methods

UV analysis of Amadori-glycated phosphatidylethanolamine in foods and biological samples

Jeong-Ho Oak, Kiyotaka Nakagawa, and Teruo Miyazawa[1]

Biodynamic Chemistry Lab, Graduate School of Life Science and Agriculture, Tohoku University, Tsutsumidori-Amemiyamachi 1-1, Sendai 981-8555, Japan

Abstract **Maillard reactions are among the most important of the chemical and oxidative changes occurring in food and biological samples that contribute to food deterioration and to the pathophysiology of human disease. Although the association of lipid glycation with this process has recently been shown, the number of lipid glycation products in food and biological materials has not been clear. In this study, we synthesized the Amadori products derived from the glycation of phosphatidylethanolamine (PE), i.e., Amadori-PEs. Dioleoyl PE was incubated with glucose and lactose for 15 days, and the resultant Amadori-PEs were purified and isolated using solid phase extraction followed by HPLC. With this procedure, essentially pure (>98% purity) Amadori-PEs glycated with glucose (Glc-PE) and with lactose (Lac-PE) were obtained and used as standards in the subsequent studies. To determine the presence of Amadori-PEs in food and biological samples, the carbonyl group of Amadori-PEs was ultraviolet (UV)-labeled with 3-methyl-2-benzothiazolinone hydrazone, and the labeled Amadori-PEs were analyzed with normal phase HPLC-UV (318 nm). The detection limit was 4.5 ng (5 pmol) for Glc-PE and 5.3 ng (5 pmol) for Lac-PE. Among the several food samples examined, infant formula and chocolate contained a high amount of both Glc-PE and Lac-PE over wide concentration ranges, such as 1.5–112 μg/g. Testing biological materials showed Amadori-PE (Glc-PE) was detectable in rat plasma.**—Oak, J-H., K. Nakagawa, and T. Miyazawa. **UV analysis of Amadori-glycated phosphatidylethanolamine in foods and biological samples.** ***J. Lipid Res.*** **2002.** 43: **523–529.**

Supplementary key words glycation • phosphatidylethanolamine • Amadori product determination • 3-methyl-2-benzothiazolinone hydrazone • HPLC-UV

The oxidative modification of lipids in foods and biological samples has been recognized as playing a central role in food deterioration and in the pathophysiology of human diseases such as atherogenesis, diabetes, aging, and others (1–3). Recently, glycation of aminophospholipids has been of interest for expanding the concept of the Maillard reaction (4, 5). Subsequent studies (6–11) have shown that phosphatidylethanolamine (PE) reacts with glucose leading, through an unstable Schiff base, to a PE-linked Amadori product (Amadori-PE) (**Fig. 1**). This product accelerates membrane lipid peroxidation (11–14). Hence, Amadori-PE is believed to be a key compound for generating oxidative stress, which may cause food deterioration as well as several diseases. However, to date the accumulation and distribution of Amadori-PE, as well as other glycated lipids in foods and biological samples, is not fully understood. This is mainly because of the difficulty in preparing authentic glycated lipids and the lack of a suitable method for specifically measuring Amadori-PE in food and biological samples.

The formation of Amadori-PE during glycation was demonstrated in the study of Lederer et al. (8), by the following procedure. PE was incubated with D-glucose and the phospholipid fraction was purified. The fraction was next treated with phospholipase D, where both phosphatidic acid and glycated ethanolamine [1-deoxy-1-(2-hydroxyethylamino)-D-fructose] were produced. Afterwards, glycated ethanolamine was converted into an ultraviolet (UV)-absorbing derivative with 3-methyl-2-benzothiazolinone hydrazone (MBTH). From a detailed spectrometric analysis of the derivative, the formation of Amadori-PE was inferred (8). These results suggest that Amadori-PE itself may react with MBTH to form the UV-labeled Amadori-PE (**Fig. 2**), which would enable the rapid and convenient determination of Amadori-PE by using HPLC. Here, we develop a HPLC method for determining Amadori-PE after its derivatization with the UV-probe, MBTH, and discuss the possible peroxidative role of Amadori-PE in food and biological samples.

Abbreviations: Amadori-PE, Amadori-glycated phosphatidylethanolamine; GC, gas chromatography; MBTH, 3-methyl-2-benzothiazolinone hydrazone; MS, mass spectrometry; PAF, platelet-activating factor; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; RBC, red blood cells; SM, sphingomyelin; TIC, total ion current; UV, ultraviolet.

[1] To whom correspondence should be addressed.
e-mail: miyazawa@biochem.tohoku.ac.jp

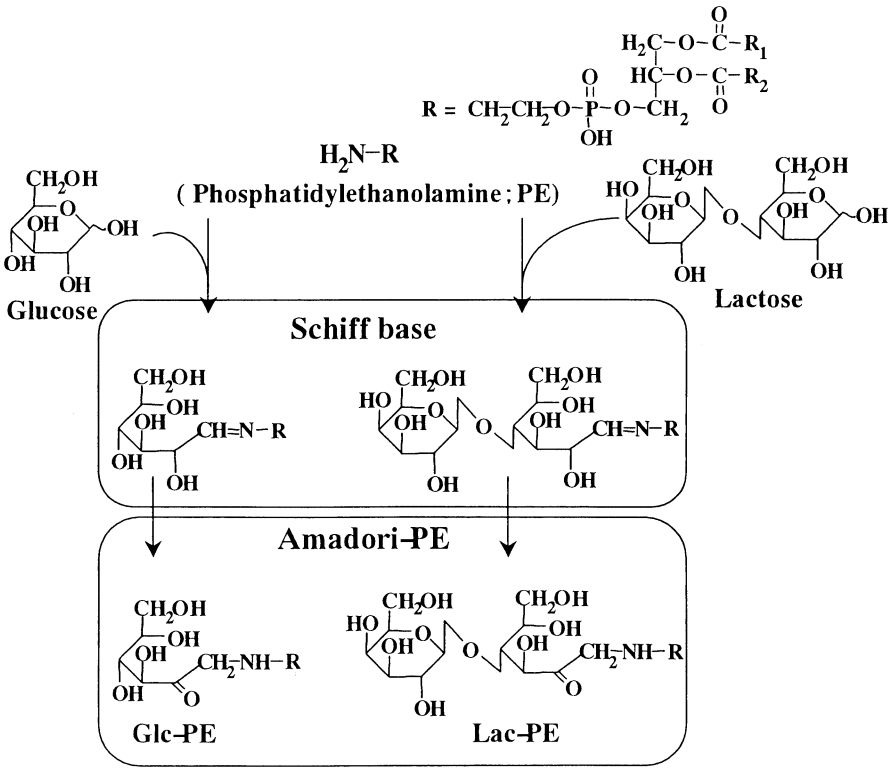

Fig. 1. Scheme for the glycation of phosphatidylethanolamine (PE). Sugar reacts with the amino group of PE to form an unstable Schiff base, which undergoes an Amadori rearrangement to yield the stable PE-linked Amadori product (Amadori-PE). Glc-PE, Amadori-compound derived from glucose and PE; Lac-PE, Amadori-compound derived from lactose and PE.

MATERIALS AND METHODS

Materials

1,2-Di(*cis*-9-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine (dioleoyl PE) was purchased from Funakoshi (Tokyo, Japan). D-Glucose and MBTH were obtained from Wako (Osaka, Japan). α-Lactose was from Sigma (St. Louis, MO). All other reagents were of analytical grade.

Preparation of standard Amadori-PEs

Amadori-PE standards (Glc-PE and Lac-PE)[2] were synthesized as previously described (11). Briefly, dioleoyl PE (27 μmol) was incubated either with D-glucose (2.0 mmol) or with α-lactose (2.0 mmol) in 30 ml of 0.1 M phosphate buffer-methanol (2:1, v/v, pH 7.4) at 37°C. After a 15 day incubation, lipid-soluble products were extracted with chloroform-methanol (2:1, v/v). The resultant extract was dissolved in 1 ml of methanol-30% ammonium hydroxide (95:5, v/v) and then loaded onto a Varian Bond Elut phenylboronic acid cartridge (Harbor City, CA) equilibrated in the same solvent. The cartridge was rinsed with an additional 3 ml of methanol-30% ammonium hydroxide (95:5, v/v), and the eluant discarded. Amadori-PEs were recovered with 5 ml of methanol, and subsequently purified by HPLC. The chemical

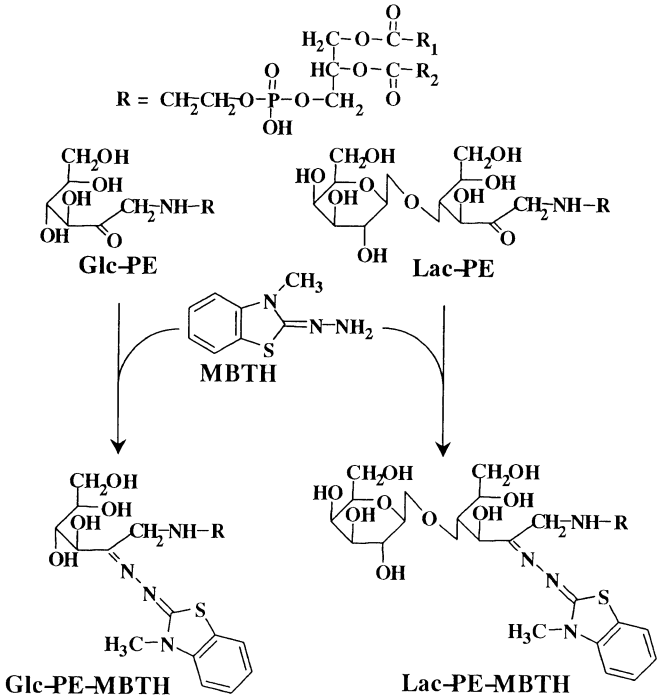

Fig. 2. Reaction pathway of Amadori-PEs with UV-labeling reagent, 3-methyl-2-benzothiazolinone hydrazone (MBTH). The carbonyl group of Amadori-PEs (Glc-PE and Lac-PE) reacts with MBTH to form the UV-labeled Amadori products. Glc-PE-MBTH, Glc-PE labeled with MBTH; Lac-PE-MBTH, Lac-PE labeled with MBTH.

[2] The nomenclature used to designate the two types of Amadori-PE are Glc-PE and Lac-PE for phosphatidylethanolamine glycated with glucose and lactose, respectively. Also, Glc-PE-MBTH and Lac-PE-MBTH represent the derivatives from the reaction of MBTH with Glc-PE and Lac-PE, respectively. The molecular species of PEs and Amadori-PEs are indicated by the total number of carbons in the acyl chain at the *sn*-2-position and the degree of unsaturation.

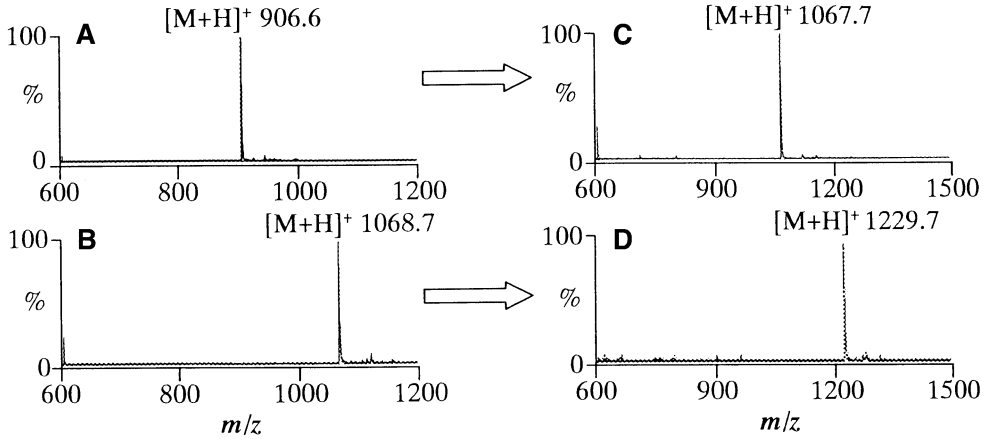

Fig. 3. Mass spectrometry (MS) spectra of Amadori-PEs. The molecular ions of Amadori-PEs (A: Glc-PE; B: Lac-PE) were not detectable after derivatization with MBTH. Consequently, the molecular ions of their derivatives (C: Glc-PE-MBTH; D: Lac-PE-MBTH) were detected on MS spectra.

structures of Amadori-PEs (Glc-PE and Lac-PE) were characterized on the basis of chromatography, one- and two-dimensional nuclear magnetic resonance spectroscopy, and electrospray mass spectrometry (MS).

Derivatization of Amadori-PE with MBTH

A 0.5–20 nmol of an Amadori-PE standard (Glc-PE or Lac-PE) was dissolved in 0.2 ml of 2.5% MBTH methanol solution. This solution was incubated at 37°C for 24 h in a nitrogen atmosphere. After the incubation, 6 ml of chloroform-methanol (2:1, v/v) and 1.5 ml of 10 mM HCl were added. After centrifuging at 1,000 × *g* for 15 min, the organic phase was collected. The extraction was repeated once more and the extracts were combined and evaporated. The residues were redissolved in 0.5 ml of chloroform-methanol (2:1, v/v), and a portion of the aliquot (1–50 μl) was subjected to either MS or UV-HPLC with on-line mass spectrometry (HPLC-UV-MS). MS analysis was performed using a Mariner electrospray ionization time of flight mass spectrometer (PerSeptive Biosystems, Framingham, MA) in the positive ion measurement mode with a spray voltage of 3,000 V, a nozzle potential of 100 V, and a nozzle temperature of 150°C. The flow rate of nebulizer gas was 0.3 ml/min. Full scan spectra were obtained by scanning masses in the *m/z* range between 400 and 1,500. For the HPLC-UV-MS system, a normal phase column (Inertsil Sil 100-5, 4.6 × 150 mm, GL Science, Tokyo, Japan) was used. The column was a linear gradient of 90% solvent A [chloroform-methanol (95:5, v/v)] to 100% solvent B [chloroform-methanol-water-acetic acid (300:170:27.5:2.5, v/v/v/v)] over 15 min, then at 100% B for 15 min. The flow-rate was adjusted to 1 ml/min and the column was maintained at 30°C. After the column eluant passed through the UV detector (UV-980, Japan Spectroscopic Co., Tokyo, Japan) at 318 nm, one of 20 volumes of the eluant was subjected to MS.

Food and biological samples

All food samples were purchased from a local supermarket in Sendai, Japan. Rat blood was obtained from 6- and 50-week-old male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan). Rat plasma was prepared by centrifuging the blood at 1,000 × *g* for 15 min at 4°C. Human milk (collected 1–2 months after birth) was obtained from healthy voluntary donors with their informed consent.

For the preparation of solid food samples, 1 g of the edible part was cut into pieces. The samples were then homogenized in a Waring blender with 40 ml of chloroform-methanol (2:1, v/v), and the total lipids were prepared by the method of Folch et al. (15). For liquid food or biological fluid samples, 1–10 ml of the sample was used for total lipid extraction. The total lipids were redissolved in an aliquot of chloroform-methanol (2:1, v/v), and were subjected to HPLC-MS to monitor the presence of Amadori-PEs. HPLC-MS analysis was performed using the same conditions as described above, except that solvent mixtures A [chloroform-methanol-acetic acid (450:47.5:2.5, v/v/v)] and B [chloroform-

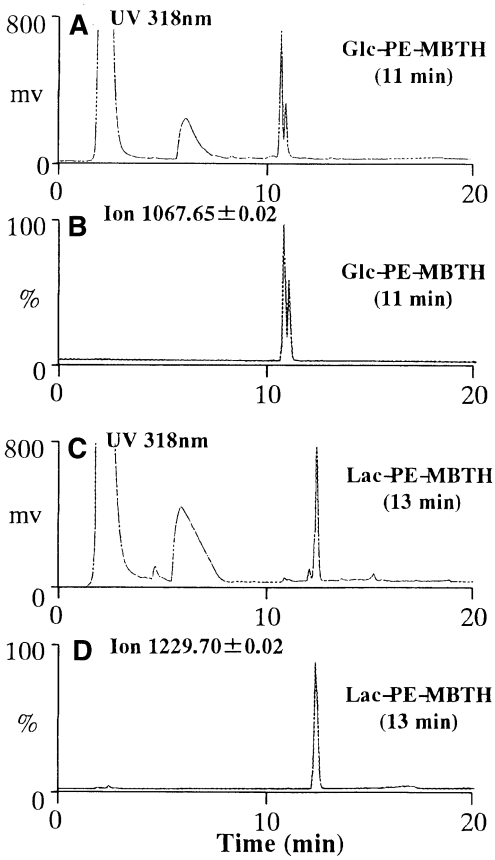

Fig. 4. UV- and single ion-chromatograms of the UV-labeled Amadori-PEs. 1.0 nmol of the standards, Glc-PE (A, B) or Lac-PE (C, D), were treated with MBTH and analyzed by HPLC-UV-MS.

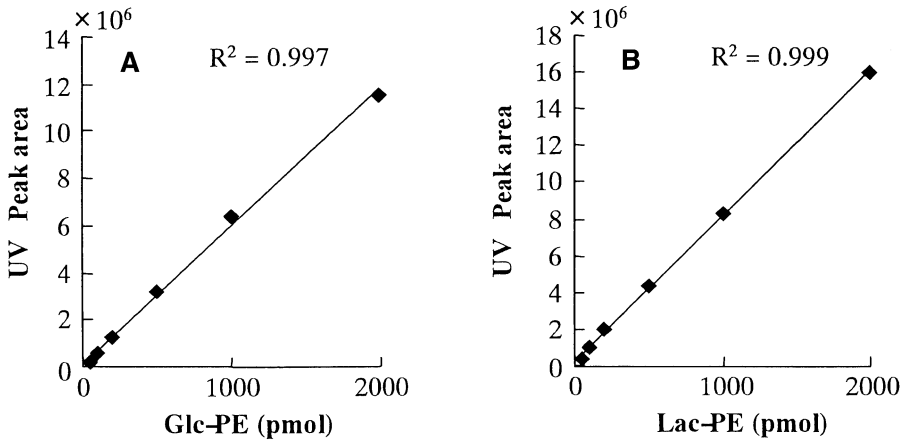

Fig. 5. Calibration curves of Amadori-PEs. Of the standards Glc-PE (A) or Lac-PE (B), 0.5–20 nmol were reacted with MBTH and analyzed by HPLC-UV.

methanol-water-acetic acid (300:170:27.5:2.5, v/v/v/v)] were used as the mobile phases. The solvent A was decreased from 100% to 0% over 30 min.

For derivatization, the resultant total lipid extract (contained 5–500 μg PE) was evaporated and treated with MBTH as described above. Labeled lipids were subjected to HPLC-UV analysis to determine Amadori-PE concentrations. The concentrations were expressed as the mean of the results from two identical samples. In addition, PE concentration was measured by using an HPLC-evaporative light-scattering detector (16), and the mol percentage of Amadori-PE to PE was calculated.

RESULTS

Direct reaction of standard Amadori-PEs with MBTH

The proposed direct reaction of Amadori-PEs with MBTH was documented by MS analysis (**Fig. 3**). After incubation of Amadori-PEs with MBTH, molecular ions of Amadori-PEs (*m/z* 906.6 $[M+H]^+$ for Glc-PE, and *m/z* 1,068.7 $[M+H]^+$ for Lac-PE) were no longer detectable and were replaced by ions identical to the MBTH-labeled Amadori-PEs (*m/z* 1,067.7 $[M+H]^+$ for Glc-PE-MBTH, and *m/z* 1,229.7 $[M+H]^+$ for Lac-PE-MBTH). Under these conditions, more than 98% of Amadori-PE reacted with MBTH over 24 h, yielding stable derivatives (data not shown). **Figure 4A** shows a typical UV chromatogram of the resultant extract from the reaction mixture of Glc-PE with MBTH. The derivative's peak was detected in the extract at a retention time of 11 min, and appeared identical (*m/z* $[M+H]^+$ 1,067.7) to Glc-PE-MBTH (Fig. 4B). When Glc-PE-MBTH was isolated and redissolved in methanol, it showed UV maximal absorption at 318 nm (data not shown). Similarly, a peak identical to Lac-PE-MBTH (*m/z* $[M+H]^+$ 1,229.7, UV maximum at 318 nm) was found at a retention time of 13 min from the reaction mixture of Lac-PE and MBTH (Fig. 4C and D). The derivatization of Amadori-PEs with MBTH was found to be linear in the range of concentrations from 18 ng (20 pmol) to 1,810 ng (2,000 pmol) for Glc-PE, and from 21 ng (20 pmol) to 2,130 ng (2000 pmol) for Lac-PE (**Fig. 5**). The detection limit was 4.5 ng (5 pmol) for Glc-PE, and 5.3 ng (5 pmol) for Lac-PE at a signal-to-noise ratio of 3.

MS and UV-analysis of Amadori-PEs present in food and biological samples

The presence of Amadori-PEs in food and biological samples was checked by HPLC-MS before testing the

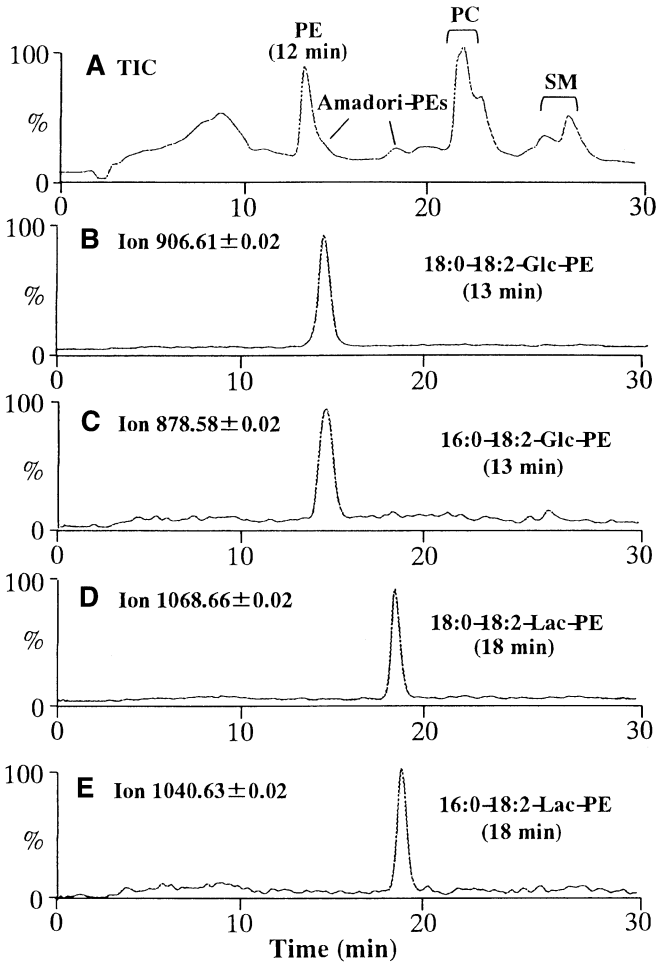

Fig. 6. HPLC-MS analysis of total lipid extract from infant formula. A: Total ion current (TIC) profile of phospholipids from infant formula. B–E: Single ion plots for the major molecular species of Glc-PE and Lac-PE. PC, phosphatidylcholine; SM, sphingomyelin.

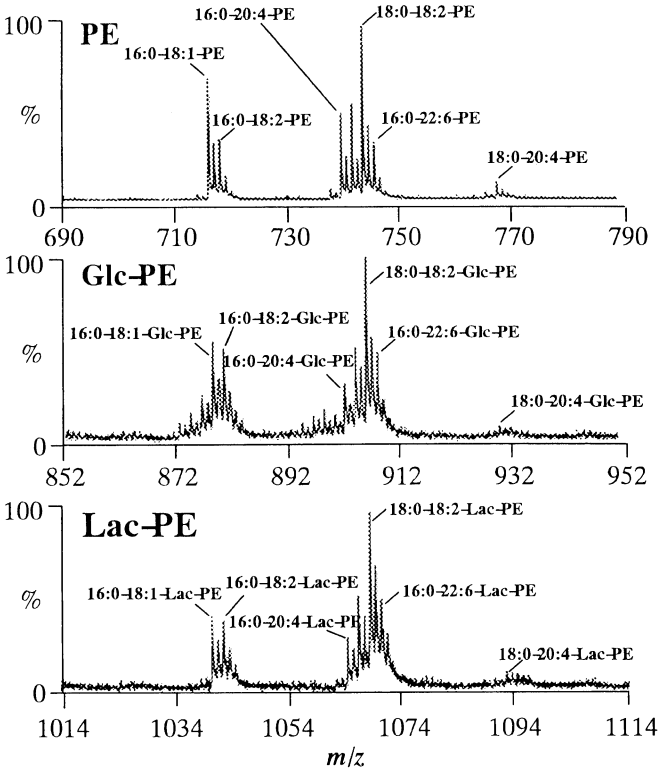

Fig. 7. MS spectra of the peaks of PE (12 min), Glc-PE (13 min), and Lac-PE(18 min) detected in the chromatogram of Fig. 6.

present method for analysis. Infant formula was tested first since it contains high amounts of sugar and lipids. As shown in **Fig. 6**, Amadori-PEs (Glc-PE and Lac-PE) could be detected in single ion-chromatograms of the lipid extract from infant formula. The MS spectra of PE, Glc-PE, and Lac-PE (**Fig. 7**) indicated that the molecular species of PE (fatty acid subclass) were randomly glycated. Applying the present UV-labeling method for the determination of Amadori-PEs in infant formula, we obtained a clear UV-chromatogram (**Fig. 8**). The peaks ascribed to UV-labeled Amadori-PEs (retention time 11 min for Glc-PE-MBTH, and 13 min for Lac-PE-MBTH) were well separated from the other unknown smaller peaks. By using standard curves (Fig. 5), the concentration of Amadori-PEs in infant formula was 32–112 μg (35–124 nmol)/g for Glc-PE, and 49–88 μg (46–82 nmol)/g for Lac-PE (**Table 1**). When 50 μg of an Amadori-PE standard was added to 1 g of infant formula and the sample analyzed, we obtained recoveries of Amadori-PE of 89% for Glc-PE and 82% for Lac-PE. Using our current labeling and detection procedure, significant amounts of Amadori-PEs were detectable in infant formula, chocolate, soybean milk, and rat plasma (Table 1).

DISCUSSION

The increasing importance of the Maillard reaction to issues of human nutrition and physiology, especially with respect to food processing, has shown the need for analytical means to detect lipid glycation compounds. Here we

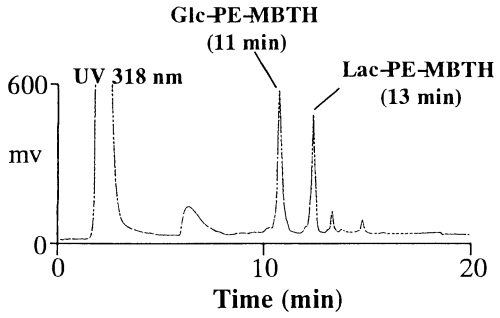

Fig. 8. UV-chromatogram of the MBTH-labeled Amadori-PEs. Total lipid extract from infant formula was treated with MBTH and subjected to HPLC-UV.

present a rapid and convenient HPLC method for determining Amadori-PE levels in food and biological samples by using the UV-labeling reagent, MBTH. This has allowed us to show, for the first time, the widespread occurrence of Amadori-PE in foodstuffs and in biological materials.

To determine Amadori-PEs by HPLC, we first identified a labeling reagent that could efficiently react with Amadori-PE directly. This was done taking into account its structure; Amadori-PE has a free carbonyl group (Fig. 1) when it exists in an acyclic form. Generally, lipids bearing a free carbonyl group are rare. Thus, the carbonyl group of Amadori-PE is a useful marker allowing for selectivity and sensitivity in its determination. On the other hand, labeling reagents such as 2,4-dinitrophenyl hydrazine and dansyl hydrazine are often used for the derivatization of carbonyl groups (17, 18). However, the reactivity of these reagents toward Amadori-PE was relatively low (data not shown),

TABLE 1. Amadori-PEs in food and biological samples as determined by HPLC-UV[a]

Samples	Glc-PE	Lac-PE	Glc-PE/PE	Lac-PE/PE
	μg (nmol)/g		*mol%*	
Infant formula				
A	32 (35)	83 (78)	3.0	6.7
B	101 (112)	88 (82)	14.3	10.5
C	112 (124)	49 (46)	21.6	11.2
Chocolate	3.9 (4.3)	1.5 (1.4)	35.3	11.3
Mayonnaise	12.2 (13.5)	ND	0.4	—
Cream powder	ND	ND	—	—
Yogurt	ND	ND	—	—
Butter	ND	ND	—	—
Margarine	ND	ND	—	—
	μg (nmol)/l			
Soybean milk	242 (268)	128 (120)	0.07	0.03
Milk	ND	79 (78)	—	0.07
Green tea	ND	ND	—	—
Coffee	ND	ND	—	—
Rat plasma				
6 weeks-old	ND	ND	—	—
50 weeks-old	238 (263)	ND	0.8	—
Human milk	ND	ND	—	—

ND, not detected.
[a] Values are the mean of a paired analysis of the same samples.

which may be attributed to the lower nucleophilicity of these labeling reagents. We therefore decided to use the more nucleophilic reagent, MBTH (Fig. 2), based on the study of Lederer et al. (8). As expected, the direct reaction of Amadori-PE with MBTH was confirmed (Fig. 3).

Secondly, the effect of temperature, pH, solvent, and incubation time was investigated to find appropriate reaction conditions. High temperature (above 60°C) and acidic and alkaline conditions all caused a decrease in the formation of MBTH-labeled Amadori-PEs (Glc-PE-MBTH and Lac-PE-MBTH) and an increase in unknown additional peaks on MS profiles (data not shown). This suggested that the derivatives were destroyed by the high reaction temperature or non-neutral condition. Among organic solvents tested, such as methanol, acetonitrile, and DMSO, the yield of derivatives was highest in methanol (data not shown). Therefore, we decided on a simple reaction condition where Amadori-PE was incubated with MBTH at 37°C in methanol. Derivatives (Glc-PE-MBTH and Lac-PE-MBTH) could be efficiently extracted with chloroform-methanol (2:1, v/v). During the extraction, addition of 10 mM HCl to the resultant reaction mixture was necessary to eliminate the excess unreacted MBTH.

A reverse-phase column is often used for phospholipid analysis, especially for the separation of the molecular species of phospholipids (19, 20). However, in this study we used a normal-phase column on which we could elute the molecular species as a single peak by using a gradient elution profile. This is an advantage for calculating the total concentration of Amadori-PEs (the sum of the molecular species of Amadori-PEs). When the extracts from the reaction mixture of Amadori-PEs with MBTH were subjected to normal phase-HPLC, peaks of UV-labeled Amadori-PEs (Glc-PE-MBTH and Lac-PE-MBTH) were clearly detected on UV- and MS-chromatograms (Fig. 4). From this result, we confirmed the fact that MBTH transforms Amadori-PE into the corresponding hydrazone, which shows a characteristic UV absorption maximum at 318 nm (Fig. 2). However, UV-labeled Amadori-PE was not eluted as a single peak. The peak displayed a small shoulder, especially for Glc-PE-MBTH (Fig. 4). Thus, reaction of Amadori-PE with MBTH gives *E/Z* isomers. Under optimized conditions, the calibration curves were linear over a wide concentration range (Fig. 5). The detection limit of the present HPLC-UV method was 4.5 ng (5 pmol) for Glc-PE, and 5.3 ng (5 pmol) for Lac-PE. Generally, the HPLC detection limit of phospholipid-derived chemical mediators, such as phospholipid hydroperoxides (21, 22) and platelet-activating factor (PAF)-like phospholipids (23), is above picomole levels. Hence, the sensitivity of the present labeling method for Amadori-PE is relatively high.

To date, there are six reports on Amadori-PE determination. The concentration of Amadori-PE in blood plasma (7, 9), red blood cells (RBC) (7, 9, 24, 25), and atherosclerotic plaques (14) from humans, and in spray-dried egg yolk powder (26) was estimated by using HPLC-MS, gas chromatography with on-line MS (GC-MS) and colorimetric methods. In the case of healthy human plasma, the proposed percentage of Amadori-PE to plasma PE was 2.3 mol% (7) and 15 mol% (9). For healthy human RBC, the percentage was 1.2 mol% (7), 11 mol% (9), 0.048 mol% (24), and 0.044 mol% (25). Therefore, there are large and significant differences among the proposed blood concentrations. According to our preliminary studies, the ionization rate varied depending on the structure of the molecular species of Amadori-PEs. This would be one reason for the variation of proposed Amadori-PE concentrations measured by HPLC-MS and GC-MS. As shown in Fig. 6 and Fig. 7, the MS technique is useful for the detection and structural analysis of Amadori-PEs in food and biological samples. However, the quantitative analysis of Amadori-PE by MS is difficult.

As shown in Fig. 8 and Table 1, the present UV-labeling method can be applied to the determination of Amadori-PEs in food and biological samples. As expected, processed foods (infant formula, chocolate, mayonnaise, milk, and soybean milk) contained a significant amount of Amadori-PEs. As these foods have high amounts of sugar and lipids, lipid glycation would occur during heat processing of these products. On the other hand, some foods (cream powder, yogurt, butter, margarine, tea, and coffee) did not contain any Amadori-PEs, probably because of low amounts of sugar or lipids and the relatively low temperatures used during processing of these products. Among the tested food samples, infant formulas have the most Amadori-PEs. The formulas contain PE (0.04–0.09%, w/w), and of this, 9.7–32.8 mol% was detected as the Amadori product. In contrast, human milk did not contain significant amounts of Amadori-PEs. Because the Amadori-PE generates superoxide anions and other reactive oxygen species under the presence of metal ions (11), the high glycation rate found in infant formulas may impair the nutritive value of the products. Amadori-PEs were below the detection limit in plasma of young rats (6 weeks old), whereas a significant amount of Glc-PE was detectable in plasma of old rats (50 weeks old). This first suggested that membrane lipid glycation increases with aging. The glycation of membrane lipids would conceivably cause membrane lipid peroxidation, which results in a deteriorated membrane structure. Such abnormalities might lead to disorder in the maintenance of cellular integrity and survival contributing to pathogenesis. On the other hand, since phosphatidylserine (PS) has an amino group, the glycation of PS may occur in the food and biological materials. However, there was no detectable glycated PS in our prepared food and biological samples (data not shown). Also, Ravandi et al. (7) were unable to detect glycated PS in human plsma or red blood cell membrane, so we focused only on assay for Amadori-PE in the present study.

To further show the involvement of lipid glycation in food deterioration and in the pathophysiology of human disease, we are now using our UV-labeling method to quantify Amadori-PE in various foods as well as in healthy and non-healthy human blood and tissue samples. In addition, our UV-labeling method may be developed to the new analytical means for determining Amadori-glycated proteins as well as Amadori-PE.

K. N. is a Japan Science and Technology Corporation (JST) Domestic Research Fellow.

Manuscript received 11 September 2001 and in revised form 3 December 2001.

REFERENCES

1. Witztum, J. L., and D. Steinberg. 1991. Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* **88:** 1785–1792.
2. St Angelo, A. J. 1996. Lipid oxidation on foods. *Crit. Rev. Food Sci. Nutr.* **36:** 175–224.
3. Kinoshita, M., S. Oikawa, K. Ayasaka, A. Sekikawa, T. Nagashima, T. Toyota, and T. Miyazawa. 2000. Age-related increases in plasma phosphatidylcholine hydroperoxide concentrations in control subjects and patients with hyperlipidemia. *Clin. Chem.* **46:** 822–828.
4. Bucala, R., Z. Makita, T. Koschinsky, A. Cerami, and H. Vlassara. 1993. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc. Natl. Acad. Sci. USA.* **90:** 6434–6438.
5. Pamplona, R., M. J. Bellmunt, M. Portero, D. Riba, and J. Prat. 1995. Chromatographic evidence for Amadori product formation in rat liver aminophospholipids. *Life Sci.* **57:** 873–979.
6. Ravandi, A., A. Kuksis, L. Marai, and J. J. Myher. 1995. Preparation and characterization of glucosylated aminoglycerophospholipids. *Lipids.* **30:** 885–891.
7. Ravandi, A., A. Kuksis, L. Marai, J. J. Myher, G. Steiner, G. Lewisa, and H. Kamido. 1996. Isolation and identification of glycated aminophospholipids from red cells and plasma of diabetic blood. *FEBS Lett.* **381:** 77–81.
8. Lederer, M. O., C. M. Dreisbusch, and R. M. Bundschuh. 1997. Amadori products from model reactions of D-glucose with phosphatidylethanolamine-independent synthesis and identification of 1-deoxy-1-(2-hydroxyethylamino)-D-fructose derivatives. *Carbohydr. Res.* **301:** 111–121.
9. Lertsiri, S., M. Shiraishi, and T. Miyazawa. 1998. Identification of deoxy-D-fructosyl phosphatidylethanolamine as a non-enzymic glycation product of phosphatidylethanolamine and its occurrence in human blood plasma and red blood cells. *Biosci. Biotechnol. Biochem.* **62:** 893–901.
10. Utzmann, C. M., and M. O. Lederer. 2000. Independent synthesis of aminophospholipid-linked Maillard products. *Carbohydr. Res.* **325:** 157–168.
11. Oak, J. H., K. Nakagawa, and T. Miyazawa. 2000. Synthetically prepared Amadori-glycated phosphatidylethanolamine can trigger lipid peroxidation via free radical reactions. *FEBS Lett.* **481:** 26–30.
12. Ravandi, A., A. Kuksis, and N. A. Shaikh. 1998. Glycated lipids present in LDL cause increased oxidation susceptibility: a novel role for glucose in LDL oxidation. *Clin. Chem.* **44:** A73.
13. Ravandi, A., A. Kuksis, and N. A. Shaikh. 1999. Glycated phosphatidylethanolamine promotes macrophage uptake of low density lipoprotein and accumulation of cholesteryl esters and triacylglycerols. *J. Biol. Chem.* **274:** 16494–16500.
14. Ravandi, A., A. Kuksis, and N. A. Shaikh. 2000. Glucosylated glycerophosphoethanolamines are the major LDL glycation products and increase LDL susceptibility to oxidation: evidence of their presence in atherosclerotic lesions. *Arterioscler. Thromb. Vasc. Biol.* **20:** 467–477.
15. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
16. Caboni, M. F., S. Menotta, and G. Lercker. 1994. High-performance liquid chromatography separation and light-scattering detection of phospholipids from cooked beef. *J. Chromatogr. A.* **683:** 59–65.
17. Conrad, C. C., P. L. Marshall, J. M. Talent, C. A. Malakowsky, J. Choi, and R. W. Gracy. 2000. Oxidized proteins in Alzheimer's plasma. *Biochem. Biophys. Res. Commun.* **275:** 678–681.
18. Visser, S. A. G., C. J. G. M. Smulders, W. W. F. T. Gladdines, H. Irth, P. H. van der Graaf, and M. Danhof. 2000. High-performance liquid chromatography of the neuroactive steroids alphaxalone and pregnanolone in plasma using dansyl hydrazine as fluorescent label: application to a pharmacokinetic-pharmacodynamic study in rats. *J. Chromatogr. B.* **745:** 357–363.
19. Singleton, J. A., M. Ruan, J. H. Sanford, C. A. Haney, and L. F. Stikeleather. 1999. Separation and characterization of peanut phospholipid molecular species using high-performance liquid chromatography and fast atom bombardment mass spectrometry. *J. Am. Oil Chem. Soc.* **76:** 49–56.
20. Lesnefsky, E. J., M. S. K. Stoll, P. E. Minkler, and C. L. Hoppel. 2000. Separation and quantitation of phospholipids and lysophospholipids by high-performance liquid chromatography. *Anal. Biochem.* **285:** 246–254.
21. Miyazawa, T., T. Suzuki, K. Fujimoto, and K. Yasuda. 1992. Chemiluminescent simultaneous determination of phosphatidylcholine hydroperoxide and phosphatidylethanolamine hydroperoxide in the liver and brain of the rat. *J. Lipid Res.* **33:** 1051–1059.
22. Miyazawa, T., K. Fujimoto, T. Suzuki, and K. Yasuda. 1994. Determination of phospholipid hydroperoxides using luminol chemiluminescence-high-performance liquid chromatography. *Methods Enzymol.* **233:** 324–332.
23. Kamido, H., A. Kuksis, L. Marai, and J. J. Myher. 1995. Lipid ester-bound aldehydes among copper-catalyzed peroxidation products of human plasma lipoproteins. *J. Lipid Res.* **36:** 1876–1886.
24. Fountain, W. C., J. R. Requena, A. J. Jenkins, T. J. Lyons, B. Smyth, J. W. Baynes, and S. R. Thorpe. 1999. Quantification of N-(glucitol)ethanolamine and N-(carboxymethyl)serine: two products of nonenzymatic modification of aminophospholipids formed in vivo. *Anal. Biochem.* **272:** 48–55.
25. Breitling-Utzmann, C. M., A. Unger, D. A. Friedl, and M. O. Lederer. 2001. Identification and quantification of phosphatidylethanolamine-derived glucosylamines and aminoketoses from human erythrocytes: influence of glycation products on lipid peroxidation. *Arch. Biochem. Biophys.* **391:** 245–254.
26. Utzmann, C. M., and M. O. Lederer. 2000. Identification and quantification of aminophospholipid-linked Maillard compounds in model systems and eggyolk products. *J. Agric. Food Chem.* **48:** 1000–1008.