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Modification of major plasma proteins by acrylamide and glycidamide: Preliminary screening by nano liquid chromatography with tandem mass spectrometry

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ABSTRACT

Environmental and food-borne acrylamide is a suspected carcinogen in humans and is associated with several cancer types. Its biological metabolite, glycidamide, is also harmful to human health. The presence of acrylamide in the living environment makes this toxic chemical an important public health issue. Acrylamide and glycidamide bind with proteins to form protein adducts in metabolic processes. These metabolic adducts can be considered environmental modifications of proteins. This study used a simple proteomic strategy to identify acrylamide and glycidamide adducts bound in major plasma proteins. After simple sample preparation, new protein modifications by acrylamide and glycidamide were identified using nano LC combined with quadruple time-of-flight (Q-TOF) mass spectrometry. This method required only 10 μL of human plasma sample for protein modification survey. Hopefully, this strategy can help to discover protein–acrylamide (or glycidamide) adducts that are biomarkers of human exposure to high-dose acrylamide. These biomarkers may also elucidate the metabolic pathways of acrylamide and glycidamide.

1. Introduction

Acrylamide, which has numerous laboratory applications, is reportedly a human carcinogen [1]. In addition to the acrylamide gel used in biomedical laboratories, this vinyl monomer is also used in the polymer industry to change aqueous solubility, adhesion, and other physical–chemical properties. Other uses of this compound include a grouting agent in construction, a flocculant for wastewater-treatment, and a thickener and film-builder in cosmetics production [2]. Acrylamide, which is also common in starch-containing foods, may be generated in the cooking process when asparagine reacts with reducing sugars [3]. The key factors in this pathway of toxic acrylamide generation, which is known as the Maillard reaction [4,5], are temperature and time. The high acrylamide concentrations that form when food is heated to high temperatures are extremely harmful to human health [6,7]. Many foods and food products (including dried and pickled foods) that contain high acrylamide levels include potato crisps, crisp bread, dried fruits, snacks, coffee, and beer [8–11]. Acrylamide is also present in cigarette smoke [12,13]. Glycidamide, the major metabolite of acrylamide, is produced by epoxidation by the cytochrome P450 [14,15]. The diverse uses of acrylamide raise important health issues. Despite the numerous sources of acrylamide in the living environment, humans exposure is mainly via ingestion of foods and drinks. Hence, acrylamide and glycidamide exist in our bodies could be considered as the "chemical body burden" issue.

Recent studies indicate that acrylamide is associated with many cancers [16], including prostate [13,17], ovarian [18,19], breast [20,21], colorectal [22,23], lung [24], brain [25], and gastrointestinal cancer [26]. High acrylamide concentrations in food may increase cancer risk in humans. Acrylamide and glycidamide, its epoxide metabolite, are highly reactive chemicals that readily form acrylamide or glycidamide–hemoglobin adducts, which are useful biological markers of acrylamide exposure [27–37].

Acrylamide and glycidamide form carcinogenic adducts with hemoglobin, the iron-containing proteins in red blood cells that transport oxygen. Theoretically, acrylamide and glycidamide also form carcinogenic modifications with other proteins. Liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) is a powerful methodology for analyzing analytes in biological samples. The merits of MS/MS are its high sensitivity for trace element analysis, its high specificity for structural identification, and its high sensitivity for detecting multiple compounds simultaneously.

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Today, the varied applications of LC–MS/MS include proteomic study [38,39], metabolomic research [40,41], biomarker discovery [42,43], and clinical diagnosis [44,45].

This study developed a simple procedure for analyzing acrylamide and glycidamide bound in the major proteins in human plasma. Large-scale studies of acrylamide– and glycidamide–protein adducts have not been performed until now. The simple analytical method used in this study combined nano LC with quadruple time-of-flight (Q-TOF) mass spectrometry to predict hypothetical protein binding sites of acrylamide and glycidamide. The method was then used to identify protein modification sites of acrylamide and glycidamide in human plasma. This study is the first to identify acrylamide and glycidamide adducts in major human plasma proteins. The proposed method may help researchers to identify new protein-toxic adduct biomarkers of dietary or environmental acrylamide exposure.

2. Experiment

2.1. Materials and reagents

Acrylamide, glycidamide, dithiothreitol (DTT), and iodoacetamide (IAA) were purchased from Sigma–Aldrich (St. Louis, MO). Chromatographic-grade acetonitrile (ACN), acetone, ammonium bicarbonate, and formic acid (FA) were purchased from Merck (Darmstadt, Germany). The deionized water used in all experiments was obtained using the Millipore Milli-Q system (Bedford, MA, USA). The 3 kDa molecular weight centrifugal cut-off filters were also purchased from Millipore. The FA and ammonium bicarbonate were added to deionized water to obtain final concentrations of 1% and 25 mM. The DTT and IAA solutions were prepared by adding DTT and IAA to 25 mM ammonium bicarbonate to obtain final concentrations of 20 and 25 mM, respectively. The acrylamide and glycidamide solutions were prepared by adding appropriate amounts of acrylamide and glycidamide to 25 mM ammonium bicarbonate to obtain final concentrations of 100 mM. Sequence-grade modified trypsin was purchased from Promega (Madison, WI, USA). The "ProteoSpin" dealbumin kit was purchased from Norgen Biotek Corporation (Thorold, ON, Canada). All other chemical and solvents were analytical grade.

2.2. Apparatus

Tandem mass spectrometry was performed using a Waters/Micromass Q-TOF Global Ultima mass spectrometer with a nanospray source (Waters, Manchester, UK). The system included three micro-liter level pumps (pumps A, B and C), an autosampler, an inline degasser, a sample cooler, and a switch valve. The LC at nano-flow was directly connected to the nanospray ion source. The separation was performed on a Micro-tech Scientific Inc. (Vista, CA, USA) 10 cm reversed-phase C18 nano-flow column (150 μm inner diameter; 375 μm outer diameter; 3 μm particle size). The desalting column (C18 PepMap, 300 μm ID, 5 mm) used for peptide enrichment and trapping was purchased from LC Packings (Sunnyvale, CA, USA).

2.3. Protein alkylation by acrylamide and glycidamide

Because acrylamide can be generated by Maillard reaction [4,5] many foods and food products contain high levels of acrylamide. Hence, the accumulation of this compound in the body via the typical daily diet should be considered a major public health issue. In this study, we hypothesized that the modification of major plasma proteins by acrylamide and glycidamide may form the proteins adducts produced by metabolic processes. The presence of acrylamide and its metabolite in the human body was evaluated in randomly collected human plasma samples. Human plasma samples collected from healthy volunteers (*i.e.*, currently on a standard diet and with no history of smoking or occupational exposure to acrylamide) were stored in heparin tubes. Human plasma (100 μL) mixed with 1 mL acetone was vortexed for 30 s and centrifuged in an Eppendorf at 10,000 rpm for 10 min. After centrifugation, the supernatant was discarded, and the protein pellets were evaporated until dry. Protein residues were dissolved by adding ammonium bicarbonate (25 mM), and the final concentration was adjusted to 5 mg mL^{-1}. For protein alkylation, major plasma protein (10 μL) was mixed with 2 μL of 100 mM acrylamide or glycidamide solution and kept at 25 °C in darkness for 60 min. Finally, 2.5 μL of freshly prepared sequence-grade modified trypsin (20 ng μL^{-1} in a 25 mM ammonium bicarbonate buffer) was added, and the solution was kept at 37 °C for another 16 h. After digestion, 10 μL of tryptic peptide solution was injected into the LC–MS/MS system for protein identification.

2.4. Sample preparation for identifying protein–acrylamide and glycidamide adducts

The dealbumin protocol recommended by the kit manufacturer was observed. Briefly, the spin column was activated by "Column Activation and Wash Buffer". After mixing the human plasma sample (10 μL) with "Column Activation and Wash Buffer" (490 μL), the mixture was added to the 500 μL of diluted plasma sample on the activated column. After the column wash step, approximately 70% of albumin was depleted from the plasma sample (albumin-containing solution). The dealbumin solution was collected and neutralized. For protein precipitation, 100 μL albumin-containing solution and dealbumin solution were mixed with 1000 μL acetone, vortexed (30 s), and centrifuged at 10,000 rpm (10 min). After centrifugation, the supernatant was discarded, and the protein pellets were evaporated to dryness. Protein residues were then re-dissolved by adding 100 μL ammonium bicarbonate (25 mM).

2.5. Preparation of sample mimicking high exposure to acrylamide and glycidamide

To prepare a sample mimicking high exposure to acrylamide and glycidamide, the 50 μL aliquot of dealbumin proteins described above was mixed with 2 μL of 100 mM acrylamide (or glycidamide) and incubated at 25 °C for 60 min. After incubation, the excess acrylamide (or glycidamide) was removed by using a 3 kDa molecular weight centrifugal cut-off filter. After filtering and then centrifuged for 1 min at 10,000 rpm, the flowthrough was discarded. Furthermore, applied 20 μL 25 mM ammonium bicarbonate solution to the filter, centrifuged for 1 min at 10,000 rpm and discarded the flowthrough (repeat this step three times). The solution mimicking high exposure to acrylamide/glycidamide was then collected from the upper layer of the filter for further enzyme digestion.

2.6. Tryptic digestion for identifying protein–acrylamide and protein–glycidamide adducts

For protein identification, a 16 μL aliquot of protein solutions (one solution mimicking high exposure, one albumin-containing solution and one dealbumin solution) and 2 μL of 20 mM DTT (in 25 mM ammonium bicarbonate) were added to an Eppendorf and kept at 25 °C for 30 min. After reduction of the protein disulfide bonds, 2 μL of 25 mM IAA (in 25 mM ammonium bicarbonate) was added. The solution was then kept at 25 °C in darkness for 30 min. Finally, 2.5 μL of freshly prepared sequence-grade modified trypsin (20 ng μL^{-1} in a 25 mM ammonium bicarbonate buffer) was added, and the solution was kept at 37 °C for 16 h. After digestion, 10 μL