Amino Acids (2013) 44:1451–1456
DOI 10.1007/s00726-011-1180-z

SHORT COMMUNICATION

The pentosidine concentration in human blood specimens is affected by heating

Masako Nakano · Midori Kubota · Shigeru Owada · Ryoji Nagai

Received: 10 August 2011 / Accepted: 22 November 2011 / Published online: 4 December 2011

Abstract Pentosidine is an advanced glycation end product, formed by oxidation and glycation that accumulates markedly during end-stage renal failure. Measurement of the pentosidine level in physiological samples is applied as a sensitive marker for the early diagnosis of renal failure. In the quantitative measurements of pentosidine reported to date, a rapid enzyme-linked immunosorbent assay (ELISA) has been widely used to estimate the plasma/serum pentosidine levels in a number of clinical samples, because high performance liquid chromatography (HPLC) methods require multiple preparation steps before the analysis. However, the currently used clinical analysis of the plasma/serum pentosidine level by ELISA requires incubation of the plasma/serum at 100°C for 15 min to inactivate the protease, which is required before the anti-pentosidine antibody can bind to the pentosidine. In the present study, we examined whether pentosidine could be generated artificially through the heating of serum. The pentosidine content, measured by HPLC, in the serum increased by heating in a temperature- and time-dependent manner. The pentosidine content was increased 1.1- to 4.2-fold by the heating process compared to unheated samples, and the increased rate was not identical for each sample. After removing low-molecular weight (<10,000) serum components, the heat-induced pentosidine formation was decreased. Furthermore, the increase in pentosidine formation was significantly inhibited by acidic conditions more than by the addition of diethylene triamine pentaacetic acid, a metal chelator. This indicates that the level of serum pentosidine will be measured more accurately by ELISA if hydrochloric acid is added during the heating process.

Keywords Advanced glycation end products (AGEs) · Pentosidine · Renal failure · Clinical marker

Electronic supplementary material The online version of this article (doi:10.1007/s00726-011-1180-z) contains supplementary material, which is available to authorized users.

M. Nakano · M. Kubota · R. Nagai (✉)
Laboratory of Biochemistry and Nutritional Science, Department of Food and Nutrition, Japan Women's University, 2-8-1 Mejirodai Bunkyo-ku, Tokyo 112-8681, Japan
e-mail: nagai-883@umin.ac.jp

S. Owada
ASAO Clinic, 1-8-10 Mampukuji, Asao-Ku, Kawasaki, Kanagawa 215-0004, Japan

Introduction

Advanced glycation end products (AGEs) are formed non-enzymatically during the Maillard (or Browning) reaction via early products such as a Schiff base and Amadori products produced by the interactions between proteins and reducing sugars (Maillard 1912). Several AGE structures, including pentosidine (Sell and Monnier 1989), N^{ε}-(carboxymethyl)lysine (CML) (Ahmed et al. 1986), N^{ε}-(carboxyethyl)lysine (CEL) (Ahmed et al. 1997), imidazolone (Konishi et al. 1994), N^{ω}-(carboxymethyl)arginine (CMA) (Iijima et al. 2000), and pyrraline (Portero-Otin et al. 1995) have been identified.

Pentosidine, one of the fluorescent AGE structures generated under oxidative conditions, accumulates in blood and with long-lived tissue proteins, such as collagens and lens proteins. Its level reflects the extent of tissue damage associated with age-related diseases, such as diabetes mellitus, atherosclerosis, and chronic renal failure. Quantitative measurement of the pentosidine levels in vivo can be achieved using high performance liquid chromatography

(HPLC), because pentosidine possesses both autofluorescence and maintains its structural stability during sample preparation steps.

The common methods used for the analysis of pentosidine in human biological samples include either HPLC or an enzyme-linked immunosorbent assay (ELISA) using an antibody against pentosidine. The competitive ELISA developed by Izuhara et al. (1999) is performed using acid hydrolysis during the sample pretreatment. Taneda and Monnier (1994) reported that plasma samples can be pretreated by enzymatic digestion with pronase E at 37°C overnight and then filtered to remove the enzyme. Moreover, Sanaka et al. (2002) reported that plasma samples should be incubated with protease to allow the anti-pentosidine antibody to optimally access the inter- or intramolecular AGE structure, and that samples need to be incubated at 100°C for 15 min to inactivate the proteolytic enzyme.

Since multiple preparation steps and time-consuming processes are required to measure pentosidine by a HPLC analysis, the ELISA has been widely used to estimate the plasma pentosidine content in a large number of clinical samples. In addition, a simple ELISA system like the authors' protocol (Sanaka et al. 2002), has provided a rapid and convenient method for detecting the pentosidine concentration in human samples. Moreover, Sanaka et al. (2002) demonstrated that pentosidine is accepted as a highly sensitive marker for the diagnosis of early stage renal failure, because there is an increase in the plasma pentosidine level prior to the increase in the serum creatinine level in patients with mild renal dysfunction. Therefore, pentosidine is used as a glycoxidation marker in vivo (Miyata et al. 1998).

However, it has long been known that AGE formation during the incubation of proteins with reducing sugars is enhanced in a temperature-dependent manner. Furthermore, AGEs such as pentosidine and CML, another glycoxidation product, were dramatically increased during incubation of plasma and glycated protein in vitro. Our previous study indicated that CML is generated from glycated human serum albumin (HSA), a model Amadori protein, by short-term heating, resulting from direct conversion from the oxidative cleavage of the Amadori products or via several reactive aldehydes (Hayashi et al. 2002). Moreover, Miyata et al. (1998) reported that an increase in plasma pentosidine was produced by the presence of pentosidine precursors, such as carbonyl compounds in plasma, by incubation of samples at 37°C for 24 h. These reports suggested that human biological samples and Amadori proteins might be converted into AGEs as a quantified artifact by heat treatment. In the present study, we examined whether pentosidine could be generated artificially through heating, and also measured the inhibitory effect of several interventions on the heat-induced pentosidine formation.

Materials and methods

Pretreatment of human serum

The study involving human blood was approved by the ethics review committee of Japan Women's University and the ASAO Clinic for human experimentation. Human serum samples were drawn after obtaining a signed consent form from each patient. Serum samples were collected from nine patients with non-diabetic renal disease who were undergoing hemodialysis at a local medical facility. Serum samples (20 µl) were divided into unheated or heated samples for pretreatment. The heat-treated samples were added to 100 µl of 200 mM phosphate buffer solution (pH 7.4) to stabilize the pH during the 15-min heating. After the pretreatment of samples by heating, they were hydrolyzed at 110°C for 18 h in sealed test tubes with an equal concentration of hydrochloric acid (HCl) (6 N, final concentration). The pretreatment cartridge was prepared as follows, according to the methods described by Tsukahara et al. (2003) with a slight modification. A cation-exchange extraction cartridge, Oasis MCX (3 ml; Waters), was used for pretreatment of samples. The cartridge was preconditioned with 1 ml of methanol and equilibrated with 1 ml of distilled water under N_2 gas flow before loading the sample. The sample was applied to the cartridge, followed by washing of the cartridge with 3 ml of 0.1 M HCl. Pentosidine was eluted from the cartridge with 3 ml of 7% ammonia solution (v/v). The eluate was evaporated to dryness under a vacuum, and then the residues were dissolved in the HPLC mobile phase to prepare 300 µl of analytical sample solutions. The solutions were filtered through a 0.45-µm millex filter (Millipore). An aliquot (20 µl) of each sample was applied to the analytical HPLC system.

Measurement of pentosidine by HPLC

The HPLC method used was reported by Yoshihara et al. (1998), with slight modifications. The HPLC system was composed of a CBM-20A liquid chromatography system (Shimadzu, Kyoto, Japan) with a Capcellpak C18 UG80 column (250 mm × 4.6 mm (i.d.); 5 µm particle size, Shiseido, Tokyo, Japan), and an RF-10A Shimadzu spectrofluorometric detector (Ex. 335 nm, Em. 385 nm). The mobile phase was solvent A, 12% acetonitrile (v/v) containing 0.2% heptafluorobutyric acid (HFBA), and solvent B, 100% acetonitrile containing 0.2% HFBA. The flow rate was maintained at 1.0 ml/min and the column was kept at 40°C. The total cycle time between two injections was 50 min, including column washing. Pentosidine was prepared by the method described by Grandhee and Monnier

(1991). Purified pentosidine was used to obtain a standard curve by methods of Miyata et al. (1996).

The effects of temperature and time on the pentosidine formation

Twenty microliters of serum with 200 mM phosphate buffer (pH 7.4) in sealed test tube were incubated at temperatures ranging from 30 to 120°C for 15 min, and additional samples were incubated at 100°C for up to 60 min. The pentosidine content was then measured by HPLC after acid hydrolysis at 110°C for 18 h.

Comparison of the pentosidine formation between Tris–HCl and phosphate buffer

One hundred millimolar Tris–HCl, including 10 mM calcium chloride ($CaCl_2$) buffer (pH 7.5) recommended as the working concentration against proteases, was used as the buffer for some samples (Sanaka et al. 2002). Serum samples (20 µl) were divided into unheated or heated samples for pretreatment. The heat-treated samples in 100 µl of 100 mM Tris–HCl including 10 mM $CaCl_2$ (pH 7.5), or 200 mM phosphate buffer (pH 7.4) in sealed test tubes were incubated at 100°C for 15 min. After acid hydrolysis at 110°C for 18 h, the pentosidine content was measured by HPLC.

The effects of dialysis of the serum on heat-induced pentosidine formation

The serum samples were dialyzed using dialysis membranes (Sanko, Tokyo, Japan) with a 10,000 molecular weight (MW) exclusion limit in 50 mM sodium phosphate buffer (PBS) (pH 7.4) at 4°C for 24 h. After dialysis, the serum was collected from the dialysis membrane and the protein concentration was measured by the bicinchoninic acid method using the Pierce BCA protein assay reagents (Pierce, Rockford, IL, USA). Equal amounts of serum and dialyzed serum proteins were placed into sealed test tubes in the presence of 200 mM phosphate buffer (pH 7.4). The samples for each group were separated into unheated and heated groups, then the heated group was incubated at 100°C for 15 min. The samples were measured for their pentosidine content by HPLC after acid hydrolysis at 110°C for 18 h.

The effects of heating on the pentosidine formation from glycated proteins

Both glucose-derived glycated BSA and ribose-derived glycated BSA were prepared as described previously (Nagai et al. 1997; Motomura et al. 2009). Briefly, BSA (50 mg/ml) with 1.6 M glucose or 1.0 M ribose in 10 ml of 50 mM PBS (pH 7.4) in the presence of 2 mM diethylenetriamine pentaacetic acid (DTPA) was incubated at 37°C for 7 days, followed by dialysis against 50 mM PBS (pH 7.4). Both glycated BSA samples were divided into unheated and heated samples, and the heated samples were incubated at 100°C for 15 min. The pentosidine content of the samples was quantified by a HPLC analysis after acid hydrolysis with 6 N HCl at 110°C for 18 h.

The effects of various interventions on the pentosidine formation in glycated BSA and serum

Glycated BSA (2 mg/ml) samples in sealed test tubes were divided into six groups, which were used as one unheated sample and five heated samples. The heated samples with or without inhibitor reagents were incubated for 15 min at 100°C in the presence of each final concentration of sodium borohydride ($NaBH_4$) (100 mM), aminoguanidine (90 mM), DTPA (1 mM), or HCl (1.2 N), followed by acid hydrolysis at 110°C for 18 h and determination of pentosidine by HPLC.

Similarly, additional serum samples (20 µl) in sealed test tubes was divided into five groups, which included one unheated sample and four of heated samples. Each heated sample was incubated for 15 min at 100°C in the presence of either distilled water, 200 mM phosphate buffer (pH 7.4), 200 mM phosphate buffer (pH 7.4) containing DTPA (1 mM), or 200 mM phosphate buffer (pH 7.4) with HCl (1.2 N). The samples were then hydrolyzed at 110°C for 18 h in sealed test tubes with an equal concentration of 6 N HCl, followed by the determination of pentosidine by HPLC.

Statistical analysis

All values are expressed as the mean values ± SD. The data were compared using a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple-group comparisons, and using Student's t tests for two-group comparisons. The p values <0.05 were considered to be statistically significant.

Results and discussion

Determination of the pentosidine content in tissues and biological fluids has been widely employed to estimate the AGE level in vivo because of its acid stability and characteristic autofluorescence. Miyata et al. (1998) demonstrated that the pentosidine content in the plasma from both healthy subjects and hemodialysis patients was increased by incubation at 37°C for 24 h. Since the present clinical

analysis of the plasma pentosidine level by ELISA requires incubation of plasma at 100°C for 15 min to inactivate the protease (Sanaka et al. 2002), pentosidine could be generated artificially through the heating process as CML (Miyata et al. 2000; Hayashi et al. 2002). Practically, Sanaka et al. (1999) reported that the plasma pentosidine levels measured using a protocol involving heating at 100°C for 15 min by ELISA (Sanaka et al. 2002) was increased compared to the results obtained by HPLC. They noted that increase percentages of pentosidine were present in the plasma of both healthy subjects and patients with pre-dialysis renal failure, those on hemodialysis, and those continuous ambulatory peritoneal dialysis (CAPD) by 11.1, 6.04, 11.8, and 24.6%. Taken together, these reports indicated that the pentosidine level in the plasma/serum is increased by heating.

To confirm these notions, we analyzed the serum pentosidine level by HPLC after the heat treatment procedure used by ELISA (Sanaka et al. 2002). The pentosidine content was significantly increased in the 80°C-treated sample compared to the 30°C-treated sample ($p < 0.05$), and increased with increasing temperature up to the 120°C ($p < 0.01$) (Fig. 1c). Furthermore, the pentosidine content steeply increased after as little as 5 min of incubation (Fig. 1d).

We also examined the changes in the pentosidine content in the serum samples from the nine different patients with or without heat treatment. As shown in Fig. 2, the pentosidine content of all of the heated groups was increased by 1.1- to 4.2-fold compared to the unheated samples. More importantly, the rate of increased pentosidine content was different for each patient (Fig. 2), implying that the serum pentosidine level is altered by heating, but that this does not occur in a uniform manner. Moreover, the increased pentosidine level in serum resulting from heating was also observed in different buffer, such as phosphate buffer and Tris–HCl buffer, the most commonly used buffers for enzymatic reactions. The pentosidine content was significantly increased after heating samples with either Tris–HCl buffer ($p < 0.05$) or phosphate buffer ($p < 0.01$) compared to the unheated samples (Table 1). Therefore, measurement of pentosidine involving heating leads the artifact pentosidine formation.

To evaluate the potential pathway of pentosidine formation, the serum was dialyzed to remove low-molecular weight components, such as glucose and amino acids. As shown in Table 1, the average pentosidine content in the serum after heat treatment was 2.9-times higher than that of the unheated serum ($p < 0.01$). On the other hand, that of the dialyzed serum was 1.9-times higher than the unheated serum ($p < 0.05$). The pentosidine content in dialyzed serum after heating was estimated to be ~38% of the level present in non-dialyzed heated serum (Table 1), suggesting

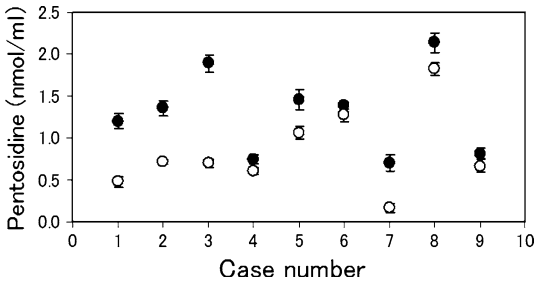

Fig. 2 A comparison of the serum pentosidine levels between unheated and heating samples. *Open circles* unheated samples, *closed circles* heated samples, $n = 3$

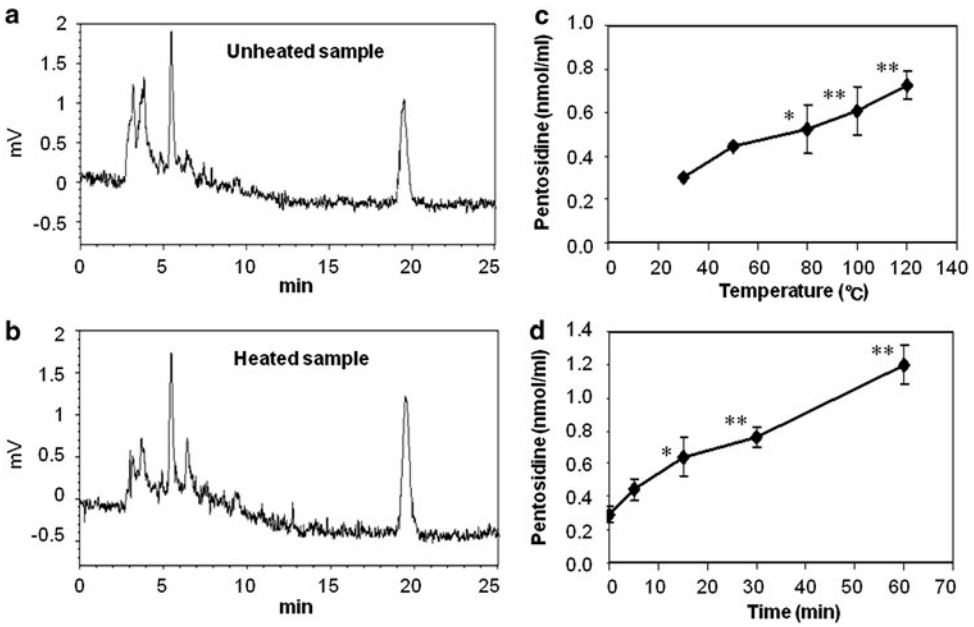

Fig. 1 *Left* a chromatogram of pentosidine obtained by a HPLC analysis. **a** Serum without heating. **b** Serum with heating. *Right* the temperature and time-dependent formation of pentosidine. **c** The effects of temperature incubated for 15 min. The data are presented as the mean values ± SD, $n = 3$. *$p < 0.05$; and **$p < 0.01$ versus 30°C. **d** The effects of time incubated at 100°C. The data are presented as the mean values ± SD, $n = 3$. *$p < 0.05$; and **$p < 0.01$ versus 0 min

Table 1 A comparison of the serum pentosidine levels treated in different buffers or serum, and treated in serum containing only protein of more than 10,000 molecular weight

Non-heating	Heating	
	Phosphate buffer	Tris–HCl buffer
Serum		
0.66 ± 0.12	1.93 ± 0.27**,##	1.04 ± 0.03*
Serum of more than 10,000 MW		
0.38 ± 0.05	0.73 ± 0.15†	–

The results are expressed in nmol per ml as the mean values ± SD of three subjects per groupThe heat-treated samples in 200 mM phosphate buffer (pH 7.4), or 100 mM Tris–HCl including 10 mM $CaCl_2$ (pH 7.5)

* $p < 0.05$ versus unheated serum, ** $p < 0.01$ versus unheated serum, ## $p < 0.01$ versus heat-treated serum in 100 mM Tris–HCl including 10 mM $CaCl_2$ (pH 7.5), † $p < 0.05$ versus unheated serum of more than 10,000 MW

that more than 60% of pentosidine was generated from low-molecular weight molecules, such as free amino acids and sugars. This observation corresponded to a previous report (Miyata et al. 1998). Thus, a large fraction of the pentosidine precursors have a molecular weight below 5,000 Da, and these molecules are the size of AGE-intermediates, such as glucose, amino acids, peptides, and carbonyl compounds.

The present study clarified that the pentosidine was generated by heating even after dialysis, suggesting that Amadori products may also be important precursors for pentosidine formation by heating. To evaluate the pentosidine formation from Amadori proteins during heat treatment, glycated BSA was prepared by incubation of BSA with glucose or ribose, and the pentosidine content was measured by HPLC. As shown in Fig. 3, the pentosidine levels were significantly increased after heating of glycated BSA compared to the unheated glycated BSA ($p < 0.01$).

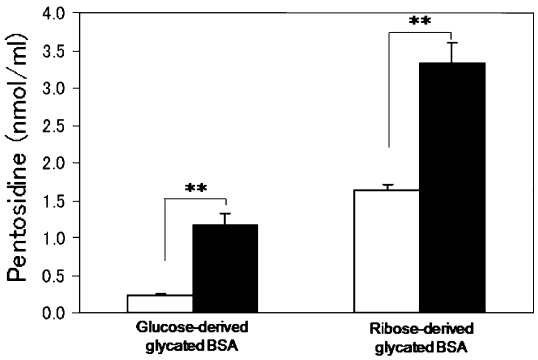

Fig. 3 A comparison of the pentosidine content generated from glycated protein-derived glucose or ribose by unheated and heated samples. The heated samples of each glycated protein were incubated at 100°C for 15 min. *Open column* unheated samples, *closed column* heated samples. The data are presented as the mean values ± SD, $n = 3$. **$p < 0.01$ versus unheated samples

The pentosidine content in the glucose-derived glycated BSA and ribose- derived glycated BSA after heat treatment were 4.7-times and 2.0-times higher than the levels from the unheated glycated BSA samples, respectively.

Since we demonstrated the heat-enhanced pentosidine formation from both Amadori products and serum, we next attempted to inhibit the pentosidine formation induced by heating using various interventions. Aminoguanidine, an inhibitor of AGE formation, acts as a strong metal chelator, as does DTPA (Sajithlal et al. 1998), and also traps carbonyl compounds (Brownlee et al. 1986). There is evidence that metal-induced oxidation of Amadori products plays a role in the formation of pentosidine (Sell and Monnier 1989; Grandhee and Monnier 1991). We observed that DTPA reduced the pentosidine content more than aminoguanidine in spite of low concentration (Fig. 4a), suggesting that the metal chelator effect is more effective for suppression of pentosidine produced by the Fenton reaction. Moreover, the addition of HCl had the highest inhibitory effect on the

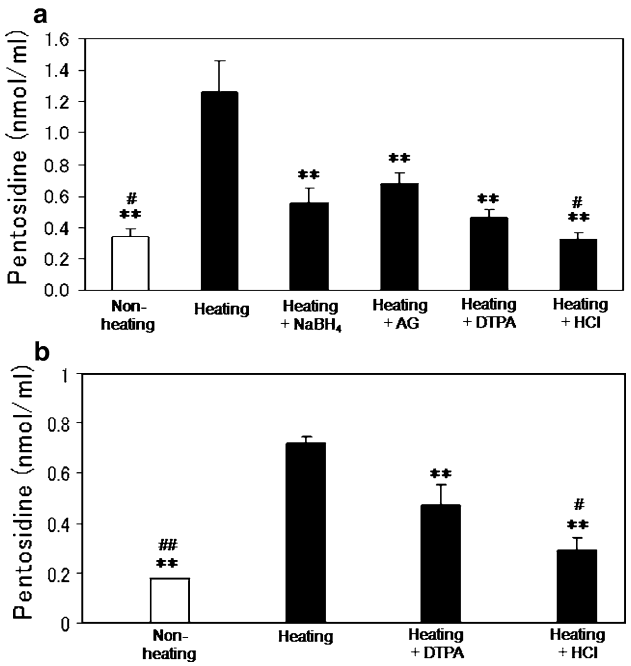

Fig. 4 The inhibitory effects of sodium borohydride ($NaBH_4$) reduction, aminoguanidine, a metal chelator, and hydrochloric acid on the pentosidine content obtained from glycated proteins or serum. The data are presented as the mean values ± SD, $n = 3$. **a** Glycated BSA was incubated at 100°C for 15 min with or without 100 mM of sodium borohydride ($NaBH_4$), 90 mM of aminoguanidine, 1 mM of diethylenetriamine pentaacetic acid (DTPA), or 1.2 N hydrochloric acid. The data are presented as the mean values ± SD. **$p < 0.01$ versus heated samples, #$p < 0.05$ versus the aminoguanidine samples. **b** Serum was incubated at 100°C for 15 min with or without 1 mM of diethylenetriamine pentaacetic acid (DTPA) or 1.2 N hydrochloric acid. The data are presented as the mean values ± SD, $n = 3$. **$p < 0.01$ versus heated samples, #$p < 0.05$; ##$p < 0.01$ versus DTPA samples

pentosidine formation induced by heating (Fig. 4a, b). The inhibitory effect of HCl led to a reduction in the pentosidine production to a level similar to that observed in the unheated samples. We used HCl since the formation of pentosidine is enhanced by alkaline conditions (Grandhee and Monnier 1991), whereas pyrraline formation is enhanced by acidic conditions (Portero-Otin et al. 1995). Several previous studies have reported that the plasma and skin collagen after acid hydrolysis were neutralized by sodium carbonate (Izuhara et al. 1999), NaOH (Taneda and Monnier 1994) and phosphate buffer (Izuhara et al. 1999), followed by measurement of the pentosidine content by ELISA, demonstrating that pentosidine can be measured by ELISA only if HCl is neutralized after heat treatment.

ELISA is a convenient and practical method for measuring AGEs in a large number of clinical samples. Moreover, since pentosidine is a stable structure even after acid hydrolysis (Grandhee and Monnier 1991; Weiss et al. 1998), the addition of HCl would be a very efficient and easy-to-use method for preventing the artificial formation of pentosidine induced by heating. Our present study may provide a more reliable method for measuring the pentosidine content in clinical samples by ELISA.

Acknowledgments We are grateful to Mime Nagai and Satoko Shimasaki for their collaborative endeavors. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 18790619 to Ryoji Nagai) from the Ministry of Education, Science, Sports and Cultures of Japan.

References

Ahmed MU, Thorpe SR, Baynes JW (1986) Identification of *N* epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. J Biol Chem 261:4889–4894

Ahmed MU, Frye EB, Degenhardt TP, Thorpe SR, Baynes JW (1997) N^{ε}-(carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. Biochem J 324:565–570

Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A (1986) Aminoguanidine prevents diabetes-induced arterial wall protien cross-linking. Science 232:1629–1632

Grandhee SK, Monnier VM (1991) Mechanism of formation of the Maillard protein cross-link pentosidine. Glucose, fructose, and ascorbate as pentosidine precursors. J Biol Chem 266: 11649–11653

Hayashi MC, Nagai R, Miyazaki K, Hayase F, Araki T, Ono T, Horiuchi S (2002) Conversion of Amadori products of the Maillard reaction to N^{ε}-(carboxymethyl)lysine by short-term heating: possible detection of artifacts by immunohistochemistry. Lab Invest 82:795–808

Iijima K, Murata M, Takahara H, Irie S, Fujimoto D (2000) Identification of *N*(omega)-carboxymethylarginine as a novel acid-labile advanced glycation end product in collagen. Biochem J 347:23–27

Izuhara Y, Miyata T, Ueda Y, Suzuki D, Asahi K, Inagi R, Sakai H, Kurokawa K (1999) A sensitive and specific ELISA for plasma pentosidine. Nephrol Dial Transplant 14:576–580

Konishi Y, Hayase F, Kato H (1994) Novel imidazolones compound formed by the advanced Maillard reaction of 3-deoxyglucosone and arginine residues in proteins. Biosci Biotechnol Biochem 58:1953–1955

Maillard LC (1912) Action des acides amines sur les sucres: formation des melanoidines par voie methodique. C R Acad Sci 154:66–68 (Paris)

Miyata T, Ueda Y, Shinzato T, Iida Y, Tanaka S, Kurokawa K (1996) Accumulation of albumin-linked and free-form pentosidine in the circulation of uremic patients with end-stage renal failure: renal implications in the pathophysiology of pentosidine. J Am Soc Nephrol 7:1198–1206

Miyata T, Ueda Y, Yamada Y, Izuhara Y, Wada T, Jadol M, Saito A, Kurokawa K, Strihou CY (1998) Accumulation of carbonyls accelerates the formation of pentosidine, an advanced glycation end product: carbonyl stress in uremia. J Am Soc Nephrol 9:2349–2356

Miyata T, Horie K, Ueda Y, Fujita Y, Izuhara Y, Hirano H, Uchida K, Saito A, de Strihou C, Kurokawa K (2000) Advanced glycation and lipidoxidation of the peritoneal membrane: respective roles of serum and peritoneal fluid reactive carbonyl compounds. Kidney Int 58:425–435

Motomura K, Fujiwara Y, Kiyota N, Tsurushima K, Takeya M, Nohara T, Nagai R, Ikeda T (2009) Astragalosides isolated from the root of astragalus radix inhibit the formation of advanced glycation end products. Agric Food Chem 57:7666–7672

Nagai R, Ikeda K, Higashi T, Sano H, Jinnouchi Y, Araki T, Horiuchi S (1997) Hydroxyl radical mediates N^{ε}-(carboxymethyl) lysine formation from Amadori product. Biochem Biophys Res Commun 234:167–172

Portero-Otin M, Nagaraj RH, Monnier VM (1995) Chromatographic evidence for pyrraline formation during protein glycation in vitro and in vivo. Biochim Biophys Acta 1247:74–80

Sajithlal GB, Chithra P, Chandrakasan G (1998) The role of metal-catalyzed oxidation in the formation of advanced glycation end products: an in vitro study on collagen. Free Radic Biol Med 25:265–269

Sanaka T, Niwayama J, Shiomi T, Kimura T, Nishimura H, Nakamura K (1999) Development of competitive ELISA methods for plasma pentosidine concentration and the analysis of the patients with chronic renal failure. Jin to touseki 47: 867–872 (Japanese)

Sanaka T, Funaki T, Tanaka T, Hoshi S, Niwayama J, Taitoh T, Nishimura H, Higuchi C (2002) Plasma pentosidine levels measured by a newly developed method using ELISA in patients with chronic renal failure. Nephron 91:64–73

Sell DR, Monnier VM (1989) Structure elucidation of a senescence cross-link from human extracellular matrix. J Biol Chem 264:21597–21602

Taneda S, Monnier VM (1994) ELISA of pentosidine, an advanced glycation end product, in biological specimens. Clin Chem 40:1766–1773

Tsukahara H, Sekine K, Uchiyama M, Kawakami H, Hata I, Todoroki Y, Hiraoka M, Kaji M, Yorifuji T, Momoi T, Yoshihara K, Beppu M, Mayumi M (2003) Formation of advanced glycosylation end products and oxidative stress in young patients with type 1 diabetes. Pediatr Res 54:419–424

Weiss MF, Rodby RA, Justice AC, Hricik DE (1998) Free pentosidine and neopterin as markers of progression rate in diabetic nephropathy. Kidney Int 54:193–202

Yoshihara K, Nakamura K, Kanai M, Nagayama Y, Takahashi S, Saito N, Nagata M (1998) Determination of urinary and serum pentosidine and its application to elder patients. Biol Pharm Bull 21:1005–1008