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Sodium dodecyl sulphate modulates the fibrillation of human serum albumin in a dose-dependent manner and impacts the PC12 cells retraction

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Abstract

Protein aggregation is impacted by many factors including temperature, pH, and the presence of surfactants, electrolytes, and metal ions. The addition of sodium dodecyl sulphate (SDS) at different concentrations may play a significant role in the human serum albumin (HSA) fibrillation pathway. Here the heat induction of HSA fibrillation incubated with different concentrations of SDS was evaluated using a variety of techniques. These included ThT fluorescence, Congo red absorbance, circular dichroism, dynamic light scattering, and atomic force microscopy (AFM). To explore HSA surface properties, the surface tension of solutions was measured using Du Noüy Ring method tensiometry. In addition, the criteria of neurite outgrowth and complexity were monitored by exposing PC12 cells to different forms of HSA amyloid intermediates. ThT fluorescence kinetic studies indicated that SDS at low concentrations induced more fibrillation of HSA, while SDS at high concentrations inhibited the fibrillation of HSA. At higher SDS concentrations hydrophobic forces had a significant role whereas at lower SDS concentrations electrostatic forces were dominant. The cell culture studies demonstrated the significant impact of SDS concentration on HSA fibrillation and subsequent neuronal cell morphology. The HSA incubated with low concentrations of SDS inhibited neurite outgrowth and complexity of the PC12 cells, whereas high concentrations of SDS had lesser effect. Thus, SDS acts as a salt at lower concentrations, while at higher concentrations acts as a chaperon, with significant impact on fibrillation of HSA.

Keywords

Fibrillation; Sodium dodecyl sulphate; Human serum albumin; Surface tension; Neuron-like PC12 cells

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1. Introduction

There are a number of proteins and peptides which are able to self-assemble into insoluble and highly ordered cross-beta sheet aggregates termed amyloid fibrils [1–3]. The protein amyloid fibrillar aggregates are associated with the pathogenesis of Alzheimer's, Parkinson's, prion diseases, and type 2 diabetes [3,4]. The functionality of amyloids such as melanosomes and hydrophobins human serum albumin (HSA) has also been clearly identified [3–6]. There is great interest in finding safe and effective anti-amyloid drugs including small organic molecules that bind and inhibit fibrillation, and enzymes which are able to participate in their proteolytic processing [7,8].

The study of amyloid formation provides insight into the conformational energy landscape of proteins involved in this process. Fibrillation generally develops under conditions in which the protein is in a partially destabilized or misfolded/unfolded state competing with the normal folding pathways [9,10]. The protein's primary sequence and the characteristics of its surrounding environment can determine the aggregation process [11,12]. Formation of the amyloid fibrils reflects an alternative to the native packing conformation, in which a polypeptide chain struggles to reduce its surface-accessible area, saturate hydrogen bonding, and reach a denatured global free energy minimum [13]. Salt ions as an environmental determinant may interact directly with charged groups of protein and cause an efficient electro-selectivity series followed by an increase in aggregation [14].

Surfactants also play a significant role in protein conformational changes generally by electrostatic and hydrophobic interactions, as well as specific bindings. These interactions can result in protein folding or unfolding depending on the concentrations of both the surfactant and protein [15]. Sodium dodecyl sulphate (SDS), as an anionic surfactant, consists of both polar and non-polar portions, and induces different characteristics on protein conformation in a concentration dependent manner [16]. At low concentrations, SDS induces the folding of HSA, but at moderate concentrations, below critical micelle concentration, causes its denaturation [17].

Human serum albumin, the major soluble protein constituent of the circulatory system, plays a significant physiological role as the carrier of proteins and regulator of blood pressure [18]. It exhibits a stable structure, which is normally resistant to the formation of fibrils since most of its sequence (>60%) is arranged in an α -helix structure with 17 disulfide bridges. The temperature-induced unfolding of HSA occurs through a sequential two-state transition in the IIA and IIIA subdomains, with a first melting temperature (T_m) at 56 °C and the second at 62 °C, and are lowered by acidic environment [19]. Thus, HSA exhibits a propensity to easily aggregate in vitro, and can be used as a suitable model to study protein aggregation.

Human serum albumin is principally characterized by its remarkable ability to bind a broad range of hydrophobic small molecules including fatty acids and a variety of drugs. These interactions occur at two primary sites [20,21], which overlap with the binding location of endogenous ligands. Changes in protein environment, such as temperature, ionic strength, and additives can increase or decrease protein aggregation [22–24]. The aggregation

pathway can be promoted under conditions that favour partly destabilized monomers and dimers, such as low pH, high temperature, and the presence of chemical denaturants [25]. For example Cu (II) as a metal ion induces the fibrillation of HSA since electrostatic shielding plays a significant role in fibrillation steps [26,27].

Oligomeric intermediates that occur during the fibrillation process are thought to have a role in pathogenicity of amyloid diseases [28]. There is a generic mechanism by which oligomers may interact with cell membrane, and perturb the integrity of membrane architecture [29]. As a consequence of “function follow form” principle, the morphology of HSA plays an important role in the living cells, and may have specific cellular function especially in specialized cells such as neurons. Cumulative evidence suggests that neurite outgrowth and complexity reflect neuronal growth, health, and response to exogenous stress [30,31]. In this study we induced fibrillation of HSA under extreme conditions (low pH and high temperature), and determined the impact of various SDS concentrations on HSA fibrillation. In addition, we characterized the surface properties of HSA during fibrillation using tensiometry in order to determine the contribution of hydrophobic forces. Finally, the impact of different concentrations of SDS on cytotoxicity of HSA fibrillar intermediates were explored in neuronal PC12 cells.

2. Materials and methods

2.1. Chemicals

Human serum albumin 99% and free of fatty acid and globulin (code 3782), Thioflavin T (ThT), Congo red (CR), Dulbecco's modified Eagle's medium (DMEM) and nerve growth factor (NGF) were from Sigma–Aldrich (St. Louis, MO, USA). Horse serum and foetal bovine serum were from Life Technologies (Carls Bad, CA, USA). Glycine, sodium phosphate, potassium phosphate and sodium chloride were from Merck (Germany).

2.2. Human serum albumin fibrillation

Human serum albumin amyloid fibrils and fibril aggregates were obtained by heat denaturation at pH 2. The samples were prepared at a concentration of 10.0 mg/mL by dissolving in glycine buffer (5 mM, pH 2.0) and incubating at 65 °C without agitation to induce fibrillation. The HSA solution concentration was determined using a Shimadzu UV–Vis–NIR recording spectrophotometer (model UV-3100, Japan) at 280 nm with a molar extinction coefficient of 35,219 M⁻¹ cm⁻¹ [32].

2.3. Thioflavin T spectroscopy

The stock solution of ThT was prepared in phosphate buffer (25 mM, pH 6) at a concentration of 2.5 mM and stored at 4 °C. Concentration of ThT was determined with a molar extinction coefficient of 26,600 M⁻¹ cm⁻¹ at 416 nm [33]. At different time intervals, an aliquot (10 µL) of incubated HSA solution was mixed with 490 µL of ThT solution (25 µM) in a quartz cuvette with 1 cm path length. We used a Cary Eclipse fluorometer (Varian, Australia) for fluorescence intensity measurements. The samples were excited at a wavelength of 440 nm, and emission was detected at 482 nm. The excitation and emission slit width were set at 5 nm.

2.4. Congo red binding assays

At each time point, 24 μL aliquot of sample was mixed with 176 μL of CR solution (20 μM), prepared in 5 mM potassium phosphate and 150 mM NaCl (pH 7.4), and incubated at room temperature for 15 min. Absorption spectrums were recorded from 400–700 nm using a UV–Vis–NIR recording spectrophotometer (Carry UV-100, Varian, Australia) and a quartz cuvette with 1 cm path-length.

2.5. Circular dichroism spectroscopy

Secondary structure during fibrillation was analyzed by far-UVCD spectra. We diluted 50 μL of sample solution 2-fold in glycine buffer (5 mM, pH 2.0) in a quartz cell with a 1 mm path length. The CD spectra measurement was recorded on a circular dichroism polarimeter (Model J-810, Jasco, Japan) by scanning the sample from 195 to 260 nm at 25°C using a bandwidth of 1 nm, a step interval of 1 nm, an average time of 0.5 s, and a slit-width of 0.02 nm. The spectra of glycine buffer were subtracted from sample spectra for data analysis.

2.6. Atomic force microscopy

The morphology of HSA fibrils/aggregates incubated with different concentrations of SDS was visualized using atomic force microscopy (AFM) images, which were recorded in non-contact mode with a loop filter of 2 Hz and force of 5.5–22.5 N/m. For each scanning 10 μL of incubated solution (diluted to the concentration of 0.002 mg/mL) was placed on a fresh plate of mica (washed with deionized water and ethanol), followed by drying with nitrogen and kept at room temperature. Each scan was done in an area of $1\text{ }\mu\text{m} \times 1\text{ }\mu\text{m}$ using an AFM (Autoprobe CP Research, Veeco, USA) and conical shape silicon tip with resonance frequency of 190–325 kHz.

2.7. Surface tension measurements

The solution surface tension was measured with a tensiometer (Krüss K100, Germany) using Du Noüy Ring method at 25°C. For each measurement, sample solutions were diluted to a concentration of 0.2 mg/mL and in a volume of at least 2 mL. Surface tension detection was used at appropriate time of incubation according to the ThT fluorescence kinetic profiles for each intermediate including native, oligomer, protofibril, and fibril, before and 1st, 3rd, and 5th days after incubation, respectively. The measurements were done for each intermediate and the average was used for data analysis.

2.8. Dynamic light scattering

Dynamic light scattering (DLS), also known as photon correlation spectroscopy, was used to determine the size of the oligomer forms of incubated protein solutions [34]. Each determination was done with a 500 μL aliquot solution diluted to the concentration of 0.1 mg/mL, using a DLS (size 90Plus, Brookhaven, USA) and quartz cuvette with 1 cm path-length.

2.9. Cell culture and PC12 cell differentiation

Rat pheochromocytoma (PC12) cells were obtained from Pasteur Institute (Tehran, Iran). PC12 cells were grown in DMEM, supplemented with 5% fetal bovine serum, 10% horse

serum, and 1% antibiotic mixture comprising penicillin–streptomycin, in a humidified atmosphere at 37°C with 5% CO₂. Cells were differentiated by incubating with nerve growth factor (NGF; 50 ng/mL) every other day for 6 days in growth medium which was changed three times a week.

2.10. Treatment conditions and morphological analysis of differentiated PC12 cells

Differentiated PC12 cells seeded in 6-wells plates were incubated with 16 mM HSA and its two amyloid intermediates (Protofibril and Fibril) for 6, 9 and 12 h. Protofibril and fibril intermediates of HSA aggregates, incubated alone and with 150 µM and 1 mM SDS, were directly added to culture medium. For morphological analysis, two random images were acquired from each well by phase contrast microscopy (Olympus-IX71, Japan). Quantification was done on a minimum of 50 cells per treatment, in which cell body was the criteria for selection. Processes were completely within the field of view, and the cell body of individual cell was distinct from neighbouring cell bodies. Cells fitting these criteria were analyzed and their neurite outgrowth and complexity parameters including cell body area, average neurite length, neurite width, number of primary neurites, bipolar morphology and the nodes/primary neurites ratio were quantified.

Cell body area was defined as the area of the cell that neurite processes originate from and contains cell organelles. Neurite length was calculated by summing the lengths of the primary process and all associated branches. To establish the average neurite width, the outlines of individual primary neurites were traced. The cell body area calculated by Cell A software and then divided by the length of the neurite. Primary neurites were defined as clear protrusions from the cell body which were greater than 10 µm in length. Cells were considered “bipolar” if they displayed a cell body with one process at each end. To evaluate neurite networks, images were analyzed using the cell counter plug-in to score all branching nodes in each image. Nodes were defined as sites where individual neurites branched or separated neurites contacted each other. All measurements were expressed as proportions, which used the number of cells displaying the characteristic of a sub-population of the total number of cells that met the selection criteria described above.

2.11. Statistical analysis

All the experiments were carried out at least three times, and the final data are represented as the mean (SD) and mean ± S.E.M. Comparison between groups was made by one-way analysis of variance (ANOVA) followed by a specific post hoc test to analyze the difference. Mean differences were considered significant when $p < 0.05$ (* or # $p < 0.05$, ** or ## $p < 0.01$ and *** or ### $p < 0.001$).

3. Results and discussion

3.1. Diminishing of HSA fibrillation with higher concentration of SDS

To gain information about the formation of fibrillar assemblies, Thioflavin T and Congo red were used as markers for detection of amyloid fibrillation, because of their binding tendency to cross beta sheet structures [35]. Fig. 1A shows the ThT fluorescence spectra of incubated HSA under acidic and high temperature conditions. The increasing shift at 482 nm peak

indicated the increase in beta sheet assemblies, in which ThT fluorescence of incubated HSA reached its maximum. Fig. 1B shows the Congo red binding evaluating fibril formation during incubation of HSA for 5 days. There was an increase in intensity of Congo red spectra with more red shift, relative to Congo red spectra alone or incubated with native HSA. Thus, there was an increase in the intensity at 540 nm, which is a characteristic of more Congo red binding to amyloid fibrils [35].

Fig. 1C shows the kinetic of amyloid aggregation for different protein-surfactant solutions by curving the emission of ThT fluorescence at 482 nm versus incubation time. The lack of lag phase indicated that the fibrillation process does not require nucleation, and incubation of HSA with ascending concentrations of SDS resulted in beta sheet assemblies which are nucleation independent. Fig. 1C also shows the lower concentration of SDS (150 μ M) resulted in increased slope and intensity of ThT fluorescence emission kinetics. This indicated the formation of more beta sheets with a higher rate relative to non-SDS condition. However, higher SDS concentration (1 mM) caused a decrease in the slope and intensity of ThT fluorescence kinetic, implying decreased formation of beta sheet structures with a lower rate.

3.2. Different SDS concentrations have different impact on secondary structure

Far-UV CD spectra of solutions of the HSA native form and those incubated for 5 days were considered to study the structures conformed under the effect of SDS and incubation conditions. The spectra of native form showed two minima at 208 and 222 nm, characteristic of helical structures [36]. When the temperature was raised to 65°C the minimum at 222 nm progressively (or partially) disappeared, and $[\theta]$ (molar ellipticity) at 208 nm was also strongly reduced (Fig. 1D). Thus, higher temperature conditions resulted in intermediates that were clearly less helical than the pre-incubated conformations. These changes were followed by different magnitudes for the three different solutions, in the absence and the presence of 150 μ M and 1 mM SDS.

The intensity losses at the 222 nm band indicated that the increase in random coil/ β -sheet conformations was accompanied by a reduction in the α -helical content of the protein structure. In order to have an appropriate insight into the impact of SDS on secondary structure of HSA, the percentages of each structure (alpha-helix, beta sheet, turn and random coil) were calculated using CDNN software. The changes between non-incubated and incubated forms for each sample are represented in Table 1. For non-SDS solution reduction of α -helical content was accompanied with an increase in the content of both α -sheet structure and random coil implying the formation of amyloid-like aggregates in the solution. For HSA incubated with 150 μ M SDS, more α -helix were converted to β -sheet/random coil structures. These results indicated that more amyloid-like structures were formed in the presence of 150 μ M of SDS compared with non-SDS solution. Incubation of HSA with 1 mM SDS caused less conversion of α -helix to β -sheet/random coil structure compared with both non-SDS and 150 μ M SDS, and less amyloid-like structure formation.

3.3. Visualizing of HSA fibrillar structures

For morphological evaluations of HSA structure, the 5 day-incubated solutions of HSA in the absence or presence of 150 μM or 1 mM SDS were used based on ThT fluorescence profiles of incubated HSA in the absence of SDS, which resulted in fibril formation. All SDS concentrations were under the SDS critical micelle concentration (CMC) prepared in glycine buffer at 65°C. The AFM images showed in the first day spherical bead-like shapes emerged indicating the formation of oligomer structures (Fig. 2A), and such species resulted in amyloid-like forms with further incubation. Fig. 2B indicates that by continuing the incubation for 5 days, the strand and un-branched fibrils were formed (typically around 20 nm thickness). For samples incubated with 150 μM SDS, more and thicker fibril-like structures were observed with the thickness of 27 nm (Fig. 2C). However, those incubated with 1 mM SDS had spherical and uncertain shapes, but clearly did not form fibrils (Fig. 2D).

3.4. Contribution of different forces in different concentrations of SDS interacting with HSA

Any change in physicochemical properties of proteins such as conformational changes results in alteration of surface properties [37]. Protein conformational stability and aggregation also correlate with changes in surface tension of solution [38]. Raising the temperature results in weakened hydrogen bonding, however, the hydrophobic interactions become stronger [39]. By breaking the cooperative hydrogen bonds, the helical structures become destabilized. However, the hydrophobic groups will be exposed to the solvent by partially unfolding of the protein, which is favourable to initiate the aggregation process. Surface tension is used for measurement of the hydrophobicity of adsorbed proteins at the air-water interface [39,40]. Decreasing surface tension of solutions is due to denatured protein structure and enhanced exposure of hydrophobic surfaces [41].

Next, we measure surface tension of sample solutions in the air-water interface for native, oligomers, proto-fibrils, and mature fibril forms. For non-SDS solution, there was a partial decrease in surface tension at first day of incubation (Fig. 3A). Following longer incubation, the surface tension of solution increased to 60.62 mN/m (3rd day) and 65.7 mN/m (5th day). In the presence of 150 μM SDS, like non-SDS condition, surface tension of solution followed by a decrease in first day of incubation and then increased on 3rd and 5th days. These results suggest that incubation of HSA in the absence or presence of 150 μM SDS, resulted in the exposure of hydrophobic surfaces to the solvent and caused lower surface tension. The exposure of such hydrophobic surfaces was replaced by hydrophilic surfaces of fibril up on longer incubation and fibril formation. Thus, the hydrophobic patches were buried in the core of fibril and resulted in an increase in surface tension of solution. Although the trends of surface tension alteration, either in the absence or presence of 150 μM SDS, were similar, their intensity were greater in solutions containing 150 μM SDS.

There were a decrease in the surface tension of solution by adding SDS, from 57.93 mN/m (non SDS) to 56.68 mN/m (150 μM SDS), and even further decreased by increasing the concentration of SDS to 1 mM (55.58 mN/m). Human serum albumin solution in the presence of 1 mM SDS followed an ascending increase in surface tension during incubation with a lower value of surface tension after 5 days of incubation. These results indicated

different behaviour of HSA surface properties in the presence of different concentrations of SDS. Dual behaviour of SDS at different concentrations has been previously demonstrated [42]. In lower SDS concentrations, electrostatic forces are dominant, and at higher concentrations hydrophobic forces have a substantial role in interaction with proteins. This is because of the amphiphilic structure of SDS, which has a hydrophobic tail and a negatively charged head group [43].

The concentrations of SDS used here were lower than its critical micelle concentration (CMC), and even lower than SDS concentrations where hydrophobic forces shown to cause protein unfolding. The effective concentrations are variable depending on the size of the target protein [16,42]. Fig. 3A shows lower surface tension of solutions in the presence of 1 mM SDS, implying the contribution of hydrophobic forces to the higher concentration of SDS. Presumably, such hydrophobic forces are the reason for decreased or hindered fibrillation of HSA through shielding of HSA monomers and inhibition of their hydrophobic interactions. At lower concentrations of SDS (150 μ M), the hydrophobic forces were minimal and electrostatic forces were likely responsible for excessive fibrillation.

Oligomers are generally small spherical species [44,45] as viewed by AFM (Fig. 2A). The size of these spherical oligomer aggregates was obtained by dynamic light scattering (Fig. 3B) after 12 h of incubation for different solutions. The diameter of particles for HSA incubated without SDS was about 62 nm. This value increased to 82 nm for HSA incubated with 150 μ M SDS. The incubation of HSA in the presence of 1 mM SDS resulted in decreased diameter of particles to ~67 nm compared with 150 μ M condition. These results indicated the formation of larger particles at lower concentration of SDS compared with higher SDS concentration (1 mM). As indicated above, at different concentrations of SDS different forces dominate, which reveal the dual behaviour of SDS. At lower concentration, SDS caused more aggregation and resulted in larger size particles compared with higher SDS concentration, where the size of particles was near non-SDS condition. However, at higher concentration of SDS the size of particles were larger than non-SDS condition, but as deduced by ThT fluorescence kinetic profile and AFM images, these kind of oligomers had less potential to form fibrillar HSA.

3.5. HSA amyloid intermediates, formed in the presence of lower SDS concentration, adversely affect neurite outgrowth in PC12 cells

The neurite outgrowth properties of PC12 cells were monitored after exposure to protofibril and fibril forms of HSA prepared in the absence or presence of different SDS concentrations. These properties included cell body area, average neurite length and average neurite width, which are indicative of neuronal cell growth. In this manner, the impact of different structures of HSA on the differentiation of neuronal cells was evaluated (Fig. 4A). These HSA structures were resulted from the presence or absence of SDS at various time points, which were chosen as the native (0 h), proto-fibrils (3 days), and mature fibrils (5 days). There was a trend for cell body area to be smaller in PC12 cells treated with HSA solutions incubated for 5 days (fibril) compared with HSA solutions incubated for 3 days (protofibril) (Fig. 4B). These treatments with various form of HSA resulted in significant differences in cell morphologies after incubation for 9 and 12 h. The HSA protein structures

gained after 3 days of incubation with 0 and 150 μM SDS showed time dependent increases in cell body area compared with control and native groups. We found that HSA incubated in the presence of 150 μM SDS for 3 days, where the proto-fibril are mainly formed, could significantly decrease average neurite length in PC12 cells over time (Fig. 4C). Here the neurites were not only shorter but also were wider than those of HSA fibril-treated cultures (Fig. 4D). Interestingly, HSA incubated in the presence of 1 mM SDS for 3 or 5 days showed less effect on PC12 cell neurite outgrowth compared with HSA incubated with 150 μM SDS.

3.6. Differential modulation of neurite complexity by HSA incubated with different concentrations of SDS

Numbers of primary neurites ($>10\ \mu\text{m}$), nodes/primary neurites ratio, and percentage of bipolar cells were measured in order to evaluate neurite complexity of PC12 cells. Time course study of each HSA amyloid intermediate in 150 μM SDS resulted in a clear time dependent reduction in numbers of primary neurites compared with native group and control PC12 cells (Fig. 5A). The PC12 cells incubated with HSA protofibril (150 μM SDS) showed 1.25-fold less primary neurites after 12 h compared with fibril group under same conditions. The number of cells with bipolar morphology, defined as cell body with only two neurites, was significantly increased by the forms of HSA incubated with 150 μM SDS (Fig. 5B). However, we observed less bipolar cells with forms of HSA incubated with 1 mM SDS.

To also investigate the final parameter of complexity, we calculated the ratio of total neurite branching nodes to total number of primary neuritis. Fig. 5C shows the nodes/primary neurites ratio in PC12 cells was gradually decreased in the presence of protofibril and fibril forms HSA prepared in the presence of 150 μM SDS. In contrast, the number of primary neurites and nodes/primary neurites ratios in PC12 cells, incubated with HSA prepared in the presence of 1 mM of SDS, were minimally affected and were similar to no SDS condition. These results are consistent with the data obtained from evaluation of neurite outgrowth.

4. Conclusions

Increasing amounts of evidence suggest that protein aggregation into cytotoxic aggregates is a pathological hallmark of many diseases named “protein conformational diseases”, such as Alzheimer’s disease in which $\text{A}\beta$ is subjected to oligomerization and aggregation [40]. The conditions of the present study (acidic pH and high temperature) induced fibrillation of HSA, and therefore, amyloid intermediates of HSA with structural characteristics similar to $\text{A}\beta$. We previously demonstrated that the low concentrations of SDS play a salt role, whereas at higher concentrations SDS has a chaperone-like function [42]. At higher concentrations, SDS interacts with proteins through its charged head group and shields exposed hydrophobic patches of proteins through its tail [16]. Thus, SDS as a chaperon hindered more hydrophobic interactions among HSA monomers, which initiate fibrillar aggregates. Reduction in surface tension implies higher neurotoxic effects [41], but in the presence of 1 mM SDS lower surface tension was contributed to the more hydrophobic forces at higher SDS concentration. Some studies have shown that lipid molecules may

cause to conformational change of proteins and even amyloid formation in vitro, and SDS was also considered as a good model for anionic phospholipids that may have a significant effect on fibril formation in vivo [46]. To the best of our knowledge this is the first time-dependent investigation of different structural forms of HSA on neurite differentiation. These results indicated that proto-fibril and fibril forms of HSA incubated with low concentrations of SDS caused neurite retraction in PC12 cells. In contrast, HSA incubated with higher concentrations of SDS significantly protected PC12 cells from the negative effects of HSA amyloid intermediates.

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Fig. 1.

Evaluating amyloid aggregation of dissolved HSA (10 mg/mL) in glycine buffer (5 mM, pH 2.0) with different concentrations of SDS incubated at 65°C. (A) ThT spectra of HSA solution in the absence of SDS. (B) Congo red binding of incubated HSA in the absence of SDS. (C) The content of fibril formation was recorded via ThT fluorescence during the incubation time for different HSA-SDS solutions. Represented data are the average of 3 independent measurements. (D) Far-UV CD spectra of solutions containing 0, 150 μ M, 400 μ M, 700 μ M, and 1 mM SDS in the presence of 10 mg/mL HSA before incubation and after 5 days of incubation. The (N) stands for native form and (I) stands for those solutions incubated for 5 days.

Fig. 2.

AFM images of HSA samples incubated for 5 days. (A) First day, spherical shapes as oligomer structures. (B) Incubated in the absence of SDS, long and mature fibrils with visible nodes were present. (C) In the present of 150 μ M SDS, and (D) in the present of 1 mM SDS. Scale bar represents 100 nm.

Fig. 3. Structural analysis of HSA products under various conditions. (A) Surface tension changes for different HSA solutions at the air–water interface after different times of incubation at 25°C. *Significantly different from control. Statistical significances were achieved when $p < 0.05$ (** $p < 0.01$). (B) Dynamic light scattering results for the size of HSA oligomer structured particles after the first day of incubation. Error bars presented standard deviation (SD).

Fig. 4.

Time dependent evaluation of morphological changes in differentiated PC12 cells exposed to amyloid intermediates of HSA incubated with different SDS concentrations. (A) The impact of protofibril and fibril forms of HSA, after incubation with different concentrations of SDS, on neurite outgrowth of differentiated PC12 cells. (B) Cell body area. (C) Average neurite length. (D) Average neurite width was quantified at different times after treatment. Error bars presented standard error of the mean (SEM). *Significantly different from control cells. Statistical significances were achieved when $p < 0.05$ (* or # $p < 0.05$ and ** or ## $p < 0.01$).

Fig. 5.

The effects of protofibril and fibril forms of HSA, after incubation with different SDS concentrations, on neurite complexity of differentiated PC12 cells. (A) Primary neurites per cell. (B) Percent of bipolar cells. (C) The ratio of nodes to primary neurites quantified at different times after treatment. Error bars presented standard error of the mean (SEM).

Significantly different from control cells. Statistical significances were achieved when $p < 0.05$ (or # $p < 0.05$ and ** or ## $p < 0.01$).

Table 1

The percentages of secondary structure variations. HSA solutions incubated with different concentrations of SDS as a surfactant.

Surfactant concentration	α -Helix	β -Sheet	Turn	Unordered
Non-SDS	-16.0404	6.8924	1.5424	7.1204
150 μ M	-21.6622	9.8758	1.8724	9.9724
1 mM	-6.7932	4.17	1.7244	0.852