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Elucidating the role of surfactant dispersed CNTs towards HSA fibrillation *in vitro* — A multiple spectroscopic approach

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ABSTRACT

Nanoparticles could act as the efficient catalyst or the inhibitor towards the fibrillation pathway of biomolecules. In the present work, spectroscopic approaches including Thioflavin T fluorescence, Congo red dye absorbance, tryptophan fluorescence emission, three-dimensional fluorescence and circular dichroism spectra has been used to detect and evaluate the fibrillation induced conformational changes in HSA in the existence of CNTs. CR dye absorbance showed the red shift from 495 to 500 nm upon binding with fibrils. Similarly, ThT fluorescence emission upon the excitation at 450 nm showed the gradual increase in the intensity that conveyed the enhanced formation of fibrils in the presence of CNTs. Trp emission maxima obtained at 344 nm gradually decreased with no prominent shift in the fibrillar samples. Changes in the 3D spectral peaks 1 and 2 of native and fibrillar solutions suggested the alteration in the aromatic amino acid residues micro-environment. CD results showed increased percentage contents of beta-sheet structures in the fibrillar solutions interacted with CNTs. Hence, the research outcome of the study conveys a promoting role of surfactant detached SW and MWCNTs against HSA fibrillation *in vitro*.

1. Introduction

Carbon nanotubes (CNTs) occupy a preeminent position among carbon-based nanomaterials. They possess unique optical, electrical and mechanical properties with the potential application in various sectors including nanocomposite, CNT based biosensors and in the electronic components such as the field emission devices and probe tips [1–5]. The conductive nature of the CNTs is due to the delocalization of the π-electrons [6]. CNTs Vander Waals interaction energy of *ca.* 500 eV/μm tends them prone to entanglement and bundling and renders their dispersion to be challenging [7]. Amphiphilic surfactants were found to be capable for dispersing CNTs with the integrity and intrinsic properties maintained [8–10] and introduced application in nanotube-based electronic devices, composites, biomedical *etc.* [11–13]. Hence, the improved scope of CNTs in diversified applications creates concern towards their potential toxic effect towards the health and environment.

Nanosize range of particles facilitates their entry into human body most efficiently through inhalation, dermal deposition and ingestion. In specific, CNT possesses high aspect ratio that could agglomerate into structures of micrometer range [14–20]. Especially, single-walled carbon nanotubes (SWCNTs) are hydrophobic and tubular nanostructures with the diameters ranging from 0.4 to 3.5 nm that aids in their entry into the cytoplasm and nucleus by passing through the lipid bilayer [28,29]. Hence, both the biosafety and the biocompatibility of the CNTs towards the *in vivo* biomedical applications should be stressed enough for understanding their interaction behavior with human body.

Current area of research in protein science has targeted towards the understanding of biological macromolecules' interaction with that of the nanoparticles. Protein fibrillation relates to several human diseases [30,31]. Few studies have suggested that the protein binding on the nanosurfaces causes the alteration in the protein folding and aggregation mechanism [21,22]. Nanoparticles including fullerene [23], CNTs [24,25], polymeric and fluorinated ones [26,27] have been reported to cause significant variation in the amyloid fibrillation pathways of biomolecules investigated based on their physicochemical properties. Nanoparticles could either inhibit or promote amyloid formation by increasing or decreasing the lag phase time for the nucleation to occur [25,26]. In turn, protein's intrinsic stability also affects the surface-mediated nucleation process. Hence, in the present work, we have investigated the effect of surfactant detached single and multi-walled carbon nanotubes against the fibrillation of human serum albumin formed *in vitro*.

2. Materials and methods

2.1. Chemicals

Single and multi-walled carbon nanotubes was purchased from Sisco Research Laboratories Private Limited, Mumbai. Tween 20,

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human serum albumin, Thioflavin T and, Congo red, the amyloid staining dyes were obtained from Sigma-Aldrich, USA. Protein solutions were prepared with phosphate buffer of 0.1 M. CNTs were dispersed using ultra sonicator bath for 30 min in the same buffer prior to the experiment.

2.2. Fibril formation

HSA fibrils were prepared by dissolving HSA in Tris-HCl buffer, with the addition of 50% (v/v) ethanol, and followed by the incubation at 37 ° C continuously for five days to attain stable oligomer formation [32,33]. To check the role of CNTs against the fibril formation, surfactant dispersed SWCNTs and MWCNTs of increasing concentrations ranging from 2 to 10 mg/l has been added to the incubated systems. Hence, HSA fibrils that were formed only in the presence of ethanol were kept as the control, and the other solutions induced with CNTs were considered as the test.

2.3. Spectroscopic and microscopic studies

To detect HSA fibril formation, the solutions were incubated with Thioflavin T and Congo red dye for 5 min before measurement. UV absorbance spectrum of the fibrillar samples was measured in the wavelength range from 200 to 600 nm using double beam spectrometer (Systronics, India), with the resolution of about 0.1 nm. ThT fluorescence has been measured with spectrophotometer F-7000 FL (Hitachi, Japan) by fixing the excitation wavelength at 450 nm and the emission spectrum reported from 465 to 600 nm. Tryptophan emission pattern of the fibrils was measured with the excitation wavelength fixed at 295 nm and the emission spectrum obtained has been reported from 300 to 500 nm. Three-dimensional spectra of fibrils have also been measured in the excitation and emission wavelength range from 200 to 900 nm. CD spectra ranging from 200 to 320 nm have been measured with Jasco J-715 CD spectrophotometer and the quantitative estimation of the secondary structural contents was determined using the Jasco software (Tokyo, Japan). Fluorescence images of fibrils were captured using a Leica DM 2500 M microscope.

3. Results and discussion

Amyloid fibril formation from alpha-globular proteins including BSA, HSA and myoglobin at the *in vitro* condition has been reported earlier [34]. Such aggregation of serum albumins would be promoted only under certain parameters that favor the formation of the partly destabilized monomers and dimmers. Incubation of HSA at low pH, higher temperature and in the existence of chemical denaturants induces its fibrillation with the conformation getting transition from alpha-helical structures to the beta-sheet conformation. Native HSA as such lacks specific properties for its predisposition into amyloid fibrils *in vitro*. It has >60% of the sequence made up of the alpha-helical structures, tightened with the intramolecular interactions such as hydrogen bonds [35,36].

In the literature, the effect of the various experimental conditions and reagents on the fibrillation of HSA has been reported. For example, Susmita et al. have studied the inhibitory effect of epigallocatechin on the fibrillation of HSA [37]. S. Movaghati et al. have studied the varying effect of SDS on the fibrillation of HSA at the lower and higher concentrations [38]. Nitin et al. have reported the effect of cationic and anionic surfactants against the fibrillation of HSA and described their disrupting role on the matured fibrillar structures [39]. Among diverse nanoparticles, SWCNTs were reported to possess the binding affinity with Alzheimer's amyloid peptides such as beta (1–40) and (1–42) [40]. SWCNTs have also been reported to reduce the beta-sheet rich oligomers' formation in the case of Alzheimer's amyloid — beta (16–22) peptide [41]. Zhao Ming Fu et al. have suggested the induced beta-barrel formation on SWCNT surfaces arising out of the interplay of the dehydration and SWCNT-peptide or the peptide-peptide interactions using molecular dynamics simulation studies [42]. On contrast, Jing Jing et al. group have explored the preventive role of graphene, CNT, and C_{60} on the islet amyloid polypeptide fragments 22–28 ($IAPP_{22-28}$) aggregation behavior by inhibiting the beta-sheet formation and further aggregation mechanism [43]. Yonghui Ghan et al. have reported the more aggregation of BSA in the presence of hydroxylated MWCNTs [44]. Hence, there exist significant controversy, in revealing the promoting or the inhibiting role of CNTs against protein fibrillation and this field of investigation require more understanding at the molecular level. In this paper, an attempt has been made to test the role of surfactant dispersed (Tween 20) single and multi-walled CNTs against the fibrillation of HSA *in vitro*. The results obtained lead us to hypothesize that there exists enhanced fibrillation in the presence of CNTs.

The fibrillation of HSA has been clearly affected by the presence of CNTs in the *in vitro* condition. Figs. 1 and 2 show the Congo red (CR) absorbance and Thioflavin T (ThT) dye fluorescence upon binding with amyloid fibrils. In general, both CR and ThT were utilized as the marker for detecting amyloid fibrillation due to their binding affinity with the beta-sheet structures. As shown in Fig. 1(a) and (b), the CR dye absorbance at 495 nm got enhanced, and red shifted to 500 nm upon addition of fibrils formed in the presence of increasing concentrations of SW and MWCNTs. Thus, the hyperchromicity of CR dye governs the increased formation of fibrils in the presence of CNTs [45].

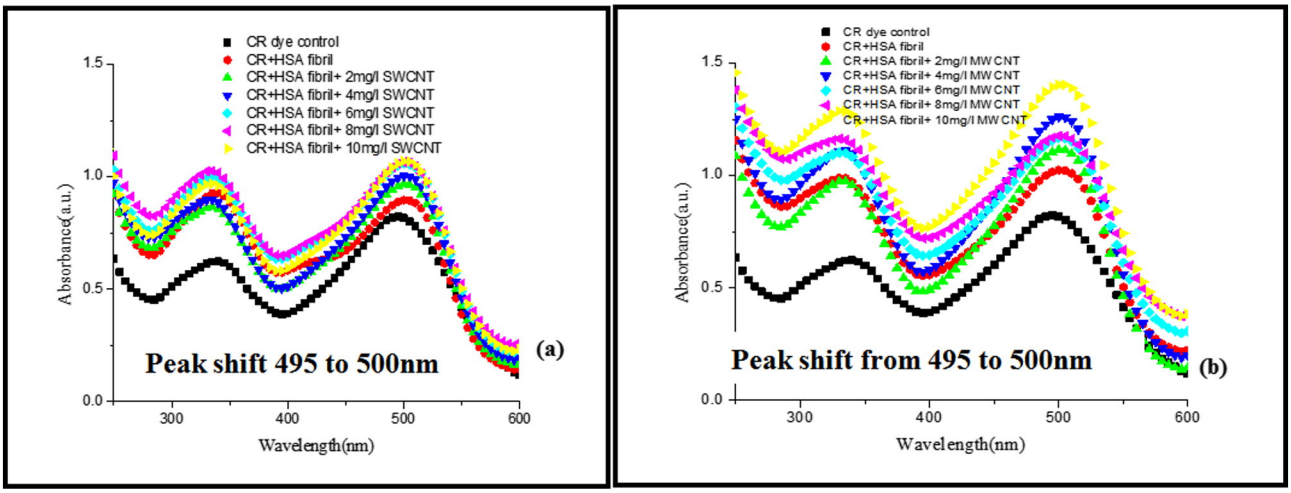

Fig. 1. UV absorbance of Congo red dye before and after incubation with fibrillar solutions.