Structure and Mechanism of Formation of Human Lens Fluorophore LM-1

RELATIONSHIP TO VESPERLYSINE A AND THE ADVANCED MAILLARD REACTION IN AGING, DIABETES, AND CATARACTOGENESIS*

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Human lens crystallins become progressively yellow-brown pigmented with age. Both fluorescent and non-fluorescent protein adducts and cross-links are formed, many of which result from the advanced Maillard reaction. One of them, LM-1, is a blue fluorophore that was earlier tentatively identified as a cross-link involving lysine residues (1). A two-step chromatographic system was used to unequivocally identify and quantitatively prepare a synthetic fluorescent cross-link with lysine residues that had identical UV, fluorescent, and chromatographic properties with both acetylated and non-acetylated LM-1. Proton, 13C NMR, and molecular mass of the synthetic compound were identical with vespertilysine A, a fluorescent cross-link discovered by Nakamura et al. (2). The fragmentation patterns of vespertilysine A and LM-1 were identical as determined by NMR/mass spectrometry. Lenticular levels of vespertilysine A increase curvilinearly with age and reach 20 pmol/mg at 90 years. Levels correlate with degree of lens crystallin pigmentation and fluorescence and are increased in diabetics, in contrast to Nε-(carboxymethyl)lysine and pentosidine. Ascorbate, D-pentoses, and D-threose, but neither D-glucose under oxidative conditions, Dl-glyceraldehyde, methylglyoxal, glyoxal, nor glycolaldehyde, are precursors. However, addition of C-2 compounds greatly catalyzes vespertilysine A formation from ribose. Thus, vespertilysine A/LM-1 is a novel product of the advanced Maillard reaction in vivo and a specific marker of a diabetic process in the lens that is different from glycoxidation.

Further studies showed a LM-1-like fluorescent compound could be synthesized from bovine serum albumin reacted with ascorbic acid or its oxidation products, or from ribose, but not glucose or fructose (1). Considerable efforts in our laboratory were devoted toward obtaining sufficient quantities of LM-1 for structural identification. Unpublished data were suggestive of one or two lysine residues linked to a heterocyclic ring. However, further studies were hampered by our unexpected inability to prepare the compound from l-lysine and D-ribose and its presence in very low concentrations in the lens. In a more recent collaborative study with Graham and Nagaraj (3), LM-1 was found to co-chromatograph, by RP-HPLC† and TLC, with a fluorescent cross-link synthesized from l-lysine and D-ribose that had been originally identified as pentodilysine in the “cross-linking region” of the chromatogram obtained with diphenylboronic acid derivatives. However, despite extensive spectroscopic data, unequivocal structural identification of pentodilysine was not achieved.

The enormous efforts that had already gone into our attempts to elucidate the structure of LM-1 combined with the unknown intrinsic value of LM-1 as a marker of the advanced Maillard reaction in vivo would have made us abandon the project. However, when LM-1 was quantitated in lenses from dogs that had been diabetic for 5 years (4), the surprising observation was made that LM-1 was elevated in dogs with moderate glycemic control, whereas pentosidine, a glycoxidation product, was elevated in only in lenses from poorly controlled animals. This suggested that LM-1 was a unique marker for a diabetic process reflecting mild hyperglycemia.

This promising ability of LM-1 to reflect mild hyperglycemia in the lens, together with the availability of newer LC/MS instruments, such as the LCQ ion-trap instrument, led us to pursue the project further. We present below our approach and data that have led us to conclude that LM-1 is identical with vespertilysine A, a recently discovered lysine cross-link of the advanced Maillard reaction (2). Its mechanism of formation and relevance to the biochemistry of the aging lens in comparison to glycation and other advanced glycation products are described.

EXPERIMENTAL PROCEDURES

Materials—All reagents were of the highest grade available. Poly-l-lysine HBr (Mn = 30,000–70,000), Nε-acetyl-l-lysine, l-lysine, ribose, glucose, ascorbic acid, arabinose, xylose, threose, glycolaldehyde, and glycolaldehyde were obtained from Sigma. Glyoxal and methylglyoxal were purchased from Fluka (St Louis, MO). Human lenses were ob-

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† The abbreviations and trivial names used are: RP-HPLC, reverse phase-high performance liquid chromatography; CML, Nε-carboxymethyllysine; HPLC, high performance liquid chromatography; MS, mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; WI, water-insoluble; WS, water-soluble.
tained from the National Disease Research Interchange (Philadelphia, PA), classified into type I–IV and bruneus based on pigmentation using Pirie classification (5). Dr. R. H. Nagaraj also kindly provided bruneus lenses. Lenses were stored at −70 °C until used.

**Reaction of Ribose with Poly-L-lysine or N^{\text{\text{\text{\textalpha}}}}-Acetyl-L-lysine or L-Lysine.—** The fluorphore from D-ribose (150 m M) and poly-L-lysine (0.02 mg/ml) or N^{\text{\text{\text{\textalpha}}}}-acetyl-L-lysine (60 g/liter) or L-lysine (60 g/liter) were dissolved in 200 ml sodium phosphate buffer, pH 7.4, filtered through 0.2-μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI), and incubated for 93 h at 37 °C for the poly-L-lysine or for 48 h at 50 °C for the amino acids. The poly-L-lysine/ribose mixture was dialyzed against 4 × 4000 ml of water for 48 h, using 5000 MW cut-off membrane tubing with a molecular weight cut-off of 12,000–14,000 (Spectrum Medical Industries, Los Angeles, CA). The diazylated poly-L-lysine and the N^{\text{\text{\text{\textalpha}}}}-acetyl-L-lysine were hydrolyzed with 6 N HCl at 100 °C for 18 h, at a concentration of 5 mg of poly-L-lysine or 30 mg of N^{\text{\text{\text{\textalpha}}}}-acetyl-L-lysine/ml of HCl.

**Isolation of a LM-1-like Fluorphore from the Reaction Mixtures.—** The fluorphore with excitation and emission maxima at 370−440 nm was isolated using a combination of reverse phase and ion exchange chromatography. The poly-L-lysine acid hydrolysate was evaporated in a Speed Vac concentrator (Savant Instruments, Hicksville, NY), and 63 mg was dissolved in 8 ml of 0.2 M pyridine formate (pH 3.25) and loaded onto a cation exchange column (3 cm × 15 cm, Dowex 50WX8–400). Fractions of 5 ml were collected at a flow rate of 1 ml/min. Fractions 1–40 were discarded before the column was equilibrated with 0.2 M pyridine formate (pH 3.25), fractions 41–80 were collected with the same solvent system as before. After analysis by HPLC, the fractions that contained the fluorescent component were pooled, evaporated, and reconstituted in water. The eluent from the column was monitored with an on-line fluorescence detector (Waters 470 scanning fluorescence detector) at 370 and 440 nm for the excitation and the emission, respectively. The chromatograms were recorded with a chromatography software (Borwin, JMBS Developpements, Le Fontainil, France). Fractions 1–120, which showed a major fluorescent peak similar to LM-1, were evaporated in a Speed-Vac concentrator and reconstituted in water.

The N^{\text{\text{\text{\textalpha}}}}-acetyl-L-lysine hydrolysate was centrifuged at 1000 × g for 10 min to remove a black pellet resulting from the acid hydrolysis. The HCl was evaporated using a Rotavapor. Methanol was used to wash the dry pellet. The pellet was dissolved in 600 ml of 0.4 M pyridine formate (pH 5.25) and loaded onto a cation exchange column (10 × 15 cm, Dowex 50WX8–400). The column was eluted at a flow rate of 0.7 ml/min with 1% acetic acid for 20 min, and washed with an increasing gradient of acetonitrile (0–45%) for 40 min. The column eluent was monitored with an on-line fluorescence detector (Waters 470 scanning fluorescence detector) at 370 and 440 nm for the excitation and the emission, respectively. The chromatograms were recorded with a chromatography software (Borwin, JMBS Developpements, Le Fontainil, France). Fractions 1–120, which showed a major fluorescent peak similar to LM-1, were evaporated in a Speed-Vac concentrator and reconstituted in water.

**HPLC Purification.—** The fluorphore was purified by HPLC with three different columns. The samples were first injected into a C-18 reverse-phase semi-preparative column (Vydac 218TP101, 10 mm × 25 cm, 10 μm; The Separation Groups, Hesperia, CA). A Waters HPLC instrument (Waters Chromatography Div., Milford, MA) with model 510 pumps, automatic injector (model 712 WISP), and a model 680 controller were used. The column was eluted at a flow rate of 0.7 ml/min with 1% acetic acid for 20 min, and washed with an increasing gradient of acetonitrile (0–45%) for 40 min. The column eluent was monitored with an on-line fluorescence detector (Waters 470 scanning fluorescence detector) at 370 and 440 nm for the excitation and the emission, respectively. The chromatograms were recorded with a chromatography software (Borwin, JMBS Developpments, Le Fontainil, France). Fractions 1–120, which showed a major fluorescent peak similar to LM-1, were evaporated in a Speed-Vac concentrator and reconstituted in water.

**RESULTS**

Isolation of Fluorescent Material from D-Ribose and Poly-L-lysine or N^{\text{\text{\text{\textalpha}}}}-Acetyl-L-lysine.—** Based on previous experiments suggesting involvement of L-lysine and d-ribose in LM-1 formation (3), we incubated poly-

**NMR—** The samples were dissolved and evaporated three times in 100% deuterium oxide (pH 7.0). The samples containing 1.7 mg/ml synthetic fluorophores and 0.02 mg/ml LM-1 were transferred to a 5-mm NMR tube. 1H NMR, 13C NMR, and heterocorrelation spectra were run at 25 °C with a 600-MHz Varian VXR spectrometer.

**Mass Spectrometry—** Mass spectrometry was performed by the LCQ ion-trap instrument available at the Veterans Administration Medical Center mass spectrometry facility (Dr. C. Hoppel). Samples were dissolved in 0.5% acetic acid with 50% methanol for direct infusion into the instrument.

**Spectroscopy—** Fluorescence spectra were recorded with a SLM-Amino spectrofluorometer (model 8100; Amino-Bowman, Rochester, NY). Absorption spectra were obtained with a Hewlett-Packard 8452A diode array spectrophotometer (Hewlett-Packard, Inc., Avondale, PA) connected to an IBM PC-AT computer (IBM Corp., Boca Raton, FL).

**In Vitro Incubation of Poly-L-lysine with Sugars, Oxoaldehydes, or Ascorbic Acid—** Sugars (glyceraldehyde, threose, xylose, ribose, arabinose or glucose), oxoaldehydes (glyoxal or methylglyoxal), or ascorbic acid were incubated with poly-L-lysine in 200 mM sodium phosphate buffer (pH 7.4). After 7 days at 37 °C, the samples were dialyzed against 2 × 4000 ml of water for 48 h, using Spectra/Por membrane tubing with a molecular weight cut-off of 3500–10,000. After dialysis, the samples containing 1.7 mg/ml synthetic fluorophores and 0.02 mg/ml LM-1 were transferred to a 5-mm NMR tube. 1H NMR, 13C NMR, and heterocorrelation spectra were run at 25 °C with a 600-MHz Varian VXR spectrometer.

**Kinetic Studies on LM-1/Vesprelysine A Formation from Poly-L-lysine Incubated with d-Ribose with Increasing Concentrations of Oxoaldehydes, Glyceraldehyde, or Glycolaldehyde—** Incubations of poly-L-lysine with ribose were carried out in 200 mM phosphate buffer, pH 7.4, with 0, 1, 5, and 10 mM glyoxal, methylglyoxal, glyceraldehyde, or glycolaldehyde. After 1, 2, and 4 days at 37 °C, samples were analyzed for the concentration of vesprelysine A.
L-lysine with D-ribose for 93 h and acid-hydrolyzed the reaction products. The mixture was passed over Dowex 50W, which was eluted with three different pyridine formate buffers. A major fluorescent material at 370/440 nm was eluted by HPLC reverse phase chromatography on C-18 column at 9–11 min (Fig. 1). This material was found to elute from the Dowex column between fractions 100 and 120 (data not shown). A similar fluorescent peak was obtained from Na-acetyl-L-lysine and D-ribose (Fig. 1, inset). In subsequent work, all studies were performed with the material prepared from Na-acetyl-L-lysine.

The fluorescent peak isolated from Na-acetyl-L-lysine was rechromatographed on a smaller Dowex column and then sequentially chromatographed with semipreparative C-18 HPLC column, cation exchange column, and C-18 analytical column. Detection was carried out both at 370/440 nm for fluorescence and UV at 220 nm. From 15 g of Na-acetyl-L-lysine hydrochloride, 500 mg of chromatographically pure fluorescent compound were obtained (yield 0.0033%).

The chromatographic properties of the pure compound were compared with LM-1 from human lenses, isolated by a combination of C-18 as described by Nagaraj and Monnier (1) and cation exchange chromatography. Co-chromatography between the synthetic and the native fluorophore was observed in both systems (Fig. 2, A and B).

**Structural Characteristics of the Synthetic Fluorophore**

The synthetic compound was analyzed by proton, 13C NMR, and mass spectrometry. The proton NMR (Fig. 3A) showed four single aromatic protons (α–d) at 8.31 (s), 8.12 (s), 7.97 (d), and 6.9 (d) ppm, two e-carbon linked protons at 4.76 (t) and 4.41 (t) ppm (e and e′), two overlapping α-carbon-linked protons at 3.80 ppm (α and α′), and the aliphatic lysyl protons at 1.3–2.3 ppm. The 13C spectrum showed 2 peaks between 170 and 180 ppm (attributed to two carboxylic groups), 7 peaks (2 of which are superimposed) between 90 and 160 ppm (from the aromatic ring), 1 water side band (at 40.4 ppm), and 10 peaks (including 4 × 2 superimposed peaks) between 20 and 60 ppm (from two aliphatic chains) (Fig. 3B). Taken together, these data suggested that the isolated compound was identical with vespelysine A (Fig. 4) described by Nakamura (6). This was confirmed by both heterocorrelation analysis (Fig. 3C), which showed which protons are linked to which carbon, and ES mass spectrometry using ion-trap methodology (LQ), which revealed m/z at 393.4 with MS/MS Fragments at 376 (loss of OH), 348 (loss of 1 COOH), 264 (loss of 1 norleucine), and 219 (the 264 fragment with additional loss of 1 COOH) (Fig. 5, A and B). When fragment 264 was analyzed by MS/MS/MS, a new fragment at m/z 135 attributed to the aromatic ring of vespelysine A was obtained (data not shown).

**Comparison of Synthetic Vesperlysine A with Native Fluorophore LM-1**

For comparative studies, LM-1 was isolated through repetitive HPLC chromatography essentially as described for vespelysine A. From a pool of 22 lenses (Pirie pigmentation stage II–IV and brunescent; age range: 44–86 years), 2.6 and 4.6 mg (based on the extinction coefficient for vespelysine A, see below) were obtained from the water-soluble and water-insoluble fractions, respectively. Fluorescence at various pHs and UV spectra are shown in Fig. 6. The fluorescence spectra of syn-
Detection of Vesperlysine A in the Human Lens—Human lenses (age: 16–86 years; n = 46) were separated into WS and WI fractions and analyzed by a two-step HPLC system using fluorescence at 440 nm (excitation at 370 nm). An age-related increase was noted in both water-soluble and water-insoluble fractions (Fig. 7A). A shallow increase of the regression line ($r^2 = 0.32, p < 0.05$) was noted in the water-soluble fraction, which reached only 2.5 pmol/mg of protein at 90 years (individual data points not shown). In contrast, a sharp curvilinear increase ($r^2 = 0.85, p < 0.001$) was noted in the insoluble fraction, which reached 20 pmol/mg at 90 years, i.e. 6–8 fold higher than in the soluble fraction. Moreover, most of the values from diabetic lenses were increased, including in those the WS fraction in which the highest diabetic value reached 10 pmol/mg (data not shown). 

Comparison of Vesperlysine A with Other Markers of the Maillard Reaction—A comparative analysis of Maillard reaction markers was carried out in the water-insoluble fractions from 46 lenses for vesperlysine A (Fig. 7A) and a subgroup of 36 lenses for the markers glycated lysine (furosine) (Fig. 7B), pentosidine (Fig. 7C), and CML (Fig. 7D). It is readily apparent that, with age, a curvilinear increase is observed for CML, pentosidine, and vesperlysine A, in contrast to furosine, the concentration of which remains unchanged, as previously reported by Patrick (7). Furthermore, only vesperlysine A and furosine are elevated beyond normal values in diabetic lenses. Finally, CML stands out as the major modification, followed by furosine, vesperlysine A, and pentosidine, the levels of which are 6, 150, and 500 times lower than CML at the end of life span, respectively.

Further correlation was sought with the degree of pigmentation of the lenses using the classification of Pirie (5) (Fig. 8). A strong relationship was noted with lens color for all advanced Maillard products, except for vesperlysine A, the concentration of which is decreased in the highly pigmented, so-called brunescent lenses. Differences between diabetic and non-diabetic lenses were significant for furosine in all lens types ($p < 0.001$), and for vesperlysine A in type III and IV lenses ($p < 0.001$).

Correlation with Total Lenticular Fluorescence—The markers described above were correlated with lens crystallin fluorescence at the emission/excitation maxima of insoluble native crystallins, which were detected at 310/430 nm. As shown in Table 1, all markers were significantly correlated with total fluorescence except furosine. Interestingly, removing the values for the diabetic lenses ameliorated the correlation coefficients for all advanced glycation end products (AGEs), and removing the brunescent lenses ameliorated the correlation coefficient of vesperlysine A and pentosidinie. Most impressively, CML had the highest correlation coefficient in normal subjects, suggesting that it is an excellent surrogate marker for the process responsible for total fluorescence at 310/430 nm. We determined that vesperlysine A accounted for at most 5% of the total fluorescence at 370/440 nm in the water-insoluble proteins of old lens crystallins.

Mechanism of Formation of Vesperlysine A—Studies on the
mechanism of formation of vesperlysine A were performed. Poly-L-lysine (9 g/liter) was incubated in 200 mM phosphate buffer with 50 mM carbonyl compound (D-glucose, D-ribose, D-arabinose, D-threose, DL-glyceraldehyde, methylglyoxal, glyoxal, and ascorbic acid) for 7 days at 37 °C. The results (Table II) show that vesperlysine A could be efficiently synthesized from ribose, threose, and ascorbate, but not from glucose, glyoxal, methylglyoxal, and glyceraldehyde. Arabinose and xylose were also precursors.

Because the heterocyclic ring of vesperlysine A requires the participation of 7 carbon atoms, we hypothesized addition of C-2 and C-3 carbonyl compounds should have a catalytic effect on vesperlysine A formation from C-5 sugars. A dramatic, dose-dependent increase in vesperlysine A was noted with
glyoxal and glycolaldehyde (Fig. 9, A and B). Although some effect was noted with di-glyceraldehyde (Fig. 9D), methylglyoxal had not catalytic effect (Fig. 9C, see value of the y scale). We tentatively attribute the very small increase observed to the presence of potential contaminant(s) reported present in commercial batch of methylglyoxal (8).

In light of the fact that glyoxal and arabinose are autoxidation products of glucose (9), repeated attempts were made to obtain vesperlysine A from glucose under aerobic conditions and high phosphate buffer (200 mM) to enhance glucose autooxidation (9). Surprisingly, none succeeded.

Vesperlysine A in Other Tissues—Vesperlysine A was quantitated in the collagen-rich acid-insoluble fraction from heart, kidney, lung, and skin, as well as in the trichloroacetic acid-precipitated fraction from 4 healthy subjects, 4 diabetic, and 4 uremic subjects. Levels were 0.86, 3.5 ± 0.58, and 5.33 ± 0.66 pmol/mg of protein, respectively.

DISCUSSION

A chromatographic peculiarity appears to underlie former failures to synthesize LM-1 from N\(^{-}\)acetyl-L-lysine and D-ribose, in that no fluorescent peak was observed at the expected retention time for LM-1 using the C-18 reverse phase column.\(^2\)

This led to the erroneous conclusion LM-1 could not possibly be a lysine dimer. Similar observations were made in the initial phase of the current study. Subsequently, we found the retention time of vesperlysine A was significantly affected by the concomitant presence of amino acids. Thus, identical retention times between LM-1 and vesperlysine A were observed only when vesperlysine A was either added to lens protein hydrolysate, or injected mixed together with, e.g., acid-hydrolyzed bovine serum albumin. The awareness of this chromatographic idiosyncrasy eventually allowed us to demonstrate full identity between LM-1 and vesperlysine A in terms of chromatographic behavior in two systems, with and without derivatization with acetic anhydride, as well as spectral analysis.

Vesperlysiners were first described in vitro by Ienaga and colleagues (2) as a family of fluorescent sugar-derived cross-links of L-lysine. In our studies, the single major fluorescent peak at LM-1 retention time corresponded to vesperlysiner A. However, a second minor peak that was also elevated in old lenses co-chromatographed at 18 min with another synthetic lysine-ribose advanced product (data not shown), suggesting other vesperlysiners might also be present in the human lens.

In depth studies on the mechanism of LM-1/vesperlysiner A formation were carried out, and the results were compared with our former study (1) and that of Nakamura (2). First, we were able to reproduce our previous findings (1) that LM-1 could be most efficiently made from D-ribose and ascorbate, but also D-threose. In our previous study, neither fructose nor glucose were precursor. In contrast to Nakamura et al., numerous attempts to make vesperlysiner A from glucose, even in presence of 200 mM non-Chelex-treated phosphate buffer, failed. This was rather unexpected in view of the fact that both D-arabinose and glyoxal are autoxidation products of glucose (9), which should act in concert as vesperlysiner A precursors, as suggested by the kinetic experiments in Fig. 9. This discrepancy suggests presence of competing reactions, possibly by glucose itself and other products for sites necessary for vesperlysiner A formation. Further possible differences include the utilization by Nakamura et al. of pentylamine instead of lysine as a reactant. Finally, we have utilized a highly specific two-column system in all our analytical studies, which may have helped achieve better separation of vesperlysiner isomers.

The inability of C-2 and C-3 sugar fragments and oxoaldehydes per se to make vesperlysiner A is in agreement with its structure. However, based on its structure, we hypothesized and confirmed that both glyoxal and glycolaldehyde are vesperlysiner A precursors when added to D-ribose. The presence of these oxoaldehydes in the lens is anticipated based on the documented accumulation of the glyoxal lysine dimer (GOLD) (11). Similarly, glyceraldehyde, which by itself was not a vesperlysiner A precursor, could moderately catalyze its formation from D-ribose. Most importantly, however, methylglyoxal was
neither a precursor nor a catalyst of vespertlysine A formation. We tentatively attribute the minute increase in vespertlysine A from ribose in presence of 10 mM methylglyoxal (Fig. 9) to contaminants, which have been reported in the commercial batch of methylglyoxal.

The inability of methylglyoxal and glucose to serve as vespertlysine A precursor in the lens is an important finding as vespertlysine A thus becomes a marker for metabolic pathways different from those represented by existing markers of the advanced Maillard reaction in vivo, i.e. the glycoxidation markers and those derived from methylglyoxal, i.e. methylglyoxyllysine (11), argpyrimidine (13), and \( \text{N}^\text{e} \)-(carboxymethyl)lysine (14).

The fact that threose, a known ascorbate degradation product (15, 16), is a potent precursor of vespertlysine A, together with our previous data showing that LM-1 could form from ascorbate oxidation products under anaerobic conditions (1), strongly suggests ascorbate is a likely precursor of vespertlysine A in the aging and diabetic lens. In support, we previously reported exposure of rat lenses to high galactose in vitro and in vivo favored the uptake of ascorbate oxidation products, which are otherwise excluded by the healthy lens, such as 2,3-dike-
Toxolanic acid. The latter was previously found to be a LM-1 precursor (1).

The proposed mechanism of non-oxidative formation of vesperlysine A is strongly supported by the data in the lens whereby the comparison with the glycoxidation markers pentosidine and CML is quite revealing (Fig. 7). First, a simple metal-catalyzed mechanism, as observed during glucose autoxidation, can be excluded on the basis that neither CML nor pentosidine are elevated in the diabetic lenses examined. Second, we formerly showed pentosidine was elevated only in lenses from poorly controlled diabetic dogs, and the sharp increase corresponded to a concomitant increase in Amadori product of glucose (4). This led to the conclusion that most pentosidine formed in diabetic human and dog lens is likely linked to a permeability breakdown of the lens. Thus, the dichotomy between the pentosidine and the vesperlysine A data in the diabetic lens leads to the conclusion that pentosidine in the diabetic lens unlikely originates from ascorbate, as originally proposed (18). In contrast, vesperlysine A/LM-1 formed readily from ascorbate oxidation products even under anaerobic conditions (1). Thus, when structural constraints and the data above are taken into consideration, ascorbate emerges as one of the most likely precursors of vesperlysine A, whereby a catalytic effect from trioses or glycolaldehyde/glyoxal cannot be excluded. The absence of elevated CML excludes lipoxidation as a vesperlysine A precursor in the diabetic lens. Similar absence of increased CML in the diabetic lens was reported previously by Lyons et al. (19). Proposed mechanistic pathways are summarized in Fig. 10.

As expected, all Maillard reaction markers strongly correlated with age and the age-related pigmentation of the lens. Again, we confirmed the previous findings that LM-1/vesperlysine A levels were comparatively lower in the brunescent lenses, i.e. a rare form of advanced cataract that is characterized by extreme pigmentation, covalent cross-linking, and insolubilization of the crystallins. Several explanations for this finding are possible, among which are the blocking of vesperlysine A formation sites by kinetically more favored reactions, and possibly also the increased photo-oxidative destruction of LM-1/vesperlysine A, which has been found to act as photosen-
sitter in UVA-irradiated lens crystallins (10).

This study is to our knowledge the first to document the existence of vesperlysine A in vivo. In view of the fact that it was either non-detectable, or present in minute quantities in other human and rat tissues, and only mildly increased in plasma proteins from patients with end stage renal disease, a condition associated with dramatic increase in many advanced glycation end products (AGEs), vesperlysine A emerges as novel Maillard reaction marker of human lenticular aging, which is most likely specific for carbonyl stress by ascorbate in the diabetic lens.

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