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Biochemical, Biophysical, and Thermodynamic Analysis of *in vitro* Glycated Human Serum Albumin

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Abstract—Glycated human serum albumin (HSA) is known to be involved in the pathogenesis of several diseases, and we have therefore investigated possible alterations in HSA on glycation. HSA was incubated for 5 and 20 weeks independently with constant glucose concentration at 37°C under aerobic conditions. Biochemical, spectral, electrophoretic, circular dichroism spectropolarimetric, and thermodynamic analyses confirmed that the structure and stability of HSA is significantly affected on glucose modification. Glycated HSA-AGE-20w showed appreciable elevation (15.8%) in β-sheet structure and decrease in α-helix (10.4%) and random coil (5.7%) structures. Slight changes have also been observed in turns (3.2%) of HSA-AGE-20w. Quenching studies with antioxidants diethylene triaminepentaacetic acid and superoxide dismutase showed inhibition in glycation to the extent of 50-65 and 30-40%, respectively. The novelty of present study is that glycation of HSA can cause induction of secondary and tertiary structure changes that may generate thermodynamically more stable high molecular weight aggregates having remarkably increased β-sheet structure than its non-glycated form. This may interfere with the normal function of HSA, thus contributing to diabetic complications.

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Key words: human serum albumin, non-enzymatic glycation, advanced glycation end products, antioxidants

Albumin is the most abundant protein in human serum, about 35-50 g/liter, and it is prone to glycation [1]. It is a three-domain heart-shaped molecule that comprises a helical structure. Each domain is composed of two subdomains that are stabilized by internal disulfide bridges revealed in its crystal structure [2].

Non-enzymatic glycation of albumin occurs at multiple sites. Glucose can attach to Lys199, Lys281, Lys439, and Lys525 as well as some other lysine and arginine residues, and also at the N-terminal residues of polypeptides [3]. Glycation pathways involve a series of complex multi-step reactions [4] that causes formation of early and advanced glycation end-products (AGEs), which are characterized by auto-fluorescence, brown color, and inter- and intramolecular cross-linking. Although the initial reactions are reversible, subsequent reactions give rise to irreversible cross-linked rearranged products of glucose with proteins, whose structural and functional properties undergo alterations [5].

Ketoamines are similar to but more reactive than Amadori products and participate in AGE formation [6]. Protein bound carbonyl intermediates have also been postulated in the proposed routes to AGEs [7]. AGEs such as pentosidine, imidazolones, and crossline are considered to be good biomarkers of AGE cross-linking in the kidney of diabetic subjects [8, 9]. Glycation of polypeptides render them toxic, perhaps due to the AGE modified cross β-sheet structure or aggregation [10].

Glycation has the potential to induce unfolding and refolding of globular proteins into cross-β structure [11]. Earlier studies on serum albumin indicated similar reaction with glucose yielding a stable glycated form of albumin, which is markedly elevated in diabetes [12, 13].

No study on the stability of protein macromolecules is complete without a measure of their tolerance towards thermal stress. The thermodynamic stability of a protein is a balance between large stabilizing forces derived from

Abbreviations: AGEs) advanced glycation end-products; BSA) bovine serum albumin; DETAPAC) diethylene triaminepentaacetic acid; DNPH) dinitrophenyl hydrazine; HSA) human serum albumin; HSA-AGE-5w) HSA glycated for 5 weeks; HSA-AGE-20w) HSA glycated for 20 weeks; NBT) nitroblue tetrazolium; SOD) superoxide dismutase; TNBS) 2,4,6-trinitrobenzenesulfonic acid.

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noncovalent intramolecular interactions and large destabilizing forces, primarily chain conformational entropy [14]. Other factors affecting protein stability besides glycosylation [15] are conformational flexibility [16] and protein–protein interactions [17].

Our study was designed to evaluate the influence of constant glucose concentration on human serum albumin (HSA) incubated for different time-periods and to explore its biochemical, structural, and thermodynamic characteristics.

MATERIALS AND METHODS

Materials. HSA, dinitrophenyl hydrazine (DNPH), bovine serum albumin (BSA), 5-aminosalicylic acid, diethylene triaminepentaacetic acid (DETAPAC), superoxide dismutase (SOD), and Millipore filter were purchased from Sigma (USA). D-Glucose was obtained from Merck (India). Nitroblue tetrazolium (NBT) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were purchased from SRL Chemicals (India). All other chemicals and reagents were of analytical grade.

Glycation. For the preparation of glycated HSA, protein at a concentration of 15 μM in 20 mM phosphate-buffered saline (PBS), pH 7.4, was filtered through a 0.2 μm Millipore filter and incubated with 0.05 M D-glucose under sterile conditions in capped vials for 5 and 20 weeks at 37°C in the dark. Solution of HSA without glucose served as control. After incubation, the solutions were extensively dialyzed against PBS and stored at –80°C before use. Protein concentration was measured spectrophotometrically at 280 nm using absorption coefficient $\varepsilon^{1\%}_{280nm} = 5.3\ M^{-1}\cdot cm^{-1}$ [18].

Assay of glycated HSA. The glycation of HSA was quantified by a published colorimetric procedure using NBT [19] with slight modification. BSA (10 mg/ml) was incubated with 0.5 M glucose for 15 days at 37°C in 20 mM PBS, which resulted in complete modification of protein with subsequent formation of ketoamines [20]. Native and glycated HSA samples (50 μl) were added to the wells of 96-well microtiter plates in duplicate. One hundred microliters of NBT reagent (250 μM in 0.1 M carbonate buffer, pH 10.35) was added to each well and incubated at 37°C for 2 h. The plate was read in a microplate reader at 550 nm. The amount of glycated HSA in the sample was calculated using the standard curve constructed with glycated BSA.

Determination of protein-bound carbonyl groups. HSA-bound carbonyl groups were estimated as described earlier [21]. Briefly, a 200-μl aliquot (containing 0.1 mg of protein) was mixed with 400 μl of 7 mM DNPH in 2 M HCl. The mixtures were run in duplicate and the control protein samples were devoid of DNPH. After incubation for 1 h at room temperature, the DNP-hydrazones were precipitated by adding 500 μl of trichloroacetic acid (4% w/v) and centrifuged for 5 min at 14,000*g*. The pellet was dispersed in ethanol–ethyl acetate (1 : 1 v/v) in order to remove unreacted DNPH and centrifuged. After four such washes, the pellet was resuspended in 0.6 ml of 6 M guanidine hydrochloride solution in 20 mM phosphate buffer already adjusted to pH 2.3 with trifluoroacetic acid. The hydrazones were dissolved completely only by freezing overnight at –20°C and thawing. From the solution, a 200-μl aliquot was taken into a microplate and read at 379 nm by the microplate reader. The results were expressed as the number of moles of carbonyl per mole of sample protein using a $\varepsilon_{379} = 22{,}000\ M^{-1}\cdot cm^{-1}$.

Fluorometry. Fluorescence was measured using a Hitachi model F2000 spectrofluorometer (Japan). Excitation wavelength of 285 nm was used and emission spectra were recorded in the range of 290-440 nm for tryptophan fluorescence. The fluorescence analysis of AGE-specific pentosidine was also taken with excitation wavelength 375 nm and the peak was observed in the range of 300-400 nm [22]. Excitation and emission slit widths were 10 nm. All solutions of proteins were of the same concentration (60 μM).

Electrophoresis. The electrophoretic behavior of HSA samples was analyzed in denaturing conditions by SDS-PAGE on 10% polyacrylamide gel according to the method of Laemmli [23] in the presence of reducing agent β-mercaptoethanol with boiling for 3 min. The protein was electrophoresed at room temperature at 50 V for 4 h, and the bands were visualized by silver staining [24]. A mixture of protein markers (10 μg/ml) of molecular weight range 14-98 kD was also electrophoresed.

Circular dichroism. CD of native and glycated HSA (2.2 μM) samples were recorded on a Jasco J-810 spectropolarimeter equipped with a temperature controlled sample cell holder attached to a NESLAB model RYE 110 water bath with an accuracy of ±0.10°C. Cuvettes of 1-10 mm pathlength were used. The CD results are expressed in millidegrees. Each spectrum was the average of three scans. CD spectra were recorded over a broad wavelength range (200 to 350 nm) at 5 mm/millidegree sensitivity. All protein solutions were prepared in 20 mM sodium phosphate buffer, pH 7.4. Relative percentages of the secondary structure elements present were estimated by using computer data processor based on the Chen and Yang equation [25].

Thermal denaturation. Native and glucose-modified HSA samples were subjected to thermal denaturation. All the samples were melted from 30 to 96°C at a rate of 1.0°C/min after 10 min equilibration at 30°C. The change in absorbance was recorded at 280 nm and percent denaturation evaluated with increase in temperature.

By assuming that unfolding of protein structure occurs due to breaking of weak noncovalent bonds, the initial and final states of thermal denaturation of protein were related to apparent equilibrium constant, defined as:

$$K_{app} = (A_{obs} - A_N)/(A_D - A_{obs}), \quad (1)$$

where A_{obs}, A_N, and A_D represent absorbance at observed, native, and fully denatured states, respectively. The free energy change, ΔG_D, for the thermal reaction was calculated using the relationship:

$$\Delta G_D = -RT \cdot \ln K_{app}, \quad (2)$$

where K_{app} is the apparent equilibrium constant, R is the gas constant (8.314 J/mol·K), and T is the absolute temperature [26].

Statistical analysis. Values are given as mean ± SD. Multiple comparisons between data were done using Sigma 6.1 software followed by the Student *t*-test. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

The ketoamine moieties formed by the glycation of albumin were measured colorimetrically by using NBT (Fig. 1). The formation of ketoamine was found to be 5.72 ± 0.3 and 5.94 ± 0.4 mol/mol for HSA-AGE-5w and HSA-AGE-20w, respectively. The control non-glycated HSA gave a negligible ketoamine concentration of 0.2 ± 0.1.

The carbonyl contents of HSA-AGE-5w and HSA-AGE-20w were found to be 0.24 ± 0.07 ($p < 0.02$) and 0.31 ± 0.08 ($p < 0.01$) mol/mol HSA, respectively (Fig. 2). As evident, long-term incubation of a sample mixture generated a somewhat higher yield of carbonyl groups. DETAPAC (1 mM) exhibited significant inhibitions of 74 and 83% in carbonyl group formation for HSA-AGE-5w and HSA-AGE-20w, respectively. However, SOD (3 μg/ml) did not show such remarkable inhibition as DETAPAC and gave 33.3 and 40.9% inhibitions in case of HSA-AGE-5w and HSA-AGE-20w, respectively.

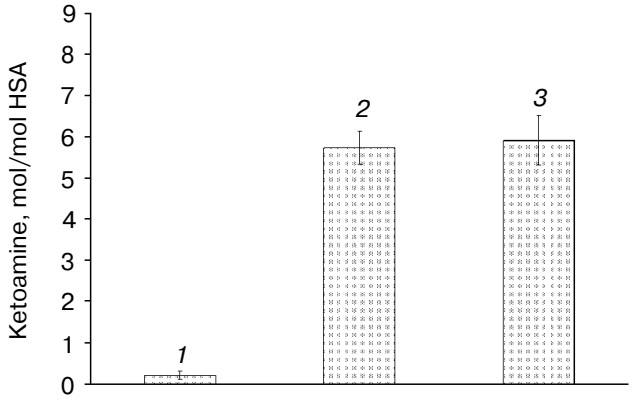

Fig. 1. Formation of ketoamine during incubation of HSA (1 mg/ml) with glucose (0.05 M) for 5 (*2*) and 20 weeks (*3*) at 37°C; *1*) HSA (control) without glucose under similar experimental conditions.

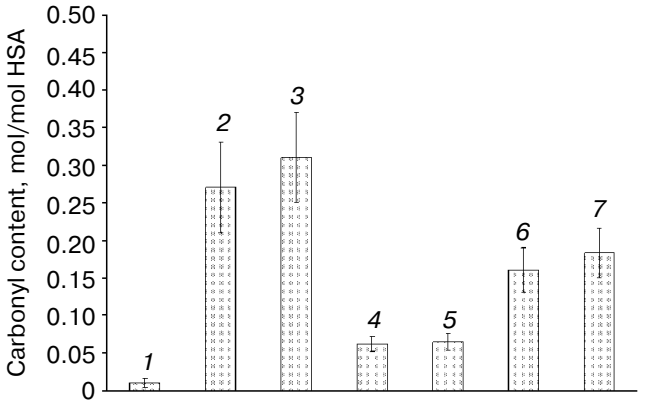

Fig. 2. Protein-bound carbonyl groups present in native and glucose (0.05 M)-modified HSA samples and effect of antioxidants DETAPAC (1 mM) and SOD (3 μg/ml). Control HSA (*1*); HSA plus glucose incubated for 5 weeks (*2*) and 20 weeks (*3*). HSA plus glucose plus DETAPAC incubated for 5 weeks (*4*) and 20 weeks (*5*). HSA plus glucose plus SOD incubated for 5 (*6*) and 20 weeks (*7*).

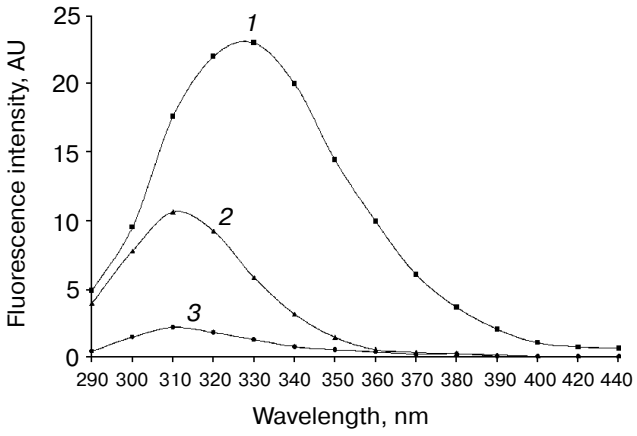

Fig. 3. Tryptophan fluorescence emission spectra of native (*1*) and glycated HSA (60 μM) incubated for 5 (*2*) and 20 weeks (*3*). All the samples were excited at 285 nm and emissions were recorded at 330 nm for native and at 310 nm for both the glycated samples.

The fluorescence emissions were measured under identical conditions. Tryptophan-specific fluorescence analyses were conducted, where both native as well as glycated samples of HSA were excited at 285 nm. The emission maxima of native and glycated samples were found to be 330 and 315 nm, respectively, indicating a blue shift of 15 nm and marked time-dependant reduction in fluorescence intensity on glycation (Fig. 3). Glycated samples were analyzed for fluorescence at the wavelength optimum for pentosidine, an AGE that is a potent cross-linking agent. Significant ($p < 0.001$) parallel increase in the pentosidine-specific fluorescence, with an increase in

incubation period of sample mixtures, proved formation of pentosidine. Inhibition in the pentosidine-specific fluorescence of HSA-AGE-20w due to antioxidant SOD (3 μg/ml) was much more significant ($p < 0.001$) than that observed in HSA-AGE-5w ($p < 0.01$). However, DETAPAC (1 mM) showed potent inhibition ($p < 0.001$) with both of the samples (Fig. 4).

For further characterization of *in vitro* glycation, HSA samples were analyzed by SDS-PAGE in the presence of β-mercaptoethanol (Fig. 5). There is an accompanying decrease in the electrophoretic mobility and smear towards higher molecular weight ranges from 65 to 98 kD (lanes *3* and *4*). HSA samples glycated in the presence of DETAPAC and SOD also showed inhibition in aggregate formation, with SOD exhibiting lesser effectiveness. HSA glycated for 20 weeks in the presence of 5-aminosalicylic acid produced almost indistinguishable pattern in comparison to native HSA.

Changes in protein conformation were also observed in far-UV CD spectra of glycated HSA samples (Fig. 6). To obtain the secondary structure details of native and glycated HSA samples, we adopted CD spectroscopy connected to a computer software program based on Chen and Yang's equation, and the results are given in Table 1. HSA-AGE-5w showed slight increase in β-sheet structure, which is remarkably elevated in HSA-AGE-20w, but showed gradual decrease in α-helix structure. There is slight increase in turns of both glycated samples, but appreciable decrease in random coil structure was found for HSA-AGE-5w and HSA-AGE-20w as compared to their native form.

Thermal denaturation of native and glycated samples of HSA was investigated between 30 to 96°C. For HSA-AGE-5w and HSA-AGE-20w, the unfolding of the protein started at around 50°C, and the melting temperatures (T_m) were computed to be 63.3 and 70.7°C, respectively. Native HSA showed typical protein unfolding evident above 40°C, and the T_m was found to be 54.7°C (Fig. 7). Increase in melting temperature by 8.6 and 16°C for HSA-AGE-5w and HSA-AGE-20w, respectively, confirms thermal stabilization with increase in glycation.

Thermal stability was also assessed using absorbance in UV region with increasing temperature (Table 2). The free energy of thermal denaturation (ΔG_D) for native HSA was found to decrease linearly from 318.15 to 343.15 K. The ΔG_D for native HSA was computed to be −89.615 kcal/deg at 318.15 K and −1.970 kcal/deg at

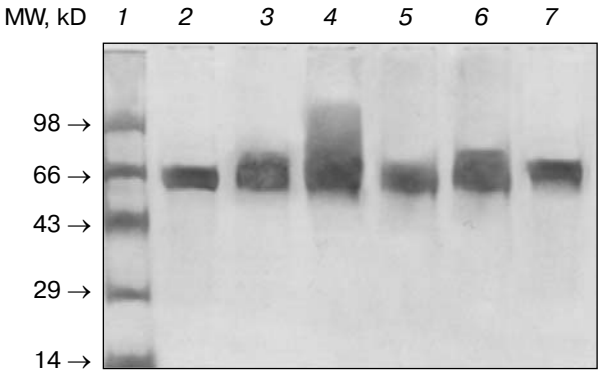

Fig. 5. SDS-polyacrylamide gel electrophoresis in the presence of β-mercaptoethanol of HSA samples. Protein samples (10 μg per lane) were loaded onto 10% polyacrylamide gel. Lanes: *1*) molecular weight markers (14-98 kD); *2*) HSA without glucose; *3*, *4*) HSA plus glucose (0.05 M) incubated for 5 and 20 weeks, respectively; *5*, *6*) 20-week glycated HSA samples incubated with DETAPAC (1 mM) and SOD (3 μg/ml), respectively; *7*) 20-week glycated samples of HSA incubated with 5-aminosalicylic acid.

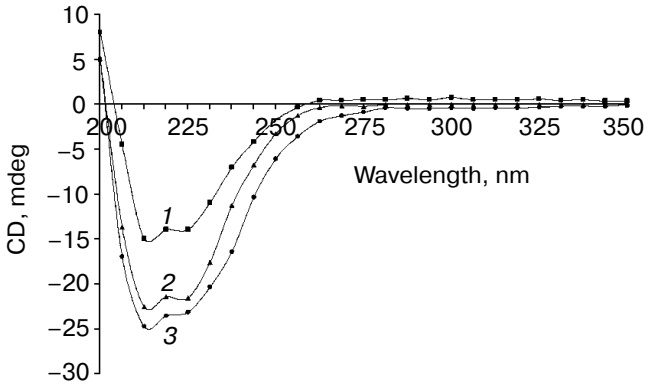

Fig. 6. CD spectra of native and glycated HSA at 25°C. The protein concentration was 2.2 μM in 20 mM sodium phosphate buffer (pH 7.4). Spectra are shown for native HSA (*1*) and glycated HSA incubated for 5 (*2*) and 20 weeks (*3*). The spectra are averages of three experiments.

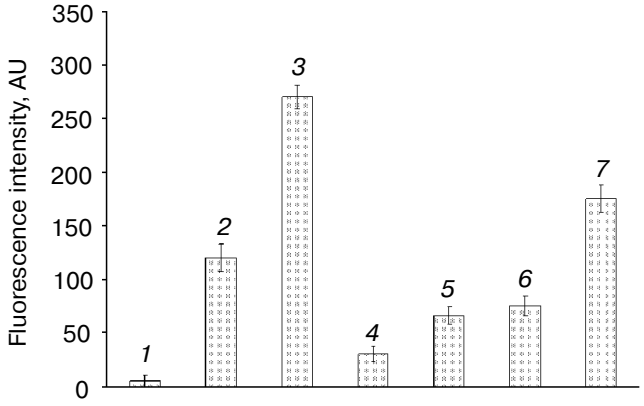

Fig. 4. Formation of pentosidine-specific fluorescence in glycated HSA incubated for 5 (*2*) and 20 weeks (*3*) at 37°C and its inhibition by DETAPAC (1 mM) and the antioxidant SOD (3 μg/ml). *1*) Control sample containing only HSA; *4*, *5*) HSA plus glucose (0.05 M) plus DETAPAC incubated for 5 (*4*) and 20 weeks (*5*); *6*, *7*) HSA plus glucose (0.05 M) plus SOD incubated for 5 (*6*) and 20 weeks (*7*).

Table 1. Secondary structure of native and glycated HSA observed by CD spectroscopy

Conformation	Native HSA	HSA-AGE-5w	HSA-AGE-20w
α-Helix	41.3 ± 1.08*	39.5 ± 0.86 (−4.3%)	37.0 ± 1.13 (−10.4%)
β-Sheet	28.4 ± 0.94	29.9 ± 0.79 (+5.2%)	32.8 ± 1.24 (+15.8%)
Random coil	11.7 ± 0.64	11.4 ± 0.71 (−4.3%)	11.0 ± 0.68 (−5.7%)
β-Turns	18.6 ± 0.76	19.2 ± 1.02 (+2.2%)	19.1 ± 0.89 (+3.2%)

Note: HSA-AGE-5w and HSA-AGE-20w, HSA incubated with glucose (0.05 M) for 5 and 20 weeks at 37°C, respectively.

* The values are in percent. Each sample was read in triplicates and the values are mean ± SD. Parentheses represent the percent change in the secondary structure from the native HSA. Percent decrease and increase are denoted by "−" and "+" sign.

343.15 K. On the other hand, an elevation in temperature (from 343.15 to 374.15 K) showed positive ΔG_D values. The ΔG_D values were computed to be 2.347 kcal/deg at 348.15 K and 10.053 kcal/deg at 371.15 K. In the case of glycated HSA, the ΔG_D was computed to be −9.793 kcal/deg and −11.553 kcal/deg at 313.15 K for HSA-AGE-5w and HSA-AGE-20w, respectively, which decreased linearly to −2.103 kcal/deg at 353.15 K and −2.113 kcal/deg at 363.15 K for HSA-AGE-20w.

DISCUSSION

Our study shows the formation of thermodynamically more stable high molecular weight aggregates as well as induction of β-sheet structure on covalent binding of glucose to HSA.

The reaction with NBT is a standard method for the detection of formed ketoamines, which allows us to characterize the binding of sugar to primary amine on HSA. The finding that the ketoamine generation after 20 weeks of incubation was almost the same as that observed in 5-week incubated sample indicates, that the maximal formation of ketoamine is attained during the initial incubation itself, as they are early non-enzymatic glycation adducts and are important precursors of AGEs [27].

Ketoamines are converted to protein carbonyl compounds via a protein enediol generating the superoxide radical [28], which once formed can be converted to the highly reactive hydroxyl radical (˙OH) via the Fenton reaction [6]. Carbonyl content is considered a reliable indicator of and is by far the most commonly used marker of protein oxidation [29]. Similarly, HSA-AGE-20w and -5w showed considerable quantities of carbonyl groups, but the number of these groups was similar with slight increase in incubation period. This may be due to the possibility that 5 weeks was enough time saturate all free amino groups with sugar, thus no or very few carbonyl groups were left to be formed with further incubation. Incubation with antioxidants DETAPAC and SOD markedly inhibits their formation. These are preventive antioxidants, which intercept oxidizing species before damage can be done. DETAPAC inhibits protein damage by inhibiting the process of glucose autooxidation and by inhibiting hydroxyl radical production [30]. SOD also shows inhibition in the generation of carbonyl groups but to a lesser extent, as the enzyme is an antioxidant catalyzing the dismutation of O_2^- to H_2O_2 [31].

The aromatic amino acids tyrosine and tryptophan exhibit the intrinsic fluorescence of proteins. The emission characteristics are sensitive to fluorophore environments within the 3-D structure of the protein [32]. HSA is a unique protein as it has 17 tyrosine residues and only one tryptophan residue [33]. Loss of tryptophan fluorescence of glycated samples can be ascribed to the destruction of the residue and/or modification of the tryptophan microenvironment on glycation [12].

Another fluorescence spectrum, obtained by exciting glycated HSA samples at 325 nm, served as a biomarker for pentosidine, a well-known AGE cross-linking agent

Table 2. Thermodynamic characteristics of native and glucose-modified HSA samples

Absolute temperature (K)	ΔG_D (kcal/deg)		
	native HSA	HSA-AGE-5w	HSA-AGE-20w
308.15	–	–	–
313.15	–	−9.793	−11.553
318.15	−8.615	−8.176	−10.211
323.15	−6.992	−7.274	−8.532
328.15	−4.880	−6.701	−6.892
333.15	−2.716	−5.760	−6.435
338.15	−2.060	−4.256	−4.442
343.15	−1.970	−3.434	−4.411
348.15	+2.347	−2.650	−3.701
353.15	+2.655	−2.103	−3.213
358.15	+3.730	+2.606	−2.513
363.15	+4.958	+3.888	−2.110
368.15	+6.063	+6.364	+6.725
371.15	+10.053	+7.536	+7.166
374.15	–	–	–

Note: HSA-AGE-5w and HSA-AGE-20w, HSA incubated with glucose (0.05 M) for 5 and 20 weeks at 37°C, respectively.

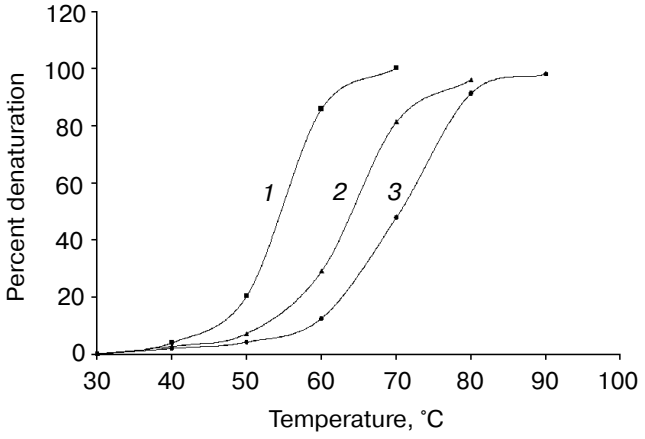

Fig. 7. Melting profile of native (*1*) and HSA glycated for 5 (*2*) and 20 weeks (*3*) at 37°C. Melting temperatures were recorded with an increment of 1°C/min.

[22]. A marked increase is seen in pentosidine-specific fluorescence with increase in incubation period. Restriction of pentosidine-specific fluorescence with DETAPAC and SOD strongly suggests that the free radicals $^{\bullet}OH$ and $O_2^{\bar{}}$ play a major role in the formation of pentosidine.

SDS-PAGE of the glycated HSA samples showed noticeable time-dependent decrease in electrophoretic mobility, which can be attributed to extensive inter- and intramolecular cross-linking of glycated HSA resulting in the formation of high molecular aggregates. However, there is no dimerization of albumin detectable due to formation of a large number of intramolecular as opposed to intermolecular cross-links. Moreover, DETAPAC and SOD, which inhibit the autooxidation of both glucose and Amadori product, decreased the glycation of HSA and thus formation of AGEs [30]. Both the antioxidants inhibited the formation of high molecular weight aggregates. As stated above, DETAPAC is a potent inhibitor of auto-oxidation and can reduce the modifications in HSA induced due to non-enzymatic glycation to a greater extent compared to SOD. HSA-AGE-20w incubated with 5-aminosalicylic acid, an anti-cross-linking agent [30], was found to cleave the AGE cross-links and did not show formation of high molecular weight aggregates. The data confirm the formation of cross-links during glycation.

Circular dichroism spectrophotometry was used to analyze conformational and secondary structural changes in protein due to glycation. In the spectral region of 200-250 nm, the CD signals of proteins are mainly due to the secondary structure [34]. Both the glycated samples showed decrease in α-helix and increase in β-sheet structure. Increase (15.8%) in β-sheet and decrease (10.4%) in the α-helix of HSA-AGE-20w was significantly higher, and HSA-AGE-5w showed only 5.2% increase in β-sheet and 4.3% decrease in α-helix structure as compared to native HSA. Therefore, glycation may cause conversion of α-helix to β-sheet structure of HSA. Moreover, the mean residual ellipticity of both glycated samples is slightly decreased at 260 nm, indicating the presence of less ordered tertiary structure as compared to native HSA. Similar to our findings earlier experimental evidences about the generation of protein aggregates also indicate that at least two different conformational changes are responsible for their formation. Actually large networks of disulfide bridges/forces are operative in assuming and propagating of the β-conformation of a protein structure, thus increasing intermolecular hydrophobic interaction involving a mechanism reminiscent of that already suggested for β-amyloid aggregation.

Additional evidence for the structural perturbations in HSA as a result of its interaction with glucose was gathered by thermal denaturation studies and computation of thermodynamic parameters. Increase in T_m and late onset of unfolding in the case of glycated HSA samples indicate their greater thermal stability compared to the native polymer [35]. Analysis of thermodynamic parameters further reiterates this finding. Native HSA displayed negative values of Gibb's free energy of denaturation (ΔG_D) till 343.15 K. The results suggest tremendous stability exhibited by native HSA. A shift in ΔG_D value from negative to positive above 343.15 K suggests the disruption of noncovalent bonds and the transition of HSA from native to denatured state. In comparison to native HSA, the glycated HSA samples displayed negative ΔG_D values beyond 343.15 K, suggesting that glycated HSA samples are thermodynamically more stable than the native form. The exothermicity was found to increase with increase in glycation and ΔG_D of glycated samples becomes more negative than the non-glycated HSA. This could be attributed to the fact the HSA has multiple positively charged amino groups interacting covalently with the negatively charged sugar residues leading to inter- and intramolecular cross linking of HSA during the course of glycation; these encompass the HSA macromolecule, thereby enhancing conformational stability.

The present study suggests that glycation of HSA causes formation of AGEs via AGE intermediates leading to significant alterations in secondary structure; slight changes in the tertiary structure were also observed, resulting in the formation of thermodynamically more stable high molecular weight aggregates having remarkably elevated β-sheet structure. Therefore, monitoring of glucose concentrations in diabetics may be beneficial in maintaining AGEs in check and reducing diabetic complications.

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