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Fibrillar disruption by AC electric field induced oscillation: A case study with human serum albumin

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ABSTRACT

The effect of oscillation induced by a frequency-dependent alternating current (AC) electric field to dissociate preformed amyloid fibrils has been investigated. An electrowetting-on-dielectric type setup has been used to apply the AC field of varying frequencies on preformed fibrils of human serum albumin (HSA). The disintegration potency has been monitored by a combination of spectroscopic and microscopic techniques. The experimental results suggest that the frequency of the applied AC field plays a crucial role in the disruption of preformed HSA fibrils. The extent of stress generated inside the droplet due to the application of the AC field at different frequencies has been monitored as a function of the input frequency of the applied AC voltage. This has been accomplished by assessing the morphology deformation of the oscillating HSA fibril droplets. The shape deformation of the oscillating droplets is characterized using image analysis by measuring the dynamic changes in the shape dependent parameters such as contact angle and droplet footprint radius and the amplitude. It is suggested that the cumulative effects of the stress generated inside the HSA fibril droplets due to the shape deformation induced hydrodynamic flows and the torque induced by the intrinsic electric dipoles of protein due to their continuous periodic realignment in presence of the AC electric field results in the destruction of the fibrillar species.

1. Introduction

The response of proteins to different forms of exogenous perturbations such as temperature [1–3], pressure [2], pH changes [4], chemical molecules/ions [5–9], laser excitation [10], and electromagnetic radiation [11–14] is a topic of major research interest. The possible effects of external electric field stress on proteins has also been investigated [15–20], but most studies are theoretical and consider a molecular dynamic (MD) approach to examine the effects of static and pulsed electric fields on the stability of different proteins. For example, MD simulations have been used to investigate the helical to β-sheet conformational transition of β-amyloid peptides on interaction with an electric field [15] whereas the generation of helical structure by disrupting native β-sheet conformation induced by an electric field has also been reported [17]. Some studies have also shown that the oscillating external electric fields with certain frequencies have greater destabilizing effects on the secondary structure of the insulin chain-B than the static electric field with the same strength [21–23]. A similar trend has been observed in a MD simulation study of chignolin under an external oscillating electric field [24]. Lugli et al. have conducted MD simulations to investigate the stability of Aβ fibrils under the perturbation of an externally applied electric field [25]. However, there are very few experimental studies related to electric field induced conformational switchover of proteins [26–28]. It may also be noted that most studies are focused on electric field induced structural dynamics of the native protein and the effect of electric field directly on the aggregated protein fibrils have not been sufficiently explored. It is worth mentioning here that alternating current (AC) electric field has been utilized in various biological applications e.g. fracture healing, tumor ablation, and inhibition of cancerous cell growth [29,30]. Despite the involvement in a variety of applications, the direct application of the AC electric field in the disintegration of amyloid fibrils has not yet been investigated. These facts reiterate the importance of studies focusing on the influence of the external electric field on amyloid fibrils. The response of amyloid fibrils under the stress induced by an AC electric field is thus of interest.

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Droplet-based microfluidics serves as a promising platform to experimentally analyze biochemical reactions using tiny droplets as containers of biological entities [31]. The basic principle of the technique is based on the phenomenon commonly known as Electrowetting on dielectric (EWOD), wherein electrical energy is used to alter the surface energy and manipulate droplet shapes on a dielectric surface. The technique has found considerable usage in a number of applications starting from cell-based assays, protein analyses, and electronic cooling etc. [32–35]. In this configuration, an insulating layer acting as a dielectric is inserted between the liquid and the counter electrode to prevent current flow. The principle of alteration of wetting properties of the liquid by modifying the interfacial tension at the three phase contact line induced by electrical stress is commonly known as electrowetting [36]. However, as the electrical wetting tension weakly depends on the polarity of the applied voltage, AC voltage may be used instead of direct current (DC) voltage [37,38]. The AC electric field has additional advantages including decrease in contact angle hysteresis [39,40], delay in contact angle saturation [41], and decrease in biomolecular adsorption at the liquid-substrate interface [42]. The continuous occurrence of wetting and de-wetting, caused by the application of AC voltage in electrowetting, leads to oscillation of the droplet. The droplet oscillation induced by AC electrowetting in the low frequency regime leads to shape deformation of the droplet, which in turn depends on the input frequency of the AC electric potential. The induced oscillatory motion can cause hydrodynamic flow inside the droplet leading to generation of significant stress. The principle of AC electrowetting has found various applications in biosensing, efficient mixing, micro-cooling, MALDI mass spectrometry etc. [43–46]. However, to the best of our knowledge, no study has been performed that takes advantage of AC electrowetting in the disruption of preformed amyloid fibrils of protein and is the motivation of our present study.

The main goal of this study is to experimentally analyze the effect of the application of an AC electric field on preformed fibrils of human serum albumin (HSA), wherein the effect of field frequencies are probed at a constant voltage. HSA, a physiologically important natively α-helical globular protein, has been shown to form fibrils with amyloid-like characteristics under specific conditions [47,48] and thus serves as a suitable model protein to analyze the effect of the applied AC electric field on the fibrils [49]. The effect of a static DC field with varying strengths on preformed fibrils of HSA has previously been reported from this laboratory. The study has shown that the HSA fibrils are significantly disrupted on exposure to a static electric field having a strength of $\sim 8 \times 10^6$ V m^{-1} for a period of 10 min [50]. In the present study, AC electric fields of various frequencies (5–100 Hz) at a fixed input voltage of the same electric field strength are applied on mature HSA fibril droplets for different periods of time (1–6 min), keeping all other experimental conditions unaltered. The effect of the application of the external AC electric field of different frequencies on fibrils is monitored by various biophysical techniques. Further to comprehend the stress generated inside the oscillating HSA fibril droplet, responsible for the disruption of the amyloid fibrils, due to the application of the AC field, the dynamic morphology change of the droplets at different frequencies is characterized by monitoring the changes in different shape-dependent parameters like droplet contact angle, droplet footprint radius, and the amplitude. Further, to assess any thermal contribution to the observed structural changes, the temperatures of the sample droplets before and after the application of electric field have also been measured.

2. Materials and methods

2.1. Materials

HSA and ThT were purchased from Sigma Chemical Co. (St Louis, MO, USA) and used as received. ITO (Indium tin oxide, In_2O_3/SnO_2) coated glasses (surface resistivity of 30–60 Ω/sq) were purchased from Sigma-Aldrich, St Louis, USA. Sylgard-184 (consisting polydimethylsiloxane (PDMS) oligomer and cross linking reagent) was purchased from Dow Corning, USA. All the other chemicals used were of analytical grade and purchased from commercial sources and used without further purification. All solutions were prepared using Milli-Q water (resistivity ~18.2 MΩ cm at 25 °C).

2.2. HSA fibril formation

HSA was dissolved in Milli-Q water and the concentration measured spectrophotometrically using a molar extinction coefficient of 35,219 M^{-1} cm^{-1} at 280 nm [51]. HSA fibrils were prepared following the reported standard procedure by incubating HSA (150 μM) at pH 7.0 (20 mM, Tris-HCl buffer) in the presence of 50% (*v/v*) ethanol at 37 °C for 24 h [50].

2.3. Surface preparation

PDMS solution, to be used as a dielectric layer, was prepared by mixing the elastomeric base and the cross linking agent in a weight ratio of 10:1, followed by desiccation. A small amount of the PDMS was then poured on the ITO coated glass substrate for coating of a thin layer of PDMS over the glass using a spin coater (Süss MicroTec) which was spun at 400 rpm for 30 s initially followed by at 4500 rpm for 70 s and cured at 95 °C overnight. To increase surface durability and hydrophobicity, the substrate was further coated with a thin layer of Teflon. The Teflon coating was performed by spin coating of Teflon solution (5 wt%), dissolved in FC-40 solvent, at 3000 rpm for 30 s, followed by curing at 130 °C for 20 min. The thickness of the PDMS and Teflon layer were found to be 12.4 ± 0.4 μm and 23.4 ± 0.2 nm respectively, as measured by a profilometer. A portion of the substrate was left uncoated for ground electrode connection.

2.4. Application of electric field

Using a controlled dispenser, a small droplet of the preformed HSA fibrillar solution was placed over the prepared substrate and positioned on a goniometer (Rame Hart, Germany) platform. The droplet and substrate were electrically connected using the EWOD setup shown in Fig. 1. One electrode was electrically grounded (connected to the uncoated portion of the ITO glass) and AC electric potential was applied to the droplet by means of a platinum wire electrode, dipped into the droplet. To apply the AC electric field with tunable frequency, the electrodes were connected to a combined AC signal generator (Agilent 33250A, 80 MHz waveform generator) and an amplifier system (Tabor Electronics 9200A, High Voltage Wide Band Amplifier). The AC power source was used to apply the alternating electric fields of various frequencies (5 to 100 Hz) on the HSA fibrillar solution droplets for different exposure times (1–6 min). The peak to peak value of the input AC voltage was maintained at 100 V. Thus the estimated electric field strength is found to be $\sim 8 \times 10^6$ V m^{-1} (the dielectric layer thickness is ~12.5 μm). The temperature and the relative humidity were maintained at 25 ± 0.5 °C and 35 ± 2% respectively during the experiment.

2.5. Thioflavin T fluorescence study

ThT fluorescence of the electric field treated HSA solutions were measured at room temperature using a Horiba Jobin Yvon Fluoromax-4 spectrofluorimeter. Aliquots, withdrawn from the sets of solutions, were diluted using 20 mM Tris-HCl buffer of pH 7.0 to achieve final protein and dye concentrations of 2 μM and 10 μM respectively. The excitation wavelength was set at 450 nm, and the emission spectra collected from 470 nm to 600 nm. Slit widths for both excitation and emission were set at 5 nm with an integration time of 0.3 s. All spectra were corrected