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Monitoring nonenzymatic glycation of human immunoglobulin G by methylglyoxal and glyoxal: A spectroscopic study

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ABSTRACT

The accumulation of dicarbonyl compounds, methylglyoxal (MG) and glyoxal (G), has been observed in diabetic conditions. They are formed from nonoxidative mechanisms in anaerobic glycolysis and lipid peroxidation, and they act as advanced glycation endproduct (AGE) precursors. The objective of this study was to monitor and characterize the AGE formation of human immunoglobulin G (hIgG) by MG and G using ultraviolet (UV) and fluorescence spectroscopy, circular dichroism (CD), and matrix-assisted laser desorption/ionization–mass spectrometry (MALDI–MS). hIgG was incubated over time with MG and G at different concentrations. Formation of AGE was monitored by UV and fluorescence spectroscopy. The effect of AGE formation on secondary structure of hIgG was studied by CD. Comparison of AGE profile for MG and G was performed by MALDI–MS. Both MG and G formed AGE, with MG being nearly twice as reactive as G. The combination of these techniques is a convenient method for evaluating and characterizing the AGE proteins.

A major complication of diabetics is their increased risk of infections [1,2]. In the human body, B-cells are responsible for protecting against foreign bodies or infections. The function of the B-cells is mediated by immunoglobulins, proteins that have an antibody function, of which immunoglobulin G (IgG)[1] is the most abundant and widely distributed. Immunoglobulins act as recognizing blocks or structures that identify a foreign body or infection and initiate an immune response [3]. Any alteration to their structure can hinder the function of immunoglobulins and affect their contribution in the humoral immunity. Nonenzymatic glycation of biomolecules is a major issue that leads to several complications in diabetics. Glycation affects protein structure and function and, thus, makes it important for characterization [4]. Extensive reports have appeared on characterizing, quantifying, and monitoring the structural changes of glycated proteins [5–7].

Immunoglobulins are rich in lysine residues, making them a potential target for glycation. Glycation of immunoglobulins occurs under physiological conditions and is increased in diabetes [8–12]. Several studies on the effects of glycation by glucose or fructose on immunoglobulin function have reported that the binding ability of glycated antibodies to their respective antigens has been impaired, thereby compromising the immune response [13–16].

Dicarbonyl compounds, glyoxal (G) and methylglyoxal (MG), are highly reactive metabolites that can act as precursors of advanced glycation endproducts (AGEs) [17–19]. Studies from our laboratory have demonstrated that G and MG can be potent glycators of DNA nucleosides and nucleotides [20,21]. In addition, there is an overproduction of MG in diabetic conditions due to excess glucose metabolism [19,22,23]. It has been reported that MG disrupts immune responses by damaging different components of the immune system [22].

The aim of this study was twofold: (i) to demonstrate the in vitro AGE formation of human IgG (hIgG) by MG and G and (ii) to characterize the AGE adducts using ultraviolet (UV) and fluorescence spectroscopy, circular dichroism (CD), and matrix-assisted laser desorption/ionization–mass spectrometry (MALDI–MS).

In this article, we describe the effect of varying glucose-derived carbonyl intermediate (G and MG) concentrations and incubation time on the in vitro AGE formation of hIgG, and we compare their reactivity using CD and MALDI–MS profiles.

Materials and methods

Chemicals and supplies

Analytical-grade G, MG, hIgG, sodium phosphate monobasic, and sodium phosphate dibasic were purchased from Sigma Chemical (St. Louis, MO, USA). All buffers were prepared with Milli-Q

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[1] *Abbreviations used:* IgG, immunoglobulin G; G, glyoxal; MG, methylglyoxal; AGE, advanced glycation endproduct; hIgG, human IgG; UV, ultraviolet; CD, circular dichroism; MALDI–MS, matrix-assisted laser desorption/ionization–mass spectrometry; HPLC, high-performance liquid chromatography; TOF, time-of-flight.

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purified distilled water (Millipore, Bedford, MA, USA). Disposable UV-transparent cuvettes (12.5 × 12.5 × 36 mm) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Unless otherwise indicated, all other reagents and solvents were of analytical grade and were purchased from Sigma–Aldrich.

Preparation of buffers and reaction mixtures

Unless otherwise indicated, 0.2 M phosphate buffer (pH 7.4) containing 0.02% sodium azide was used in all reactions. Reaction mixtures included varying amounts of G and MG (i.e., 5, 20, and 40 mM) in the presence of a constant amount of IgG (5 mg/ml). Controls included MG or G at 5-, 20-, and 40-mM concentrations and hIgG (5 mg/ml) in the absence of sugar. All reaction mixtures and controls were incubated in the dark at 37 °C for 30 days and then frozen until analysis.

UV and fluorescence spectroscopy

The acquisition of UV spectra was performed at a wavelength of 280 nm with an Ultrospec 2100 pro UV/visible spectrophotometer (GE Healthcare) and fluorescence spectra at an excitation of 340 nm and an emission wavelength of 420 nm using an LS 55 luminescence spectrometer (PerkinElmer) equipped with a thermal cell to maintain the temperature of all samples at 25 ± 1 °C. Unless otherwise indicated, all UV/fluorescence readings were performed in triplicate.

MALDI–time-of-flight–MS

MALDI measurements were performed on a Protein Chip System (Ciphergen Biosystems, Copenhagen, Denmark) operating in the positive linear mode. A nitrogen laser illuminates the sample and initiates the process of ionization and desorption inside the protein chip reader. Sinapinic acid, dissolved in acetonitrile/water (50:50, v/v) at a concentration of 50 mM, was used as the matrix. Samples for measurement were dissolved in 0.1% formic acid at a concentration of approximately 0.1 mg/ml. Equal amounts of sample and matrix solutions were added, and approximately 1–2 μl of the mixture was deposited on the gold plate and allowed to dry before introduction into the mass spectrometer. Analysis was done using the protein chip software.

Circular dichroism

CD studies were carried out on a Jasco J-810 spectropolarimeter equipped with a Peltier thermoelectric-type temperature control system and flow-through high-performance liquid chromatography (HPLC) cell. The instrument was controlled by Jasco's Spectra Manager software. The concentration of hIgG was adjusted to 200 μg/ml with 0.2 M phosphate buffer (pH 7.4). The measurement range was 190–260 nm, and the temperature was kept constant at 25 °C. Cells having a path length of 0.1 cm were used, and analysis

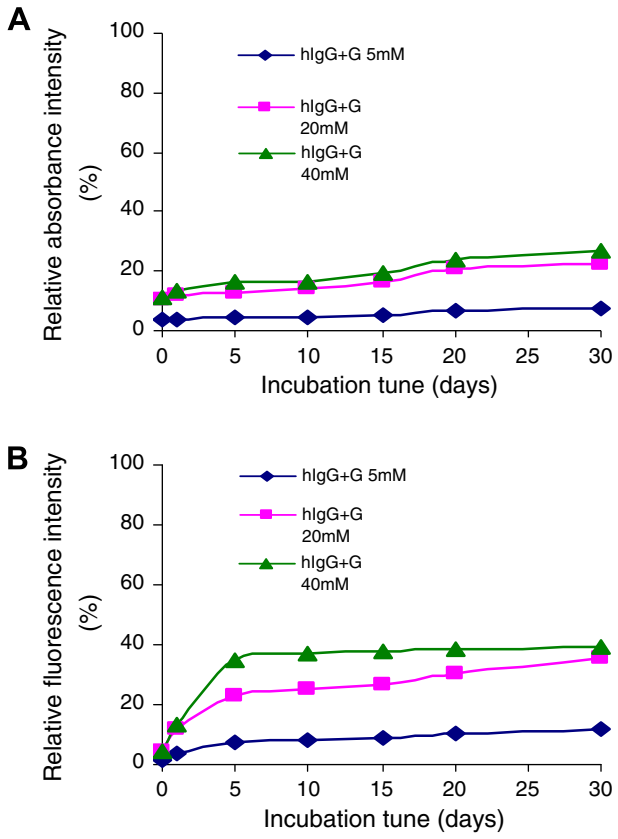

Fig.1. UV and fluorescence spectral profiles of hIgG (5 mg/ml) with glyoxal (5, 20, and 40 mM) at 37 °C for 30 days. (A) UV spectral profiles measured at 280 nm. (B) Fluorescence spectral profiles measured at an excitation wavelength of 340 nm and an emission wavelength of 420 nm. Each point in panels (A) and (B) represents the average of triplicate measurements, and every reading was found to be within 5% of its counterpart triplicate. All readings were compared with the highest reading acquired with methylglyoxal (hIgG + MG 40 mM 30 day) set at 100%.

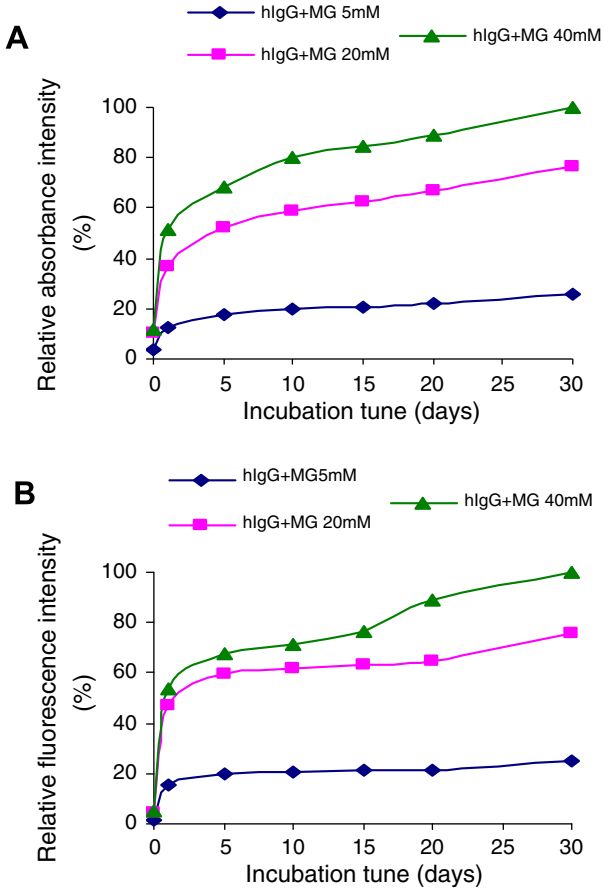

Fig.2. UV and fluorescence spectral profiles of hIgG (5 mg/ml) with methylglyoxal (5, 20, and 40 mM) at 37 °C for 30 days. (A) UV spectral profiles measured at 280 nm. (B) Fluorescence spectral profiles measured at an excitation wavelength of 340 nm and an emission wavelength of 420 nm. Each point in panels (A) and (B) represents the average of triplicate measurements, and every reading was found to be within 5% of its counterpart triplicate. All readings were compared with the highest reading that was set at 100%.