Extending the Spectrum of $\alpha$-Dicarbonyl Compounds in Vivo*

Christian Henning†, Kristin Liehr‡, Matthias Girndt§, Christof Ulrich¶, and Marcus A. Glomb††

From the †Institute of Chemistry-Food Chemistry and the ‡Department of Internal Medicine II, Martin-Luther-University Halle-Wittenberg, Kurt-Mothes-Strasse 2, 06120 Halle/Saale, Germany

Received for publication, March 7, 2014, and in revised form, August 25, 2014 Published, JBC Papers in Press, August 27, 2014, DOI 10.1074/jbc.M114.563593

Background: $\alpha$-Dicarbonyls are central intermediates in the formation of advanced glycation end products (AGEs).

Results: A quantitation method for the complete spectrum relevant in vivo was established.

Conclusion: Non-enzymatic chemistry of glucose and L-ascorbic acid as precursors and $\alpha$-dicarbonyl intermediates play an important role in vivo.

Significance: Knowledge of plasma levels of $\alpha$-dicarbonyls is crucial to understand the complex pathways of AGE formation in vivo.

Maillard $\alpha$-dicarbonyl compounds are known as central intermediates in advanced glycation end product (AGE) formation. Glucose is the primary source of energy for the human body, whereas L-threo-ascorbic acid (vitamin C) is an essential nutrient, involved in a variety of enzymatic reactions. Thus, the Maillard degradation of glucose and ascorbic acid is of major importance in vivo. To understand the complex mechanistic pathways of AGE formation, it is crucial to extend the knowledge on plasma concentrations of reactive key $\alpha$-dicarbonyl compounds (e.g. 1-deoxyglucosone). With the present work, we introduce a highly sensitive LC-MS/MS multimethod for human blood plasma based on derivatization with $\alpha$-phenylenediamine under acidic conditions. The impact of workup and reaction conditions, particularly of pH, was thoroughly evaluated. A comprehensive validation provided excellent linearity over a wide concentration range (r² = 0.9999) with good precision (R.S.D. < 10%, n = 7). The accuracy of the method was thoroughly validated by spiking human plasma with more than 40 $\alpha$-dicarbonyl compounds.

Regarding this topic, foods and peritoneal dialysis fluids are sources of reactive precursors of glycation. Through a series of reactions, these precursors are converted to reactive key $\alpha$-dicarbonyls that, in turn, are involved in a variety of enzymatic reactions. Thus, the Maillard degradation of glucose and ascorbic acid is of major importance in vivo. To understand the complex mechanistic pathways of AGE formation, it is crucial to extend the knowledge on plasma concentrations of reactive key $\alpha$-dicarbonyl compounds (e.g. 1-deoxyglucosone). With the present work, we introduce a highly sensitive LC-MS/MS multimethod for human blood plasma based on derivatization with $\alpha$-phenylenediamine under acidic conditions. The impact of workup and reaction conditions, particularly of pH, was thoroughly evaluated. A comprehensive validation provided excellent linearity over a wide concentration range (r² = 0.9999) with good precision (R.S.D. < 10%, n = 7). The accuracy of the method was thoroughly validated by spiking human plasma with more than 40 $\alpha$-dicarbonyl compounds.

To examine the impact of workup and reaction conditions, particularly of pH, the plasma samples were derivatized with $\alpha$-phenylenediamine under acidic conditions. A comprehensive validation provided excellent linearity over a wide concentration range (r² = 0.9999) with good precision (R.S.D. < 10%, n = 7). The accuracy of the method was thoroughly validated by spiking human plasma with more than 40 $\alpha$-dicarbonyl compounds.

To examine the impact of workup and reaction conditions, particularly of pH, the plasma samples were derivatized with $\alpha$-phenylenediamine under acidic conditions. A comprehensive validation provided excellent linearity over a wide concentration range (r² = 0.9999) with good precision (R.S.D. < 10%, n = 7). The accuracy of the method was thoroughly validated by spiking human plasma with more than 40 $\alpha$-dicarbonyl compounds.
(2,3-DKG), threosone, 3-deoxythreosone (3-DT), and xylosone in human lens. In human blood plasma, however, only glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG), and DHA have been described in detail so far (26–33). The implemented analytical methods vary not only in the inevitable derivatization procedure but also in the chromatographic technique used. The common alternative approach for the quantitation of DHA is the measurement of the difference of ASA before and after a reduction step (34, 35). Consequently, concentrations of published plasma levels of healthy subjects differ in a wide range and are thus not comparable (e.g. for GO from 220 to 1150 pmol/ml for MGO from 120 to 650 pmol/ml and for DHA from 550 to 6800 pmol/ml (mean values)).

Glomb and Tschirnich compared common derivatization approaches (36). According to them, the use of aromatic o-di- amines (e.g. o-phenylenediamine (OPD)) is prerequisite for the analysis of highly reactive α-DCs, such as 1-deoxyglucosone (1-DG). The detection of these short lived intermediates is limited by the rate of condensation of the reagent with the carbonyl moiety. However, these trapping reagents impose high oxidative stress on the system investigated and could lead to artifact formation, especially of α-DCs that originate from oxidative pathways (e.g. 3-arabino-hexos-2-ulos (glucosone)).

The work group of Thornalley (31) developed a reliable method for the detection of MGO as 6,7-dimethoxy-2-methylquinoline in human plasma. They stressed the importance of pH control to avoid the degradation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) to MGO, both central intermediates in several metabolic pathways in living organisms.

Although many methods for the detection of a few selected α-DCs were described already, a comprehensive method including all relevant compounds in vivo has been missing up to now. The particular analytical challenge in this regard is the very diverging polarity of the target substances but also the baseline separation of isomeric pairs with identical molecular masses (e.g. 1-/3-deoxypentosone (1-/3-DP)).

We developed and validated a highly sensitive LC-MS/MS multiform method suitable for routine analysis based on the derivatization with OPD, including 15 α-dicarbonyl compounds and two α-keto-carboxylic acids. Both substance categories are hereafter referred to as α-DCs for reasons of simplicity. In detail, the method covers 1-DG, 3-DG, glucosone, \( \text{N}^4-(3,6\text{-dideoxyhexos-2-ulos-6-yl})\)-lysine (Lederer’s glucosone), DHA, 2,3-DKG, 4,5-dihydroxy-2,3-pentanedione (1-DP), 4,5-dihydroxy-2-oxopentanone (3-DP), 3,4-dideoxypentosone (3,4-DDP), pentos-2-ulos (pentosone), 1-deoxythreosone (1-DT), 3-DT, threosone, MGO, ethanediol (glyoxal, GO), 2-oxopropanoic acid (pyruvic acid), oxoethanoic acid (glyoxylic acid), and ethanediol acid (oxalic acid) and was used to screen an initial set of plasma samples from 15 healthy human subjects and 24 uremic patients undergoing hemodialysis (HD patients). The results are discussed with respect to the sources and mechanistic relationships of the α-DCs detected.

**EXPERIMENTAL PROCEDURES**

**Materials and Plasma Samples**—Chemicals of the highest grade available were obtained from Sigma-Aldrich and Fisher unless otherwise indicated. NMR solvents were purchased from ARMAR Chemicals (Leipzig/Doetlingen, Germany). The quinoline derivatives of the α-DCs will be marked hereafter with the suffix “-Q”. 1-DG-Q, 3-DG-Q, glucosone-Q, Lederer’s glucosone-Q, 1-DP-Q, 3-DP-Q, 3,4-DDP-Q, pentosone-Q, 1-DT-Q, 3-DT-Q, threosone-Q, pyruvic acid-Q, glyoxylic acid-Q, oxalic acid-Q, and 3-DG were synthesized according to our previous work (37–39). The identities of target compounds were verified by nuclear magnetic resonance (NMR) experiments.

Written informed consent was obtained from all patients. The study was approved by the Ethics Committee of the Medical Faculty of the Martin Luther University Halle-Wittenberg. Blood samples were obtained from 15 healthy subjects with normal renal function and 24 non-diabetic patients undergoing hemodialysis using EDTA as an anticoagulant (2 mg/ml whole blood). In HD patients, samples were obtained predialysis before the midweek treatment session. Hemodialysis was performed three times weekly for 4–5 h using polysulfone dialyzers. All patients were treated with bicarbonate hemodialysis (acid concentrate type 257, 8.4% sodium bicarbonate type 200, MTN Neubrandenburg GmbH, Neubrandenburg, Germany) with ultrapure water quality (by reverse osmosis and sterile filters). Plasma was derived by centrifugation (3000 × g, 10 min, 4 °C) within 20 min of collection and immediately subjected to the assay procedure described below. HbA1c, creatinine, and C-reactive protein were measured by routine methods at the central laboratory of Martin-Luther-University Clinical Center, Halle (Saale, Germany).

2-(2′(R),3′(R),4′-Trihydroxybutyl)quinoxaline (3-DGal-Q)—3-Deoxy-\( \text{d} \)-threo-hexos-2-ulos (3-deoxygalactosone; 3-DGal) was synthesized according to the literature (40) with the exception of the 3-DGal bis(benzoyl hydrazone) cleavage. Here, instead of benzoaldehyde, sodium nitrite was used, following the procedure of Henseke and Bauer (41). Purification of crude 3-DGal-Q was achieved by preparative high performance liquid chromatography with ultraviolet detection (HPLC-UV). NMR results were in line with those of Hellwig et al. (40).

3-(\( \text{o} \)-erythro-Glycerol-1-yl)-quinoxaline-2-carboxylic Acid o-Aminoanilide (DHA Precursor-Q)—DHA (250 mg; 1.44 mmol) was suspended in methanol (25 ml), and OPD was added (308.5 mg; 2.85 mmol). The reaction mixture was heated for 2 h at 40 °C and allowed to cool. The precipitated solid was isolated by filtration; washed with water, ethanol, and ether; and dried (yield: 282.6 mg, 76%). Recrystallization from ethanol gave yellow needles.

\(^1\)H NMR (500 MHz, DMSO-\( \text{d}_{6} \)): \( \delta \) (ppm) = 3.44–3.55 (m, 2H), 4.03 (m, 1H), 5.42 (m, 1H), 6.63 (m, 1H), 6.8 (dd, \( J = 8.0, 1.4 \) Hz, 1H), 7.0 (m, 1H), 7.36 (dd, \( J = 8.0, 1.5 \) Hz, 1H), 7.96 (m, 2H), 8.17 (m, 1H), 8.23 (m, 1H). \(^{13}\)C NMR (100 MHz, DMSO-\( \text{d}_{6} \)): \( \delta \) (ppm) = 63.3, 72.0, 74.5, 116.4, 116.6, 122.9, 126.4, 127.2, 128.9, 129.5, 131.0, 132.0, 139.6, 141.2, 143.2, 147.4, 156.0, 165.1. HR-MS: \( \text{m/z} \) 393.09645 (found); \( \text{m/z} \) 393.09596 (calculated for \( \text{C}_{19}\text{H}_{12}\text{O}_{4}\text{N}_{4}\text{K} \left[ \text{M} + \text{K}^{+} \right] \)).

3-(\( \text{o} \)-erythro-Glycerol-1-yl)-quinoxaline-2-carboxylic \( \gamma \)-Lactone (DHA-Q)—A suspension of precursor DHA-Q (500 mg) in water (10 ml) was treated with 0.1 N hydrochloric acid (25 ml) and stirred at room temperature. The reaction process was followed by thin layer chromatography (TLC; EtOAc, UV detection). The reaction mixture was extracted with EtOAc (3 ×...
**α-Dicarbonyl Compounds in Vivo**

35 ml, the organic layers were combined, and the solvent was evaporated. The residue was purified by column chromatography (silica gel 60, 63–200 μm (Merck), EtOAc). Fractions including the compound with R_f 0.24 (TLC: EtOAc, UV detection) were collectively concentrated in vacuo to afford 180 mg (66%) of light yellow needles. NMR results were in line with those of Nemet and Monnier (25). HR-MS: m/z 269.05301 (found); m/z 269.05328 (calculated for C_{12}H_{11}O_{5}N_{2} \ [M + Na]^+).

2,3-DKG—2,3-DKG as sodium salt was synthesized according to the method of Otsuka et al. (42). The product was obtained as a white hygroscopic precipitate. It was characterized by the LC-MS/MS method mentioned below after derivatization with OPD and directly utilized for quinoxaline synthesis.

3-((1S,2R)-1,2,3-Trihydroxypropyl)-quinoxaline-2-carboxylic Acid (2,3-DKG-Q)—The corresponding quinoxaline of 2,3-DKG was obtained according to Nemet and Monnier (25). Purification of the quinoxaline solution was done by preparative HPLC-UV (t_r = 62 min). Fractions including the pure compound were collectively evaporated under reduced pressure, dissolved in water, and lyophilized to afford 2,3-DKG-Q as a light yellow powder in quantitative yield. NMR data were in line with the literature. HR-MS: m/z 263.06749 (found); m/z 263.06735 (calculated for C_{12}H_{10}O_{4}N_{2} [M – H]^-).

Isotopically Labeled 2-Hydroxy-3-methyl-2,3-^{13}C_{2}-quinoxaline (Pyruvic Acid-^{13}C_{2}-Q)—Pyruvic acid-^{13}C_{2}-Q was synthesized according to the method of Arun et al. (43). Briefly, a solution of OPD in distilled water (306.9 mg in 8 ml of ultrapure water, 2.84 mM) was added to 1,2-^{13}C_{2}-pyruvic acid (250.0 mg in 8 ml ultrapure water, 2.84 mM) dropwise with constant stirring. The precipitated pale yellow colored compound was filtered, washed with water, and lyophilized. The crude sample was recrystallized from 50% ethanol absolute (404.7 mg, 88%). NMR data were in line with the literature. HR-MS: m/z 163.0779 (found); m/z 163.0776 (calculated for C_{7}^{13}C_{2}H_{6}ON_{2} [M + H]^+).

**Time Course of the OPD Reaction of 3-DG, MGO, GO, Glyoxal, DHA Precursor-Q, and 2,3-DKG under Assay Conditions**—All compounds (20 μM) were incubated under assay conditions in amber glass vials under argon atmosphere at 22 °C in various buffer solutions containing 0.55 mM OPD and 3.4 mM EDTA. The buffers used were potassium phosphate buffer (0.4 M, pH 7.0), sodium formate buffer (0.4 M, pH 3.0), and perchloric acid (0.4 M, pH <0). Vials were tightly sealed by a screw cap with integrated polytetrafluoroethylene/silicone septum. Reaction mixtures were subjected to LC-UV analysis as described below not only after 24 h but immediately after the addition of OPD and after a 1-h incubation time to account for possible MGO impurities of the starting materials. The yield of MGO was determined against the authentic reference α-DC-Q standards.

**Assay of α-DCs in Plasma**—Sodium formate buffer (2 M, pH 3.0, 200 μl), the internal standard pyruvic acid-^{13}C_{2}-Q (1.25 μM, 100 μl) and the derivatizing agent OPD (2.75 mM, 200 μl) were added to 500 μl of blood plasma. The sample was incubated for 24 h in the dark at room temperature under argon atmosphere. TFA (2 M, 250 μl) was added, and the incubation continued for 1 h under the same conditions. The pH of the sample was then adjusted to pH 3.0 with ammonium hydroxide (4 M, 415 μl). Water was added (85 μl) to give a total sample volume of 1750 μl. The protein precipitate was separated by centrifugation (16,000 × g and 20 °C). The supernatant (storage at −80 °C) was administered to LC-MS/MS analysis.

**HPLC-UV**—A Besta HD 2-200 pump (Wilhelmsfeld, Germany) was used at a flow rate of 15 ml/min. Elution of material was monitored by a UV detector (Jasco UV-2075 with a preparative flow cell (Gross-Umstadt, Germany)). The detection was done with 3-DGal-Q under the assumption of an equal extinction coefficient.

**Accurate Mass Determination (High Resolution MS)**—The high resolution positive and negative ion electrospray ionization mass spectra (electrospray ionization-high resolution MS) were taken on a Bruker Apex III Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with an infinity cell, a 7.0-tesla superconducting magnet, a radio frequency-only hexapole ion guide, and an external off-axis electrospray source (Apollo; Agilent, Santa Clara, CA).
Nitrogen was used as a drying gas at 150 °C. The samples were dissolved in methanol, and the solutions were introduced continuously via a syringe pump at a flow rate of 120 μl/min. The native and the spiked plasma sample were subjected to plasma workup samples and incubations were analyzed in single batches to exclude interassay variations.

To obtain fragmentation spectra of α-DCs-Q in plasma workup solutions, target material was first enriched by repeated collection from the above HPLC system. After solvent evaporation in a vacuum concentrator (Savant SpeedVac Plus SC 110 A combined with a Vapor Trap RVT 400, Thermo Fisher Scientific GmbH (Dreieich, Germany)), the residue was dissolved in pyruvic acid-13C2 as an internal standard.

Interferences. Potential losses during the workup procedure and intrabatch changes of instrument sensitivity were corrected with pyruvic acid-13C2 as an internal standard.

To determine recovery rates, the respective alpha-DCs-Q were added at four different concentrations to three parallel sets of blood samples of one subject. The native and the spiked plasma sample were subjected to the assay of α-DCs in plasma as described above. The recovery rate was estimated as the quotient of (spiked α-DC-Q amount – amount α-DC-Q in native plasma sample)/amount of added α-DC-Q × 100%.

**Validation of the Quantitation Method**—Intraassay coefficients of variation were determined by repeated workup and analysis of a plasma sample (n = 6). In addition, the limit of detection and limit of quantitation with all steps of the analysis included were estimated according to DIN 32645 (n = 6, confidence level p = 0.95, k = 3) (44). To determine recovery rates, the respective alpha-DCs-Q were added at four different concentrations to three parallel sets of blood samples of one subject. The native and the spiked plasma sample were subjected to the assay of α-DCs in plasma as described above. The recovery rate was estimated as the quotient of (spiked α-DC-Q amount – amount α-DC-Q in native plasma sample)/amount of added α-DC-Q × 100%.

**TABLE 1**

| Quinoxaline and letter assignment (see Fig. 2) | Retention time | Precursor ion | Product ion 1<sup>a</sup> | Product ion 2<sup>b</sup> | Product ion 3<sup>b</sup> |
|-----------------------------------------------|---------------|--------------|----------------|----------------|----------------|
| min amu<sup>c</sup> | m/z | DP | eV | m/z | eV | CXP | m/z | eV | CXP | m/z | eV | CXP |
| 2,3-DKG (a) | 9.5 | 265.1 | 40 | 185.1 | 23.0 | 11.0 | 221.1 | 17.0 | 17.0 | 161.0 | 27.0 | 11.0 |
| Glucosone (b) | 9.9 | 251.1 | 50 | 173.1 | 24.0 | 16.0 | 161.1 | 30.0 | 13.0 | 233.2 | 19.0 | 16.0 |
| Oxalic acid (c) | 10.5 | 162.9 | 70 | 90.1 | 42.0 | 15.0 | 145.1 | 25.0 | 10.0 | 117.1 | 31.0 | 7.0 |
| Pentosone (d) | 11.2 | 221.1 | 50 | 173.1 | 22.0 | 14.0 | 161.0 | 27.0 | 13.0 | 132.1 | 45.0 | 10.0 |
| 1-DG (e) | 11.9 | 235.2 | 55 | 175.1 | 28.0 | 12.0 | 187.1 | 27.0 | 11.0 | 217.2 | 18.0 | 16.0 |
| DHA (f) | 12.0 | 247.1 | 55 | 229.0 | 18.0 | 20.0 | 185.3 | 20.0 | 15.0 | 171.1 | 33.0 | 10.0 |
| 3-DG (g) | 13.9 | 235.2 | 55 | 199.1 | 24.0 | 17.0 | 145.0 | 31.0 | 9.0 | 157.1 | 12.0 | 30.0 |
| Threosone (h) | 14.0 | 191.2 | 45 | 173.1 | 17.5 | 14.0 | 144.1 | 37.0 | 11.0 | 161.2 | 24.0 | 12.0 |
| 1-DP (i) | 16.8 | 205.1 | 50 | 187.1 | 21.0 | 14.0 | 145.1 | 27.0 | 12.0 | 169.2 | 30.0 | 11.0 |
| Glycolic acid (j) | 17.1 | 147.1 | 65 | 129.0 | 26.5 | 9.0 | 119.1 | 30.0 | 10.0 | 102.1 | 39.0 | 8.5 |
| 3-DP (k) | 17.1 | 205.1 | 50 | 187.2 | 19.5 | 10.0 | 158.1 | 34.0 | 14.0 | 174.1 | 32.5 | 13.0 |
| Lederer’s glucosone | 18.4 | 363.3 | 65 | 187.1 | 27.0 | 16.0 | 159.1 | 29.0 | 10.0 | 219.1 | 24.0 | 12.0 |
| 3-DT (l) | 19.2 | 175.1 | 55 | 157.2 | 24.0 | 10.0 | 129.2 | 35.0 | 9.0 | 145.0 | 25.5 | 10.0 |
| DHA precursor (m) | 19.5 | 355.3 | 52 | 109.2 | 19.0 | 8.0 | 247.2 | 15.0 | 16.0 | 337.2 | 15.0 | 12.0 |
| Pyruvic acid (n) | 19.5 | 160.9 | 60 | 133.1 | 28.0 | 10.0 | 91.9 | 40.0 | 7.0 | 64.9 | 54.0 | 11.0 |
| Pyruvic acid-13C2 (o) | 19.5 | 163.1 | 60 | 134.0 | 28.0 | 10.0 | 92.1 | 40.0 | 7.0 | 64.9 | 54.0 | 11.0 |
| 1-DT (p) | 19.6 | 175.1 | 45 | 157.1 | 22.0 | 7.0 | 156.1 | 35.0 | 15.0 | 89.1 | 55.0 | 5.0 |
| 3,4-DDP (q) | 20.5 | 189.1 | 55 | 171.2 | 25.0 | 15.0 | 145.1 | 25.0 | 11.0 | 102.1 | 52.0 | 16.0 |
| GO (r) | 20.9 | 131.1 | 32 | 77.0 | 40.0 | 6.0 | 104.0 | 30.0 | 8.0 | 65.0 | 45.0 | 5.0 |
| MGO (t) | 22.3 | 145.0 | 50 | 77.0 | 41.0 | 5.0 | 118.1 | 30.5 | 7.0 | 65.0 | 45.0 | 5.0 |

<sup>a</sup> MRM transition used for quantitation (quantifier).
<sup>b</sup> MRM transition used for confirmation (qualifier).
<sup>c</sup> DP, declustering potential.
<sup>d</sup> CE, collision energy.
<sup>e</sup> CXP, collision cell exit potential.
<sup>f</sup> amu, atomic mass units.
Development of an LC-MS/MS Method for 17 Relevant Dicarbonyl Compounds as Their Corresponding Quinoxalines—The complex matrix and the low concentration of most target analytes hamper the use of UV detectors for qualitative and quantitative analysis. Tandem mass spectrometric detection in the multiple-reaction monitoring mode was prerequisite for the detection of the 17 relevant α-DCs in blood plasma. To achieve highly sensitive detection utilizing collision-induced dissociation of the protonated molecules, the compound-specific orifice potentials and fragment-specific collision energies had to be determined by authentic reference material (Table 1).

HPLC separation was based on a RP-18 phase with methanol as the organic eluent. Optimization of gradient, column temperature, and ion pair reagent led to almost baseline separation of all critical quinoxalines possessing the same molecular weight, which therefore could not be distinguished via MS detection (1-DG-Q versus 3-DG-Q versus 4-DG-Q versus 3-DGal-Q, 1-DP-Q versus 3-DP-Q, 1-DT-Q versus 3-DT-Q). Fig. 2 shows the typical chromatogram of a plasma sample after derivatization with OPD.

Verification of the Derivatization Procedure—The reactivity of α-DCs required conversion into stable quinoxaline derivatives. After the blood sample is drawn from the living subject, the complex system blood is prone to significant alterations regarding its biological activity and chemical composition, especially under exposure to oxygen. Immediate plasma generation and instant start of the derivatization procedure under well defined conditions is therefore prerequisite for reproducible quantitative results.

To prevent underestimation of rather slow reacting compounds, OPD was chosen as the derivatization agent. It has a comparatively high conversion rate of α-DCs to the respective quinoxaline derivatives. To ensure complete derivatization, selected native α-DCs (GO, MGO, glyoxylic acid, pyruvic acid, oxalic acid, and 3-DG) were incubated with OPD under stan-

### Table 3

Levels of all relevant α-dicarbonyl compounds in human blood plasma

| Quinoxaline      | Healthy subjects | HD patients |
|------------------|------------------|-------------|
|                  | Mean ± S.D. | Range | Mean ± S.D. | Range |
| Glucosone        | 46 ± 11 | 28–67 | 96 ± 49<sup>a</sup> | 57–276 |
| 1-DG             | 22 ± 3  | 16–28 | 30 ± 16 | 12–82 |
| 3-DG             | 43 ± 5  | 35–56 | 65 ± 20<sup>b</sup> | 36–125 |
| Lederer’s glucosone | <LOQ   | <LOQ    | 7.0 ± 2.5<sup>c</sup> | <LOQ–13 |
| Pentosone        | 15 ± 10<sup>a</sup> | 2.6–7.8 | 11 ± 5<sup>c</sup> | <LOQ–23 |
| 1-DP             | 3.6 ± 1.2<sup>a</sup> | 9–17 | 6.2 ± 2.6<sup>c</sup> | 3.2–15 |
| 3-DP             | 11 ± 2  | 9–17 | 33 ± 9<sup>c</sup> | 20–53 |
| Threonosine      | 5.4 ± 0.7<sup>a</sup> | 4.2–6.6 | 9.0 ± 3.5<sup>c</sup> | 4.2–19 |
| 1-DT             | 3.1 ± 0.3 | 2.6–3.8 | 3.8 ± 1.0 | 2.0–6.6 |
| 3-DT             | 10 ± 2 | 8–14 | 45 ± 11<sup>c</sup> | 28–75 |
| MGO              | 61 ± 7 | 51–76 | 219 ± 129<sup>c</sup> | 42–617 |
| GO               | 491 ± 47 | 405–564 | 1273 ± 980<sup>c</sup> | 400–4914 |
| Pyruvic acid     | 7250 ± 2549 | 2818–12,038 | 35,874 ± 19,080<sup>c</sup> | 8399–97,935 |
| Glyoxylic acid   | 1264 ± 353 | 783–1942 | 2031 ± 608<sup>c</sup> | 1026–3514 |
| Oxalic acid      | 40 ± 21 | 18–82 | 36 ± 12 | 16–70 |
| 3,4-DDP          | 6.0 ± 1.0 | 4.4–7.7 | 19 ± 8<sup>c</sup> | 6–42 |
| DHA precursor    | 687 ± 351<sup>a</sup> | 337–1303 | 1925 ± 1160<sup>c</sup> | 308–4829 |
| DHA              | 15,400 ± 4019<sup>a</sup> | 6809–23,967 | 12,538 ± 7183<sup>b</sup> | 2737–26,680 |
| 2,3-DKG          | 1741 ± 674<sup>a</sup> | 567–2669 | 411 ± 238<sup>c</sup> | 145–937 |

<sup>a</sup> LOD < x < LODQ.

<sup>b</sup> The sum of DHA-Q and DHA precursor-Q account for approximately 38% of the true DHA content.

<sup>c</sup> Values adjusted as described under “Discussion.”
standard assay conditions, and the formation of respective quinoxalines was monitored between 0.5 and 48 h. The results presented in Fig. 3 revealed complete reaction (±5%) within 11 h, with the short-chained substances reacting much more rapidly, which is in accordance with our previous findings (36). The exception was oxalic acid, which showed no reaction with OPD. Furthermore, the stability of all quinoxaline compounds included in our method was monitored with authentic references under the derivatization conditions for 24 h. No degradation was observed.

In addition, the derivatization of a plasma sample was monitored by repeated injection of aliquots and LC-MS/MS analyses of the complete α-DC spectrum. Besides the risk of underestimation of α-DCs in trace amounts, avoiding overestimation of certain dicarbonyl quinoxalines is an even bigger challenge. For example, de novo formation of glucosone-Q and GO-Q during derivatization with OPD are described in the literature (22, 36, 45). The main reasons are the strong oxidative conditions imposed on the system by the addition of OPD in combination with oxygen and trace amounts of metal ions. However, with our derivatization procedure, a stable plateau for all analytes was reached within the relevant incubation time, indicating no or negligible de novo formation in the plasma matrix. In this regard, it is important to note that the incubation took place under argon atmosphere to minimize the impact of oxygen. Furthermore, EDTA as a metal ion-chelating agent was present in the assay.

**FIGURE 1.** Verification of 1-DG by CID of m/z 235.2 [M + H]+. A, authentic reference; B, plasma workup.

**FIGURE 2.** LC-MS/MS chromatogram of a plasma sample after standard workup procedure. For clarity, only the quantifier mass transition for each analyte of the scheduled multiple-reaction monitoring is shown. For letter assignments (a–r), see Table 1.

**FIGURE 3.** Reactions of selected dicarbonyl compounds with OPD in formate buffer (0.4 M, pH 3.0) at room temperature: 3-DG (●), MGO (●), GO (□), pyruvic acid (▼), glyoxylic acid (▲), and oxalic acid (hexagons).
The plasma samples were diluted in formate buffer (pH 3.0). McLellan and Thornalley (31) applied different derivatization procedures to biological systems and discovered significant interferences from G3P and DHAP by spontaneous elimination of phosphate to form MGO during sample processing, depending on the chosen pH value. The rate of reaction increased with increasing pH. To avoid the degradation of G3P and DHAP, we conducted the derivatization step under strong acidic conditions with perchloric acid. However, under these harsh conditions, we discovered significant degradation of 3-DG-Q, 1-DP-Q, 3-DT-Q, and threosone-Q (data not shown). Especially 3-DG-Q with a half-life of 80 h was rapidly degraded to form the diastereomer 3-DGal-Q (Fig. 4). Mittelmaier et al. (46) already investigated the formation of 3-DGal in peritoneal dialysis fluids under sterilization conditions. They postulated a reaction mechanism that includes a reversible dehydration of free 3-DG leading to 3,4-dideoxyglucosone-3-ene (3,4-DGE). Subsequently, 3,4-DGE undergoes hydration to form 3-DGal. Obviously, this type of reaction also takes place with the corresponding quinoxalines under harsh acidic assay conditions. Whereas with the native α-DC, the carbonyl moiety is the driving force, with the quinoxaline, it is the extension of conjugated unsaturation.

We monitored the degradation of 3-DG-Q in sodium formate solution at different pH values (data not shown). Based on our findings, we chose more gentle conditions and employed a formate buffer (0.4 M, pH 3.0). Here, no degradation of 3-DG-Q at 21 °C for 48 h was observed. In addition, formation of MGO caused by oxidative degradation of nucleic acids and related compounds during the derivatization process is accelerated by strong acids like perchloric acid but negligible with milder acidic treatment (47, 48).

To ensure reliable MGO quantitation with the method described herein, we conducted control experiments at different pH conditions with 100 and 200 μM concentrations of G3P and DHAP, respectively (Table 4). The chosen concentrations were based on the experiments conducted by McLellan and Thornalley (31). The conversion rate at pH 3.0 after 24 h in mol % was below 2% for G3P and below 8% for DHAP, independent from the starting concentration of both. The minor interferences found were inevitable, and the results were comparable with the well established method of McLellan. To ensure the absence of contaminants in water or chemicals used in the assay possibly leading to overestimation, a reactant blank was incubated along with the plasma samples.

Mechanistic Relationship of DHA, 2,3-DKG, and Their Corresponding Quinoxalines under Assay Conditions—Investigation of the derivatization reaction of DHA in sodium formate buffer (0.4 M, pH 3.0) with OPD (0.55 mM) gave rather surprising results shown in Fig. 5 and in the scheme in Fig. 6. Initially, a precursor compound (DHA precursor-Q) with two molecules of OPD was formed. The precursor then converted to DHA-Q. However, after 24 h, no plateau was reached, and the precursor compound was still detectable in significant amounts. A quantitative reaction of DHA with OPD was only achieved after more than 6 days, which is not feasible for the present analytical assessment. In addition, oxidative conditions imposed by OPD lead to formation of DHA from ASA. When 20 μM ASA was incubated under assay conditions, 50 mol % of the ASA was converted to DHA-Q after 6 days, whereas after 24 h, de novo formation of DHA-Q from ASA was negligible (<2%).

In contrast, conversion of 2,3-DKG to its corresponding quinoxaline is completed after 8 h (data not shown). Obviously, DHA is the least reactive of all α-DCs assessed (with the exception of oxalic acid). Consequently, it is important to note that the plasma levels of DHA-Q and DHA precursor-Q given in Table 3 have to be added and account for ~38% of the true DHA plasma content.

To monitor the conversion of DHA precursor-Q to DHA-Q, the precursor was synthesized independently. The authentic
reference DHA precursor-Q was then incubated under assay conditions (Fig. 7). A half-life of 5 h was observed. After 24 h, 81% of DHA precursor-Q was converted to DHA-Q. Residual amounts of DHA precursor-Q (10% based on initial concentration) remained and had to be considered for the calculation of DHA concentration. Interestingly, also small amounts of 2,3-DKG-Q were detected (7%). A reverse reaction of the quinoxalines to form free DHA with subsequent hydrolytic opening of the lactone ring system to yield 2,3-DKG and its corresponding quinoxaline 2,3-DKG-Q can be excluded. Thus, DHA-Q has to be hydrolyzed slowly to form 2,3-DKG-Q directly. To further investigate the relationship of the two quinoxalines, 2,3-DKG-Q was incubated under assay conditions, including the protein precipitation step with TFA after 24 h. The results in Fig. 8 show conversion of 2,3-DKG-Q to DHA-Q, which was significantly triggered at the lower pH during the 1-h precipitation step. 2,3-DKG-Q yields ~25% DHA-Q after the complete workup procedure. Obviously, there is a steady state between DHA-Q and 2,3-DKG-Q with preferred DHA-Q formation as the more stable product under acidic conditions.

Validation of the Proposed Method—The limit of detection and limit of quantitation with all steps of the analytical method included were calculated as described under “Experimental Procedures.” The limit of detection differs from 0.5 to 42.2 pmol/liter. The accuracy of the method was determined as the recovery rate at three different concentration levels and ranged between 82 and 120%. Repeatability was expressed as the coefficient of variation and was 10% or better. The complete validation data are shown in Table 5.

DISCUSSION

Up to now, only GO, MGO, 3-DG, and DHA were quantitated in human plasma using established methods, although the spectrum of α-DCs in vivo is supposed to be far more complex. Major endogenous sources are the degradation of blood glucose and ascorbic acid. As a result, the corresponding degradation products already identified in model experiments should also be present in vivo. Beyond that, lipid peroxidation as well as enzymatic and non-enzymatic pathways irrespective of Maillard reactions contribute to the content of α-DCs. It is speculated that even exogenous sources like foods add to the plasma level of α-DCs.

Because derivatization with OPD is prone to both underestimation of α-DC levels and de novo formation during the derivatization procedure, recovery levels for each compound...
were thoroughly evaluated, and conversion conditions were kept strictly constant. A blank was prepared for each derivatization period to provide quantitative conversion set to monitor the quality of all incorporated reagents. The derivatization along the entire carbon backbone makes this procedure to cover the total amount of free and reversibly bound \(-\text{DCs}\) in a reliable way. Although, the definition of \(-\text{DCs}\) except DHA and oxalic acid, which will be further known as \(-\text{DCs}^*\), is still open to discussion.

**TABLE 5** Coefficient of variation (CV), recovery rates, limit of detection (LOD), and limit of quantitation (LOQ) (all steps of the analysis included) of plasma samples

| Quinoxaline     | CV $^{a}$ | Recovery $^{b}$ (μmol/ml) | LOD $^{c}$ (μmol/ml) | LOQ $^{d}$ (μmol/ml) |
|-----------------|-----------|---------------------------|----------------------|-----------------------|
| Glucosone       | 6         | 0.109                     | 6.1                  | 18.3                  |
| 1-DG            | 4         | 0.112                     | 2.2                  | 6.6                   |
| 3-DG            | 5         | 0.097                     | 5.4                  | 16.2                  |
| Lederer’s glucosone | —         | 0.114                     | 3.5                  | 10.5                  |
| Pentosone       | 7         | 0.098                     | 8.3                  | 24.9                  |
| 1-DP            | 9         | 0.092                     | 2.5                  | 7.5                   |
| 3-DP            | 8         | 0.093                     | 3.5                  | 10.5                  |
| Threosone       | 3         | 0.090                     | 4.2                  | 12.6                  |
| 1-DT            | 3         | 0.091                     | 0.5                  | 1.5                   |
| 3-DT            | 3         | 0.098                     | 2.0                  | 6.0                   |
| MGO             | 8         | 0.106                     | 3.0                  | 9.0                   |
| GO              | 9         | 0.098                     | 6.9                  | 20.7                  |
| Pyruvic acid    | 2         | 0.099                     | 14.1                 | 42.3                  |
| Glyoxalic acid  | 4         | 0.092                     | 42.2                 | 126.6                 |
| Oxalic acid     | 7         | 0.089                     | 6.8                  | 20.4                  |
| 3,4-DKP         | 3         | 0.110                     | 0.8                  | 2.4                   |
| DHA precursor   | —         | —                        | 2.9                  | 8.7                   |
| DHA             | 10        | 0.091                     | 22.9                 | 68.7                  |
| 2,3-DKG         | 6         | 0.119                     | 6.1                  | 18.3                  |

$^{a}$ Repeatability conditions, $n = 6$.

$^{b}$ Replicate analyses, $n = 3$.

$^{c}$ Estimation not possible because analyte was below the limit of detection.

$^{d}$ Estimation not possible because compound is the intermediate in DHA-\(-\text{Q}\) formation.

of the triose phosphates G3P and DHAP are known sources for MGO. Oxidation of acetone in the catabolism of ketone bodies, oxidation of aminoacetone in the catabolism of threonine, degradation of proteins glycated by glucose, and degradation of ASA also contribute to the total plasma content of MGO. GO is formed by DNA oxidation, lipid peroxidation (52), sugar autoxidation (53), and oxidative degradation of glycated proteins (11). GO and MGO are both detoxified mainly by the glyoxalase system with glutathione as a cofactor to give glycolate and lactate, respectively (55). Because \(C_2^*\) and \(C_3^*\) fragments originate by various non-enzymatic and enzymatic pathways besides the breakdown of glucose and ASA, they must be evaluated as very vague parameters from the mechanistic point of view. The same applies for pyruvic acid as a central intermediate in several metabolic processes, which explains its high concentration in plasma.

The \(C_2^*\)-dicarbonyl compound glycosone and its analogues 1-DG and 3-DG do not arise from ascorbic degradation and therefore are markers for glucose-derived \(\alpha\)-\(-\text{DCs}\) in the context of Maillard chemistry (see scheme in Fig. 9). 3-DG is formed non-oxidatively from the Amadori product of glucose via 1,2-enolization and dehydration, whereas 2,3-enolization yields 1-DG (56). Oxidation of the Amadori compound leads to glucosone (37). Besides from the Maillard reaction, an important endogenous route leading to 3-DG formation from glucose is the enzymatic polyol pathway (57). Although the bioconversion of glucose into glucosone by pyrolysis of oxalate for synthetic purposes is described in the literature (58), no such enzymatic pathway has been identified *in vivo* so far.

3-DG is reviewed in the literature as the most abundant \(\alpha\)-dicarbonyl *in vivo* (59) but possesses only a very limited glycation reactivity (37). Thus, the chemistry of 3-DG has to be considered as of minor relevance regarding Maillard processes under physiological conditions. This is supported by the fact that 3-DG-derived \(-\text{AGEs}\) (e.g., \(N^\delta-[5\text{-hydro-5-(2,3,4-trihydroxybutyl)-4-imidazol-2-y]lornithine (3-DG-H1), 6-(2-formyl-5-hydroxyethyl-1-pyrrolyl)-l-norleucine (pyrraline), and \(N^\gamma-2\text{--[[4S-5-ammonio-5-oxido-5-oxopentyl]amino]-5-[(2S,3R)-2,3,4-trihydroxybutyl]-3,5-dihydro-4H-imidazol-4-ylidene]-l-lysinate (DODIC or DOGDIC)]\)) are only of minor quantitative importance in blood plasma. 1-DG and glucosone were identified as the central intermediates leading to \(C_2^*\) and \(C_3^*\)-fragments, respectively (60). Their reductone structure with an \(\alpha\)-oxo-enediol moiety boasts significantly higher reactivity. More generally, this applies to all analog \(C_2^*\) and \(C_3^*-\text{dicarbonyls}\). As reported before, the half-life of 1-DG is about 0.5 h under physiological conditions (61) versus 8 h for glucosone (37) and 40 h for 3-DG (36). Hence, 1-DG is by far the most reactive and thus important \(\alpha\)-DC intermediate regarding glucose-derived \(-\text{AGEs}\). In particular, amine-induced \(\beta\)-cleavage in the presence of lysine leads directly to carboxylic acid amides, a novel class of amide-\(-\text{AGEs}\) (14, 62) that are of quantitative importance *in vivo* (9).

Lederer’s glucosone was not detected in the plasma of healthy subjects, although it is a relative stable non-reductone structure like 3-DG. However, unlike 3-DG, the required enolization along the entire carbon backbone makes this \(\alpha\)-DC susceptible to multiple degradation processes. The existence of...
Lederer’s glucosone in vivo is evident from the detection of its AGE follow-up structure glucosepane at low levels in human blood and extracellular matrix (63, 64).

DHA is a C₆-dicarbonyl structure exclusively assigned to the degradation of ASA, formed by oxidation. As mentioned above, the DHA level in plasma can only be roughly estimated by the analytical assay described herein, mainly because of the incomplete conversion to its corresponding quinoxaline in the given time period. However, DHA in aqueous solution hydrolyzes irreversibly to 2,3-DKG, which is the central intermediate of ASA degradation (65). Therefore, it is important to differentiate between DHA as part of the redox equilibrium ASA-DHA, mediated in vivo by enzymatic pathways, and 2,3-DKG as the direct precursor of fragmentation products with a carbon backbone smaller than C₆.

The yields of 2,3-DKG-Q from DHA in Fig. 5 and from DHA precursor-Q in Fig. 7 after 24 h were almost equal, which certainly excludes de novo formation of 2,3-DKG via hydrolyzation of DHA. In addition, we confirmed that ASA and DHA did not yield any C₄- or C₅-dicarbonyl degradation compounds during the incubation period. Indeed, the plasma level of 2,3-DKG can be estimated fairly accurately, taking into account the comprehensively investigated relationship of DHA-Q and 2,3-DKG-Q under assay conditions. Based on our results, 6.5% of DHA-Q was converted to 2,3-DKG-Q during the derivatization time of 24 h (Figs. 7 and 8). On the other hand, 25% of the formed 2,3-DKG-Q was converted to DHA-Q. The values given in Table 3 for 2,3-DKG are already adjusted accordingly. The adjustments described above are only valid if DHA-Q is in significant excess, which is given by the situation in blood plasma. Only in this case, the change in DHA-Q level due to the conversion is negligible compared with that of 2,3-DKG-Q.

α-DCs with a carbon skeleton smaller than C₆ arise from the degradation of glucose as well as of ASA. As established in previous papers of our group (39, 66), the C₄-dicarboxyls threosone, 1-DT, and 3-DT are formed from both 1-DG and 3-DG.
α-Dicarbonyl Compounds in Vivo

2,3-DKG via β-dicarboxylic cleavage with an C4-enediol as the reactive intermediate. Oxidation of the latter leads to threosone, whereas dehydration at C3 results in 3-DT. The enediol may also undergo isomerization to give 1-DT in an equivalent reaction. As a consequence under deaeration, which represents the situation in vivo, 3-DT was the prominent structure because the reductone 1-DT has to be considered a much more reactive and, thus, short lived intermediate.

It has to be noted that differentiation between 1-threo-pentos-2-ulose (arising from ASA and often referred to as xylosose) and the C4-stereoisomer d-erythro-pentos-2-ulose (arising from glucose) was not possible. For the applied LC-MS/MS method, MRM parameters were determined with 1-threo-pentos-2-ulose. In model incubations of glucose and lysine, a signal with retention time and mass transitions identical to those of 1-threo-pentos-2-ulose was detected (data not shown). By definition, with glucose as precursor, the detected compound has to be d-erythro-pentos-2-ulose. Thus, both structures coelute and are therefore summarized under the term pentosone regardless of its origin. Yet, the plasma levels of pentosone were comparatively high and did not fit into the picture. Decarboxylation is a well established mechanism of ASA degradation and leads to C5-compounds, including pentosone (65, 67), but under physiological conditions, the formation of C4-dicarbonyls from 2,3-DKG is favored (25, 39). Glucose-derived pentosone stems from glucosone by the same mechanism of hydrolytic cleavage with an C4-enediol as the precursor. After isomerization and hydration, formic acid is yielded. Oxidation leads to pentosone, whereas water elimination yields 3-DP or, after 2,3-enolization, 1-DP (60). Considering the need for an oxidation step in order to obtain pentosone from both 2,3-DKG or glucosone, formation of 1-DP and 3-DP should be favored. The results therefore strongly suggest an additional alternative source for pentosone formation in vivo.

In Maillard chemistry, glyoxylic acid is assigned to disaccharide chemistry (38) but can also arise from oxidation of glyoxal (68) and degradation of DHA (65, 69). However, this cannot account solely for the plasma levels, which were 2.5-fold higher than GO and in the same range as 2,3-DKG. An alternative source is the degradation of hydroxyproline with the subsequent glyoxylate metabolism in the human organism, which is rather complex, involving several enzymatic and non-enzymatic reactions, and has been subject to recent investigation (54).

Oxalic acid can originate via β-dicarboxylic fragmentation as well as via oxidative α-DC cleavage from 2,3-DKG and is the main degradation product of the latter (39). In addition, oxalate is also part of the glyoxylate metabolism mentioned above. However, under assay conditions, the dicarboxylic acid oxalic acid is not converted to its corresponding quinoxaline. This is expected, because at the chosen workup pH, the carboxylic acid groups do not show sufficient carbonyl activity to react with OPD. Consequently, there must be alternative precursors for oxalic acid-Q formation to oxalic acid itself. 3,4-DDP is a known intermediate of maltose degradation but was found in neither the glucose nor ASA reaction systems (38). Hence, the origin of the detected 3,4-DDP-Q remains unknown.

The presented LC-MS/MS method provides for the first time the opportunity to identify and quantitate the complete spectrum of relevant α-DCs in plasma of healthy human subjects in a single chromatographic run. For 14 compounds, the plasma levels were determined. Three compounds were below the limit of quantitation but were unequivocally identified. 10 substances have not been reported for human plasma samples before. To evaluate the clinical relevance of the assay described herein, an initial set of 24 uremic patients undergoing hemodialysis was analyzed. Uremia is related to an increase in oxidative and carbonyl stress and thus should lead to a clear shift in the dicarbonyl spectrum. Indeed, most α-DCs were considerably higher in HD patients. Glucose-derived glucosone exhibited a 2-fold increase, which is expected under conditions of elevated oxidative stress. In contrast, 1-DG does not require an oxidation step for its formation and remained nearly at the level of healthy subjects. Interestingly, plasma levels of 2,3-DKG were considerably decreased in HD patients. This must be explained by a significantly accelerated degradation via oxidative pathways. GO and MGO are further compounds of published interest in regard to certain chronic diseases like uremia. A 3-fold elevation of GO and a 4-fold increase of MGO were observed in uremic plasma. This is in line with the literature (27, 29, 32), although the absolute values differ significantly, depending on the respective study. The assessment of α-DC plasma levels depends strongly on the analytical approach, specifically on workup conditions, derivatization procedure, and chromatographic method. Most importantly, the derivatization was conducted in the presence of protein to assess both free and reversibly bound α-DCs. Therefore, a direct comparison of the plasma levels of the present study and those reported previously is not possible.

Nevertheless, validation of the present method for the detection of α-DC compounds in plasma has been carried out extensively regarding the formation of artifacts and mechanistic relationships to exclude false quantitative data. In general, the elevated levels of α-DCs found explain the elevated levels of AGEs in uremia. The newly developed method has now to be extended to follow-up studies with patients with various complications. The results for a wide range of highly reactive carboxyl intermediates will help us to understand the complex mechanisms and factors that influence α-DC formation and consequently open new perspectives regarding the formation and relevance of AGE chemistry in vivo.

Acknowledgment—We thank Dr. J. Schmidt (Leibnitz Institute of Plant Biochemistry, Halle, Germany) for performing accurate mass determination.

REFERENCES
1. Monnier, V. M., Mustata, G. T., Biemel, K. L., Reihl, O., Lederer, M. O., Zhenyu, D., and Sell, D. R. (2005) Cross-linking of the extracellular matrix by the maillard reaction in aging and diabetes: an update on “a puzzle nearing resolution”. Ann. N.Y. Acad. Sci. 1043, 533–544
2. Baynes, J. W. (2001) The role of AGEs in aging: causation or correlation. Exp. Gerontol. 36, 1527–1537
3. Tessier, F. J., and Birlouez-Aragon, I. (2012) Health effects of dietary Maillard reaction products: the results of ICARE and other studies. Amino
1. Glomb, M. A., and Monnier, V. M. (1995) Mechanism of protein modification by glyoxal and glycoaldehyde, reactive intermediates of the Maillard reaction. J. Biol. Chem. 270, 10017–10026

2. Glomb, M. A., and Lang, G. (2001) Isolation and characterization of glyoxal-al-glyoxal oximes. J. Agric. Food Chem. 49, 1493–1501

3. Smuda, M., and Glomb, M. A. (2013) Fragmentation pathways during Maillard-induced carbohydrate degradation. J. Agric. Food Chem. 61, 10198–10208

4. Smuda, M., Voigt, M., and Glomb, M. A. (2010) Degradation of 1-deoxy-o-erythro-hexo-2,3-diolen in the presence of lysine leads to formation of a carboxylamide amide. J. Agric. Food Chem. 58, 6458–6464

5. Ahmed, N., and Thornalley, P. J. (2002) Chromatographic assay of glycation products in human serum albumin glycated in vitro by derivatization with 6-aminoquinolyl-ν-hydroxy succinimidyl-carbamate and intrinsic fluorescence. Biochem. J. 364, 15–24

6. Rabbani, N., and Thornalley, P. J. (2012) Methyglyoxal, glyoxalase 1 and the dicarbonyl proteome. Amino Acids 42, 1133–1142

7. Linden, T., Musi, B., Järkelid, L., Forsbäck, G., Kjellstrand, P., Deppisch, R., and Wieslander, A. (2001) Glucose degradation products in peritoneal dialysis fluids may have both local and systemic effects: a study of residual fluid and mesothelial cells. Perit. Dial. Int. 21, 607–610

8. Tauer, A., Bender, T. O., Fleischmann, E. H., Niwa, T., Jöres, A., and Pischetsrieder, M. (2005) Fate of the glucose degradation products 3-deoxyglucoisone and glyoxal during peritoneal dialysis. Mol. Nutr. Food Res. 49, 710–715

9. Ankrat, N. A., and Appiah-Opong, R. (1999) Toxicity of low levels of methylglyoxal: depletion of blood glutathione and adverse effect on glucose tolerance in mice. Toxicol. Lett. 109, 61–67

10. Degen, J., Hellwig, M., and Henle, T. (2012) 1,2-Dicarbonyl compounds in commonly consumed foods. J. Agric. Food Chem. 60, 7071–7079

11. Arribas-Lorenzo, G., and Morales, F. J. (2010) Analysis, distribution, and dietary exposure of glycolaldehyde and methylglyoxal in cookies and their relationship with other heat-induced contaminants. J. Agric. Food Chem. 58, 2966–2972

12. Gensberger, S., Mittelmaier, S., Glomb, M. A., and Pischetsrieder, M. (2012) Identification and quantification of six major α-dicarbonyl process contaminants in high-fructose corn syrup. Anal. Bioanal. Chem. 403, 2923–2931

13. Schalkwijk, C. G., Posthuma, N., ten Brink, H. J., ter Wee, P. M., and Teerlink, T. (1999) Induction of 1,2-dicarbonyl compounds, intermediates in the formation of advanced glycation end-products, during heat-sterilization of glucose-based peritoneal dialysis fluids. Perit. Dial. Int. 19, 325–333

14. Materials Testing Standards Committee NA 062 of the Deutsches Institut fuer Normung (German Institute for Standardization) (1994) DIN 32645; Chemical Analysis: Decision Limit, Detection Limit and Determination Limit; Estimation in Case of Repeatability; Terms, Methods, Evaluation.
α-Dicarbonyl Compounds in Vivo

Beuth Verlag, Berlin

45. Mittelmaier, S., Fünfrocken, M., Fenn, D., Fichert, T., and Pischetsrieder, M. (2010) Identification and quantification of the glucose degradation product glucosone in peritoneal dialysis fluids by HPLC/DAD/MSMS. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 878, 877–882

46. Mittelmaier, S., Fünfrocken, M., Fenn, D., and Pischetsrieder, M. (2011) 3-Deoxygalactactosone, a new glucose degradation product in peritoneal dialysis fluids: identification, quantification by HPLC/DAD/MSMS and its pathway of formation. Anal. Bioanal. Chem. 399, 1689–1697

47. Chaplen, F. W., Fahl, W. E., and Cameron, D. C. (1996) Detection of methylglyoxal as a degradation product in animal components treated with strong acid. Anal. Biochem. 236, 262–269

48. Chaplen, F. W., Fahl, W. E., and Cameron, D. C. (1998) Evidence of high levels of methylglyoxal in cultured Chinese hamster ovary cells. Proc. Natl. Acad. Sci. U.S.A. 95, 5553–5558

49. Lo, T. W., Westwood, M. E., McLellan, A. C., Selwood, T., and Thornalley, P. J. (1996) Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N-α-acetyllarginine, N-α-acetylcysteine, and N-α-acetyltysine, and bovine serum albumin. J. Biol. Chem. 269, 32309–32305

50. Dhar, A., Desai, K., Liu, J., and Wu, L. (2009) Methylglyoxal, protein binding and biological samples: are we getting the true measure? J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877, 1093–1100

51. Chaplen, F. W., Fahl, W. E., and Cameron, D. C. (1996) Method for determination of free intracellular and extracellular methylglyoxal in animal cells grown in culture. Anal. Biochem. 238, 171–178

52. Fu, M. X., Requena, J. R., Jenkins, A. I., Lyons, T. J., Baynes, J. W., and Thorpe, S. R. (1996) The advanced glycation end product, N-ε-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. J. Biol. Chem. 271, 9982–9986

53. Wells-Knecht, K. J., Zyzak, D. V., Litchfield, J. E., Thorpe, S. R., and Baynes, J. W. (1995) Mechanism of antioxidative glycosylation: identification of glyoxal and arabinose as intermediates in the antioxidative modification of proteins by glucose. Biochemistry 34, 3702–3709

54. Vistoli, G., De Maddis, D., Cipak, A., Zarkovic, N., Carini, M., and Aldini, G. (2013) Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radic. Res. 47, 3–27

55. Thronalley, P. J. (1998) Glutathione-dependent detoxification of α-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. Chem. Biol. Interact. 111, 137–151

56. Niwa, T. (1999) 3-Deoxyglucosone: metabolism, analysis, biological activity, and clinical implication. J. Chromatogr. B Biomed. Sci. Appl. 731, 23–36

57. Mittelmaier, S., Fu "nfrocken, M., Fenn, D., and Pischetsrieder, M. (2011) Detection of free intracellular and extracellular methylglyoxal as a degradation product in animal components treated with strong acid. Anal. Biochem. 236, 262–269

58. Fu, M. X., Requena, J. R., Jenkins, A. I., Lyons, T. J., Baynes, J. W., and Thorpe, S. R. (1996) The advanced glycation end product, N-ε-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. J. Biol. Chem. 271, 9982–9986

59. Vistoli, G., De Maddis, D., Cipak, A., Zarkovic, N., Carini, M., and Aldini, G. (2013) Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radic. Res. 47, 3–27

60. Glomb, M. A., Gobert, J., and Voigt, M. (2010) Dicarbonyls from Maillard degradation of glucose and maltose. ACS Symp. Ser. 1042, 35–44

61. Glomb, M. A., and Pfähler, C. (2000) Synthesis of 1-deoxy-α-erythrose-2,3-diulose, a major hexose Maillard intermediate. Carbohydr. Res. 329, 515–523

62. Glomb, M. A., and Pfähler, C. (2001) Amides are novel protein modifications formed by physiological sugars. J. Biol. Chem. 276, 41638–41647

63. Biemel, K. M., Friedl, D. A., and Lederer, M. O. (2002) Identification and quantification of major maillard cross-links in human serum albumin and lens protein. Evidence for glucosepane as the dominant compound. J. Biol. Chem. 277, 24907–24915

64. Sell, D. R., Biemel, K. M., Reihl, O., Lederer, M. O., Strauch, C. M., and Monnier, V. M. (2005) Glucosepane is a major protein cross-link of the senescent human extracellular matrix. Relationship with diabetes. J. Biol. Chem. 280, 12310–12315

65. Shin, D. B., and Feather, M. S. (1990) The degradation of L-ascorbic acid in neutral solutions containing oxygen. J. Carbohydr. Chem. 9, 461–469

66. Voigt, M., and Glomb, M. A. (2009) Reactivity of 1-deoxy-α-erythrose-2,3-diulose: a key intermediate in the Maillard chemistry of hexoses. J. Agric. Food Chem. 57, 4765–4770

67. Reihl, O., Lederer, M. O., and Schwack, W. (2004) Characterization and detection of lysine-arginine cross-links derived from dehydroascorbic acid. Carbohydr. Res. 339, 483–491

68. Rossner, J., Velisek, J., Pudil, F., and Davidek, J. (2001) Strecker degradation products of aspartic and glutamic acids and their amides. Czech J. Food Sci. 19, 41–45

69. Takagi, M., and Morita, N. (1987) Active oxygens and the peroxidation of linoleic acid catalysed by degraded species of ascorbic acid. Bioelectrochem. Bioenerg. 18, 171–178