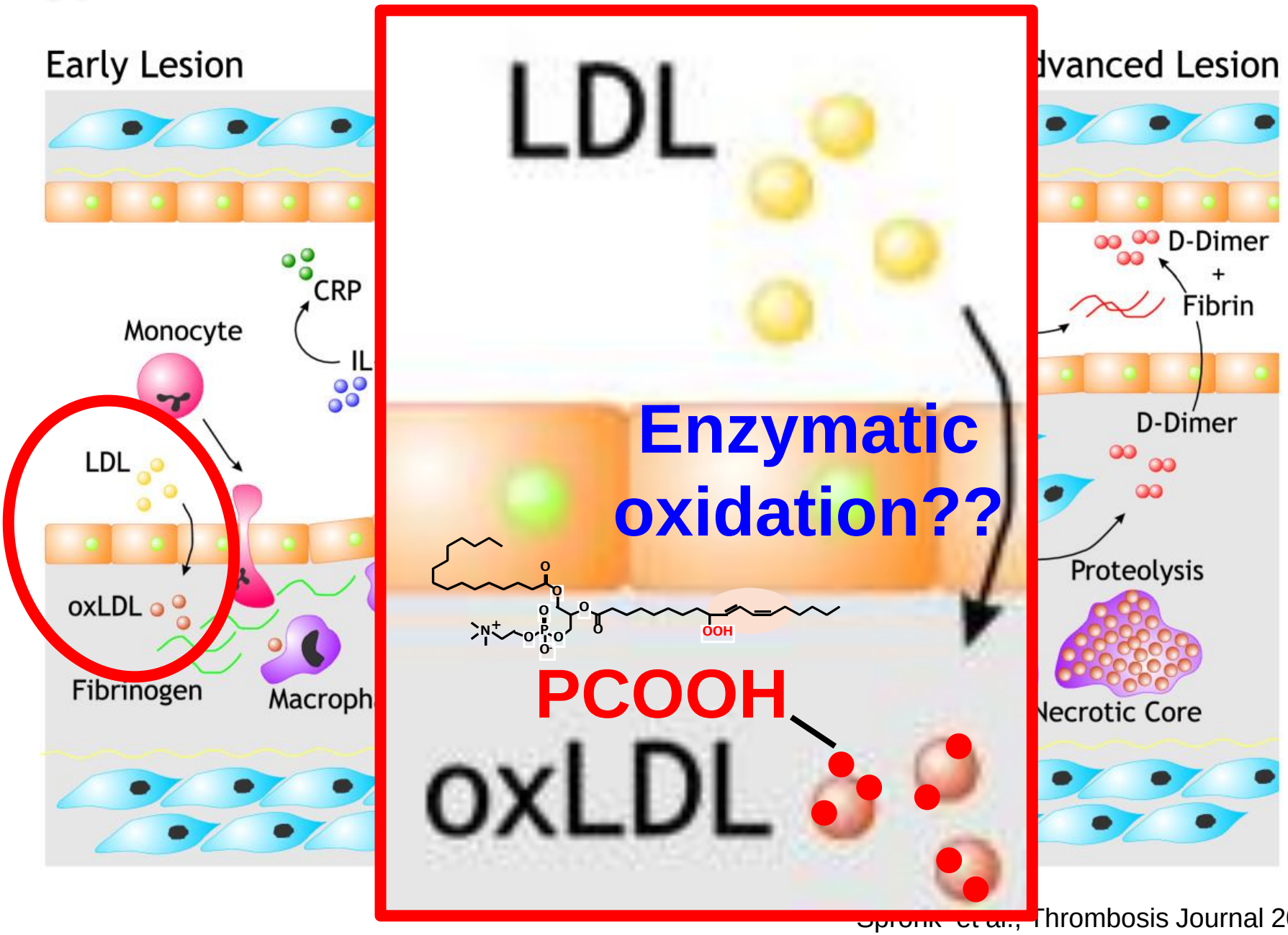
Auto-oxidation, Enzymatic oxidation

Hyperlipidemia



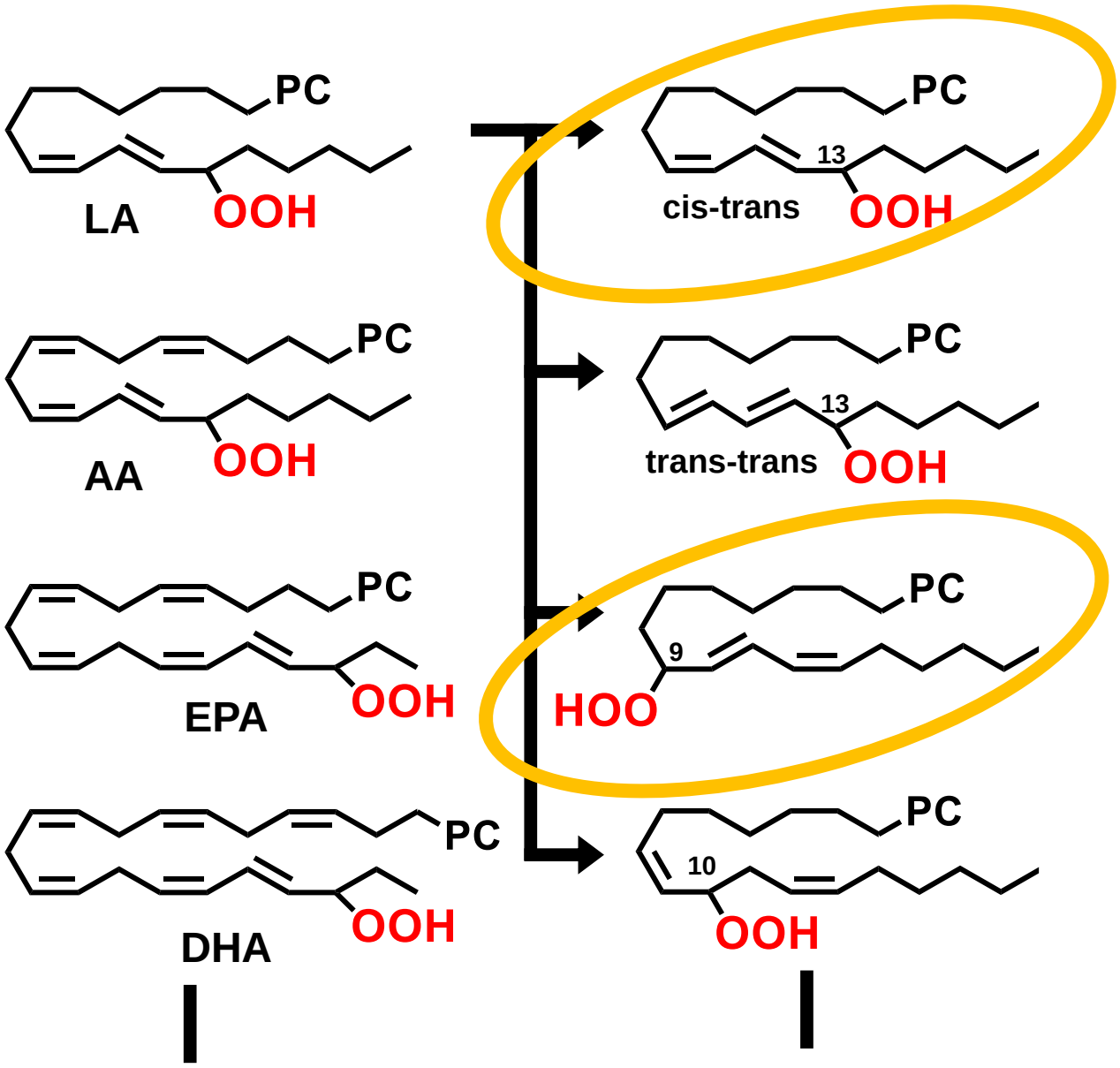
Auto-oxidation, Enzymatic oxidation

Hypothesis

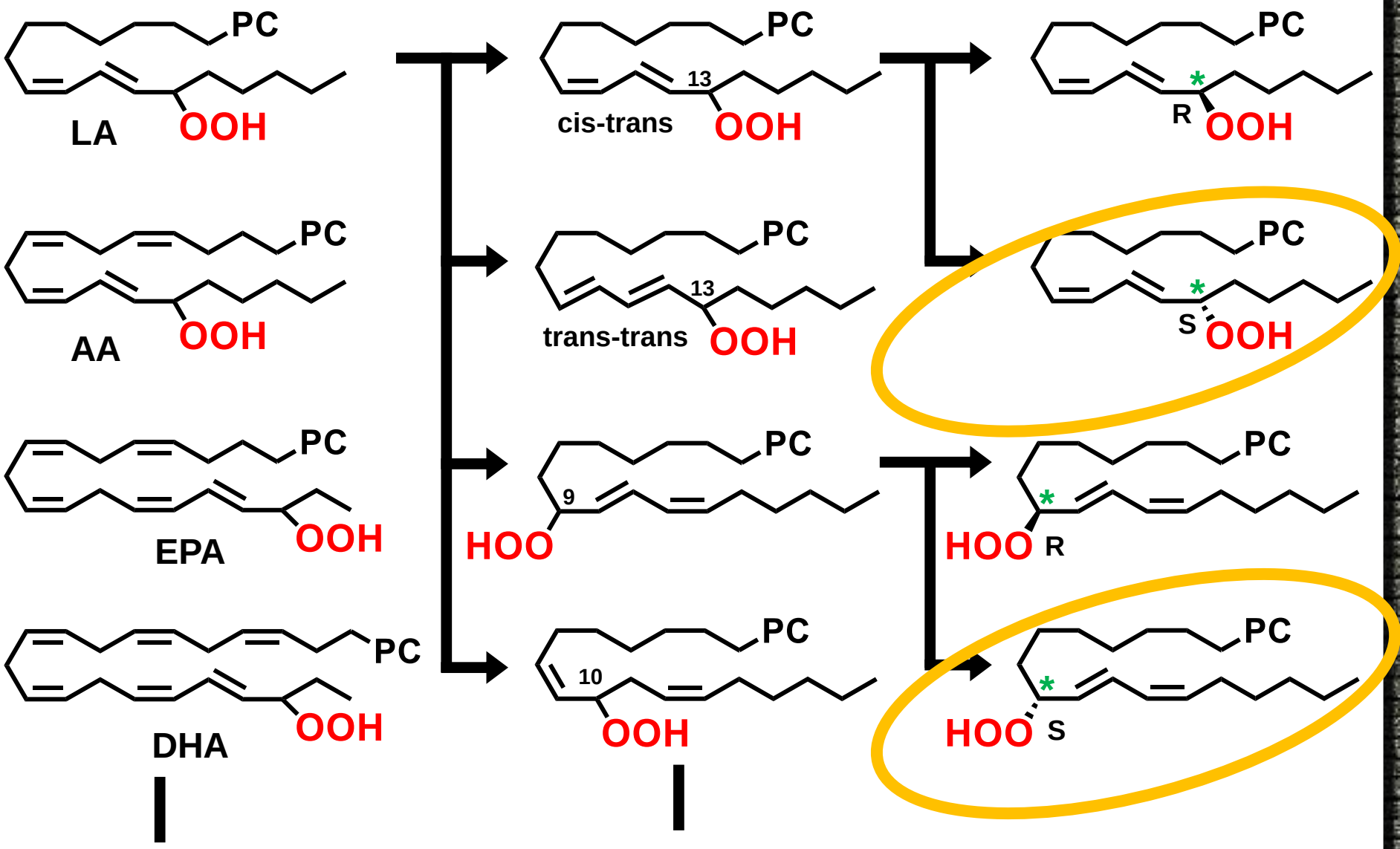


Sprink et al., Thrombosis Journal 2004

Hypothesis


PC
LA
OOH
PC
13
cis-trans
OOH
PC
AA
OOH
PC
13
trans-trans
OOH
PC
EPA
OOH
PC
9
HOO
PC
DHA
OOH
PC
10
OOH

Hypothesis

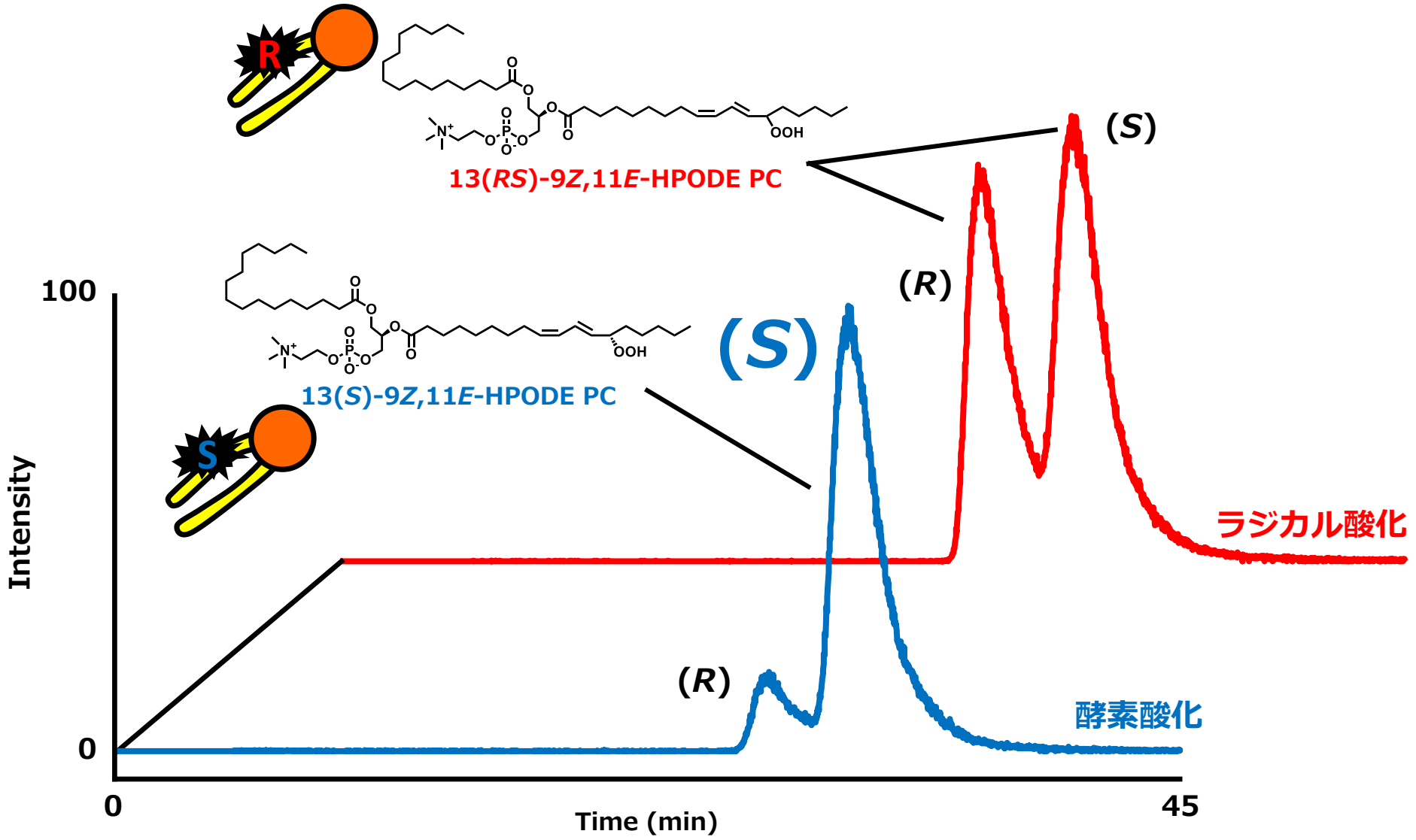


立体異性分離（*RS*分離）可能な条件を見出した

Chiral column	Condition 1			Condition 2			Condition 3			Condition 4			Condition 5			Condition 6			Condition 7			Condition 8			Condition 9		
	Rs	t1	t2	Rs	t1	t2	Rs	t1	t2	Rs	t1	t2	Rs	t1	t2	Rs	t1	t2	Rs	t1	t2	Rs	t1	t2	Rs	t1	t2
IA	0.0	30	30	0.0	22	22	0.0	17	17	0.0	16	16	nd	nd	nd	0.0	16	16	0.0	3	3	0.0	2	2	not-tested		
IB	0.0	30	30	0.0	25	25	0.0	27	27	0.0	25	25	nd	nd	nd	nd	nd	nd	0.0	5	5	0.0	2	2	not-tested		
IC	0.0	30	30	0.0	27	27	0.0	7	7	0.0	7	7	nd	nd	nd	nd	nd	nd	0.0	8	8	0.0	3	3	not-tested		
ID	0.0	16	16	0.0	14	14	0.0	6	6	0.0	6	6	nd	nd	nd	0.0	15	15	0.0	3	3	0.0	2	2	not-tested		
IE	nd	nd	nd	nd	nd	nd	0.0	15	15	0.0	16	16	nd	nd	nd	nd	nd	nd	0.0	8	8	0.0	3	3	not-tested		
IF	nd	nd	nd	nd	nd	nd	0.0	30	30	0.0	27	27	nd	nd	nd	nd	nd	nd	0.0	5	5	0.0	3	3	not-tested		
AD-RH	0.0	12	12	0.0	6	6	0.0	5	5	0.0	51	51	not-tested			not-tested			not-tested			not-tested			not-tested		
AS-RH	nd	nd	nd	nd	nd	nd	0.0	5	5	0.0	5	5	not-tested			not-tested			not-tested			not-tested			not-tested		
AY-RH	nd	nd	nd	0.0	8	8	0.0	6	6	0.0	6	6	not-tested			not-tested			not-tested			not-tested			not-tested		
OD-RH	0.0	18	18	0.0	15	15	0.0	9	9	0.0	9	9	not-tested			not-tested			not-tested			not-tested			not-tested		
OJ-RH	nd	nd	nd	nd	nd	nd	0.0	5	5	0.0	5	5	not-tested			not-tested			not-tested			not-tested			not-tested		
OZ-RH	0.0	3	3	nd	nd	nd	0.0	13	13	0.0	13	13	not-tested			not-tested			not-tested			not-tested			not-tested		
AD-H	not-tested			not-tested			not-tested			not-tested			0.0	35	35	0.0	6	6	0.0	2	2	0.0	2	2	not-tested		
AY-H	not-tested			not-tested			not-tested			not-tested			0.0	11	11	0.0	8	8	0.0	2	2	0.0	2	2	not-tested		
OD-H	not-tested			not-tested			not-tested			not-tested			0.0	18	18	0.0	5	5	0.0	2	2	0.0	2	2	not-tested		
OJ-H	not-tested			not-tested			not-tested			not-tested			0.0	6	6	0.0	6	6	0.0	2	2	0.0	1	1	not-tested		
OZ-H	not-tested			not-tested			not-tested			not-tested			0.0	7	7	0.0	8	8	0.0	3	3	0.0	3	3	not-tested		
OP (+)	not-tested			not-tested			not-tested			not-tested			not-tested			not-tested			not-tested			not-tested			1.6	24	27

Rs（分離度）≧1.5で完全分離; t1, 第1ピーク溶出時間; t2, 第2ピーク溶出時間

キラルLC-MS/MS分析によるPCOOHの立体異性体解析



J. Ito, et al., *J. Chromatogr. A*, 1386:53-61 (2015)

酵素酸化とラジカル酸化・一重項酸素酸化を明確に区別できる

Journal of Chromatography A, 1386 (2015) 53–61

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Direct separation of the diastereomers of phosphatidylcholine hydroperoxide bearing 13-hydroperoxy-9*Z*,11*E*-octadecadienoic acid using chiral stationary phase high-performance liquid chromatography

Junya Ito[a], Kiyotaka Nakagawa[a,*], Shunji Kato[a], Takafumi Hirokawa[b], Shigefumi Kuwahara[b], Toshiharu Nagai[c], Teruo Miyazawa[a]

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[c] *Tsukishima Foods Industry Co., Ltd., Tokyo, 134-8520, Japan*

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Diastereomer
HPLC-Q-TOF/MS
Phosphatidylcholine hydroperoxide

ABSTRACT

Increasing evidence suggests that phospholipid peroxidation plays important roles in the pathogenesis of various diseases, such as atherosclerosis. With regard to the biochemical processes that initiate phospholipid peroxidation *in vivo*, enzymatic conversion of phosphatidylcholine to phosphatidylcholine hydroperoxide (PCOOH) by lipoxygenase (LOX) may play a crucial role. This will become clear if we can analyze PCOOH bearing hydroperoxy fatty acids with *S*-stereoconfiguration. In this study, we therefore attempted such an analysis. Initially, we used LOX, linoleic acid and Lyso phosphatidylcholine, and synthesized PCOOH bearing 13*S*-hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13(*S*)-9*Z*,11*E*-HPODE). PCOOH bearing racemic 13-9*Z*,11*E*-HPODE was also prepared. We used liquid chromatography equipped with CHIRALPAK OP (+) (poly (*o*-pyridyl diphenylmethacrylate) coated on silica), a UV detector and a quadrupole-time-of-flight mass spectrometer, and achieved diastereomer separation of PCOOH stereoisomers with excellent resolution and peak shape. This is the first study reporting the diastereomer separation of PCOOH. The present method will be beneficial in developing a better understanding of the biochemical processes that initiate oxidative stress (PCOOH formation) *in vivo*, which may lead to further elucidation of the involvement of PCOOH in the development of diseases. In addition to clinical applications, the present method may also be effective in the evaluation of enzymatic oxidative food deterioration.

Anal Bioanal Chem (2016) 408:7785–7793
DOI 10.1007/s00216-016-9882-4

RESEARCH PAPER

A novel chiral stationary phase HPLC-MS/MS method to discriminate between enzymatic oxidation and auto-oxidation of phosphatidylcholine

Junya Ito[1] · Kiyotaka Nakagawa[1] · Shunji Kato[1] · Takafumi Hirokawa[2] · Shigefumi Kuwahara[2] · Toshiharu Nagai[3] · Teruo Miyazawa[1,4]

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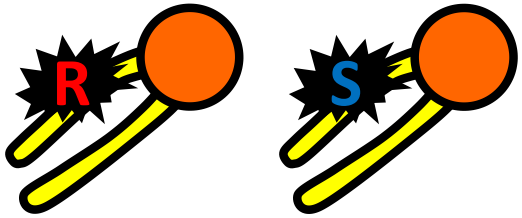
Abstract To elucidate the role of enzymatic lipid peroxidation in disease pathogenesis and in food deterioration, we recently achieved stereoselective analysis of phosphatidylcholine hydroperoxide (PCOOH) possessing 13*S*-hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13(*S*)-9*Z*,11*E*-HPODE) using HPLC-MS/MS with a CHIRALPAK OP (+) column. Because enzymatic oxidation progresses concurrently with auto-oxidation, we need to distinguish them further. Here, we attempted such an analysis. First, we used lipoxygenase, linoleic acid, and lysophosphatidylcholine (LPC) to synthesize the enzymatic oxidation product 13(*S*)-9*Z*,11*E*-HPODE PC, and the auto-oxidation products 13(*RS*)-9*Z*,11*E*-HPODE PC and 13(*RS*)-9*E*,11*E*-HPODE PC, which were used as standards to test the ability of various columns to separate the enzymatic oxidation product from auto-oxidation products. Separation was achieved by connecting in series two columns with different properties: CHIRALPAK OP (+) and CHIRALPAK IB-3. The CHIRALPAK OP (+) column separated 13(*R*)-9*Z*,11*E*-HPODE PC and 13(*S*)-9*Z*,11*E*-HPODE PC, whereas CHIRALPAK IB-3 enabled separation of 13(*S*)-9*Z*,11*E*-HPODE PC and 13(*RS*)-9*E*,11*E*-HPODE PC. The results for the analysis of both enzymatically oxidized and auto-oxidized lecithin (an important phospholipid mixture in vivo and in food) indicate that our method would be useful for distinguishing enzymatic oxidation and auto-oxidation reactions. Such information will be invaluable for elucidating the involvement of PCOOH in disease pathogenesis and in food deterioration.

Keywords Chiral stationary phase · Chiral column · Phosphatidylcholine hydroperoxide · Lipid oxidation · LC-MS/MS

Abbreviations

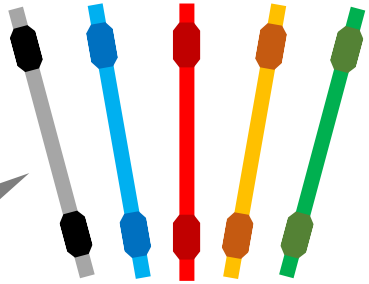
16:0 LPC 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine

IMMSを用いた立体異性体の分析法の構築



Lipid hydroperoxides

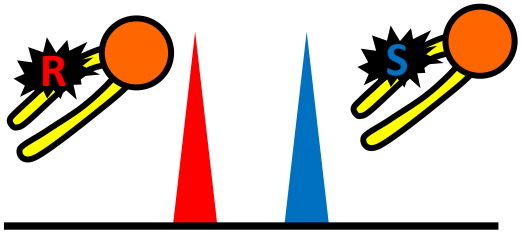
- Chiral
- C18
- Silica
- etc…



Columns

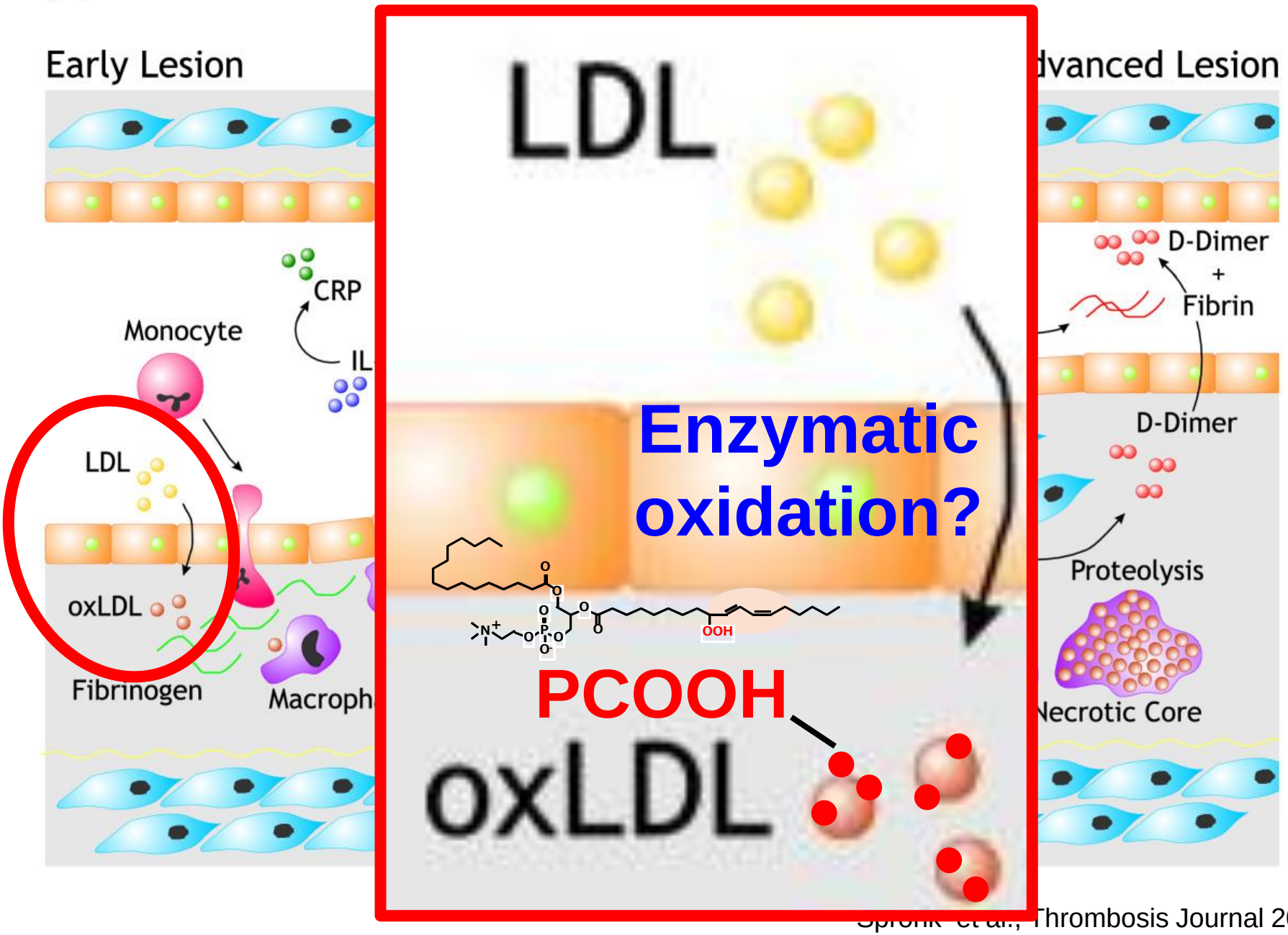
QTRAP® 6500+

Ion mobility-mass spectrometry (IMMS)



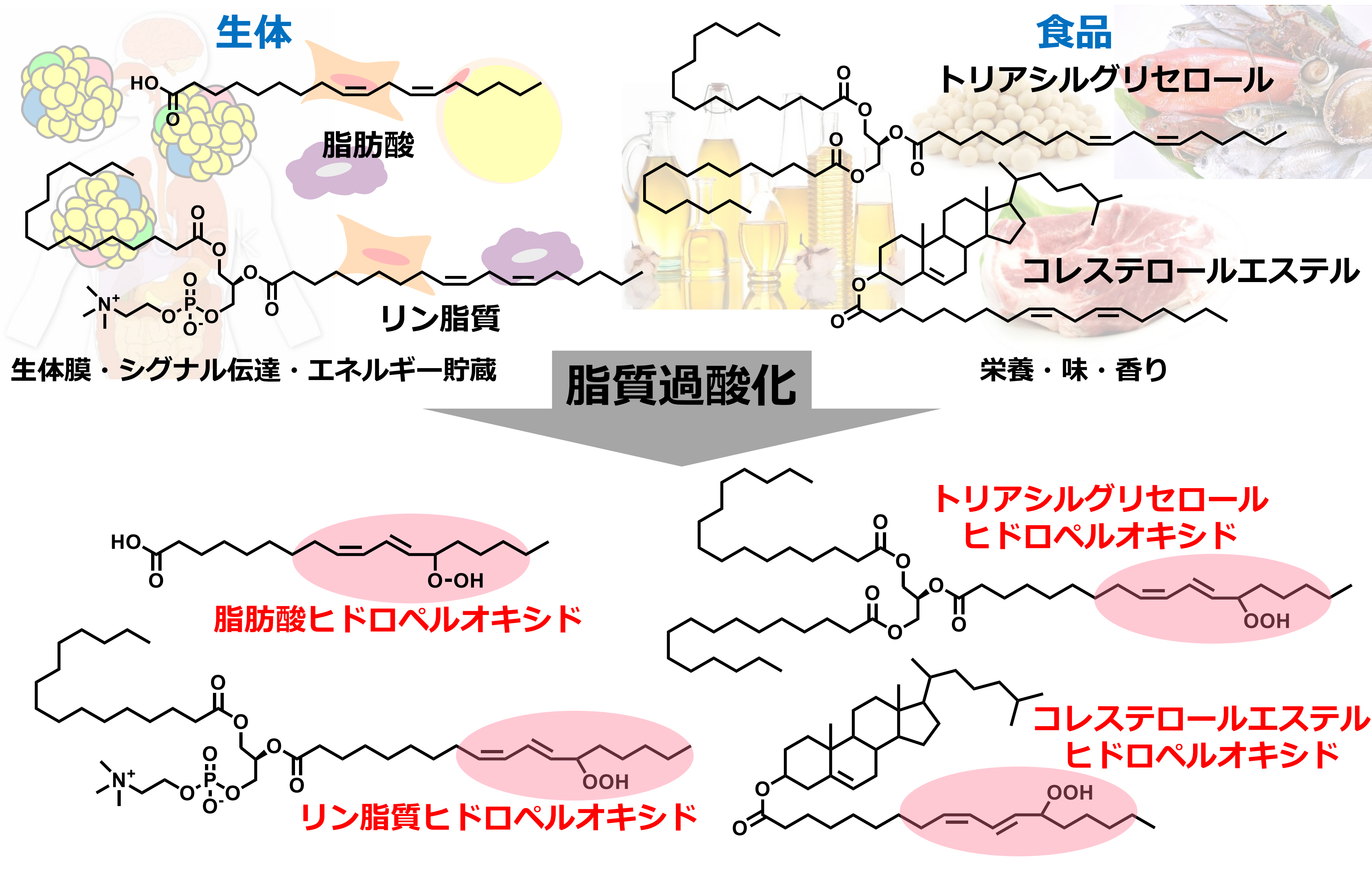
LC-IMS-MS/MS法を構築する

Hypothesis

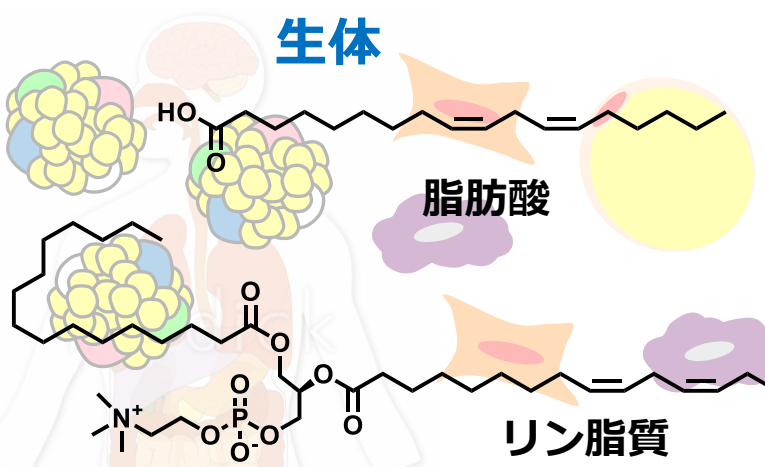


Sprink et al., Thrombosis Journal 2004

脂質過酸化は生体老化や食品劣化に大きく関与する



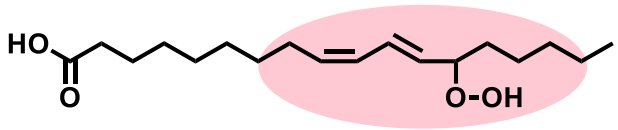
まとめ



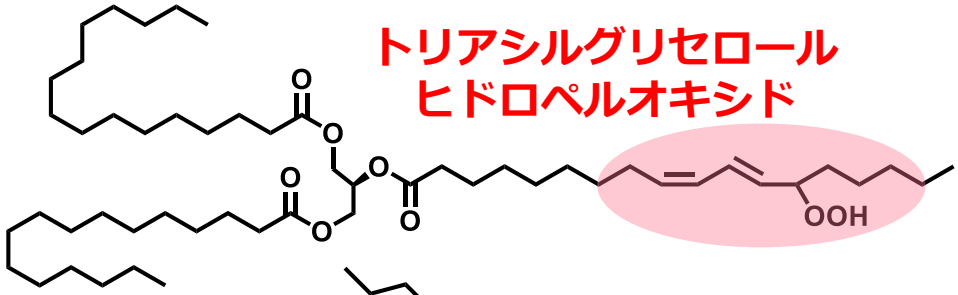
自動酸化
（ラジカル酸化）

酵素酸化
（リポキシゲナーゼ：LOX）

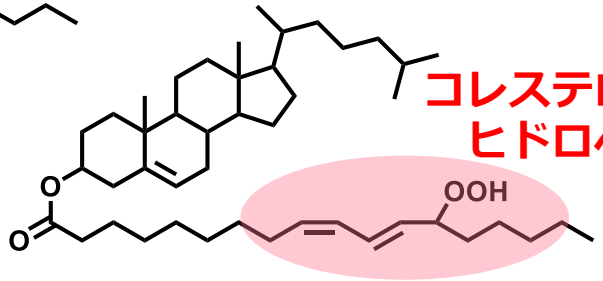
光酸化
（一重項酸素酸化）



脂肪酸ヒドロペルオキシド



リン脂質ヒドロペルオキシド



生体や食品の脂質過酸化機構を解明し、生体老化や食品劣化を予防する

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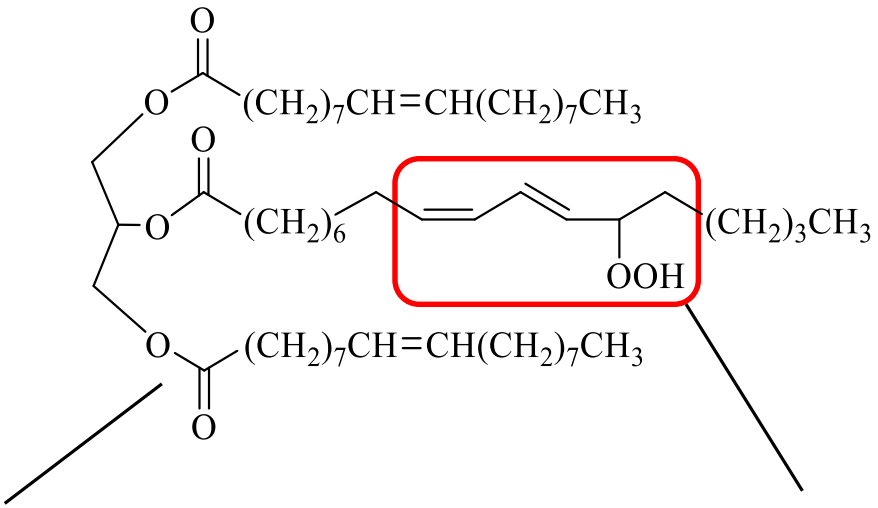
Determination of triacylglycerol oxidation mechanisms in canola oil using liquid chromatography-tandem mass spectrometry

Shunji Kato[1,2], Naoki Shimizu[1], Yasuhiko Hanzawa[1], Yurika Otoki[1], Junya Ito[1], Fumiko Kimura[3], Susumu Takekoshi[2], Masayoshi Sakaino[4], Takashi Sano[4], Takahiro Eitsuka[1], Teruo Miyazawa[5,6], Kiyotaka Nakagawa[1*]

Triacylglycerol (TG), the main component of edible oil, is oxidized by thermal- or photo- oxidation to form TG hydroperoxide (TGOOH) as the primary oxidation product. Since TGOOH and its subsequent oxidation products cause not only the deterioration of oil quality but also various toxicities, preventing the oxidation of edible oils is essential. Thereby understanding oxidation mechanisms that cause the formation of TGOOH is necessary. Since isomeric information of lipid hydroperoxide provides insights about oil oxidation mechanisms, we focused on dioleoyl-(hydroperoxy octadecadienoyl)-TG (OO-HpODE-TG) isomers, which are the primary oxidation products of the most abundant TG molecular species (dioleoyl-linoleoyl-TG) in canola oil. To secure highly selective and sensitive analysis, authentic OO-HpODE-TG isomer references (i.e. hydroperoxide positional/geometrical isomers) were synthesized and analyzed with HPLC-MS/MS. With the use of the method, photo- or thermal- oxidized edible oils were analyzed. While dioleoyl-(10-hydroperoxy-8E,12Z-octadecadienoyl)-TG (OO-(10-HpODE)-TG) and dioleoyl-(12-hydroperoxy-9Z,13E-octadecadienoyl)-TG (OO-(12-HpODE)-TG) were characteristically detected in photo-oxidized oils, dioleoyl-(9-hydroperoxy-10E,12E-octadecadienoyl)-TG and dioleoyl-(13-hydroperoxy-9E,11E-octadecadienoyl)-TG were found to increase depending on temperature in thermal-oxidized oils. These results prove that our methods not only evaluate oil oxidation in levels that are unquantifiable with peroxide value, but also allows for the determination of oil oxidation mechanisms. From the analysis of marketed canola oils, photo-oxidized products (i.e. OO-(10-HpODE)-TG and OO-(12-HpODE)-TG) were characteristically accumulated compared to the oil analyzed immediately after production. The method described in this paper is valuable in the understanding of oil and food oxidation mechanisms, and may be applied to the development of preventive methods against food deterioration.

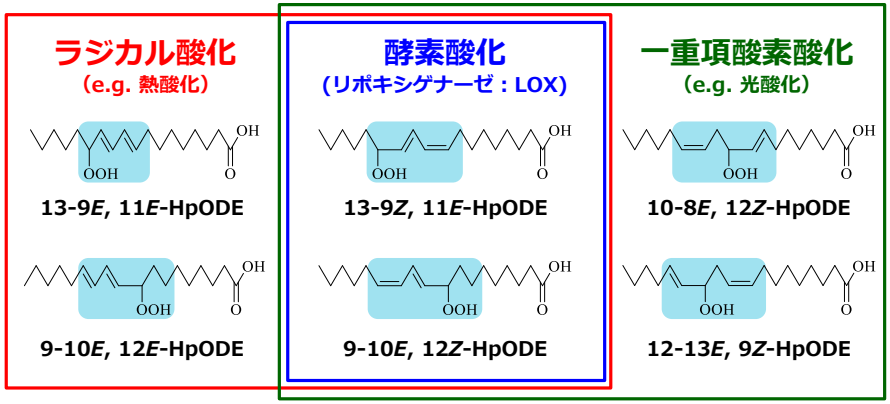
TGOOH解析に必要な情報



①構成脂肪酸

オレイン酸、リノール酸
リノレン酸、EPA、DHA…
組み合わせ

②酸素結合位置（異性体）



定量性と汎用性を考慮した２つの方法を構築

TGOOH標品の調製

リノール酸

↓ 酸化（ラジカル＋光酸化）

↓ 精製（分取HPLC）

リノール酸ヒドロペルオキシド（HpODE）異性体

- 9-hydroperxy-octadeca-10*E*, 12*Z*-dienoic acid
- 9-hydroperxy-octadeca-10*E*, 12*E*-dienoic acid
- 10-hydroperxy-octadeca-8*E*, 12*Z*-dienoic acid
- 12-hydroperxy-octadeca-13*E*, 9*Z*-dienoic acid
- 13-hydroperxy-octadeca-9*E*, 11*E*-dienoic acid
- 13-hydroperxy-octadeca-9*Z*, 11*E*-dienoic acid

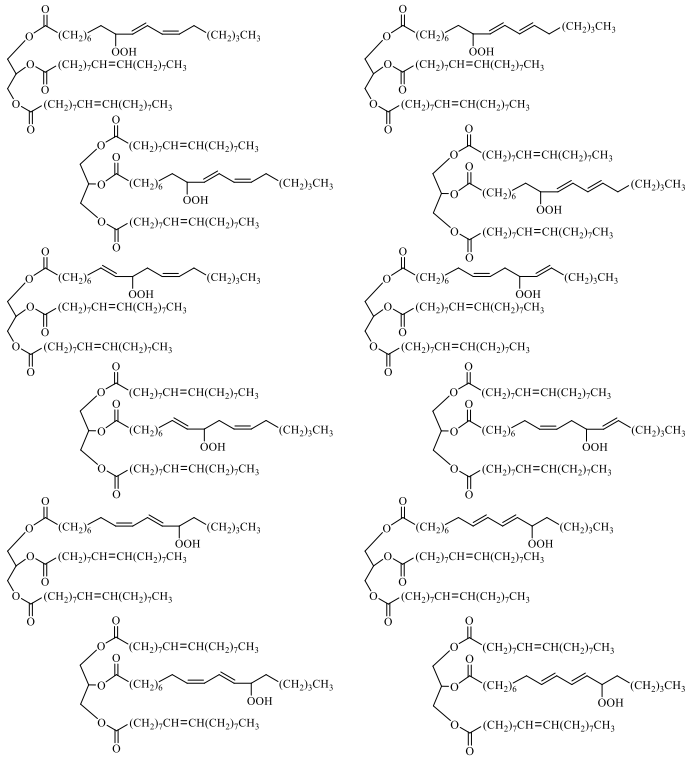
↓ ヒドロペルオキシド基の保護 (PPTS, MxP)

LAOOMxP

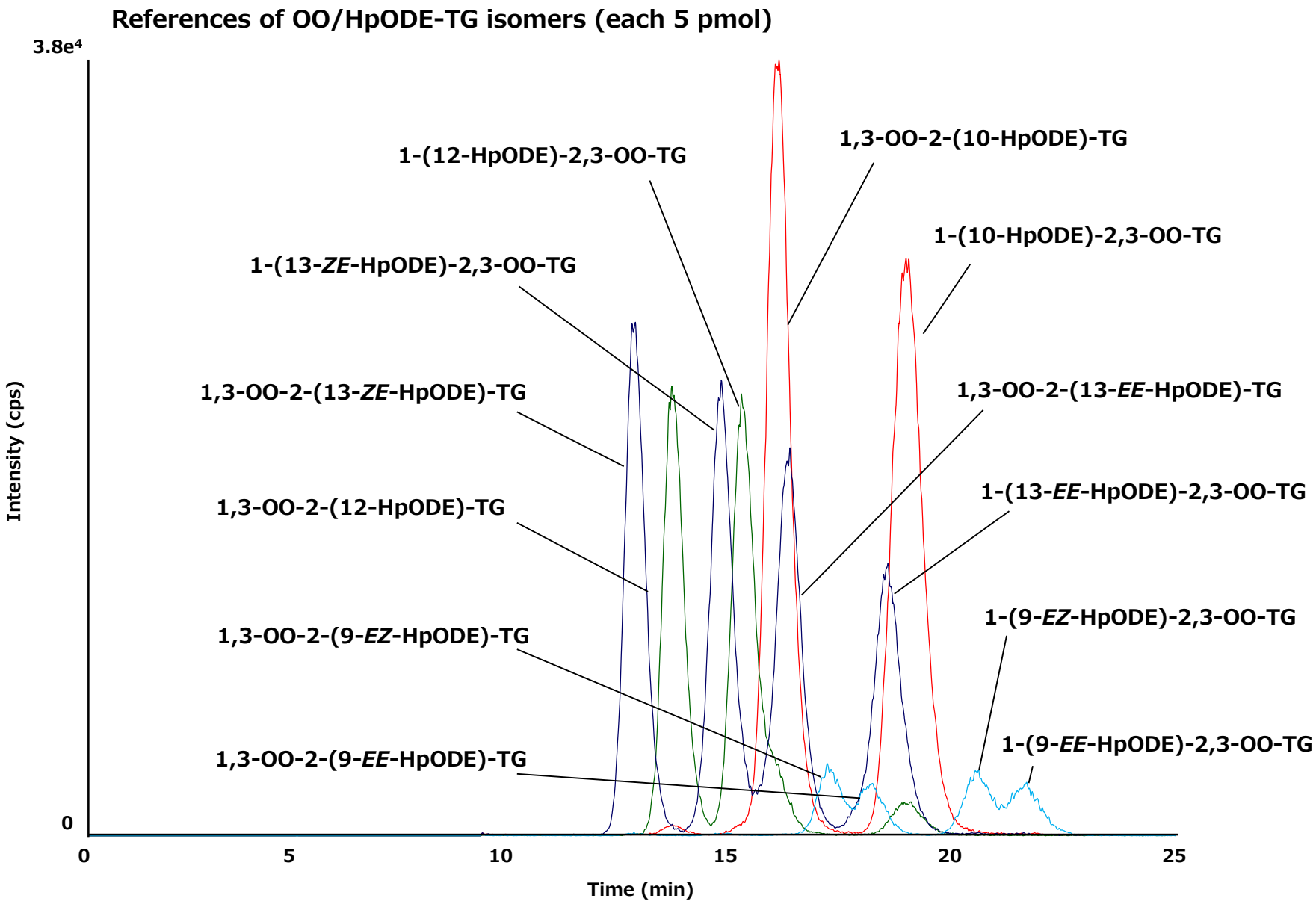
↓ エステル化 Dioleoyl glycerol, DCC, DMAP

↓ 脱保護

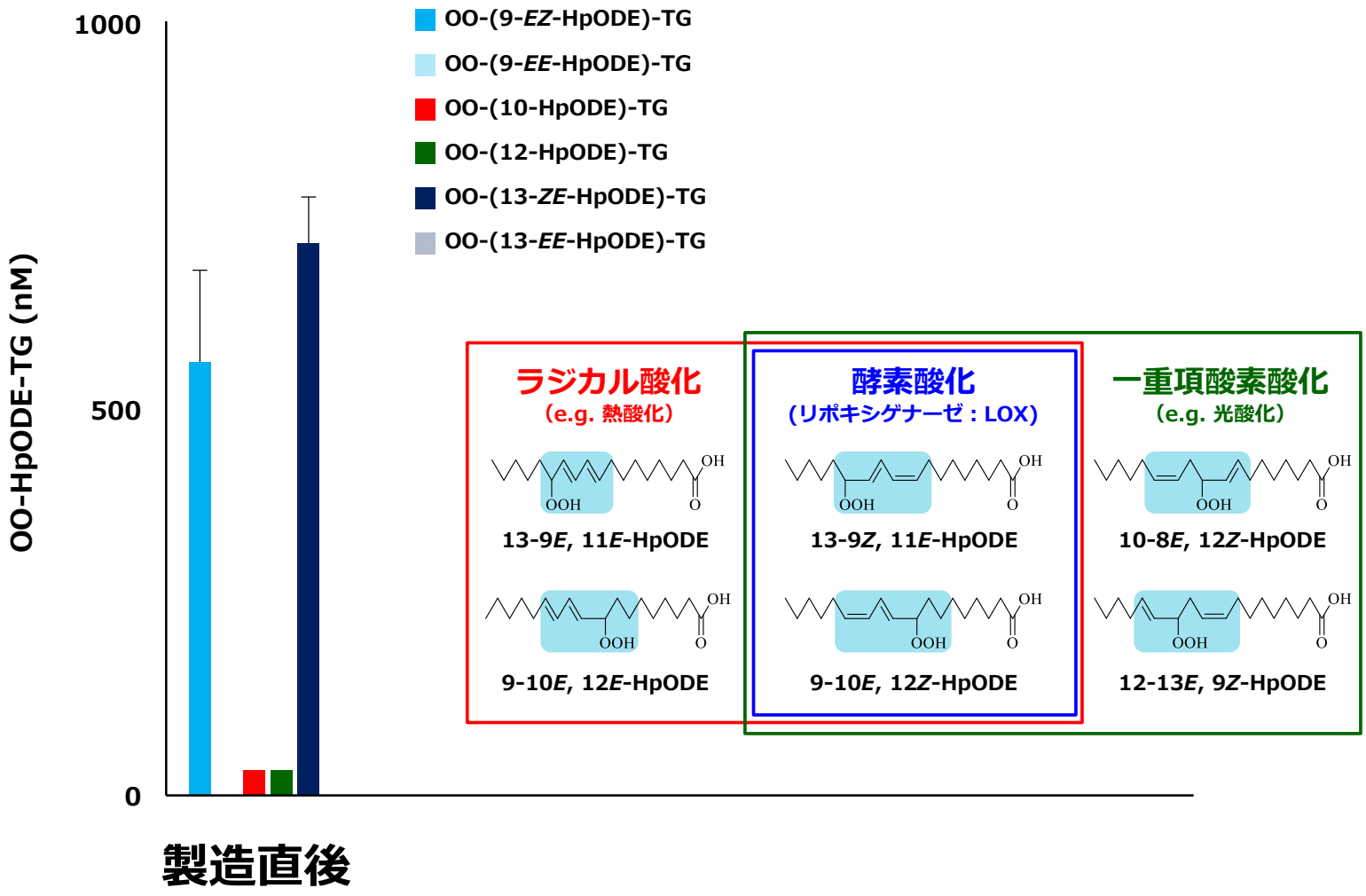
OOL/OLO type TGOOH isomers



TGOOH異性体 (標準品)

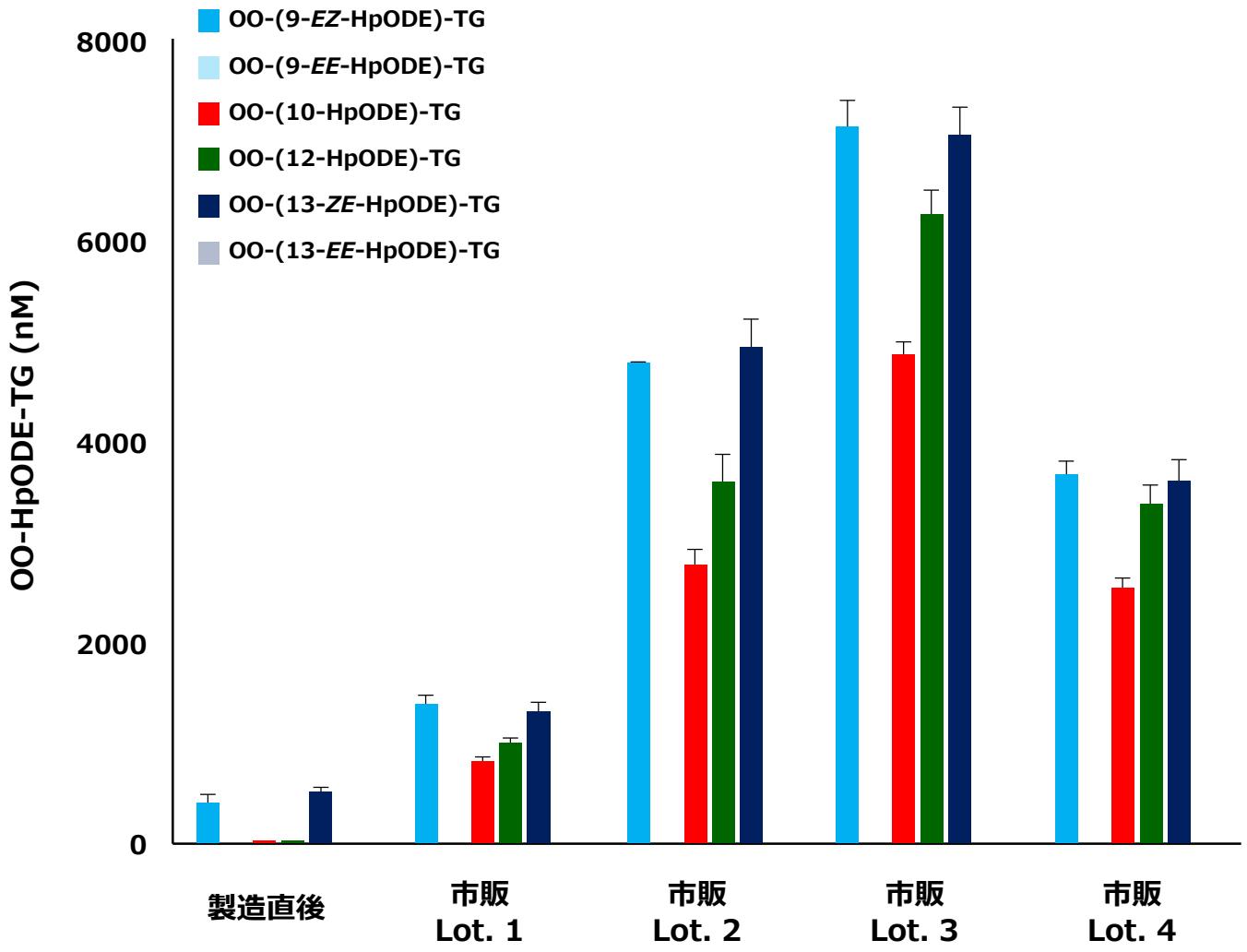


サンプル解析



新鮮な油脂（POV=0）に含まれるTGOOHも
高感度に定量が可能

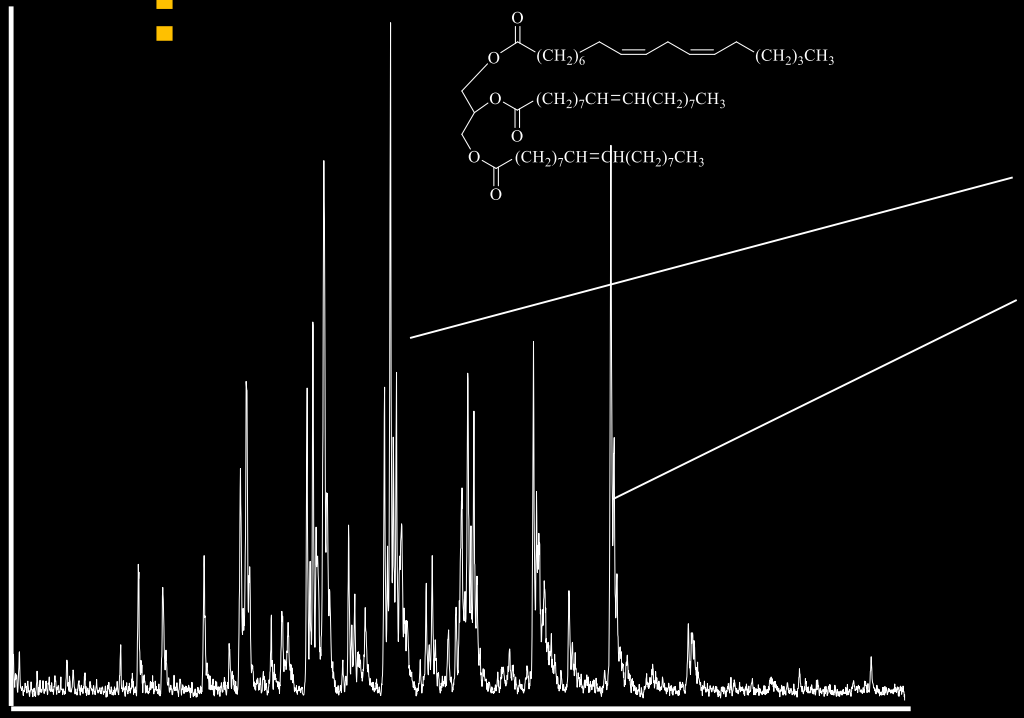
サンプル解析



店頭に並んでいる間は主に光酸化が亢進

様々な油脂へと応用

Olive oil
Fish oil
Rice bran oil
Sesame oil
⋮



Oleic acid
α-linolenic acid
γ-linolenic acid
DHA
⋮

公益社団法人
日本食品科学工学会
第65回大会

会　期　2018年8月22日（水）～24日（金）

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大会会頭　池田 郁男（東北大学大学院農学研究科）

基調講演
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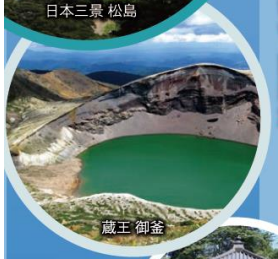
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瑞鳳殿

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