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Biophysical insight into the anti-amyloidogenic behavior of taurine

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ABSTRACT

In this work, we investigated the inhibitory ability of taurine on the aggregation of Human serum albumin (HSA) and also examined how it controls the kinetic parameters of the aggregation process. We demonstrated the structural alterations in the HSA after binding to the taurine at 65 °C by exploiting various biophysical techniques. UV–vis spectroscopy was used to check the turbidometric changes in the protein. Thioflavin T fluorescence kinetics was subjected to explore kinetic parameters comparing the amyloid formation in the presence of varying concentration of taurine. Further, Congo red binding and ANS binding assays were performed to determine the inhibitory effect of taurine on HSA fibrillation process and surface hydrophobicity modifications occurring before and after the addition of taurine with protein, respectively. Far UV CD and Dynamic Light Scattering (DLS) confirmed that taurine stabilized the protein α-helical structure and formed complex with HSA which is further supported by differential scanning calorimetry (DSC). Moreover, microscopic imaging techniques were also done to analyze the morphology of aggregation formed. Taurine is also capable of altering the cytotoxicity of the proteinaceous aggregates. Molecular docking study also deciphered the possible residues involved in protein and drug interaction.

1. Introduction

Protein misfolding and aggregation is most interesting and challenging topic in industry and human health [1,2]. Amyloids are well defined protein aggregates which are responsible for more than 20 diseases in humans such as Alzheimer, Parkinson, diabetes II and Huntington, etc. [2–5]. Soluble protein aggregates possess cell cytotoxic effect and insoluble aggregates may be undesirable for drug products therefore prevention or retardation is an essential task [6,7]. In addition to protein associated with diseases other proteins such as lysozyme, serum albumins, insulin, etc. also form amyloids under suitable conditions like high temperature, low pH, in presence of surfactants, oxidation, ionic strength and crowding agents [8–10]. Number of evidences are available that proves that morphological and histochemical properties of disease associated or disease unrelated proteins are very similar which suggests that fibril formation is the intrinsic property of all polypeptide [11].

Currently, the research is being done that provoke aggregation inhibition of proteins *in vitro* and *in vivo*. Small molecules such as polyphenols, metal ions, vitamins, nucleotides and synthetic peptides have been reported as anti-aggregation agents [12–14].

Human serum albumin is a globular protein composed of 585 amino acid residues and 17 disulphide bonds. It is the most abundant plasma protein and serves as a carrier protein for the large number of small molecules, fatty acids and drugs [15]. Interaction of HSA with large number of molecules is widely studied because of its role as a carrier molecule. Although HSA is very stable protein but possesses propensity to aggregate provided suitable conditions *in vitro* [9]. Recently, a lot of work has been reported dealing with aggregation inhibition of serum albumin *in vitro* which can serve as a model for designing anti amyloidogenic drugs [16]. Here, we are reporting first time inhibitory effect of taurine on thermally induced aggregation of human serum albumin.

Taurine (2-amino-ethanesulfonic acid) is a ubiquitous small sulphur containing amino acid found in almost all mammals [17]. It can be obtained from egg, meat and seafood or alternatively can be

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synthesised inside the body from methionine and cysteine in presence of vitamin B6 [18]. In humans the main source of taurine is diet and endogenous synthesis is very low further its concentration varies from 1 to 2 mM. Taurine involves in various physiological functions such as balance of neurotransmitters, fat digestion, bile salt formation, calcium transport, osmoregulation and anti-inflammation [19]. Taurine deficiency is associated with epilepsy, depression hyperactivity and anxiety [20]. Moreover, taurine and its analogue also have been reported to be anti-diabetic, inhibit tumor cell growth, neuroprotective and it also reduces neonatal mortality [21–23].

The aim of the present study is to investigate the effect of taurine on the fibrillation of human serum albumin *in vitro*. Diverse array of methodologies ranging from spectroscopy to imaging and molecular docking such as UV–vis spectroscopy, fluorescence spectroscopy, circular dichroism (CD), ThT and Congo red dye binding assay, fluorescence microscopy, dynamic light scattering (DLS) and transmission electron microscopy (TEM) have been employed to unravel inhibitory activity of taurine against fibrillation of HSA. Finally, cell cytotoxicity was measured using MTT assay; for this purpose PC 12 and SH-SY5Y cell lines were used. The results demonstrated that Taurine can inhibit human serum albumin fibril formation *in vitro* moreover it also reduced the cell cytotoxic effect of aggregates. We foresee that our research could pave the way for future work on the designing of suitable molecules against aggregation associated diseases.

2. Materials

Human serum albumin, Thioflavin T (ThT), (ANS), Congo red and taurine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.1. Methods

2.1.1. Preparation of HSA solution

A stock solution of 500 μM was made in 20 mM phosphate buffer pH 7.4 and extensively dialyzed against the same buffer and concentration was determined using a UV–vis spectrophotometer (Perkin Elmer Lambda25) $E^{1\%}_{cm} = 5.3$ at 280 nm. For the preparation of amyloid 100 μM HSA was used and samples were incubated at 65 °C in the presence of 50 mM NaCl for 120 h in a circulating shaking water bath [24]. Rest of the study carried out diluting the protein with the same condition to 10 μM.

2.1.2. pH measurement

pH was determined using Mettler Toledo Seven Easy pH meter (model S20) which was routinely calibrated with standard buffers. The experiments were performed in the 20 mM pH 7.4 sodium phosphate buffer. All preparations used in the experiments were filtered through 0.45 μm Millipore Millex-HV PVDF filter.

2.1.3. ThT fluorescence spectroscopic measurements

A stock solution of ThT was prepared in double distilled water and filtered with 0.2 μm Millipore filter. The concentration of ThT was measured using molar extinction coefficient $\varepsilon_M = 36{,}000\ M^{-1}\ cm^{-1}$ at 412 nm [25]. The protein samples (10.0 μM), in the absence as well as presence of varying concentration of Taurine, were incubated for 120 h at 65°. Post incubation, samples were supplemented with 10 μM of ThT solution and were further incubated for 30 min in the dark. The ThT was excited at 440 nm and spectra were recorded from 450 to 600 nm. The excitation and emission slit widths were set at 10 nm. All data are fitted by using following equation in Sigma plot [26].

$$F = F_i + m_i t + \frac{F_f + m_f t}{1 + e^{-[\frac{t-t_0}{\tau}]}} \quad (1)$$

where F is the fluorescence intensity at time t, and t_0 is the time to attain 50% of maximal fluorescence intensity. $(F_i + m_i t)$ and $(F_f + m_f t)$ represent the initial base line related to the induction time and final constant line, respectively. The apparent rate constant for fibril growth is given by $1/\tau$, and the lag time is calculated by $t_0 - 2\tau$.

2.1.4. Turbidity measurements

Turbidity measurements were performed on a Perkin Elmer double beam UV–vis spectrophotometer model lambda 25 in a cuvette of 1 cm path length. The turbidity of HSA sample incubated in pH 7.4 phosphate buffer with 50 mM NaCl for 120 h at 65 °C in the absence and presence of varying concentration of taurine, ranging from 0 to 1.5 mM was determined by monitoring the change in absorbance at 350 nm. Respective blank corrections were done prior to all experiments. The equilibrium data obtained from turbidity measurements was fitted using Sigma plot 12.0 to single exponential equation [27]:

$$A = A_0 e^{-\Lambda[I]} \quad (2)$$

where A_0 and A are the turbidities at 350 nm in the absence and presence of inhibitor, A is the inhibition constant and $[I]$ is the concentration of inhibitor.

2.1.5. Congo red binding assay

Congo red was dissolved in a 20 mM phosphate buffer (pH 7.4) consisting of 50 mM NaCl and filtered through 0.45 μM membrane filter. The concentration was determined using ε_M $45{,}000\ M^{-1}\ cm^{-1}$ at 498 nm. The protein concentration was fixed at 10 μM. CR (10 μM) were mixed at a molar ratio of 1:1 with protein in the absence and presence of Taurine (incubated for 120 h at 65 °C) and kept for 15 min. The absorbance spectra (400–700 nm) of the samples were recorded with a UV–vis spectrophotometer (Perkin Elmer Lambda 25) in a 1 cm path length cuvette.

2.1.6. ANS fluorescence measurements

The steady-state fluorescence measurements were performed on Schimadzu spectrophotometer (RF-5301 PC). Both excitation and emission slits were set at 5 nm. For ANS binding experiment, protein samples (incubated for 120 h at 65 °C) at pH 7.4 were incubated with 50 fold molar excess of ANS for 30 min at 25 °C in dark. The excitation wavelength for ANS fluorescence was set at 380 nm and the emission spectra were recorded from 400 to 600 nm. The protein concentration was fixed at 10 μM.

2.1.7. Far-UV CD measurements

The circular dichroic measurements were performed on a JASCO spectropolarimeter (J-815) with a thermostatically controlled cell holder attached to a peltier with multitech water circulator. The experiments were carried out with HSA (10 μM) incubated at 65 °C for 120 h in the absence and presence of 750 μM of taurine and HSA (10 μM) incubated at 25 °C for 24 h in the absence and presence of 750 μM of taurine. Spectra were scanned in the range of 200–250 nm in a cuvette of 0.1 cm path length. Each spectrum was an average of three scans. In all CD measurements, the HSA concentration was invariable. The results were expressed as MRE (mean residue ellipticity) in deg cm^2 $dmol^{-1}$, which is given by:

$$\text{MRE} = \frac{\theta_{obs}(mdeg)}{10 \times n \times Cp \times l} \quad (3)$$

where θ_{obs} is the observed ellipticity in degrees, C_p the molar fraction and l is the length of the light path in centimeter. The spectra