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Monitoring the effect of glucosamine and glyceraldehyde glycation on the secondary structure of human serum albumin and immunoglobulin G: An analysis based on circular dichroism, thermal melting profiles and UV–fluorescence spectroscopy

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Abstract

Glucosamine and glyceraldehyde can nonenzymatically interact with proteins to form advanced glycation endproducts (AGEs). The objective of this investigation was to determine the effects of nonenzymatic glycation by glucosamine and glyceraldehyde on the secondary structure of human serum albumin (HSA) and human IgG and to also demonstrate the in vitro formation of AGEs by these two sugars under different conditions. The formation of AGEs was monitored by capillary electrophoresis, UV and fluorescence spectroscopy. The changes in the secondary structure of HSA and IgG were determined by circular dichroism (CD) and thermal melting (Tm) profiles of the native and glycated proteins. CD and Tm studies revealed that at 40 mM sugar concentration, glucosamine had a stabilizing effect on HSA and destabilized IgG whereas glyceraldehyde destabilized both the α-helical and β-pleated conformations of HSA and IgG, respectively. HSA and IgG structures were also negatively impacted with 40 mM glucose.

Keywords: Advanced glycation endproducts (AGEs); Human serum albumin; Immunoglobulin G; Glucosamine; Glyceraldehyde

1. Introduction

The carbonyl groups of reducing sugars can readily interact with the free amino groups of a protein to form an imine (Schiff base) with the latter then rearranging to generate an Amadori product. Once formed, an Amadori product can undergo cyclization, dehydration, condensation and oxidation to generate a heterogeneous class of compounds referred to as advanced glycation endproducts (AGEs) [1]. As AGEs have been shown to form both in vitro and in vivo, factors influencing their formation have received considerable attention [2,3]. Moreover, with AGEs playing a major role in the pathophysiology of diabetes there has emerged an interest in evaluating the effect of nonenzymatic glycose insults on the function and structure of proteins [4,5]. Also, there has been a plethora of literature on different methods for detecting, separating, quantifying and for monitoring the structural changes of glycated proteins [6–9].

One common method for examining structural changes has relied on CD and Tm profiles whereby conformational alterations have been followed by comparing the CD and Tm characteristics of native proteins with those of the glycated protein counterparts In one study, for example, Muhamadi-Nejad and colleagues used CD and Tm profiles to demonstrate that low concentrations of glucose destabilized HSA while high concentrations (e.g., 27.5 mM) preserved and stabilized the α-helical structure of the protein [10].

The aim of this study was two-fold. First, it was to demonstrate the in vitro formation of AGEs by glucosamine and glyceraldehyde and secondly, to determine the effects of glycation

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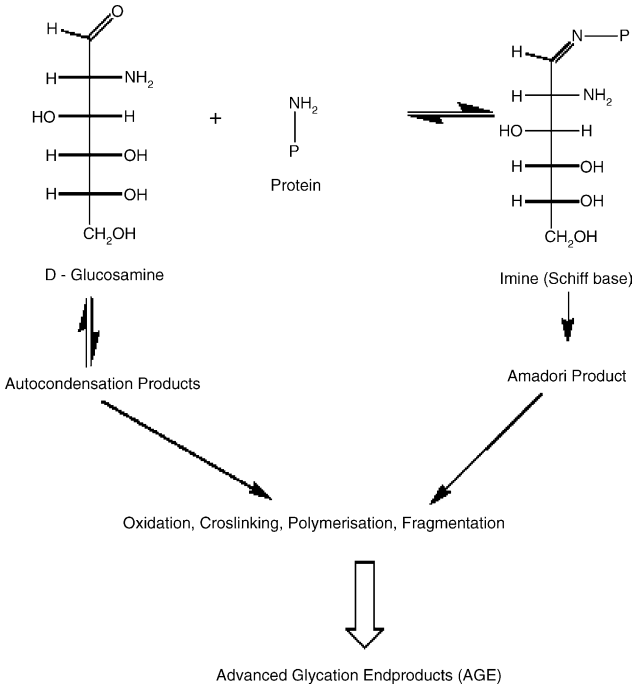

Fig. 1. AGE pathway.

by these two sugars on the secondary structure of human serum albumin (HSA) and human IgG. Why focus on glucosamine? Glucosamine is an unregulated compound that is typically taken in high doses as a dietary supplement for the treatment of osteoarthritis [11–15]; it readily reacts with proteins and forms nonenzymatically induced auto-condensation products that with time transform into AGEs (Fig. 1) [16]. Like glucosamine, glyceraldehyde (GA) is a highly reactive sugar and because it can form in vivo as a by product of the fructose metabolism pathway, its long term effects on proteins can provide valuable information.

In this article we describe the effect of varying sugar concentration and incubation time on the in vitro AGE formation of HSA and human IgG by glucosamine and glyceraldehyde and compare the circular dichroism (CD) and thermal melting (Tm) profiles with those formed by glucose.

2. Experimental

2.1. Chemicals and reagents

Analytical grade DL-glyceraldehyde (GA), D-glucose (Glc), D-glucosamine (GlcN), human serum albumin, human immunoglobulin G, sodium tetraborate, sodium phosphate monobasic, sodium phosphate dibasic were purchased from Sigma Chemical Company (St. Louis, MO, USA). All buffers were prepared with Milli-Q purified distilled water (Millipore, Bedford, MA, USA). Disposable UV-transparent cuvettes (12.5 mm × 12.5 mm × 36 mm) were obtained from Fisher Scientific (New Lawn, NJ, USA).

2.2. Preparation of reaction mixtures

Unless otherwise indicated, 0.2 M phosphate buffer pH 7.2 containing 0.02% sodium azide was used in all reactions. The term sugar has been used broadly to include any of the sugars from the list consisting of GA, Glc or GlcN. Reaction mixtures included varying amounts of sugar (i.e., 2, 20 or 40 mM) in the presence of constant amounts of protein (e.g., either 35 mg/ml HSA or 10 mg/ml IgG).

The sugar concentrations in the study were selected from a broad range of values to determine the effect of glycose concentration on AGE formation. Glucose concentration in normal and healthy individuals is about 2–5 mM in serum. In early diabetics this value can reach up to 20 mM, and in cases of chronic and uncontrolled diabetes can approach 40 mM. The protein concentrations used in this study were based on the physiological concentrations of HSA and IgG in human serum.

Controls included either sugar only (40 mM) or HSA (35 mg/ml) or IgG (10 mg/ml) in the absence of sugar. All reaction mixtures and controls were incubated in the dark at 37 °C for 15 days and were frozen until analysis.

2.3. UV–vis and fluorescence spectroscopy

UV readings were acquired at 280 nm with a Lambda 900 Perkin-Elmer spectrometer (Shelton, CT, USA). The acquisition of fluorescence spectra were accomplished at an excitation of 340 nm and an emission wavelength of 420 nm using an LS 55 luminescence spectrometer (Perkin-Elmer Instrumentation) 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 on duplicate samples.

2.4. Circular dichroism

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 HPLC cell. The instrument was controlled by Jasco's Spectra Manager™ software. The protein concentrations for HSA and IgG were adjusted to 100 and 200 μg/ml, respectively in the presence of a 2.5 mM phosphate buffer, pH 7.2. The measurement range was 190–260 nm and temperature was kept constant at 25 °C. Cells having path lengths of 0.1 cm were used and 10 scans were co-added at a scanning speed of 200 nm/min.

2.5. Thermal melting experiments

The thermal melting measurements were carried out on a DU 800 Spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA) connected to Peltier temperature controller. All samples were heated from 20 to 90 °C at a scan rate of 1 °C/min and absorbance readings were monitored at 280/222 nm for HSA-sugar samples and 280/217 nm for the IgG-sugar samples. The protein concentrations for HSA and IgG were adjusted to 100 and 200 μg/ml, respectively with a 2.5 mM phosphate buffer pH 7.2.