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Accumulation of Carbonyls Accelerates the Formation of Pentosidine, an Advanced Glycation End Product: Carbonyl Stress in Uremia

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Abstract. Advanced glycation end product (AGE) formation is related to hyperglycemia in diabetes but not in uremia, because plasma AGE levels do not differ between diabetic and nondiabetic hemodialysis patients. The mechanism of this phenomenon remains elusive. Previously, it was suggested that elevation of AGE levels in uremia might result from the accumulation of unknown AGE precursors. The present study evaluates the *in vitro* generation of pentosidine, a well identified AGE structure. Plasma samples from healthy subjects and nondiabetic hemodialysis patients were incubated under air for several weeks. Pentosidine levels were determined at intervals by HPLC assay. Pentosidine rose to a much larger extent in uremic than in control plasma. Pentosidine yield, *i.e.*, the change in pentosidine level between 0 and 4 wk divided by 28 d, averaged 0.172 nmol/ml per d in uremic *versus* 0.072 nmol/ml per d in control plasma ($P < 0.01$). The difference in pentosidine yield between uremic and control plasma was maintained in samples ultrafiltrated through a filter with a 5000-Da cutoff value and fortified with human serum albumin (0.099 *versus* 0.064 nmol/ml per d; $P < 0.05$). Pentosidine yield was higher in pre- than in postdialysis plasma samples (0.223 *versus* 0.153 nmol/ml per d; $P < 0.05$). These results suggest that a large fraction of the pentosidine precursors accumulated in uremic plasma have a lower than 5000 Da molecular weight. Addition of aminoguanidine and OPB-9195, which inhibit the Maillard reaction, lowered pentosidine yield in both uremic and control plasma. When ultrafiltrated plasma was exposed to 2,4-dinitrophenylhydrazine, the yield of hydrazones, formed by interaction with carbonyl groups, was markedly higher in uremic than in control plasma. These observations strongly suggest that the pentosidine precursors accumulated in uremic plasma are carbonyl compounds. These precursors are unrelated to glucose or ascorbic acid, whose concentration is either normal or lowered in uremic plasma. They are also unrelated to 3-deoxyglucosone, a glucose-derived dicarbonyl compound whose level is raised in uremic plasma: Its addition to normal plasma fails to increase pentosidine yield. This study reports an elevated level of reactive carbonyl compounds ("carbonyl stress") in uremic plasma. Most have a lower than 5000 Da molecular weight and are thus partly removed by hemodialysis. Their effect on pentosidine generation can be inhibited by aminoguanidine or OPB-9195. Carbonyl stress might contribute to AGE modification of proteins and thus to clinically relevant complications of uremia.

The nonenzymatic Maillard reaction progressively modifies proteins by forming Schiff base and Amadori adducts between protein amino group and glucose- or ascorbate-derived carbonyl group. It eventually yields irreversible advanced glycation end products (AGE) (1,2). The process develops slowly with aging (3) and is markedly enhanced in diabetic patients (4–6), as a result of sustained hyperglycemia.

Plasma AGE rise even more dramatically in uremia regardless of the presence or absence of diabetes (5–7). They have been implicated in the development of several complications of end-stage renal failure, such as dialysis-related amyloidosis, bone resorption, and atherosclerosis (8–13).

The mechanism of their increase in uremia remains to be elucidated. In a previous study, we focused on one of the several AGE structures, pentosidine (14), which accumulates in uremic plasma, predominantly in the albumin fraction (7). Since glucose levels were normal, we suggested the accumulation in uremic plasma of other, unknown precursors of pentosidine (7).

In the present study, we demonstrate the accumulation of pentosidine precursors in uremic plasma and provide evidence that they are reactive carbonyl compounds. The level of precursors is ascertained by measuring pentosidine generation during *in vitro* incubation of both normal and uremic plasma. Pentosidine yield is markedly higher in uremic than in normal samples. Plasma precursors are further character-

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ized by measuring pentosidine yield in predialysis plasma ultrafiltrate as well as in postdialysis sample. The reduction of the pentosidine yield by the addition of aminoguanidine or OPB-9195, both of which inhibit the Maillard reaction, suggests that the plasma precursors are reactive carbonyl compounds. Direct evidence for raised levels of reactive carbonyl compounds in uremic plasma is provided by the observation that ultrafiltrated plasma exposed to 2,4-dinitrophenylhydrazine yields twice as much hydrazones in uremic than in control plasma. We suggest that the accumulation of reactive carbonyl compounds in uremic plasma is best described as "carbonyl stress."

Materials and Methods

Plasma Samples

Fresh heparinized plasma samples were obtained from 16 healthy Japanese subjects (seven men, 57.8 ± 6.1 yr old) with normal renal function, from 39 nondiabetic Japanese patients (21 men, 59.3 ± 8.8 yr old) given cuprophane dialysis, and from three nondiabetic Caucasian patients (1 man, 50.0 ± 2.4 yr old) given polyacrylonitrile dialysis. Informed consent was obtained from all patients. Normal renal function was defined as a serum creatinine level <1.0 mg/dl (88.4 μmol/L) and the absence of proteinuria by a negative Albustix (Bayer-Sankyo, Tokyo, Japan). Hemodialysis was performed either with a cellulose membrane (surface area 1.0 to 2.0 m^2; $n = 39$) and a dialysate containing 2 g/L (11.1 mmol/L) glucose, 30 mmol/L bicarbonate, and 8 mmol/L acetate or with a polyacrylonitrile (AN69) membrane (surface area 1.25 to 2.05 m^2; $n = 3$) and a glucose-free dialysate containing 35 mmol/L bicarbonate and 4 mmol/L acetate. The protein concentration was determined using BioRad protein assay reagents (Richmond, CA) with human albumin as a standard.

In some experiments, plasma samples were ultrafiltrated twice through a filter with a 5000-Da cutoff value (Ultrafree CL-LCC; Nihon Millipore, Tokyo, Japan) and fortified with essentially fatty acid-free grade human serum albumin (HSA) (Sigma, St. Louis, MO) to a final concentration of 30 mg/ml.

In Vitro Incubation Experiments

Normal and uremic plasma samples and ultrafiltrates (1.0 ml) were sterilized with a 0.22-μm pore filter and incubated under air in sealed sterile 1.5-ml plastic tubes at 37°C for various times. In some experiments, normal plasma was fortified with 3-deoxyglucosone synthesized according to the method of Kato *et al.* (15). Thirty milligrams of essentially fatty acid-free grade bovine serum albumin (BSA) (Sigma) was also incubated with either 100 mM glucose or 20 mM ascorbic acid (Wako Pure Chemicals, Osaka, Japan) in 5.0 ml of 0.1 M sodium phosphate buffer, pH 7.4, under the same conditions. In some experiments, the sample incubation was performed in the presence of 1, 10, or 100 mM aminoguanidine (Tokyo Chemical Industry, Tokyo, Japan) (16,17) or OPB-9195 (a gift from Fujii Memorial Research Institute, Otsuka Pharmaceutical, Ohtsu, Japan) (18).

Pentosidine Measurement by HPLC Assay

Pentosidine was assayed by HPLC assay as described previously (7,19). In brief, the sample was lyophilized, hydrolyzed in 100 μl of 6N HCl for 16 h at 110°C under nitrogen, followed by neutralization with 100 μl of 5N NaOH and 200 μl of 0.5 M sodium phosphate buffer, pH 7.4, filtered through a 0.5-μm filter, and diluted with phosphate-buffered saline. A sample (corresponding to 25 μg of proteins) was injected into an HPLC system and fractionated on a C18 reverse-phase column. The effluent was monitored at an excitation emission wavelength of 335/385 nm using a fluorescence detector (RF-10A; Shimadzu). Synthetic pentosidine was used to obtain a standard curve. The identity of the substance in the specimens, detected at the same retention time as authentic pentosidine, was confirmed as pentosidine by fast atom bombardment-mass spectrometry by determination of its molecular mass (m/z 379.4), which was in good agreement with the theoretical value for pentosidine (379.4394 Da for chemical mass). Limits of detection were 2 pmol of pentosidine per milligram of plasma protein.

Carbonyl Measurement

Plasma samples were ultrafiltered through a filter with a 5000-Da cutoff value. For determination of carbonyl compound content, 20 μl of 0.1% 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl was added to 100 μl of plasma ultrafiltrated through a filter with a 5000-Da cutoff value. After standing for 30 min at room temperature in the dark, the mixture was washed with 500 μl of pentane, and 10 μl of 37% formaldehyde was added to react with excess DNPH for 30 min at room temperature in the dark (20). The mixture was washed 3 times with 130 μl of chloroform, and absorbance was measured at 360 nm. The carbonyl content was calculated as nmol/ml using a molar absorption coefficient of 22,000 $M^{-1} \cdot cm^{-1}$ (21). Samples processed similarly but without DNPH treatment were used as blanks. In this method, the glucose-derived carbonyl group also reacts with DNPH. The glucose-derived carbonyl content in plasma samples was therefore calculated on the basis of the plasma glucose concentration and a standard curve of the carbonyl content obtained in distilled water containing various concentrations of glucose. The level of plasma carbonyl compounds other than glucose was then calculated as the difference between total plasma carbonyl and glucose-derived carbonyl level.

Statistical Analysis

Data are expressed as means ± SD. Unpaired t test was used for a statistical evaluation of significant difference between the two groups.

Results

Predialysis Uremic and Normal Plasma Samples

Incubation of plasma samples *in vitro* under air yielded increasing amounts of pentosidine residues on proteins. As shown in Figure 1, pentosidine concentration in the medium rose linearly with the duration of incubation. The yield of pentosidine was thus calculated *per* ml and *per* day of incubation as the difference in pentosidine concentration between time 0 and 4 wk, divided by 28. Pentosidine yield was significantly ($P < 0.01$) higher in uremic (0.172 nmol/ml per d) predialysis plasma than in control plasma (0.072 nmol/ml per d) (Figure 1A). It is of interest that in uremic plasma, the pentosidine content increase after a 4-wk incubation was highly correlated with the initial level of pentosidine ($r = 0.564$, $P < 0.05$) (Figure 2).

To determine the extent to which pentosidine precursors in plasma were bound to protein, substances with a molecular weight >5000 Da, which include albumin, were removed from plasma by ultrafiltration, and the ultrafiltrate was fortified with HSA (containing 5 pmol pentosidine *per* mg of albumin) before incubation. As shown in Figure 1B, pentosidine formation remained higher in uremic than in normal plasma ultrafil-

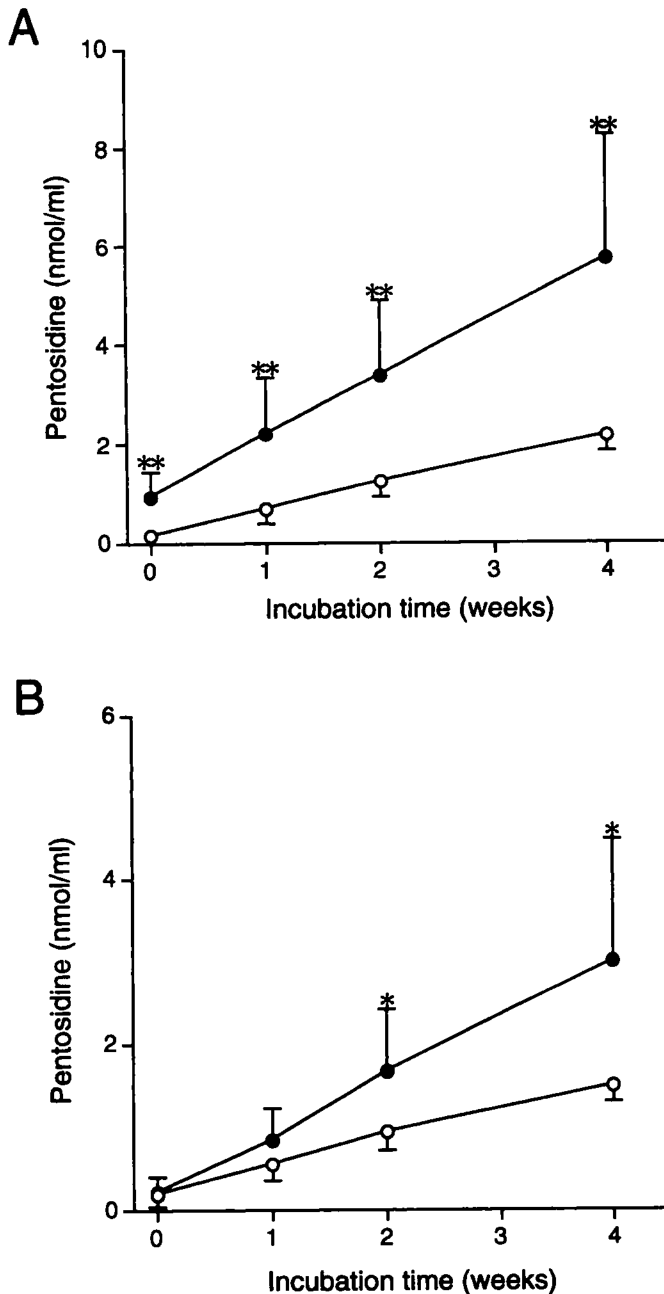

Figure 1. (A) Generation of pentosidine upon incubation of plasma from healthy subjects and hemodialysis patients. Plasma samples were obtained (n = 5) from healthy subjects (○) and nondiabetic hemodialysis patients (●). $^{**}P < 0.01$, compared with normal plasma. (B) Generation of pentosidine upon incubation of normal and uremic ultrafiltrated plasma (<5000 Da) fortified with human serum albumin (HSA). Plasma samples (n = 5) from healthy subjects (○) and nondiabetic hemodialysis patients (●) were ultrafiltrated twice through a filter with a 5000-Da cutoff value and fortified with HSA (30 mg/ml). $^{*}P < 0.05$, compared with normal plasma. Plasma samples and ultrafiltrates were incubated at 37°C under air. Pentosidine levels were determined at intervals by HPLC assay. Data are expressed as means ± SD.

trate ($P < 0.05$). However, the yield of pentosidine was lower in plasma ultrafiltrate than in whole plasma both in uremic (0.099 *versus* 0.172 nmol/ml per d, $P < 0.05$) and in healthy subjects (0.064 *versus* 0.072 nmol/ml per d, $P < 0.05$). This indicates that in uremia, at least 60% of the pentosidine generated *in vitro* originates from substances with a molecular weight <5000 Da.

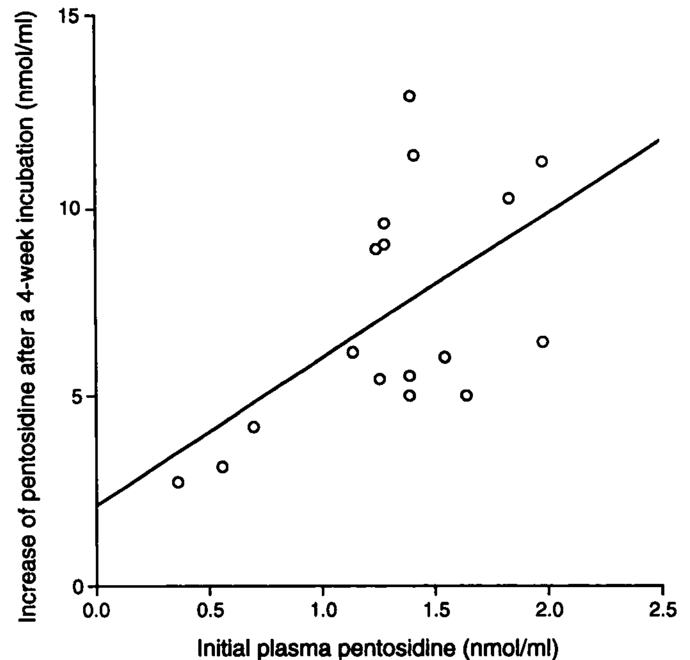

Figure 2. Correlation between the initial level of pentosidine and the increase of pentosidine after a 4-wk incubation of uremic plasma. Pentosidine generation after a 4-wk incubation is linearly related to the initial level of pentosidine (n = 17, r = 0.564, $P < 0.05$). The equation of the line is $y = 3.863x + 2.152$.

Effect of Hemodialysis

Pentosidine yield during incubation was compared in pre- and postdialysis plasma samples from patients given cellulose dialysis. Pentosidine yields fell significantly ($P < 0.05$) during dialysis (0.223 and 0.153 nmol/ml per d in pre- and postdialysis samples, respectively) (Figure 3A). The difference of pentosidine yield between pre- and postdialysis samples was still observed after ultrafiltration and addition of HSA ($P < 0.05$) (0.074 and 0.041 nmol/ml per d in pre- and postdialysis samples, respectively) (Figure 3B). Pentosidine yields in the plasma were also measured at different time intervals in three patients treated with a highly porous polyacrylonitrile (AN69) membrane. It fell from 0.509 nmol/ml per d predialysis to 0.281 nmol/ml per d after 2 h and remained stable after 4 h (0.302 nmol/ml per d) (Figure 4).

Inhibition by Aminoguanidine and OPB-9195

Both aminoguanidine and OPB-9195 are inhibitors of the Maillard reaction. Incubation of BSA (pentosidine level below detection limit) for 4 wk with either glucose or ascorbate yielded pentosidine (31 and 218 pmol/mg of protein, respectively) (Table 1). Since pentosidine is formed through carbonyl amine chemistry between protein amino and guanidino groups and glucose- or ascorbate-derived carbonyl groups (22,23),

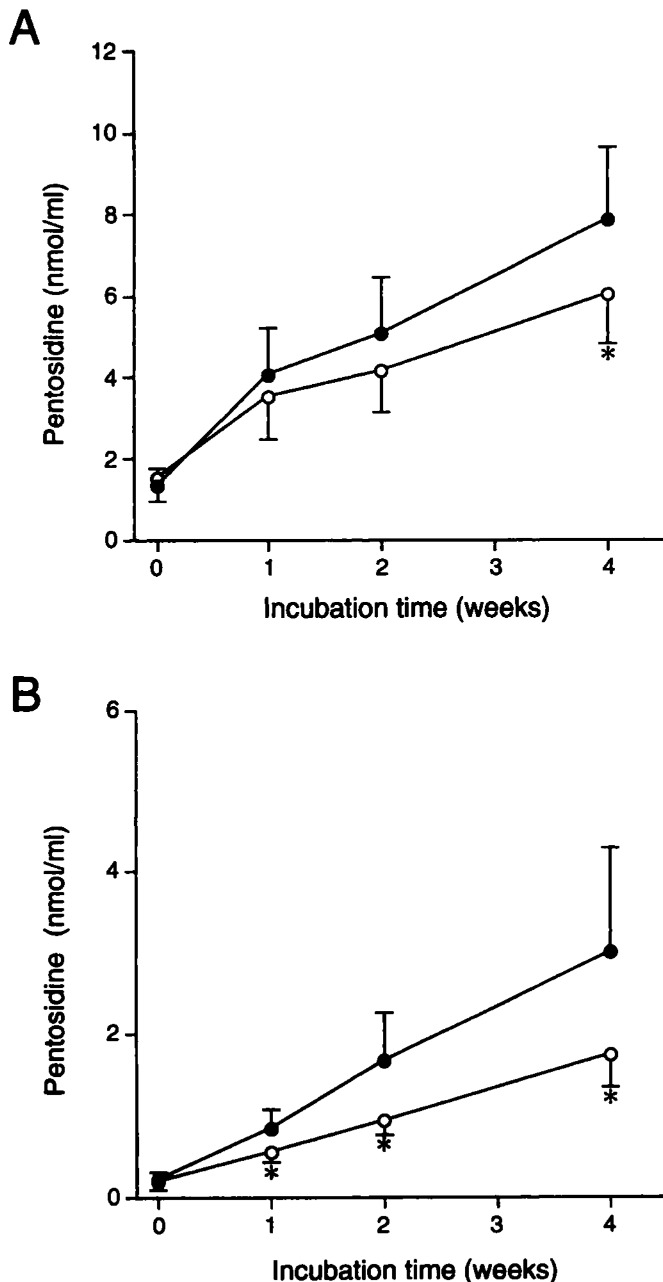

Figure 3. (A) Generation of pentosidine upon incubation of pre- and postcellulose dialysis plasma. Plasma samples were obtained before (●) and after (○) dialysis on a cellulose membrane (n = 15). *P < 0.05, compared with predialysis plasma. (B) Generation of pentosidine upon incubation of ultrafiltrated pre- and postcellulose dialysis plasma (<5000 Da) fortified with HSA. Pre- (●) and post- (○) dialysis plasma samples (n = 5) were ultrafiltrated twice through a filter with a 5000-Da cutoff value and fortified with HSA (30 mg/ml).*P < 0.05, compared with predialysis plasma. Plasma samples and ultrafiltrates were incubated at 37°C under air. Pentosidine levels were determined at intervals by HPLC assay. Data are expressed as means ± SD.

these results confirm that the pentosidine yield measured in this assay is derived largely from glucose- or ascorbate-derived carbonyl groups. Addition of increasing amounts of aminoguanidine or OPB-9195, both inhibitors of Maillard reaction, inhibited pentosidine generation during incubation of BSA. Half-maximal inhibition of glucose-derived pentosidine formation was obtained with approximately 25 mM aminoguanidine and approximately 3.7 mM OPB-9195, and that of ascorbate-derived pentosidine formation at approximately 1.9 mM aminoguanidine and approximately 0.45 mM OPB-9195. In both instances, OPB-9195 appeared somewhat more effective than aminoguanidine.

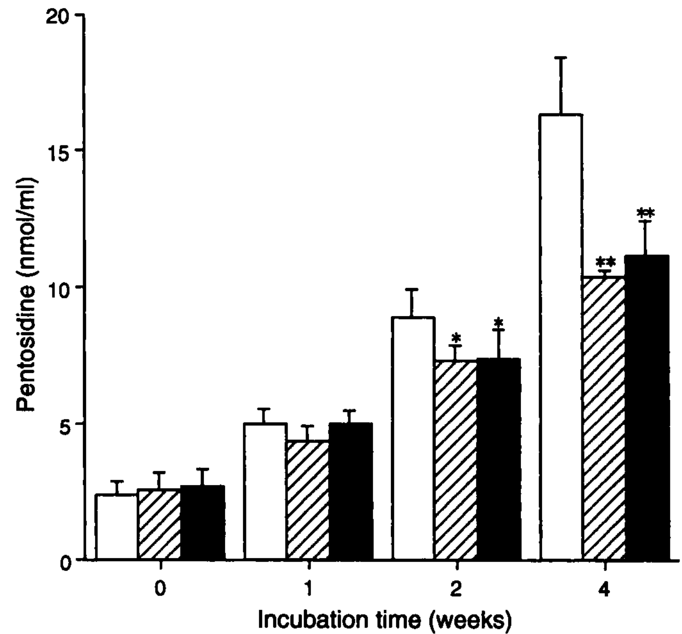

Figure 4. Generation of pentosidine upon incubation of pre-, during-, and postpolyacrylonitrile (AN69) dialysis plasma. Pre- (□), 2-h (▨), and 4-h (■) plasma samples (n = 3) were incubated at 37°C under air, followed by determination of pentosidine levels at intervals by HPLC assay. Data are expressed as means ± SD. *P < 0.05 and **P < 0.01, compared with predialysis plasma.

Addition of aminoguanidine or OPB-9195 also inhibited pentosidine formation during incubation of normal and uremic plasma (Table 2). Half-maximal inhibition of pentosidine formation was obtained with a concentration of ~7.5 mM and ~21 mM aminoguanidine and ~1.0 mM and ~2.8 mM OPB-9195 in both normal and uremic plasma, respectively. After an 8-wk incubation, pentosidine yield was similar in uremic plasma with added aminoguanidine or OPB-9195 (4.95 and 3.50 nmol/ml, respectively) and in normal plasma without inhibitors (5.78 nmol/ml).

Plasma Levels of Carbonyl Compounds in Uremic and Normal Plasma

The reaction of DNPH with carbonyl compounds yields stable DNP adducts (hydrazone) (20) that can be measured by a spectrophotometric assay (21).The level of total carbonyl compounds measured by this method in plasma ultrafiltrates is markedly higher in predialysis (n = 22) than in control plasma (n = 11) (18.04 ± 4.26 *versus* 7.76 ± 1.30 nmol/ml, P < 0.001). After subtraction of the glucose contribution (see Ma-

Table 1. Inhibition of glucose- or ascorbate-derived pentosidine formation by aminoguanidine or OPB-9195[a]

Concentration of Inhibitor (mM)	Pentosidine Level (pmol/mg of protein)			
	Glucose		Ascorbate	
	Aminoguanidine	OPB-9195	Aminoguanidine	OPB-9195
0	30.7 ± 4.5	30.7 ± 4.5	218.3 ± 26.7	218.3 ± 26.7
1	27.7 ± 4.9	29.0 ± 5.1	140.3 ± 32.0[b]	64.3 ± 6.7[c]
10	17.5 ± 2.0[b]	11.3 ± 2.7[c]	52.7 ± 13.3[c]	15.0 ± 3.5[c]
100	2.3 ± 1.2[c]	8.0 ± 1.0[c]	6.7 ± 2.5[c]	13.3 ± 3.0[c]

[a] Bovine serum albumin was incubated at 37°C for 4 wk with either 100 mM glucose or 20 mM ascorbate in 0.1 M phosphate buffer, pH 7.4, in the presence of several concentrations of either aminoguanidine or OPB-9195 (n = 3). Data are expressed as means ± SD.
[b] $P < 0.01$, compared with the absence of inhibitors.
[c] $P < 0.001$, compared with the absence of inhibitors.

Table 2. Inhibition of pentosidine formation upon incubation of normal and uremic plasma by aminoguanidine or OPB-9195[a]

Concentration of Inhibitor (mM)	Pentosidine Level (nmol/ml)			
	Healthy Subjects		Hemodialysis Patients	
	Aminoguanidine	OPB-9195	Aminoguanidine	OPB-9195
0	5.78 ± 0.69	5.78 ± 0.69	17.77 ± 2.55	17.77 ± 2.55
1	4.51 ± 0.56	2.88 ± 0.48[c]	17.15 ± 5.37	10.22 ± 2.61[b]
10	2.21 ± 0.37[c]	0.43 ± 0.13[c]	10.15 ± 2.46[b]	3.80 ± 1.03[c]
50	0.70 ± 0.12[c]	0.37 ± 0.08[c]	4.95 ± 1.47[c]	3.50 ± 0.90[c]

[a] Normal or uremic plasma samples (n = 3) were incubated at 37°C for 8 wk in the presence of several concentrations of either aminoguanidine or OPB-9195. Data are expressed as means ± SD.
[b] $P < 0.05$, compared with the absence of inhibitors.
[c] $P < 0.01$, compared with the absence of inhibitors.

terials and Methods), calculated carbonyl compound level decreases averaged 14.28 ± 4.24 and 3.60 ± 1.35 nmol/ml, respectively ($P < 0.001$). Hemodialysis with a cellulose membrane reduced the total carbonyl compound level from 18.04 ± 4.26 to 9.18 ± 2.19 nmol/ml ($P < 0.001$). Simultaneously, however, the glucose-rich dialysate raised serum glucose from 95.5 ± 16.1 predialysis to 125.8 ± 22.8 mg/dl postdialysis, thus obscuring the effect of dialysis on carbonyl level. After subtraction of the glucose contribution, calculated carbonyl compound level fell from 14.28 ± 4.24 to 4.28 ± 1.92 nmol/ml ($P < 0.001$). This 70% dialysis-induced reduction is larger than the 31% decrease observed with the pentosidine yield approach, which failed to take into account the specific contribution of changes in serum glucose.

Role of 3-Deoxyglucosone as a Precursor of Pentosidine

3-Deoxyglucosone is a glucose-derived compound with two carbonyl groups, which accumulates in uremic plasma (24). To test whether 3-deoxyglucosone stimulates pentosidine formation in uremia, we fortified normal plasma with various concentrations of 3-deoxyglucosone and incubated it for 4 wk (Table 3). Interestingly, 3-deoxyglucosone, even at concentrations 10 times above that measured in uremic plasma, failed to augment the pentosidine yield. Thus, the carbonyl compounds involved in pentosidine formation in uremia are different from 3-deoxyglucosone.

Table 3. Effect of increasing amounts of 3-deoxyglucosone on pentosidine level after a 4-wk incubation of normal plasma[a]

3-Deoxyglucosone (nmol/ml)	Pentosidine Level (nmol/ml)
0	2.25 ± 0.280
5	2.64 ± 0.647[b]
10	2.29 ± 0.353[b]
20	2.06 ± 0.304[b]
100	2.50 ± 0.700[b]

[a] Plasma samples (n = 5) from healthy subjects were fortified with several concentrations of 3-deoxyglucosone and incubated at 37°C for 4 wk under air. The pentosidine levels were determined by HPLC assay. Data are expressed as means ± SD.
[b] Not significant *versus* in the absence of 3-deoxyglucosone. Note that the plasma concentration of 3-deoxyglucosone in patients on hemodialysis is approximately 10 nmol/ml (24).

Discussion

Identification of the mechanisms involved in AGE genesis in uremia is a prerequisite for the delineation of therapeutic strategies. In this study, we provide evidence for the accumulation in uremic plasma of precursors of a well defined advanced glycation end product, pentosidine. We had previously suggested that, in the absence of hyperglycemia, uremic serum probably contained AGE precursors (7,25). We now document the accumulation of such precursors since incubation of uremic plasma under air yields more than twice as much pentosidine as normal plasma.

Incubation of plasma ultrafiltrated through a 5000-Da cutoff filter discloses that a large fraction of the precursor molecules have a molecular weight below 5000 Da. The ultrafiltrate keeps 90% of its original pentosidine formation potential when it originates from normal plasma and 60% when it is derived from uremic samples. The significant proportion of low molecular weight precursors present in uremic plasma is further demonstrated by the effect of dialysis: During a session, the pentosidine yield upon plasma incubation falls by 31% with a low-flux cellulose membrane and 45% with a high-flux polyacrylonitrile membrane. These observations probably underestimate the fall in pentosidine precursors during dialysis since plasma level of glucose, another precursor, may rise at the end of dialysis session with a glucose-containing dialysate.

Of interest, the pentosidine yield of incubated plasma was lowered after 2 h of dialysis on high-flux AN69 and remained stable during the subsequent 2 h. In this study, changes in glucose levels were negligible because a glucose-free dialysate was used. Such a pattern is reminiscent of that observed either for compounds largely removed by membrane adsorption or for substances with a delayed mobilization from an extravascular pool such as phosphate (26). Whatever the explanation, it is clear that pentosidine precursors are removed from uremic plasma by hemodialysis just as other uremic toxins.

The close correlation observed between the pentosidine yield during incubation and the preincubation level of pentosidine suggests that the latter is determined mainly by the amount of precursors accumulated in the plasma. Pentosidine levels might thus be taken as a marker of the plasma level of precursor molecules.

The nature of the precursors molecules remains to be identified. It is known from *in vitro* studies that pentosidine is derived mainly from carbonyl precursors originating either from glucose or from ascorbate (22,23). These compounds generate Schiff base adducts and subsequently Amadori products eventually yielding AGE, including pentosidine. In the present study, we confirm the generation of pentosidine during incubation of BSA with either 100 mM glucose or 20 mM ascorbate and demonstrate its inhibition by aminoguanidine or OPB-9195. Aminoguanidine as well as OPB-9195 inhibit glycoxidation (17). Both contain a hydrazine nitrogen atom that reacts with carbonyl groups, directly or via the base upon hydrolysis, to form hydrazone (unpublished observations). Their ability to lower pentosidine formation in this model supports the role of carbonyl precursors in the generation of pentosidine.

Of even greater significance is our observation that both aminoguanidine and OPB-9195 also inhibit pentosidine formation during the incubation of uremic and normal plasma. Even at high concentrations, neither compound returns pentosidine concentration to values observed at equivalent concentrations in nonuremic plasma, a finding suggesting that, at such high substrate levels, inhibition is not complete as might be expected from a competitive antagonist. Still, it is noteworthy that both aminoguanidine and its somewhat more effective analogue, OPB-9195, return pentosidine generation to that observed in nonuremic plasma without inhibitor. Altogether, these results strongly suggest that the pentosidine precursors accumulated in uremic plasma are reactive carbonyl compounds.

Whether the present results based on pentosidine generation apply to all AGE compounds remains to be discussed. Very recently, Hou *et al.* reported that aminoguanidine inhibits AGE formation on β2-microglobulin incubated *in vitro* with 50 or 100 mM glucose (27). Although the generated AGE compound was not identified in this study, these results are well in line with the view that reactive carbonyl compounds play a critical role in AGE formation. Our demonstration of a similar effect in uremic whole plasma, without any added carbonyl precursors such as glucose, supports the notion that AGE accumulation in uremia results from the retention of reactive carbonyl compounds.

Direct evidence for the accumulation of reactive carbonyl compounds in uremic plasma accrues from our observation that ultrafiltered plasma exposed to DNPH yields twice as much hydrazones in uremic than in control plasma: DNPH characteristically reacts with carbonyl compounds to yield hydrazones, *i.e.*, DNP adducts (20). Removal of carbonyl precursors by hemodialysis is confirmed by our observation that, after correction for changes in serum glucose levels, the level of reactive carbonyls is markedly reduced by hemodialysis.

The present study has not identified the various reactive carbonyls accumulating in uremic plasma. Their nature remains to be elucidated. As mentioned previously, it cannot be glucose or ascorbate, whose levels are known to be either normal or even decreased in uremic plasma (25). 3-Deoxyglucosone, a compound generated in the Maillard reaction, might be a better candidate as it accumulates in uremic plasma (24). This does not seem to be the case because its addition to normal plasma, even at concentrations higher than those observed in uremic patients, fails to raise the pentosidine yield.

The reason for the elevation of the carbonyl compounds in uremic serum remains to be elucidated. It might be due to the retention of metabolites by the failing kidney due either to a defective catabolism through glomerular filtration and subsequent tubular reabsorption as suggested for pentosidine itself (7,28) or to an impaired removal through urinary excretion. Alternatively, the possibility should be considered that uremia is associated with an imbalance between oxidant and antioxidant systems characterized by increased lipid, ascorbate, and protein oxidation (25,29,30) and identified as oxidative stress.

The latter might augment the generation of carbonyl compounds just as it seems to do for pentosidine generation from ascorbate (22,23,25).

As stated earlier, various sources of carbonyl compounds might contribute to the generation of different AGE structures. Carbohydrate- and ascorbate-derived carbonyls are known to yield not only pentosidine but also carboxymethyllysine (31). Similarly, carbonyls derived from unsaturated fatty acids such as arachidonic acid may yield carboxymethyllysine (32) as well as other protein adducts, advanced lipoxidation end products, such as 4-hydroxynonenal, and malondialdehyde (33). The accumulation in uremic plasma of reactive carbonyl compounds derived not only from carbohydrates but also from lipids might be termed "carbonyl stress." We suggest that it has potentially damaging consequences for proteins due not only to AGE but also to advanced lipoxidation end products, manifested clinically by complications ranging from dialysis-related amyloidosis to atherosclerosis.

Acknowledgments

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