JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Bound Malondialdehyde in Foods: Bioavailability of the *N*-2-Propenals of Lysine

JULIO GIRÓN-CALLE,* MANUEL ALAIZ, FRANCISCO MILLÁN, VALENTINA RUIZ-GUTIÉRREZ, AND EDUARDO VIOQUE†

Instituto de la Grasa (Consejo Superior de Investigaciones Científicas), Avenida Padre García Tejero 4, 41012 Sevilla, Spain

The lipid peroxidation product malondialdehyde is mostly bound to proteins in foods as an *N*-2-propenal derivative that is released as *N*-ε-(2-propenal)lysine by digestive enzymes. *N*-2-Propenals have been identified as the major forms of malondialdehyde in urine. To determine whether available lysine can be released from the *N*-2-propenals of lysine in vivo, two preparations containing *N*-ε-(2-propenal)lysine and *N*-α-(2-propenal)lysine or *N*,*N*′-di-(2-propenal)lysine were synthesized using radioactively labeled lysine and were administered to rats by gastric intubation and intraperitoneal injection. Both preparations were absorbed from the digestive tract, although not as efficiently as free lysine, but most of the radioactivity was excreted in urine. The radioactive label was also readily excreted after intraperitoneal injection. It is concluded that the *N*-2-propenals of lysine are fairly stable in vivo, so that, although they are absorbed from the gut, most of the absorbed material is not metabolized and is readily excreted as nonavailable lysine.

KEYWORDS: Malondialdehyde; lysine; *N*-2-propenals; lipid peroxidation; food proteins; absorption; bioavailability

INTRODUCTION

The peroxidative decomposition of lipids in foods containing polyunsaturated fatty acids has detrimental effects on the nutritional, toxicological, and functional properties of foods (*1*−*5*). Among the products of lipid peroxidation that have been more extensively studied is malondialdehyde (MDA). This aldehyde can serve as an index of peroxidative lipid decomposition and is often analyzed by reaction with thiobarbituric acid (*6*−*9*). MDA readily reacts with functional groups present in proteins, nucleic acids, and phospholipids, especially amino groups (*10*, *11*). The main products of the reaction with amino groups in neutral or acidic aqueous media are *N*-2-propenals (*12*, *13*), as well as 4-substituted 1,4-dihydropyridine-3,5-dicarbaldehyde when alkanals are also present (*14*, *15*). *N*-2-Propenals can form Schiff's bases by reacting with a second amino group forming *N*,*N*′-1-amino-3-iminopropene groups, which are fluorescent and less stable structures (*16*). Reactions with proteins and nucleic acids have been described (*12*, *17*−*22*). MDA has been found to be a toxic, carcinogenic, and mutagenic agent, although initial reports of its damaging effects might have been overstated to some extent (*11*, *23*−*25*). The levels of MDA as measured by reaction with thiobarbituric acid are increased in pathological conditions such as ischemia-reperfusion injury, atherosclerosis, and diabetes (reviewed in (*9*)).

Peroxidation of polyunsaturated lipids in foodstuffs during processing and/or storage leads to losses of available lysine as determined by chemical and in vivo methods (*26*−*30*). In experiments in which the presence of free and bound forms of MDA was investigated in enzymatic hydrolysates of foods of animal origin, the aldehyde was mostly found in the form of *N*-ε-(2-propenal)lysine (*31*) (**Figure 1**). Therefore, it appears that the organism is exposed to MDA in the diet mostly in the form of *N*-2-propenal derivatives of lysine. *N*-2-Propenal derivatives are usually reported as MDA because they are readily hydrolyzed under the acidic treatment that is required for the thiobarbituric acid test. Chromatographic separation before reaction with the thiobarbituric acid reagent is needed to discriminate between bound and free MDA (*9*, *32*).

N-2-Propenals were identified as the main urinary metabolites of MDA (*33*), but it remained to be determined whether these derivatives can be hydrolyzed after absorption from the digestive tract. *N*-2-Propenals of lysine in urine do not necessarily represent the same *N*-2-propenals that are taken up with the diet, because *N*-2-propenals are also endogenously generated (*37*). The *N*-2-mono- (*34*) and -dipropenal (*35*) derivatives of lysine were not hydrolyzed in incubations with liver, kidney, and intestinal mucosa homogenates, supporting the view that these derivatives are quite stable in tissues. In the present work, radioactive labeling of lysine in *N*-2-propenal derivatives has been used to assess incorporation into tissues, as well as excretion, by following the distribution of the radioactive label. The goal is to determine whether these derivatives are stable or can be metabolized in vivo, leading to release of lysine. In these

* Corresponding author: Julio Girón-Calle, Instituto de la Grasa (Consejo Superior de Investigaciones Científicas), Avenida Padre García Tejero 4, 41012 Sevilla, Spain. Phone: (34)954611550. Fax: (34)954616790. E-mail: jgiron@cica.es.

† Dr. Eduardo Vioque is deceased.

10.1021/jf025681r CCC: $22.00
Published on Web 09/11/2002

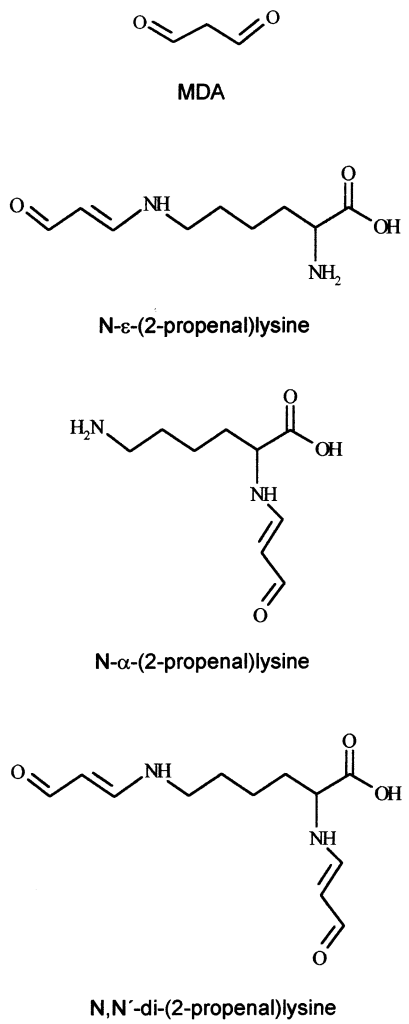

Figure 1. MDA and the *N*-2-propenals that result from its reaction with lysine.

experiments, incorporation and excretion of the radioactive label similar to the incorporation and excretion that is observed after administration of free lysine would suggest release of lysine from the administered *N*-2-propenals. Alternatively, patterns of incorporation and excretion of radioactivity different from those corresponding to free lysine would indicate that the *N*-2-propenals are not hydrolyzed in vivo and are not a source of free lysine.

MATERIALS AND METHODS

Materials and Animals. The sodium salt of MDA was prepared as described by Kikugawa and Ido (*14*) using 1,1,3,3-tetramethoxypropane and Dowex 50W-X8 resin obtained from Fluka (Bucks, Switzerland). MN-Kieselgel 60 (0.063−0.2 mm particle size) for column chromatography and Allugram analytical plates with fluorescent indicator for thin-layer chromatography were purchased from Macherey and Nagel (Duren, Germany). Ready Safe liquid scintillation fluid and BTS 450 tissue solubilizer were purchased from Beckman (Palo Alto, CA). L-[4,5-^{3}H]Lysine monohydrochloride was purchased from Amersham (Uppsala, Sweden).

^{13}C and ^{1}H nuclear magnetic resonance spectroscopy was carried out with Varian XL-200 and Bruker AC 300 instruments using tetramethylsilane as the internal standard. A Bomem MB-120 instrument was used for infrared spectroscopy.

Male Wistar rats were obtained from IFFA CREDO (Lyon, France) and kept in standard conditions for at least 1 week before experiments.

Preparation of Lysine *N*-2-Propenals. The *N*-2-propenals of lysine were prepared by reaction of L-lysine monohydrochloride (1.5 mmol) and MDA sodium salt (1.5 mmol) in methanol (20 mM) as previously described (*34*). The reaction products were chromatographed on a Kieselgel 60 column (40 × 1.8 cm, 45 g) using a chloroform/methanol stepwise elution gradient (3:2, 1:1, 2:3, 1:2, 1:3; 200 mL each per 1.5 mmol of the reagents). The eluted fractions were examined by TLC using silica gel plates with fluorescent indicator and *n*-propanol/water (8:2) as the developing solvent. UV light (254 nm) and 2-thiobarbituric acid spray reagent (*31*) were used for visualization of the MDA-containing lysine derivatives. A chromatographically pure compound with R_f 0.60 (7% yield) and a mixture of two compounds with R_f 0.47 and 0.37 (22% yield) were obtained. The compound with R_f 0.60 was *N*,*N*′-di-(2-propenal)lysine (DPL) as determined by ^{13}C and ^{1}H nuclear magnetic resonance: ^{1}H NMR (dimethyl sulfoxide-d_6) δ 1.30 (m, 2H), 1.49 (m, 2H), 1.68 (m, 2H), 2.99 (t, 2H enamine), 3.14 (m, 2H imine), 3.58 (t, H), 5.07 (m, 2H enamine), 5.22 (m, 2H imine), 7.30 (m, 2H), 8.80 (m, 2H imine), 8.89 (m, 2H enamine), 7.73 (H amine), 7.90 (H amine); ^{13}C NMR (dimethyl sulfoxide-d_6) δ 22.9, 27.8, 31.3, 43.1, 56.5, 100.0, 158.7, 174.3, 189.6. UV and IR data were also in agreement with this structure (see below).

The two compounds with lower R_f values were separated by carrying out a second chromatography of the mixture using a Kieselgel column as described above. Fractions that afforded single bands of R_f 0.47 and 0.37 on TLC showed identical ultraviolet and infrared spectroscopic properties, characteristic of the *N*-2-propenal derivatives of lysine: UV (ethanol) λ max 280 nm; IR (KBr) ν 3238 cm^{-1} (R_f 0.47) and 3252 cm^{-1} (R_f 0.37) (OH, NH), 1601 cm^{-1} (HN−C═C−C═O), 1406 cm^{-1} (C−N). The identities of these two compounds, the two *N*-2-monopropenals of lysine, *N*-α-(2-propenal)lysine and *N*-ϵ-(2-propenal)-lysine, was established by ^{13}C and ^{1}H NMR spectroscopy (*34*). This separation of the two monopropenals was carried out only for identification purposes, because they were administered together for the in vivo experiments.

To obtain radioactively labeled products L-[4,5-^{3}H]lysine monohydrochloride was added to the reaction mixtures. Radiochemical purity was assessed by scanning TLC plates using a Berthold model LB2820-1 linear plate analyzer. The radiochemical purity of the ^{3}H-labeled preparations that were administered to the experimental animals was 98.5% or better.

Biodistribution Studies. Rats weighing approximately 180 g were made to fast overnight before the experiments and kept in individual metabolic cages for urine and feces collection. Gastric intubation of the samples diluted in 1.5 mL of water was carried out using an 18 G round-tip needle. The samples were diluted in 0.4 mL of saline solution for intraperitoneal injection. After anesthesia with diethyl ether, blood was collected from the heart for plasma preparation, and organs and tissues were removed. Intestine contents were collected by perfusion with saline solution. Microsomal fractions of fresh livers were prepared by differential centrifugation according to Sherr et al. (*36*, *37*).

Samples were prepared for liquid scintillation counting according to the recommendations of the scintillation liquid manufacturer (Beckman, Palo Alto, CA) for the specific type of biological sample. This included treatment with hydrogen peroxide and acidification with acetic acid to prevent chemiluminescence. Samples of tissue, feces, plasma, digestive tract content, and microsomal fractions had to be predigested using a quaternary base solubilizer (BTS 450 tissue solubilizer). The accuracy of the liquid scintillation measurements was assured by repeating the determination after adding an internal standard in selected samples.

Statistics. Data are given as mean ± SEM. One-way analysis of variance (ANOVA) followed by the Tukey test for several groups was used to determine significance of differences between groups. A probability of $p > 0.05$ was considered significant.

RESULTS

Preparation of Lysine Propenals. Reaction of lysine with MDA in aqueous (*13*, *38*) or methanolic (*34*) media provides a mixture of the three possible lysine *N*-2-propenals, namely, *N*,*N*′-di-(2-propenal)lysine (DPL) and the lysine monopropenals *N*-ϵ-(2-propenal)lysine and *N*-α-(2-propenal)lysine (MPL) (**Figure 1**). For the present study, a preparation of chromatographi-

Table 1. Distribution of Radioactivity after Intubation into the Stomach of Lysine, MPL, or DPL[a,b]

	% of administered dpm		
	lys	MPL	DPL
urine	11.3 ± 2.0	30.8 ± 3.4[c]	21.9 ± 5.1
feces	15.1 ± 2.1	26.4 ± 1.0[c]	36.5 ± 3.3[c]
digestive content	2.7 ± 0.8	0.8 ± 0.6	1.0 ± 0.2
feces + digestive contents	17.8 ± 1.9	27.2 ± 0.6[c]	37.5 ± 3.4[c]
liver	14.91 ± 2.50	2.14 ± 0.19[c]	0.74 ± 0.17[c]
small intestine	7.75 ± 0.62	1.05 ± 0.23[c]	0.73 ± 0.12[c]
plasma[d]	2.63 ± 0.46	0.36 ± 0.06[c]	0.23 ± 0.03[c]
kidney	1.48 ± 0.23	0.26 ± 0.02[c]	0.08 ± 0.02[c]
large intestine	1.47 ± 0.10	0.29 ± 0.01[c]	0.26 ± 0.06[c]
stomach	1.25 ± 0.15	0.15 ± 0.02[c]	0.10 ± 0.01[c]
lung	0.72 ± 0.05	0.20 ± 0.04[c]	0.09 ± 0.03[c]
brain	0.62 ± 0.02	0.16 ± 0.06[c]	0.07 ± 0.01[c]
gluteus muscle[e]	0.53 ± 0.12	0.13 ± 0.01[c]	0.07 ± 0.03[c]
heart	0.38 ± 0.02	0.08 ± 0.00[c]	0.06 ± 0.01[c]
tissues total	31.74 ± 2.6	4.81 ± 0.4[c]	2.43 ± 0.1[c]
total	60.8 ± 4.1	62.8 ± 3.6	61.8 ± 1.6

[a] 12.5 μmol of lysine equivalents of the three ^{3}H-labeled preparations (lysine and MPL 1.92×10^6 cpm, DPL 5.47×10^6 cpm) were administered. Urine and feces were collected in metabolic cages for 24 h, after which rats were sacrificed for tissue sampling. [b] Values represent mean ± SEM ($n = 3$). [c] Significantly different ($p < 0.05$) when compared with lysine. [d] Values corresponding to total plasma, assuming 3.5 mL/100 g of body weight. [e] Values adjusted for 1 g of muscle, approximate weight of the tissue fragments that were taken.

cally pure DPL and a preparation containing ϵPL/αPL 7:3 (w/w) (MPL) were obtained by synthesis in methanolic solution and adsorption chromatography. Spectroscopic data of these compounds were in accord with what has been previously described for enaminal-type adducts (*13*). The ^{1}H NMR analysis of DPL showed the same *trans*-enamine−*trans*-imine tautomerism that was previously described for ϵPL (*34*, *38*) (see spectroscopic data in Materials and Methods). An apparent interconversion between the two monopropenals isomers was observed upon storage of the samples in aqueous or methanolic solution. Draper and co-workers proposed the formation of an intermediary cyclic Schiff's base to explain the same observation (*38*, *39*).

Excretion and Tissue Incorporation after Oral Administration. To avoid reaction with other components of the diet, and also to ensure consistent administration of a certain amount of sample, the *N*-2-propenals of lysine were administered by intubation into the stomach instead of by mixing them into the diet of the animals. The distributions of radioactivity in urine, feces, and tissues after administration of unaltered lysine, MPL, and DPL are shown in **Table 1** as percentages of administered counts per minute (cpm). Radioactivity in urine versus time since administration is shown in **Figure 2**.

Radioactivity in the urine collected during the first 5 h was several times higher after administration of MPL or DPL than after administration of free lysine. More than half of the radioactivity that was collected in urine after administration of MPL and DPL was excreted in this period of time (**Figure 2**). Elimination of radioactivity in feces was also higher for MPL and DPL than for lysine (**Table 1**).

Whereas almost one-third of the radioactivity corresponding to administered lysine (31.7%) was found in the tissues and organs that were collected, only 4.8% of the counts corresponding to MPL and 2.4% of those corresponding to DPL were incorporated into tissues. Accordingly, the bioavailabilities of lysine contained in these two fractions were estimated to be no higher than 15% (4.8/31.7 × 100) and 8% (2.4/31.7 × 100), respectively, of that of the unaltered amino acid. Considering

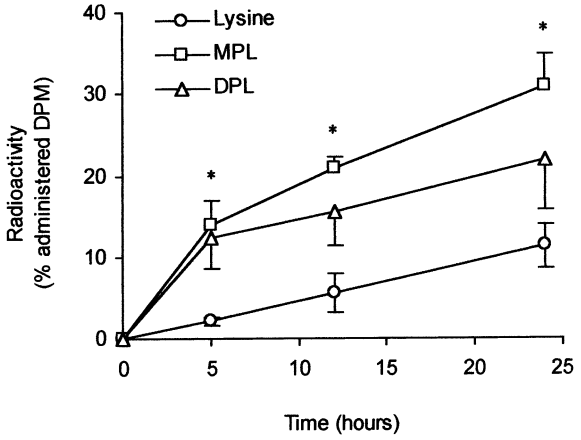

Figure 2. Radioactivity in urine collected after intubation into the stomach. Urine was collected in metabolic cages at three different times after intubation of the samples into the stomach. Data represent mean ± SEM ($n = 3$). * indicates significant difference ($p < 0.05$) when compared with lysine.

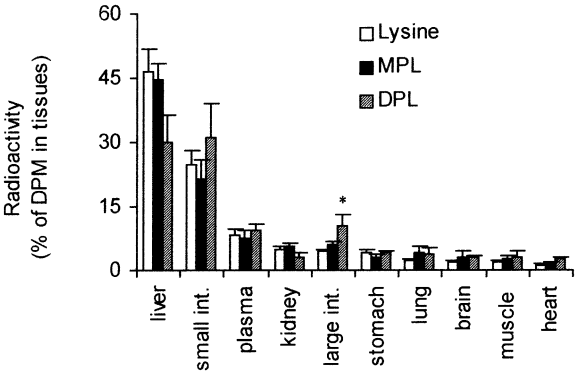

Figure 3. Distribution of the radioactivity incorporated into tissues. Radioactivity is expressed as percentage of total radioactivity in tissues to facilitate comparison among the three samples in different tissues. Data represent mean ± SEM ($n = 3$). All but one of the differences between lysine and any of the *N*-2-propenals, or between MPL and DPL, were not statistically significant ($p > 0.05$).

that the *N*-2-propenals might have been accumulated in a nonavailable form in the tissues, e.g., by reaction of the *N*-2-propenals with proteins or other components of tissues as such lysine derivatives, the true availability might be even lower.

The distribution of radioactivity as a percentage of the total radioactivity incorporated in tissues is shown in **Figure 3** to facilitate comparison among the three samples in different tissues. Thus, a correction for the different amounts of sample absorbed from the digestive tract is made. It is apparent that incorporation into tissues of the absorbed material is essentially the same for lysine, MPL, and DPL. Only in the large intestine is a significantly different incorporation of one of the *N*-2-propenal samples, DPL, observed.

Incorporation into Liver Microsomes after Intraperitoneal Injection. To determine regeneration of free lysine available for protein synthesis, the radioactivity in the hepatic microsomal fraction of rats injected intraperitoneally with the radiolabeled samples was determined. Radioactivity in plasma and urine was determined as well (**Table 2**). After administration of free lysine, 0.48% of the radioactivity was found in the microsomal fraction, showing incorporation of the amino acid into newly synthesized protein, but no radioactivity distinguishable from the background was found in the microsomal fraction of rats injected with MPL

Table 2. Radioactivity in Urine, Plasma, and the Hepatic Microsomal Fraction after Intraperitoneal Injection of Lysine, MPL, or DPL[a,b]

	% of administered dpm		
	lys	MPL	DPL
microsomes[c]	0.48 ± 0.01	0.02 ± 0.01[d,e]	0.00 ± 0.01[d,e]
plasma[f]	2.52 ± 0.20	0.08 ± 0.01[d,e]	0.05 ± 0.02[d,e]
urine	4.10 ± 2.08	34.40 ± 7.44[d]	20.69 ± 4.78

[a] 6.25 μmol of lysine equivalents of the three ^{3}H-labeled preparations (lysine and MPL 0.96×10^6 cpm, DPL 2.74×10^6 cpm) were injected, and urine was collected. After 7 h, rats were sacrificed for liver and plasma extraction. [b] Values represent mean ±SEM ($n = 3$). [c] Whole hepatic microsomal fractions counted. [d] Significantly different ($p < 0.05$) when compared with lysine. [e] Not significantly different than background ($p > 0.05$). [f] Values corresponding to total plasma, assuming 3.5 mL/100 g body weight.

or DPL. These two samples were readily excreted in urine, and only residual amounts of radioactivity were found in plasma.

DISCUSSION

Draper and co-workers studied the excretion of bound and free MDA in rats to which different MDA-containing samples had been given. Administration of ϵPL or even free MDA caused very little excretion of the free aldehyde in urine (*38*, *40*). Most of it was found as ϵPL (*39*) and *N*-α-acetyl-*N*-ϵ-(2-propenal)lysine. Administration of MDA-treated BSA (*38*) or diets promoting lipid peroxidation (*32*, *41*) resulted in excretion of the same forms of MDA. These studies clearly established that the *N*-2-propenals of lysine are the main form of excretion of MDA, but it still remained to be established whether these structures can be hydrolyzed in vivo. The fact that MDA is excreted mostly as *N*-2-propenals in urine does not exclude the possibility that *N*-2-propenals might be hydrolyzed in vivo, because the *N*-2-propenals of MDA can also be formed endogenously. Thus, *N*-2-propenals of lysine were found in the urine of fasted rats (*38*). The *N*-2-propenals that are observed in urine after administration of MDA or related compounds or diets might originate from the direct excretion of administered *N*-2-propenals, or alternatively, they might be formed endogenously and be derived from hydrolysis of administered bound MDA.

In the present work, the use of a radioactive label in the *N*-2-propenal samples allowed direct tracking of the excretion and distribution into tissues. Because the lysine moiety carries this label, regeneration of free lysine by hydrolysis of *N*-2-propenals should lead to an incorporation of radioactivity the same way that administration of the control, free lysine, leads to incorporation of radioactivity. Our results indicate that no more than 8 and 15% of administered MPL and DPL, respectively, is converted to free lysine. This implies that transit through the stomach is not enough to hydrolyze the *N*-2-propenals efficiently. Stronger acidic treatment, for instance, hydrolysis in 6 N HCl as carried out for amino acid analysis, is needed for quantitative release of free lysine (*21*). Most of the MPL and DPL that are absorbed from the digestive tract are excreted in urine, presumably as unaltered *N*-2-propenals of lysine or in the form of some other metabolite such as the *N*-α-acetyl derivative described by McGirr et al. (*38*).

The results obtained after peritoneal injection are consistent with the results obtained after intubation into the stomach, showing rapid clearance from plasma and excretion of the radioactive label. Thus, the formation of *N*-2-propenals in foods represents a loss of available lysine. This is a detrimental effect of the formation of *N*-2-propenals of lysine in foods due to peroxidative decomposition of polyunsaturated lipids. On the other hand, it also indicates that not much free MDA, if any at all, can be released in vivo from this type of lysine derivative. This might constitute a positive effect of the formation of *N*-2-propenals, which, in this way, can be considered as a form of benign, readily eliminated MDA. All of this information is consistent with previous research by others and by us concerning the excretion of bound forms of malondialdehyde (*33*) and the stability of the *N*-2-propenals of lysine in incubations in vitro with tissue homogenates (*34*, *35*). *N*-2-Propenals are also formed by reaction of MDA with nucleic acids. Interestingly, the reactivity of ϵPL with nucleic acids was found to be much lower than the reactivity of free MDA (*22*).

It remains to be established to what extent the small incorporation of radioactivity into tissues after oral administration of the *N*-2-propenals of lysine represents regeneration of available lysine. It could also represent reaction of these derivatives as such with proteins in tissues. Determination of radioactivity in the hepatic microsomal fraction after intraperitoneal administration was carried out to determine whether free lysine can be released from DPL and MPL. Nevertheless, only 0.48% of the radioactivity administered as lysine was incorporated in the time frame studied, so that the absence of radioactivity after administration of DPL or MPL is not statistically significant for this purpose.

In addition to being formed in foods, MDA is also formed in vivo, and *N*-2-propenal derivatives appear to also be products of the reaction of MDA with proteins in living tissues. This is supported by the fact that the same kinds of MDA derivatives mentioned above were found in the urine of fasted rats (*38*). Administration of free or bound forms of MDA increases the amount of these molecules over certain basal levels, considered to represent endogenously formed MDA (*24*, *38*, *40*). In vivo protein modification by reaction with MDA has also been shown by immunochemical techniques (*10*, *42*−*46*). In this context, our results indicate that the *N*-2-propenals of lysine are fairly stable in vivo, suggesting that modification of proteins by reaction with MDA to form *N*-2-propenals might be irreversible and repaired only by substitution of the modified proteins.

ABBREVIATIONS USED

MDA, malondialdehyde; DPL, *N*,*N*′-di-(2-propenal)lysine; ϵPL, *N*-ϵ-(2-propenal)lysine; αPL, *N*-α-(2-propenal)lysine; dpm, disintegrations per minute; MPL, lysine *N*-2-monopropenals.

ACKNOWLEDGMENT

This work was supported by CICYT Grants ALI88-0169, ALI91-0409, and AGL2001-0526 and by a fellowship from the Ministerio de Educación y Ciencia (J.G.). We thank Drs. F. J. Hidalgo and I. Hermosín for help with NMR studies and Dr. J. Vioque for reviewing the manuscript.

LITERATURE CITED

(1) Eriksson, C. E. Lipid oxidation catalysts and inhibitors in raw materials and processed foods. *Food Chem.* **1981**, *9*, 3−19.
(2) Karel, M. Lipid oxidation, secondary reactions, and water activity of foods. In *Autoxidation in Foods and Biological Systems*; Simic, M. G., Karel, M., Eds; Plenum Press: New York, 1980; pp 191−206.
(3) Kubow, S. Toxicity of dietary lipid peroxidation products. *Trends Food Sci. Technol.* **1990**, *1*, 67−70.
(4) Pokorny, J. Effect of lipid degradation on the taste and odor of food. *Nahrung* **1990**, *34*, 887−897.
(5) German, J. B. Food processing and lipid oxidation. *Adv. Exp. Med. Biol.* **1999**, *459*, 23−50.
(6) Siu, G. M.; Draper, H. H. A survey of the malonaldehyde content of retail meats and fish. *J. Food Sci.* **1978**, *43*, 1147−1149.

(7) Guillén-Sans, R.; Guzmán-Chozas, M. The thiobarbituric acid (TBA) reaction in foods: a review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 315−330.
(8) Draper, H. H.; Squires, E. J.; Mahmoodi, H.; Wu, J.; Agarwal, S.; Hadley, M. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radical Biol. Med.* **1993**, *15*, 353−363.
(9) Janero, D. R. Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biol. Med.* **1990**, *9*, 515−540.
(10) Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde, and related aldehydes. *Free Radical Biol. Med.* **1991**, *11*, 81−128.
(11) Draper, H. H.; Dhanakoti, S. N.; Hadley, M.; Piché, L. A.Malondialdehyde in Biological Systems. In *Cellular Antioxidant Defense Mechanisms*; Chow, C. K., Ed.; CRC Press: Boca Raton, FL, 1988; pp 97−109.
(12) Crawford, D. L.; Yu, T. C.; Sinnhuber. R. O. Reaction of malonaldehyde with protein. *J. Food Sci.* **1967**, *32*, 332−335.
(13) Nair, V.; Vietti, E.; Cooper, C. S. Degenerative chemistry of malonaldehyde. Structure, stereochemistry, and kinetics of formation of enaminals from reaction with amino acids. *J. Am. Chem. Soc.* **1981**, *103*, 3030−3036.
(14) Kikugawa, K.; Ido, Y.; Mikami, A. Studies on peroxidized lipids. VI. Fluorescent products derived from the reaction of primary amines, malondialdehyde and monofunctional aldehydes. *J. Am. Oil Chem. Soc.* **1984**, *61*, 1574−1581.
(15) Nair, V.; Offerman, R. J.; Turner, G. A.; Pryor, A. N.; Benzinger, N. C. Fluorescent 1,4-dihydropyridines. The malondialdehyde connection. *Tetrahedron* **1988**, *44*, 2793−2803.
(16) Chio, K. S.; Tappel, A. L. Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids. *Biochemistry* **1969**, *8*, 2821−2826.
(17) Buttkus, H. The reaction of malonaldehyde with myosin. *J. Food Sci.* **1967**, *32*, 432−434.
(18) Chio, K. S.; Tappel, A. L. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. *Biochemistry* **1969**, *8*, 2827−2832.
(19) Shin, B. C.; Huggins, J. W.; Carraway, K. L. Effects of pH, concentration and aging on the malonaldehyde reaction with proteins. *Lipids* **1972**, *7*, 229−233.
(20) Summerfield, F. W.; Tappel, A. L. Detection and measurement by high-performance liquid chromatography of malondialdehyde cross-links in DNA. *Anal. Biochem.* **1984**, *143*, 265−271.
(21) Uchida, K.; Sakai, K.; Itakura, K.; Osawa, T.; Toyokuni, S. Protein modification by lipid peroxidation products: formation of malondialdehyde-derived N-ϵ-(2-propenal)lysine in proteins. *Arch. Biochem. Biophys.* **1997**, *346*, 45−52.
(22) Plastaras, J. P.; Riggins, J. N.; Otteneder, M.; Marnett, L. J. Reactivity and mutagenicity of endogenous DNA oxopropenylating agents: base propenals, malondialdehyde, and N-ϵ-oxopropenyllysine. *Chem. Res. Toxicol.* **2000**, *13*, 1235−1242.
(23) Esterbauer, H. Cytotoxicity and genotoxicity of lipid-oxidation products. *Am. J. Clin. Nutr.* **1993**, *57*, 779S−785S; discussion 785S−786S.
(24) Draper, H. H.; McGirr, L. G.; Hadley, M. The metabolism of malondialdehyde. *Lipids* **1986**, *21*, 305−307.
(25) Draper, H. H.; Hadley, M. Malondialdehyde derivatives in urine. *Basic Life Sci.* **1988**, *49*, 199−202.
(26) El-Lakany, S.; March, B. E. A comparison of chemical changes in freeze-dried herring meals and a lipid−protein model system. *J. Sci. Food Agric.* **1974**, *25*, 889−897.
(27) Nielsen, H. K.; De Weck, D.; Finot, P. A.; Liardon, R.; Hurrell, R. F. Stability of tryptophan during food processing and storage. 1. Comparative losses of tryptophan, lysine and methionine in different model systems. *Br. J. Nutr.* **1985**, *53*, 281−292.
(28) Nielsen, H. K.; Finot, P. A.; Hurrell, R. F. Reactions of proteins with oxidizing lipids. 2. Influence on protein quality and on the bioavailability of lysine, methionine, cyst(e)ine and tryptophan as measured in rat assays. *Br. J. Nutr.* **1985**, *53*, 75−86.
(29) Nielsen, H. K.; Loliger, J.; Hurrell, R. F. Reactions of proteins with oxidizing lipids. 1. Analytical measurements of lipid oxidation and of amino acid losses in a whey protein−methyl linolenate model system. *Br. J. Nutr.* **1985**, *53*, 61−73.
(30) Hurrell, R. F.; Finot, P. A. Food processing and storage as a determinant of protein and amino acid availability. *Exp. Suppl.* **1983**, *44*, 135−156.
(31) Piche, L. A.; Cole, P. D.; Hadley, M.; van den Bergh, R.; Draper, H. H. Identification of N-ϵ-(2-propenal)lysine as the main form of malondialdehyde in food digesta. *Carcinogenesis* **1988**, *9*, 473−477.
(32) Piche, L. A.; Draper, H. H.; Cole, P. D. Malondialdehyde excretion by subjects consuming cod liver oil vs a concentrate of n-3 fatty acids. *Lipids* **1988**, *23*, 370−371.
(33) Draper, H. H.; Csallany, A. S.; Hadley, M. Urinary aldehydes as indicators of lipid peroxidation in vivo. *Free Radical Biol. Med.* **2000**, *29*, 1071−1077.
(34) Girón, J.; Alaiz, M.; Vioque, E. High-performance liquid chromatographic determination of N-ϵ-(2-propenal)lysine in biological samples after derivatization with diethylethoxymethylenemalonate. *Anal. Biochem.* **1992**, *206*, 155−160.
(35) Girón Calle, J. Estudio estructural y de biodisponibilidad de compuestos de reacción entre lisina y aldehidos procedentes de oxidación lipídica. Ph.D. Thesis, Departamento de Bioquímica, Bromatología y Toxicología, Universidad de Sevilla, Sevilla, Spain, 1992.
(36) Lake, B. G. In *Biochemical Toxicology a Practical Approach*; Snell, K., Mullock, B., Eds; IRL Press: Oxford, U.K., 1987; pp 183−213.
(37) Sherr, B.; Lee, C. M.; Jelesciewicz, C. Absorption and metabolism of lysine Maillard products in relation to utilization of L-lysine. *J. Agric. Food Chem.* **1989**, *37*, 119−122.
(38) McGirr, L. G.; Hadley, M.; Draper, H. H. Identification of N-α-acetyl-ϵ-(2-propenal)lysine as a urinary metabolite of malondialdehyde. *J. Biol. Chem.* **1985**, *260*, 15427−15431.
(39) Draper, H. H.; Hadley, M.; Lissemore, L.; Laing, N. M.; Cole, P. D. Identification of N-ϵ-(2-propenal)lysine as a major urinary metabolite of malondialdehyde. *Lipids* **1988**, *23*, 626−628.
(40) Draper, H. H.; Polensek, L.; Hadley, M.; McGirr, L. G. Urinary malondialdehyde as an indicator of lipid peroxidation in the diet and in the tissues. *Lipids* **1984**, *19*, 836−843.
(41) Mahmoodi, H.; Hadley, M.; Chang, Y. X.; Draper, H. H. Increased formation and degradation of malondialdehyde-modified proteins under conditions of peroxidative stress. *Lipids* **1995**, *30*, 963−966.
(42) Lee, H. S.; Shoeman, D. W.; Csallany, A. S. Urinary response to in vivo lipid peroxidation induced by vitamin E deficiency. *Lipids* **1992**, *27*, 124−128.
(43) Huang, G. S.; Wang, Z. P.; Wang, S. C.; Sun, T. J.; Chu, R.; Mao, S. J. Intracellular generation of MDA−LYS epitope in foam cells. *Life Sci.* **1999**, *65*, 285−296.
(44) Palinski, W.; Rosenfeld, M. E.; Yla-Herttuala, S.; Gurtner, G. C.; Socher, S. S.; Butler, S. W.; Parthasarathy, S.; Carew, T. E.; Steinberg, D.; Witztum, J. L. Low-density lipoprotein undergoes oxidative modification in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1372−1376.
(45) Uchida, K.; Fukuda, A.; Kawakishi, S.; Hiai, H.; Toyokuni, S. A renal carcinogen ferric nitrilotriacetate mediates a temporary accumulation of aldehyde-modified proteins within cytosolic compartment of rat kidney. *Arch. Biochem. Biophys.* **1995**, *317*, 405−411.
(46) Mooradian, A. D.; Lung, C. C.; Shah, G.; Mahmoud, S.; Pinnas, J. L. Age-related changes in tissue content of malondialdehyde-modified proteins. *Life Sci.* **1994**, *55*, 1561−1566.

Received for review May 16, 2002. Revised manuscript received July 31, 2002. Accepted August 4, 2002.

JF025681R