Role of the Maillard Reaction in Aging of Tissue Proteins: Advanced Glycation End Product-Dependent Increase in Imidazolium Cross-Links in Human Lens Proteins

Elisabeth Brinkmann Frye
Thorsten P. Degenhardt
Suzanne R. Thorpe
John W. Baynes

*University of South Carolina - Columbia, john.baynes@sc.edu*

Follow this and additional works at: https://scholarcommons.sc.edu/chem_facpub

Part of the Chemistry Commons

Publication Info
Published in *Journal of Biological Chemistry*, Volume 273, Issue 30, 1998, pages 18714-18719.
This research was originally published in the Journal of Biological Chemistry. Frye EB, Degenhardt TP, Thorpe SR, Baynes JW. Role of the Maillard Reaction in Aging of Tissue Proteins: Advanced Glycation End Product-Dependent Increase in Imidazolium Cross-Links in Human Lens Proteins. *Journal of Biological Chemistry*. 1998; 273:18714-18719. © the American Society for Biochemistry and Molecular Biology.
CARBOHYDRATES, LIPIDS, AND OTHER NATURAL PRODUCTS: Role of the Maillard Reaction in Aging of Tissue Proteins: ADVANCED GLYcation END PRODUCT-DEPENDENT INCREASE IN IMIDAZOLIUM CROSS-LINKS IN HUMAN LENS PROTEINS

Elisabeth Brinkmann Frye, Thorsten P. Degenhardt, Suzanne R. Thorpe and John W. Baynes

J. Biol. Chem. 1998, 273:18714-18719.
doi: 10.1074/jbc.273.30.18714

Access the most updated version of this article at http://www.jbc.org/content/273/30/18714

Find articles, minireviews, Reflections and Classics on similar topics on the JBC Affinity Sites.

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 18 of which can be accessed free at http://www.jbc.org/content/273/30/18714.full.html#ref-list-1
Role of the Maillard Reaction in Aging of Tissue Proteins

ADVANCED GLYcation END PRODUCT-DEPENDENT INCREASE IN IMIDAZOLIUM CROSS-LINKS IN HUMAN LENS PROTEINS

(Received for publication, February 3, 1998, and in revised form, May 4, 1998)

Elisabeth Brinkmann Frye‡, Thorsten P. Degenhardt‡, Suzanne R. Thorpe‡§, and John W. Baynes‡¶

From the ‡Department of Chemistry and Biochemistry and the ¶Department of Ophthalmology, University of South Carolina, Columbia, South Carolina 29208

Dicarbonyl compounds such as glyoxal and methylglyoxal are reactive dicarbonyl intermediates in the nonenzymatic browning and cross-linking of proteins during the Maillard reaction. We describe here the quantification of glyoxal and methylglyoxal-derived imidazolium cross-links in tissue proteins. The imidazolium salt cross-links, glyoxal-lysine dimer (GOLD) and methylglyoxal-lysine dimer (MOLD), were measured by liquid chromatography/mass spectrometry and were present in lens protein at concentrations of 0.02–0.2 and 0.1–0.8 mmol/mol of lysine, respectively. The lens concentrations of GOLD and MOLD correlated significantly with one another and also increased with lens age. GOLD and MOLD were present at significantly higher concentrations than the fluorescent cross-links pentosidine and dityrosine, identifying them as major Maillard reaction cross-links in lens proteins. Like the N-carboxyalkyllysines N¹-(carboxymethyl)lysine and N¹-(carboxyethyl)lysine, these cross-links were also detected at lower concentrations in human skin collagen and increased with age in collagen. The presence of GOLD and MOLD in tissue proteins implicates methylglyoxal and glyoxal, either free or protein-bound, as important precursors of protein cross-links formed during Maillard reactions in vivo during aging and in disease.

The Maillard reaction is a complex series of reactions between reducing sugars and amino groups on proteins, which lead to browning, fluorescence, and cross-linking of protein (1, 2). Advanced glycation end products, formed during later stages of the Maillard reaction, accumulate in long-lived tissue proteins, such as tissue collagens and lens crystallins, and may contribute to the development of complications in aging, diabetes, and atherosclerosis (3, 4). Glyoxal (GO),¹ methylglyoxal (MGO), and deoxyglucosones belong to a series of dicarbonyl compounds, identified as intermediates in the Maillard reaction. GO is formed on autoxidation of glucose under physiological conditions (5) and also as a product of lipid peroxidation (6). MGO is formed nonenzymatically by spontaneous decomposition of triose phosphate intermediates in glycolysis (7) and by amine-catalyzed sugar fragmentation reactions (8, 9). It is also a product of metabolism of acetone (10) and threonine (11). Both GO and MGO are detoxified by the glutathione-dependent glyoxalase pathway, yielding hydroxyacetic acid and α-lactate, respectively (12–14). MGO can also be detoxified by the NADPH-dependent enzyme aldose reductase, yielding 1,2-propanediol (15). The concentration of MGO is elevated in the blood of diabetic patients in vivo (16, 17), and the metabolites of MGO detoxification, acetol and 1,2-propanediol, are also increased in blood during diabetic ketoacidosis (10).

GO and MGO are reactive toward amino, guanidino, and sulfhydryl functional groups in protein (18, 19), leading to browning, denaturation, and cross-linking of proteins. Besides unidentified brown and fluorescent products, the reaction of GO and MGO with lysine and arginine residues in protein yields well-characterized compounds, such as the N-(carboxyalkyl)lysines, N¹-(carboxymethyl)lysine (CML) (20) and N¹-[1-(1-carboxyethyl)lysine (CEL) (21), and imidazolones and dehydroidimazolones (19, 22, 23). Imidazolones have been detected in tissue proteins only by immunological methods, whereas CML and CEL have been measured by gas chromatography/mass spectrometry (GC/MS) and increase in skin collagen and lens protein with age (21, 24–26). Two other products of the reaction of GO or MGO with lysine, glyoxal-lysine dimer (GOLD) and methylglyoxal-lysine dimer (MOLD) (Fig. 1), were originally characterized in reactions of GO or MGO, respectively, with hippuryllysine (27, 28). Nagaraj and colleagues (29) recently detected and measured MOLD in human serum proteins by reverse phase high performance liquid chromatography assay (RP-HPLC) and showed that cross-linking of serum proteins by MOLD was increased in diabetes. In the present study, we describe the reaction of MGO with the model protein bovine pancreatic RNase and quantify the role of MGO in cross-linking of the protein. Using a liquid chromatography/mass spectrometry (LC/MS) assay with ¹⁵N-labeled GOLD and MOLD internal standards, we also measure the levels of GOLD and MOLD in lens protein and show that the concentrations of both cross-links increase in concert with chronological age in lens protein and skin collagen. Quantitative analysis indicates that MOLD is the major chemically characterized cross-link formed in lens protein during the Maillard reaction.

EXPERIMENTAL PROCEDURES

Materials—Unless otherwise indicated, all chemical reagents were of the highest quality obtainable from Sigma, including MGO (40% aqueous solution) and RNase A (type XII-A). (Carboxymethyl)trimethylamino-
Imidazolium Cross-links in Lens Proteins

Lysine residues in protein.

Imidazolium Salt (GOLD, MOLD)

Histidine, which was unaltered during reaction of the protein with MGO and was recovered quantitatively during this procedure, was used as the internal standard for quantitation of Lys, Arg, and MOLD.

HPLC Procedures—Amino acid analysis was performed using a Pickering sodium ion exchange column (25 cm × 4.6 mm) and sodium bicarbonate/formic acid mobile phase (Pickering Laboratories Inc., Chandler, AZ). The derivatization reagent was applied to the instrument as described by the manufacturers. Amino acids were quantified by post-column derivatization with o-phthalaldehyde and fluorescence detection (20, 32). Hippuryl-amino acids were analyzed by RP-HPLC either on a 25 cm × 4.6 mm Zorbax SB C-18 HPLC column (MAC-MOD Analytical Inc., Chadds Ford, PA) or on a 15 cm × 3 mm Waters C-18 Symmetry column (Waters Corporation, using a detection wavelength of 305 nm. The gradient consisted of solvent A (0.05% acetic acid, 0.05% formic acid, and 0.1% triethylamine in water) and solvent B (75% solvent A in acetonitrile). The gradient program for the Zorbax column was: 0–50 min, 0–5% solvent B; 50–160 min, 5–100% solvent B; 160–190 min, wash with 100% acetonitrile, at a flow rate of 1 ml/min; and for the Symmetry column: 0–30 min, 0–10% solvent B; 30–100 min, 10–100% solvent B; 100–105 min, wash with 100% acetonitrile, at a flow rate of 0.5 ml/min. PTC amino acids were analyzed on a 15 cm × 4.6 mm-inner diameter 218T54 protein and peptide C-18 column (VYDAC/The Separations Group, Hesperia, CA) using a detection wavelength of 246 nm. The mobile phase consisted of solvent A (12.5 mM sodium phosphate, pH 6.2) and solvent B (30% solvent A and 70% acetonitrile). The gradient was programmed as follows: 0–40 min, 25% solvent B; 40–50 min, 50–100% solvent B; this was followed by a 10-min washing step with 100% acetonitrile, flow rate 1.2 ml/min.

Measurement of MGO Concentrations—MGO was measured using Girard’s Reagent T in 0.5 mM formic acid, pH 2.9, as described by Mitchell and Birnboim (33), using absorbance at 292 nm for quantitation.

Electrophoresis—SDS-PAGE was conducted under reducing conditions using a 4% stacking gel and a 15% separating gel as described by Laemmli (34).

Preparation of Human Lens Protein and Skin Collagen for Analysis of MOLD and GOLD—Human lenses were obtained from the South Carolina Lions Eye Bank (Columbia, SC) and stored at −70 °C until use. Lenses were decapsulated, homogenized and dialyzed against deionized water, as described previously (26, 35). Total lens protein was hydrolyzed in 6 N HCl at 110 °C for 24 h. The hydrolysate was dried and then applied to an SP-Sephadex cation exchange resin to recover basic amino acids, washed, and eluted, as described above. The eluent was dried by centrifugal evaporation and reconstituted in buffer A (sodium eluent, pH 3.15) for amino acid analysis. Fractions in the time interval including lysine, MOLD, and GOLD (15–25 min, 6 ml total volume) were collected. These pooled fractions were diluted with deionized water and acidified with 4 N HCl to pH 1–2, applied to a 2 ml-DOWEX-50W cation (sodium form), and desalted by washing with 70 ml of 0.5 N HCl; then the basic amino acids were eluted with 4 N HCl. The eluent was dried and derivatized for PTC amino acid analysis as described above.

Human skin collagen was isolated by full thickness biopsy from the upper buttock, as described previously in detail (24). Briefly, the skin flap was excised, rinsed with saline, and then incubated in 0.5% trypsin (type XI; Sigma) in 0.05 N HCl at 37 °C for 24 h with 1 mM NaCl in 10 mM phosphate buffer, pH 7.4, and with 0.5 M acetic acid to remove soluble proteins, and then extracted with chloroform:methanol (2:1) to remove any residual lipid. The collagen was hydrolyzed and analyzed as described above for lens proteins.

LC/MS Analysis of GOLD and MOLD in Human Lenses and Skin Collagen—For LC/MS analysis about 2 mg of lens protein was mixed with 3 nmol of 15N4-MOLD and 2.5 nmol of 15N4-GOLD or 4 mg of young (18 years) and old (85 years) pools of human skin collagen with 1.5 nmol of 15N4-MOLD and 1.25 nmol of 15N4-GOLD. The samples were hydrolyzed, processed, and derivatized with PITC, as described above.

Gradient HPLC/MS analysis was performed using a 100 × 1-mm Hypersil ODS column (Keystone Scientific Inc., Bellefonte, PA) with a 2 × 1-mm guard column. The gradient consisted of solvent A (90% water, 10% methanol, and 0.3% glacial acetic acid) and solvent B (20% water, 80% methanol, and 0.3% glacial acetic acid). The gradient was: 100% solvent A for 5 min, then to 100% solvent B at 15 min, and hold at 100% solvent B for 20 min; the flow rate was 50 μl/min. The ion source was an Analytica of Branford Inc. electrospray model 103443 (Branford, CT), operating with the following settings: capillary voltage, −2500 V; end capillary voltage, −3700 V; capillary temperature, −3700 °C; source temperature, 275 °C; needleless pressure, 38 p.s.i.; lens 1–6 voltages, 147.8, 25.6, 25.4, 2.7, 0, and −49.1 V. The mass spectrometer was a VG TRIO triple quadrupole mass analyzer (Beverly, MA) operating at: dwell time, 150 μs; delay time, 20 μs; and photomultiplier voltage, 600 V. The masses monitored were 417 amu for FTC-Lys+H⁺, 597 amu for (PTC)4-GOLD, 601 amu for (PTC)3-15N4-GOLD, 611 amu

![Fig. 1. Products of the reaction of glyoxal and methylglyoxal with lysine residues in protein.](Image)
Formation of Imidazolium Cross-links during the Reaction of MGO with RNase—Because MGO is present in higher concentrations in biological systems than GO (14), we concentrated on the reactions of MGO with protein. Reactions of MGO with the model protein RNase were studied under physiological conditions (pH 7.4, 37 °C). MGO was reacted with RNase, which has 10 lysine residues, at molar ratios of MGO:Lys, 1:1 and 2:1. As shown in Fig. 3, MOLD was readily detectable in MGO-modified RNase by RP-HPLC of the PTC derivative. The identity of the MOLD was confirmed by co-elution with an authentic standard and by its molecular mass of (PTC)$_2$-MOLD (611 amu) measured by ESI-MS. The kinetics of the formation of MOLD described in Fig. 4A were consistent with the kinetics of the disappearance of MGO from the reaction mixture (Fig. 4B). The half-life of MGO in the presence of the protein was ~4 h at both concentrations, whereas in phosphate buffer alone it disappeared with a half-life of ~30 h (Fig. 4B). The yield of MOLD increased 4–5-fold on doubling of the MGO concentration, an observation confirmed in two independent experiments, consistent with either a rate-limiting second order process or the requirement for 2 mol of MGO/mol of MOLD formed. In contrast, lysine and arginine decreased to similar extents at both MGO concentrations, yielding maximal modification of 5–6 of 10 lysine and 3 of 4 arginine residues in RNase (Fig. 4, C and D). The possibility that some free lysine and arginine were generated during acid hydrolysis cannot be excluded; however, the extent of the lack of reactivity of 3–4 lysine residues was confirmed by reaction with trinitrobenzenesulfonic acid (data not shown). Although ≥50% of lysine residues were modified in these reactions with formation of 20 or 95 mmol MOLD, only 1 or 3.6%, respectively, of the lysine loss could be accounted for as MOLD. CEL, measured by GC/MS (data not shown), accounted for an additional 1.3 or 1.8% of the total lysine modification (21). Thus, MOLD and CEL accounted for ≤5% of the modification of lysine residues in the protein. Browning cross-linking, and formation of fluorescent products also occurred, indicating that other unidentified products were formed. As shown in Fig. 5, the time course of cross-linking of RNase, estimated by SDS-PAGE, was consistent with the rate of formation of MOLD (Fig. 4A).

MGO-modified RNase was fractionated into monomer,
dimer, and polymer by chromatography on Sephadex G-75 (Fig. 6). Fractions were pooled, as indicated in the figure, and then analyzed for their MOLD content. As shown in the inset to Fig. 5, the MOLD content of RNase increased with the extent of polymerization of the protein. These data indicate that MOLD contributes to both inter- and intramolecular cross-linking of lysine residues in RNase; however, the yield of MOLD in the RNase dimer fraction (Fig. 6, inset) accounted for only about 5% of intermolecular cross-links, indicating that other cross-links were also formed.

Detection of MOLD and GOLD in Vivo—

Human lens proteins were analyzed for their MOLD and GOLD content using the RP-HPLC analytical procedure described in Fig. 3. The lens proteins were analyzed as a function of age, because both CEL and CML accumulate in these proteins with age (21, 26). As shown in Fig. 7, both MOLD and GOLD were detectable by RP-HPLC in the hydrolysate of lens protein. The peak identities were confirmed by co-elution with an authentic standard, but the reliability of the assay was questionable because of the low levels of MOLD and GOLD in lens proteins and their possible co-elution with other trace compounds in protein. To obtain more reliable results, we applied an LC/MS procedure to analyze lens protein hydrolysates that were derivatized with PITC. Heavy labeled MOLD and GOLD were prepared from 15N2-lysine for use as internal standards. Nonlabeled PTC 2-MOLD and PTC2-GOLD were detected at the masses 611 and 597 amu, respectively, and their heavy labeled counterparts at 615 and 601 amu, respectively. Fig. 8 shows an RP-HPLC/SIM-ESI chromatogram of PITC-derivatized lens protein, confirming detection of both GOLD and MOLD in lens protein. The standard curves (inset) were prepared by adding increasing amounts of natural GOLD and MOLD to a fixed amount of internal standard.

Using internal standardization with heavy labeled MOLD and GOLD, these compounds were measured in a group of lens proteins by HPLC-ESI-SIM-MS. As shown in Fig. 9, both MOLD and GOLD correlated significantly with age in human lens protein ($r^2 = 0.69$ and 0.75, respectively; $p < 0.001$). The MOLD and GOLD content of lens protein also correlated with
each other ($r^2 = 0.69$, $p < 0.001$). The concentration of MOLD in lens protein was approximately 5 times higher on average than that of GOLD. Measurements of MOLD in lens protein, determined by the LC/MS assay, were consistent with the results of RP-HPLC analysis, as described in Fig. 7, but concentrations measured by LC/MS were 25–80% of those estimated by RP-HPLC, consistent with errors in quantification or interference by co-eluting compounds in the HPLC assay. GOLD could not be measured in lens proteins by HPLC alone. In preliminary experiments, MOLD and GOLD were also detected in human skin collagen. Traces of both cross-links ($<0.02$ mmol/mol of Lys) were detectable in a pool of collagen from young donors (18 years old), but MOLD and GOLD were present at concentrations of 0.38 and 0.04 mmol/mol of Lys, respectively, in a pool from older donors (85 years old). The lower level of cross-links in collagen is consistent with the correspondingly lower levels of CML and CEL in skin collagen, compared with lens proteins (21, 24).

**DISCUSSION**

In these and other studies, we have identified CML and GOLD (27) and CEL and MOLD (28) as products of the reaction of GO and MGO with hippuryllysine and with RNase, respectively. Our studies on reactions with MGO confirm the observations of Nagaraj et al. (29) who described the formation of an MGO-derived imidazolium cross-link (MOLD, imidazolysine) in model systems and detected MOLD in human serum proteins by an HPLC method. Skovsted et al. (36) have also described another imidazolium cross-link, termed DOLD, which is formed by reaction of 3-deoxyglucosone with protein, although this compound has not yet been detected in tissue proteins. Dicarbonyl compounds, such as GO, MGO, and 3-deoxyglucosone, are also known to form imidazolones and dehydroimidazolones on reaction with arginine residues in proteins (19, 22, 23); however, the relative reactivity of Lys and Arg residues with dicarbonyl compounds has not been rigorously evaluated, in part because of the lack of appropriately sensitive and specific analytical methods for (dehydro)imidazolones.

In the present study, we describe an LC/MS assay for specific, accurate, and simultaneous measurement of both MOLD and GOLD in tissue proteins and show that both compounds increase in concert with one another in human lens protein during normal aging. Levels of MOLD measured by this technique were significantly lower than levels measured by HPLC alone, undoubtedly because of the greater specificity of the HPLC-ESI-SIM-MS technique, and in our hands, the HPLC technique was not reliable for estimation of the lower concentrations of GOLD in tissue proteins. MOLD and GOLD together account for only 0.2% of chemical modification of lysine residues in a senescent lens ($0.2$ mmol of GOLD + $0.8$ mmol of MOLD/mol of Lys at age $80 \times 2$ mol of Lys/cross-link). In contrast CML and CEL are present at nearly 10-fold higher concentrations ($4$ mmol of CML + $4$ mmol of CEL/mol of lysine), representing $0.8\%$ of lysine modification (21). Regardless of their low concentrations, MOLD and GOLD are present at significantly higher concentrations in lens proteins than the fluorescent cross-links pentosidine and dityrosine. At age 80, MOLD, GOLD, pentosidine, and dityrosine represent approximately 800, 200, 4, and 3 $\mu$mol/mol of lysine in lens protein (37, 38). In our 85-year-old skin collagen pool, MOLD and GOLD are present at 400 and $40 \mu$mol/mol lysine, respectively, compared with $40 \mu$mol pentosidine/mol lysine (24). At 100 mol of Lys/mol of triple stranded collagen, MOLD is present at 0.04...
mol/mol of collagen (0.08% of lysine residues). In contrast, enzymatically formed cross-links are much more abundant (1–5 mol/mol of collagen) (39). Thus, it is unlikely that GOLD or MOLD would have a significant impact on collagen cross-linking in aging, unless they are present at critical sites in the collagen molecule.

Our proposed mechanism for imidazolium cross-link formation includes the initial reaction of the dicarbonyl with two lysine molecules forming a labile Schiff base, diimine cross-link, and then recruitment of a second molecule of MGO for the cyclization reaction (27, 28). Glomb and Monnier (40) have described the formation of a diimine structure in reactions of protein with GO in vitro. Yim et al. (41) also propose that the reaction of dicarbonyls with amino groups yields radical intermediates, including a dicarbonyl dialkylamine radical cation and a dicarbonyl radical anion, which then react to form the stable trimeric glycation end products. Although it is possible that GOLD and MOLD were among the major advanced glycation end products formed in these and our in vitro studies in the presence of an excess of dicarbonyl compounds, it is likely that in biological systems at low GO and MGO concentrations, other aldehydes, including formaldehyde, acetaldehyde, glucose, or glycolytic intermediates would serve as carbon donors for the cyclization reaction. Thus, the pathway to formation of GOLD and MOLD in vivo may be unusually complex and difficult to unravel. As we and others have shown, there are many possible routes and precursors of CML and CEL in vitro (5, 21, 37, 40, 42), and it is possible that other precursors, e.g. ascorbate or polysaturated fatty acids in lipids, are more significant sources of GOLD and MOLD in vivo.

In our studies on the cross-linking of RNase by MGO, we show that MOLD is involved in both intermolecular and intramolecular cross-linking reactions. MOLD accounts for only a small percentage (~5%) of the cross-links in the dimer fraction of RNase (Fig. 6), indicating that there are other unidentified cross-links, possibly involving amino acids other than lysine. The ratio of carboxyalkyllysines:imidazolium salts and of intramolecular:intermolecular cross-links by imidazolium salts undoubtedly varies with protein structure and the packing density of protein monomers. RNase has three lysine residues (1, 7, 41) clustered in or near the active site of the protein, which are also major sites for modification of the protein by glucose (43). The adjacency of these lysine amino groups may favor intramolecular cross-linking of the protein. In other proteins, glycation occurs preferably at lysine-lysine sequences (44), which should favor intramolecular cross-linking, or at lysine residues in other basic amino acid sequences (45, 46) and at lysine residues apposed to acidic amino acids (46, 47), which may favor intermolecular cross-linking. Proteins on membrane surfaces, which are packed more densely than soluble proteins, may also be more susceptible to intermolecular cross-linking reactions. The close packing may also accelerate the age-dependent, intermolecular cross-linking, aggregation, and insolubilization of lens proteins during the Maillard reaction. Studies on the distribution of GOLD and MOLD in various lens protein fractions and on the specificity of carboxyalkylation and cross-linking of proteins by GO and MGO should provide insight into the role of these reactions in alteration of tissue proteins in aging and disease.

Acknowledgment—We thank Dr. William E. Coughlin, University of South Carolina Mass Spectrometry Laboratory for conducting the mass spectrometry analyses.

REFERENCES
1. Hodge, J. E. (1953) J. Agric. Food Chem. 1, 928–943
2. Ledl, F., and Schleicher, E. (1990) Angew. Chem. Int. Ed. Engl. 29, 565–594
3. Vlassara, H. (1994) J. Lab. Clin. Med. 124, 19–20
4. Thorpe, S. R., and Baynes, J. W. (1996) Drugs Aging 9, 69–77
5. Wells-Knecht, K. J., Zyzak, D. V., Litchfield, L. E., Thorpe, S. R., and Baynes, J. W. (1985) Biochemistry 24, 3702–3709
6. Leidl-Stahlhofen, A., Hannemann, R., and Spiteller, G. (1995) Chem. Phys. Lipids 77, 113–117
7. Richard, J. P. (1991) Biochemistry 30, 4581–4585
8. Hase, T., Mase, S., and Namiki, M. (1986) Agric. Biol. Chem. 50, 1959–1964
9. Hayashi, T., and Namiki, M. (1986) Agric. Biol. Chem. 30, 1965–1970
10. Reichard, G. A., Skutches, C. L., Hoeldtke, R. D., and Owen, O. E. (1986) Diabetes 35, 685–674
11. Ray, M., and Ray, S. (1987) J. Biol. Chem. 262, 5974–5977
12. Thornalley, P. J. (1994) Mol. Aspects Med. 14, 287–371
13. Thornalley, P. J. (1994) Ammon Acids (Vienna) 6, 15–23
14. Thorpe, S. R., and Baynes, J. W. (1993) J. Biol. Chem. 268, 4366–4369
15. Thorpe, S. R., Hooper, N. I., Jennings, P. E., Florkowski, C. M., Jones, A. F., Loidl-Stahlhofen, A., Hannemann, R., and Spiteller, G. (1995) Chem. Phys. Lipids 77, 113–117
16. Thornalley, P. J., Hooper, N. I., Jennings, P. E., Florkowski, C. M., Jones, A. F., Loidl-Stahlhofen, A., Hannemann, R., and Spiteller, G. (1995) Chem. Phys. Lipids 77, 113–117