Site specific modification of the human plasma proteome by methylglyoxal

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Michael J. Kimzey [a,1], Owen R. Kinsky [a,1], Hussein N. Yassine [b], George Tsaprailis [a], Craig S. Stump [b,c], Terrence J. Monks [a,*], Serrine S. Lau [a,*]

[a] Southwest Environmental Health Sciences Center, Department of Pharmacology & Toxicology, College of Pharmacy, The University of Arizona, Tucson, AZ 85721, United States
[b] Department of Medicine, The University of Arizona, Tucson, AZ 85721, United States
[c] Southern Arizona VA Health Care System, Tucson, AZ 85723, United States

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ABSTRACT

Increasing evidence identifies dicarbonyl stress from reactive glucose metabolites, such as methylglyoxal (MG), as a major pathogenic link between hyperglycemia and complications of diabetes. MG covalently modifies arginine residues, yet the site specificity of this modification has not been thoroughly investigated. Sites of MG adduction in the plasma proteome were identified using LC–MS/MS analysis in vitro following incubation of plasma proteins with MG. Treatment of plasma proteins with MG yielded 14 putative MG hotspots from five plasma proteins (albumin [nine hotspots], serotransferrin, haptoglobin [2 hotspots], hemopexin, and Ig lambda-2 chain C regions). The search results revealed two versions of MG-arginine modification, dihydroxyimidazolidine (R + 72) and hydroimidazolone (R + 54) adducts. One of the sites identified was R257 in human serum albumin, which is a critical residue located in drug binding site I. This site was validated as a target for MG modification by a fluorescent probe displacement assay, which revealed significant drug dissociation at 300 μM MG from a prodan–HSA complex (75 μM). Moreover, twelve human plasma samples (six male, six female, with two type 2 diabetic subjects from both genders) were analyzed using multiple reaction monitoring (MRM) tandem mass spectrometry and revealed the presence of the MG-modified albumin R257 peptide. These data provide insights into the nature of the site-specificity of MG modification of arginine, which may be useful for therapeutic treatments that aim to prevent MG-mediated adverse responses in patients.

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1. Introduction

Methylglyoxal (MG) is a reactive glucose metabolite formed by the spontaneous degradation of triosephosphates, oxidative metabolism of ketone bodies, and catabolism of threonine (Ahmed et al., 2005b). Accumulation of MG by increased formation and/or decreased metabolism creates a state of carbonyl stress (Xue et al., 2012). Excess MG is linked to the pathophysiology of many chronic diseases, including diabetes and complications associated with diabetes (Brownlee, 2001; Beisswenger et al., 2005). One of the critical pathogenic consequences of hyperglycemia in diabetes is a deficit in the removal of reactive dicarbonyls, such as MG (Sheetz and King, 2002). Many factors can trigger the accumulation of MG in vivo, including aging, hyperglycemia, inflammation, oxidative stress, and uremia (Ahmed and Thornalley, 2007).

MG is a reactive dicarbonyl electrophile that forms adducts with guanidine and amino groups of proteins, guanosyl bases of nucleic acids, and amino groups of basic phospholipids. MG reacts up to 20×10^3 times faster with proteins than glucose (Ahmed and Thornalley, 2007). One of the most quantitatively and functionally important MG adducts is the methylglyoxal-derived hydroimidazolone (MG-HI) adduct on arginine, producing a loss of positive charge via formation of a neutral hydroimidazolone (Scheme 1). The pKa of MG-HI is 4.58, a drastic change from the arginine side chain pKa of 12.48 (Wang et al., 2012). MG will also adduct to the side chains on lysine and cysteine residues, but adducts formed on these residues are transient and exhibit faster off-rate kinetics (Lo et al., 1994). Depending on the protein, approximately 0.1% of arginines in plasma proteins bear the MG-HI modification, as determined by exhaustive digestion and quantitation using MS/MS (Duran-Jimenez et al., 2009). Moreover, the relative abundance of MG adducts is estimated to be as high as one MG-derived adduct on 3–13% of all proteins, assuming the average protein contains 26 arginines (Ahmed et al., 2005b). There remains debate over the exact physiological concentration range of MG, since MG is a reactive electrophile, with >99% bound either reversibly or irreversibly with protein (Dhar et al., 2009). The rate of MG formation is estimated

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Mass
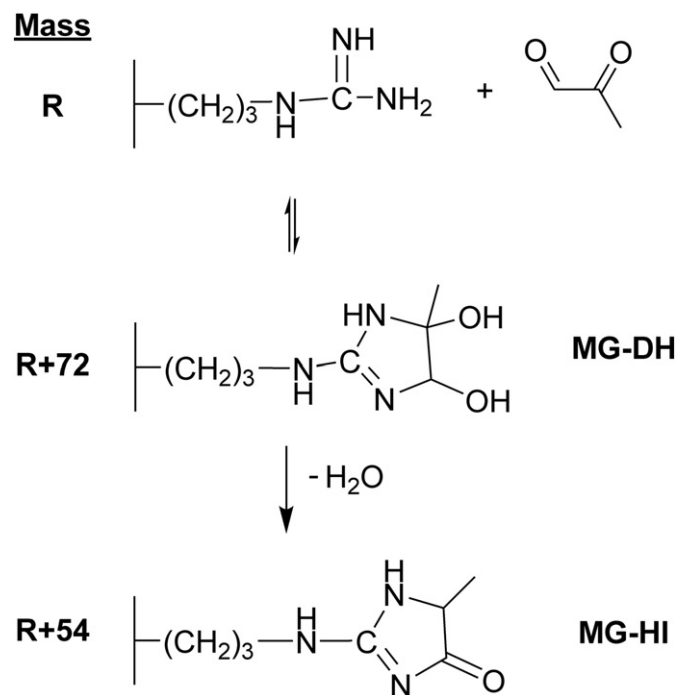
Scheme 1. Chemistry of MG-arginine adduction. MG primarily reacts with arginine residues to form neutral ring structures − MG-DH (dihydroxyimidazolidine, R + 72) and MG-HI (hydroimidazolone, R + 54).

to be 120 μM/day under normoglycemic conditions (Thornalley, 1993). Different analytical platforms to measure the MG-derivatives yield widely different plasma concentrations, ranging from 40 nM to 4.5 μM (Khuhawar et al., 2008; Dhar et al., 2009; Rabbani and Thornalley, 2011; Rabbani and Thornalley, 2014; Thornalley and Rabbani, 2014). These reports also indicate that diabetic patients exhibit higher concentrations of MG relative to normoglycemic patients. Prolonged exposure to low concentrations of endogenous MG throughout the half-life of plasma proteins may have a profound adverse effect in situations of poor glycemic control and increased dicarbonyl stress.

Adduction of proteins by MG is likely to be of physiological importance because of the high levels of MG residues in cellular and extracellular proteins relative to other advanced glycation end products (AGEs) (Ahmed et al., 2005a). Such adducts could be functionally important, especially since arginine residues occur at high frequency in ligand and substrate recognition sites of transport proteins, receptors and enzyme active sites (Thornalley et al., 2003; Kimzey et al., 2011; Bose et al., 2013; Morgan et al., 2013). Of all 20 amino acids, arginine has the highest probability of being located in these active sites, with approximately 20% of active sites containing at least one arginine (Gallet et al., 2000). Receptor binding domain analysis of 80,000 protein sequences estimate that this frequency is a 3.8-fold greater than a completely random distribution would predict (Gallet et al., 2000).

The current study represents a comprehensive proteomics analysis designed to identify potential MG modification sites in human plasma. The strategy permits the sensitive and high-throughput identification of arginine sites in the plasma of human patients. Knowledge of sites of MG modification in the plasma proteome is a prerequisite for determining possible biological and toxicological sequelae of such modifications.

2. Materials and methods

2.1. Materials

HPLC grade solvents were purchased from Sigma-Aldrich unless otherwise noted. Sequencing grade trypsin was purchased from Promega (Fitchburg, WI). Prodan (6-propionyl-2-dimethylaminonaphthalene) was a product of AnaSpec Inc. (Fremont, CA, catalog #88212, lot #64774). Fatty acid-free human serum albumin (catalog # A3782) and 40% methylglyoxal solution were obtained from Sigma-Aldrich. Lipidex-1000 was acquired from PerkinElmer (Waltham, MA).

2.2. Subject selection

Study design and subject recruitment was approved by the University of Arizona Institutional Review Board through the Human Subjects Protection Program (project number 07-0812-01). All subjects provided informed consent. Subjects were recruited from the University Medical Center, University Physicians Healthcare-Kino, Southern Arizona VA Health Care System, and El Rio diabetes and primary care clinics. From this subject pool, a cross-section of 12 subjects was selected for this preliminary study. Six male and six female, with two type 2 diabetes subjects each, were selected. Subjects were not age-matched.

2.3. Sample handling and storage

Blood was collected into heparin coated vacutainer tubes and immediately placed on ice. Blood samples were centrifuged at 4 °C and plasma was aspirated and stored at − 80 °C in 200 μL aliquots. The total time between blood collection and sample storage was less than 1 h.

2.4. Plasma protein fractionation and modification

Plasma (50 μL) from two healthy subjects was diluted to 600 μL with TBS (Tris-buffered saline) pH 7.4 and centrifuged through a 0.2 μm pore size spin filter to remove particulates. The samples were incubated with a concentration of 500 μM MG at 37 °C for 24 h in order to best detect MG-adducted peptides via LC/MS–MS methods. Unmodified plasma from the same patients was used as a baseline control. The samples were buffer exchanged into 100 mM ammonium bicarbonate pH 7.4 using Vivaspin centrifuge concentrators (MWCO 3 K).

2.5. Tryptic digestion

Modified plasma was reduced with DTT (20 mM in 100 mM ammonium bicarbonate pH 7.4) for 30 min at 55 °C and alkylated with iodoacetamide (55 mM in 100 mM ammonium bicarbonate pH 7.4) for 30 min at room temperature in the dark. Protein was then digested with trypsin (protein to trypsin at 50:1 w/w ratio) overnight at 37 °C. Peptides were desalted using HyperSep C18 columns (Thermo Scientific), lyophilized, and re-suspended in 10 μL of 1% TFA immediately prior to LC–MS/MS.

2.6. LC–MS/MS for plasma proteins

LC–MS/MS analysis of in-solution trypsin digested-proteins (Shevchenko et al., 1996) was carried out using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY), following ZipTip (Millipore, Billerica, MA) C18 sample clean-up according to the manufacturer's instructions. Peptides were eluted from a C18 precolumn (100-μm id × 2 cm, Thermo Fisher Scientific) onto an analytical column (75-μm ID × 10 cm, C18, Thermo Fisher Scientific) using a 5% hold of solvent B (acetonitrile, 0.1% formic acid) for 5 min, followed by a 5–7% gradient of solvent B over 5 min, 7–15% gradient of solvent B over 45 min, 15–35% gradient of solvent B over 60 min, 35–40% gradient of solvent B over 28 min, 40–85% gradient of solvent B over 5 min, 85% hold of solvent B for 10 min and finally a return to 5% in 1 min and another 10 min hold of 5% solvent B. All flow rates were 400 nL/min. Solvent A consisted of water and 0.1% formic acid. Data dependent scanning was performed by the Xcalibur v 2.1.0