Thermochimica Acta 594 (2014) 24–30

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

Comparative study of thermal domains analyzing of glycated and non-glycated human serum albumin

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ARTICLE INFO

Article history:
Received 27 July 2014
Received in revised form 27 August 2014
Accepted 28 August 2014
Available online 29 August 2014

Keywords:
Human serum albumin
Glycation
Protein domains
Thermal unfolding
Protein stability
Differential scanning calorimetry (DSC)

ABSTRACT

Protein resistance against denaturant agent is a useful property in industrial applications. In the current research, the domains thermal unfolding of glycated human serum albumin (GHSA) and human serum albumin (HSA) were studied under incubation at physiological conditions for 35 days. The domains thermal unfolding of GHSA and HSA were evaluated using differential scanning calorimetry (DSC), circular dichroism (CD) and UV–vis spectroscopy. The results showed that the first energetic domain of GHSA remained after cooling back from 80 °C, while the first energetic domain of HSA disappeared at this temperature. Moreover, the second energetic domain of GHSA kept on after cooling back from 90 °C, but it disappeared in HSA at this temperate. Also, the secondary structure recovery after cooling back in GHSA was higher than HSA. Therefore, according to the obtained results, glucose can act as a stabilizer for HSA domains and prominently domain III because it has more lysine residue for glycation process.

1. Introduction

Protein biological function depends on its native structure. The loss of this folded structure leads to inactivate of a protein. Resistance of a protein against a denaturant agent is a useful property in industry and medicine [1,2]. The study of protein unfolding is the best way to achieve knowledge about the protein folding process. Generally, the protein unfolding happened by two ways: chemical unfolding by organic small molecules and thermal unfolding by changing the effect of temperature [3]. Protein unfolding can be reversible or irreversible. The unfolding reversibility of many proteins has been studied using different denaturant agents (i.e., chemical reagents and temperature) [4].

An irreversible protein unfolding commonly happened in two steps: the first step is reversible unfolding of the native protein (N), and the second one is irreversible change of the unfolded protein (U) to yield a final state (F) which is unable to fold back to the native form. This behavior is well described by the Lumry–Eyring model [5] as the following simplified scheme:

$$N \leftrightarrow U \rightarrow F$$

Glycation is a spontaneous process (non-enzymatic) between free amino groups of protein (i.e., lysine and arginine residues) and carbonyl groups of reducing sugars (i.e., glucose, fructose and ribose) through nucleophilic addition reaction [6]. This process has three stages: early, intermediate, and late [7]. The early stage (reversible reaction) produces Schiff base during many hours, the intermediate stage (reversible reaction) produces Amadori compounds in many weeks [8] and the late stages (irreversible reaction) produces advanced glycation end products (AGEs) through oxidation, dehydration and cyclization [9]. Human serum albumin (HSA) is an abundant blood plasma protein and it is a good model for protein studying about conformation, folding, and ligand-binding of protein [10]. It has multifunctional activities such as antioxidant property and carrier of endocrine compounds and drugs [11]. It also maintains the osmotic pressure and pH, and plays as a role in coagulation and thrombosis [12].

Abbreviations: HSA, human serum albumin; GHSA, glycated human serum albumin; DSC, differential scanning calorimetry; CD, circular dichroism.

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http://dx.doi.org/10.1016/j.tca.2014.08.034
0040-6031/

HSA has three homologous domains which each domain is divided into two sub-domains of A and B having 6 and 4 α-helices, respectively [13]. HSA has 58 lysine residues (from 585 total residues), which Lys-525, Lys-439, Lys-281, Lys-199, Lys-233, Lys-317, Lys-351, Lys-12 and Lys-534 have affinity to interact with carbonyl groups of other compounds such as carbohydrates (glucose) [14]. There are 35 cysteine residues in HSA which 34 of them build 17 disulfide bridges. But Cys-34 is the only cysteine residues that do not participate in any disulfide bridges [14].

The chemical and thermal unfolding of HSA has been investigated in previous studies as a model for protein folding and ligand binding [15]. The reversibility of HSA unfolding has been studied through binding of the fluorescent ligand salicylate [16].

Based on a previous study, the HSA unfolding indicates through the following scheme:

$$N \leftrightarrow E \rightarrow I \rightarrow U$$

where N is the native state, E is the expanded form, I is an intermediate where domain II is unfolded but domain I is intact, and U is the unfolded protein [17–19].

Due to importance of the HSA as a suitable model which has been reported for several studies of proteins, the objectives of present study are: (i) to investigate the domains thermal unfolding and thermal reversibility of glycated HSA (GHSA) at different temperatures, and (ii) to compare the obtained results with untreated HSA.

2. Materials and methods

2.1. Materials

Human serum albumin (HAS, 96%, essentially fatty acid free) was purchased from the Sigma–Aldrich Company. β-D-glucose and 2,4,6-trinitrobenzene sulfonic acid (TNBSA; 0.01%) were purchased from the Fluka Company (Biochemika, Switzerland). All other chemicals were of analytical grade and were used without further purification.

2.2. Preparation of AGE–HSA

HSA at a final concentration of 40 mg/mL (similar to the physiological concentration in the blood) was dissolved in buffer consisting of 50 mM potassium phosphate (pH 7.4), 1 mM EDTA and 1 mM sodium azide. Glycation process was initiated by adding 16.5 mM β-D-glucose to the HSA solution. The incubation of HSA with all reagents was performed under sterile at 37 °C, pH 7.4 and dark environment (physiological-like conditions) for 35 days. The duration of 35 days was selected for incubation because this duration provides sufficient time for the completion of the last stage of HSA glycation to produce AGEs [20]. At the end of each incubation, all samples were dialyzed against 50 mM sodium phosphate buffer (pH 7.4) at 4 °C for 48 h, and then stored at −30 °C. Bicinchoinic acid (BCA) protein assay was used for the determination of protein concentration using a standard curve [21]. The standard curve was generated using bovine serum albumin (BSA). Incubation of each sample was repeated three times and average of results has been reported.

2.3. Free lysine content assay

In other to determine the glycation reaction, the free lysine content was evaluated by TNBSA assay [22]. Briefly, 0.25 mL of the 0.01% (w/v) solution of TNBSA was added to 0.5 mL of protein solution (0.2 mg/mL in 0.1 M sodium bicarbonate, pH 8.5). This mixture was incubated at 37 °C for 2 h. Then, 0.25 mL of 10% SDS and 0.125 mL of 1 N HCl were added to each sample. Finally, the absorbance of the protein solution was recorded at 335 nm. The result is reported as the number of modified lysine residue of HSA (τ) [23].

$$\tau = \frac{(OD_{control} - OD_{modified}) \times 58}{OD_{control}}$$

2.4. Circular dichroism spectropolarimetry (CD)

The far-UV CD measurements were done using J-810 with a 1 mm path length at 25, 60, 70, 80 and 90 °C. Also, the far-UV spectra achieve for every sample after cooling back from 60, 70, 80 and 90 °C at 25 °C. All protein concentrations of HSA and glycated HSA (GHSA) were 0.2 mg/mL in the presence of 50 mM sodium phosphate buffer, pH 7.4. Results of the CD measurements were converted to $[\theta]_\lambda$, the mean residue ellipticity (degree $cm^2\ mol^{-1}$) at wavelength λ (nm) by the following Eq. (1):

$$[\theta]_\lambda = \frac{\theta_\lambda M_0}{10 \times c \times l} \quad (1)$$

where $[\theta]_\lambda$ is ellipticity (milli-degree) at λ, M_0 is the mean residue weight, c is the protein concentration (mg/mL), and l is path length (cm). The CD-spectra were deconvoluted based on the spectra ranging from 200–260 nm using the CDNN CD spectra deconvolution software (version 2.1).

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimeter (DSC) experiments were carried out in Nano-DSC II (model 6100, Calorimetry Sciences Corporation, USA) with a heating rate of 2 °C/min for all scans and rescans from 25 to 95 °C. The heating rate was adequate for thermal equilibrium. A pressure of 2 atm was maintained during all DSC runs. All solutions were degassed prior to DSC runs. The baseline preparation was done using buffer in both sample and reference cells. The concentration of HSA and GHSA samples were 1.5 mg/mL in 50 mM sodium phosphate buffer of pH 7.4.

For investigating the heat effect on the HSA and GHSA structure at different temperature, four unfolding pathways were adopted: (i) increasing the temperature from 25 to 60 °C and then cooling back to 25 °C, (ii) increasing the temperature from 25 to 70 °C and then cooling back to 25 °C, (iii) increasing the temperature from 25 to 80 °C and then cooling back to 25 °C and (iv) increasing the temperature from 25 to 90 °C and then cooling back to 25 °C. So, the initial scan was performed until 60, 70, 80 and 90 °C for each sample and the rescan of all samples was obtained after cooling back to 25 °C. The percent of thermal reversibility was calculated after buffer subtraction using the following standard equation [24]:

$$\text{Thermalreversibility}\% = \left(\frac{\Delta H_{cal,rescan}}{\Delta H_{cal,scan}}\right) \times 100 \quad (2)$$

The extent of thermal reversibility depends on a number of factors such as temperature which the protein was heated during the first scan [25].

For further analysis of DSC profile, the excess heat capacity of all samples used was deconvoluted a two-state model with two independent transitions by CPCalc software [26].

3. Results

3.1. Free lysine content assay

The results of free lysine content assay (TNBSA test) show about 6 number of HSA Lys residues modified after 35 days of incubation time.