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REGULAR ARTICLE

Detergent-induced aggregation of an amyloidogenic intrinsically disordered protein

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Abstract. Intrinsically disordered proteins (IDPs) belong to an important class of proteins that do not fold up spontaneously. The conformational flexibility of IDPs allows them to adopt a wide range of conformations depending upon their biochemical environment. Many IDPs undergo profound conformational conversion that is often coupled to amyloid aggregation in the presence of negatively charged lipid membranes. Here, we show the effect of a well-known anionic lipid mimetic, sodium dodecyl sulfate (SDS), on the aggregation mechanism of a model amyloidogenic IDP, namely, bovine κ-casein. In the absence of SDS, the aggregation kinetics of reduced and carboxymethylated (RCM) κ-casein followed a nucleation dependent polymerization model that comprises both lag- and assembly phases. On the contrary, in the presence of sub-micellar concentration of SDS, the aggregation kinetics did not exhibit a lag phase and appears to follow a non-nucleation pathway. Additionally, the morphologies of the aggregates formed in the absence and presence of SDS were found to be different. In the absence of SDS, κ-casein aggregation proceeded to typical amyloid fibrils, whereas, in the presence of SDS, the aggregation yielded large oligomers. Our results provide important molecular insights into the aggregation mechanism that can be utilized for the designing of novel protein/amyloid based nanomaterials with desired properties.

Keywords. Amyloid formation; fluorescence spectroscopy; intrinsically disordered proteins; protein aggregation.

1. Introduction

Protein aggregation resulting in amyloid formation is associated with a variety of deadly human diseases.[1–7] Recently it has been realized that highly ordered amyloid aggregates can also serve as promising template for the fabrication of nano-structures for a wide variety of applications such as in bio-nanotechnology[8–11] and food processing industry.[12,13] Typically, protein aggregation is favored under the conditions that lower the stability of the native form.[14] An enormous effort has been made to elucidate the mechanism of amyloid formation.[15,16] However, the precise mechanism of amyloid formation still remains elusive due to the inherent complexities associated with the process. One of the important factors that can trigger protein aggregation is the presence of surfaces with certain physicochemical features that favor aggregation by profoundly influencing the stability of the native state of a protein.[17,18] The recruitment of proteins or peptides by synthetic or biological surfaces can result in the conformational

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Dedicated to Professor Uday Maitra on the occasion of his 60th birth anniversary.

transition of the native (functional) state to the non-native, aggregation prone (non-functional/pathological) state.[17,18] Therefore, there is a growing interest in investigating the physicochemical characteristics of interaction between protein and synthetic or natural surfaces, and aggregation of proteins on these surfaces such as lipid membranes. The protein-membrane interactions are believed to play a key role in protein misfolding.[19–21] In fact, many of the pathological amyloid deposits have been found to interact with membranes thereby causing membrane disruption[22–30] and many of these pathological amyloids are often formed by intrinsically disordered proteins (IDPs),[22,24,25] a class of proteins that lack the ability to undergo autonomous folding.[31,32] Amphiphilic sodium dodecyl sulfate (SDS) provides a membrane mimetic environment and hence have been extensively used for studying the role of membrane in amyloid formation by various proteins, including IDPs.[33–36]

In this study, we have investigated the influence of SDS as a membrane mimetic on the aggregation and fibrillation of a model amyloidogenic IDP, namely, bovine κ-casein. κ-casein is a milk protein and is a member of a protein family called caseins. Caseins belong to the functional class of IDPs known as scavengers.[37] They are the major protein elements of mammalian milk and play an important role in the cellular uptake of calcium and phosphate ions.[38,39] Additionally, caseins are well recognized for their capability to act as molecular chaperones.[40–44] The chaperone-like activity of $\alpha_{s1}-$ and β-casein also has considerable physiological advantages in preventing the amyloid formation from two amyloidogenic caseins, $\alpha_{s2}-$ and κ-casein, under physiological conditions.[45–47] The amyloid formation by κ-casein is linked with mammary cancer.[48,49] Since κ-casein is a milk protein, therefore, in order to understand the process of amyloid formation from κ-casein, it is important to consider the influence of various components of milk on amyloid formation. One of the important components of milk is lipids that comprise approximately 4% w/v of milk.[50] In addition, the membrane of mammary epithelial cells is composed of phospholipid bilayer.[51] Therefore, studying the effect of lipids or lipid mimetics on κ-casein is of physiological relevance to elucidate the mechanism of κ-casein aggregation. Here, we have used SDS, an anionic detergent, to mimic membrane environment in κ-casein fibrillation. *In vitro* protein aggregation studies have revealed that the kinetics of fibrillation is often altered in the presence of lipids/detergents.[52,53] In many cases, they accelerate the fibrillation process.[33,34] However, there are cases where they can have inhibitory effect on fibrillation.[54,55] Here we show that SDS alters the pathway and morphology of κ-casein aggregation.

2. Experimental

2.1 *Materials*

κ-casein (from bovine milk), dithiothreitol (DTT), iodoacetic acid, guanidinium chloride (GdmCl), Tris HCl, sodium chloride (NaCl), sodium dodecyl sulfate (SDS), 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANS) and sodium hydrogen phosphate (monobasic) were procured from Sigma (St. Louis, MO) and used as received. 5-((((2-iodoacetyl) amino)ethyl)amino) naphthalene-1-sulfonic acid (IAEDANS) was purchased from Molecular Probes, Invitrogen Inc. All solutions were prepared in Milli-Q water. The pH of the buffers was adjusted using a Metrohm pH meter at ∼25 °C.

2.2 *Circular dichroism (CD) measurements*

The CD measurements were carried out on Chirascan Spectrophotometer (Applied Photophysics, UK) using a 1 mm path length quartz cell, and the spectra obtained were corrected for buffer background signal. The buffer corrected spectra were then smoothened using Pro Data software. All the measurements were made at room temperature.

2.3 *Steady-state fluorescence measurements*

For all the experiments, except for the data shown in Figure 1A, κ-casein was reduced and carboxymethylated (RCM) using DTT and iodoacetic acid using the protocol described previously[56,57] and the stock solution of κ-casein was stored under denatured condition (6 M GdmCl in pH 7, 50 mM phosphate buffer). For Trp steady-state fluorescence and CD measurements, the protein stock was diluted into the native buffer (pH 7.2, 50 mM phosphate buffer) to obtain a final concentration of 20 μM. For SDS binding experiments, a fresh stock of 10 mM SDS was prepared in Milli-Q water and was used for making further dilutions. For AEDANS fluorescence measurements, reduced and denatured κ-casein was labeled with a 50 molar excess of IAEDANS under denatured condition (6 M GdmCl in pH 7.6, 100 mM Tris buffer, 2 h at 37 °C). The labeled protein was then passed through a PD-10 column to remove excess dye and was further concentrated using AMICON ultra (3 kDa cutoff; from Millipore). The concentration of the labeled protein was estimated using $\varepsilon_{337nm} = 6100\ M^{-1}\ cm^{-1}$ for AEDANS.[58] The AEDANS labeled κ-casein was stored under denatured condition (6 M GdmCl in pH 7, 50 mM phosphate buffer).

All the steady-state fluorescence measurements were carried out on Fluoromax-4 (Horiba Jobin Yvon, NJ) at ∼25 °C. The samples were excited at 295 nm (Trp), 450 nm (ThT) and 375 nm (ANS and AEDANS). The steady-state fluorescence anisotropies were measured at 350 nm (Trp) and 480 nm (ANS and AEDANS). The steady-state fluorescence anisotropy (r_{ss}) is given by the following relationship:

$$r_{ss} = \frac{I_{\parallel} - I_{\perp}G}{I_{\parallel} + 2I_{\perp}G} \quad (1)$$

where $I_{\parallel}$ and $I_{\perp}$ are fluorescence intensities collected using parallel and perpendicular geometry, respectively. For Trp fluorescence kinetics measurements, κ-casein in native buffer was taken as baseline and then SDS (100 μM) was added to this solution. The SDS solution was manual mixed and the time of mixing was typically 5–10 seconds. For monitoring ThT and ANS fluorescence kinetics upon addition of SDS (100 μM); ThT (20 μM) and ANS (20 μM), respectively were already present in the buffer containing κ-casein (20 μM). All the measurements were made at room temperature.

2.4 *Aggregation reaction*

The aggregation reaction was initiated using 200 μM of RCM κ-casein in 50 mM phosphate buffer (pH 7.2). The reaction mixture was continuously stirred at a speed of 300 rpm and the temperature was maintained at 37 °C. The aliquots (10 μM) were taken out at several time points and Trp fluorescence was measured. For measuring ThT and ANS fluorescence, 10 μM of ThT and ANS, respectively were added to the aliquots taken out from the aggregation mixture. The final protein concentration for measurement was 10 μM. All the final dilutions were made in 50 mM phosphate buffer (pH 7.2). For carrying out aggregation reaction with AEDANS labeled κ-casein, 20 μM of AEDANS labeled κ-casein was added to 180 μM of RCM κ-casein. The aliquots were taken out at different time points and the AEDANS fluorescence was measured without any further dilution. The parameters used for fluorescence measurements are mentioned above. The fluorescence data at different points were then plotted and fitted with the following equation described previously for nucleation-dependent polymerization.[59]

$$y = y0 + \frac{a}{1 + \exp\left(-\frac{x - x0}{b}\right)} \tag{2}$$

where y is the fluorescence at time x, y_0 is the initial fluorescence value, x_0 is the time when fluorescence reaches 50% of its maximum value, and a is the maximum fluorescence at stationary phase. The lag time is given by $(x_0 - 2b)$.

2.5 *Time-resolved fluorescence measurements*

The fluorescence lifetime data were acquired using a time-correlated single photon counting (TCSPC) setup (Fluorocube, Horiba Jobin Yvon, NJ). All the measurements were made at room temperature. The peak count was fixed to 10,000 and the excitation and emission polarizers were oriented at the magic angle (54.7°). For time-resolved fluorescence anisotropy measurements, the peak difference was 10,000 counts and the orientation of the emission polarizer was 0° and 90° with respect to the excitation polarizer for parallel fluorescence intensities ($I_{\parallel}$) and perpendicular fluorescence intensities ($I_{\perp}$), respectively. The emission monochromator for ANS was fixed at 480 nm with a bandpass of 12 nm and 375 nm laser diode was used as excitation source. The instrument response function (IRF) was collected using Ludox (colloidal silica). The width (FWHM) of IRF was ~250 ps. The fluorescence intensity decay curves were deconvoluted taking IRF into account using

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \tag{3}$$

2.6 *Atomic force microscopy (AFM) imaging*

AFM images were acquired on an Innova atomic force microscope (Bruker). The AFM was operated in tapping mode. For imaging, the silicon nitride cantilever probe with radius ~8 nm was used. The samples (typically diluted 1000–2000 fold with aggregation reaction buffer filtered through a 0.22 μm syringe filter) were deposited on a freshly cleaved muscovite mica (Grade V-4 mica from SPI, PA). The mica surface was incubated with the sample for 15–20 min before washing it with filtered Milli-Q water and dried under a gentle stream of nitrogen gas. The images were collected in NanoDrive (v8.03) software at a resolution of 1024 x 1024 pixels. The collected AFM images were further processed and analyzed using WSxM version 4 develop 11.6 software.[60]

3. Results and Discussion

3.1 *κ-Casein aggregation under non-reducing and reducing conditions*

Under (native) physiological conditions, κ-casein exists as collapsed disordered globules.[57] In order to follow the aggregation of κ-casein, ThT fluorescence assay was used. Since κ-casein contains two cysteine residues that can potentially form intra- and inter-molecular disulfides, we carried out aggregation reaction using a high concentration of κ-casein (200 μM), under reducing (using DTT as a reducing agent) as well as under non-reducing conditions, to decipher the role of disulfides in κ-casein aggregation. A time-dependent increase in ThT fluorescence with a typical initial lag phase, was observed with the reduced form of κ-casein, which is in line with our previous study with RCM κ-casein[61] (Figure 1A). However, in the absence of reducing agent, no time-dependent change (for >48 h) in ThT fluorescence was observed which suggests that intermolecular disulfide bonding prevents or slows down κ-casein fibrillation (data not shown). This observation is in agreement with previous studies on κ-casein fibrillation.[47] However, the aggregation kinetics observed by us for RCM κ-casein is different from previous studies that showed the absence of lag phase.[47,62] This discrepancy could arise due to the early oligomer formation when reaction is initiated from RCM κ-casein in native buffer.[47,62] In our case, we initiated the aggregation reaction by transfering the protein from the denatured state to the native buffer that

minimizes the formation of early oligomers. Therefore, in our case the aggregation reaction proceeeded via a nucleation-dependent kinetics comprising a lag phase and an assembly phase.

3.2 *Mechanism of κ-casein aggregation using multiple fluorescence probes*

Next, we followed the aggregation kinetics using multiple fluorescent probes to discern the molecular events involved in the aggregation process. Fluorescence spectroscopy being a highly sensitive and multi-parametric technique is extensively used for studying protein aggregation.[63] The steady-state fluorescence spectrum provides insights into the local environment around the fluorophore and information about an overall size of the protein can be obtained from steady state fluorescence anisotropy measurements.[63] In order to probe the conformational changes taking place during aggregation, we took advantage of the fact that κ-casein has two cysteines and a single Trp residue. We used Trp as intrinsic fluorophore and AEDANS as extrinsic fluorophore by labeling the thiols of Cys residues with IAEDANS. Additionally, we used ANS, a hydrophobic environment sensitive dye, to follow the aggregation process. The free form of ANS is almost non-fluorescent in water with an emission maximum at ∼515 nm. However, it becomes fluorescent upon binding to hydrophobic pockets and undergoes a significant blue shift to ∼475 nm which is mostly accompanied by a rise in intensity.[64] Due to the ability of ANS to bind to hydrophobic regions/clusters, it is often used in aggregation studies to follow the initial oligomerization and growth steps during fibrillation. All the probes showed the existence of a nucleation-dependent amyloid assembly pathway with a characteristic lag phase, similar to the ThT fluorescence kinetics. The lag time and the rate constants for the aggregation reaction were recovered from the time-dependent plots of all the fluorescence readouts using a previously described relationship[59]

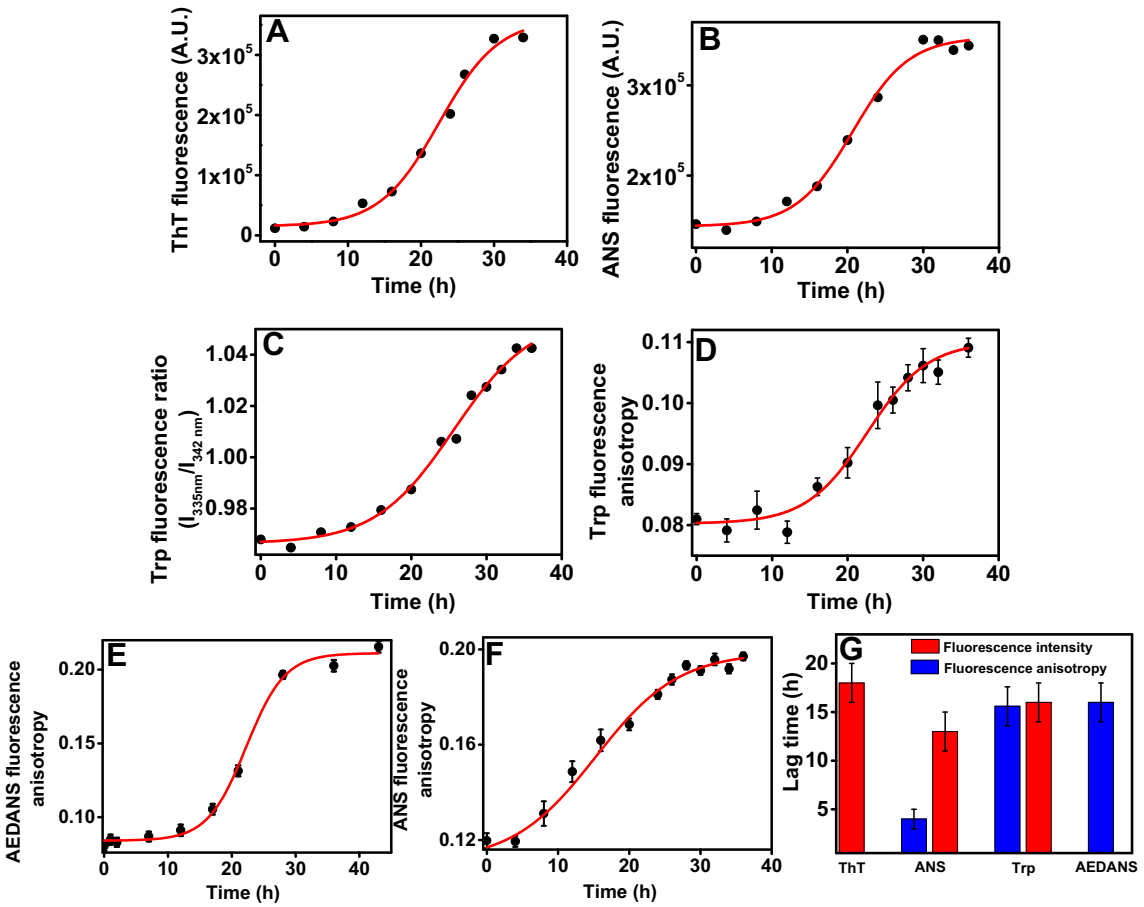

Figure 1. Aggregation kinetics of RCM κ-casein (200 μM) monitored using, (A) ThT fluorescence, (B) ANS fluorescence, (C) Ratio of Trp fluorescence intensity at 335 nm and 342 nm, (D) Trp fluorescence anisotropy, (E) AEDANS fluorescence anisotropy, and (F) ANS fluorescence anisotropy. (G) The lag time recovered from fitting the time-dependent fluorescence data shown in (A-F) using equation 2 (For details, see Section 2). All the measurements were made at room temperature.

(Figure 1). The lag time observed for all the fluorescence readouts was found to be comparable, except for the ANS fluorescence anisotropy. Additionally, the growth phase kinetics was found to be similar for all the fluorescence readouts, except for ANS anisotropy. The similarity in the rates indicate that the structural reorganization, particularly in the region harboring Trp and Cys, and aggregation occur simultaneously. The kinetics of the increase in the ANS fluorescence anisotropy was faster during amyloid formation. The monomeric form of κ-casein exists as collapsed disordered globules[57] and hence has a very weak affinity for ANS. However, as these collapsed globules coalesce during aggregation process, an increase in ANS fluorescence anisotropy as well as in fluorescence intensity with a concomitant blue shift was observed. The shorter lag phase observed for ANS anisotropy is suggestive of early oligomerization. After characterizing the kinetics of κ-casein aggregation under physiological conditions, we next embarked upon studies aimed at delineating the effect of SDS on the aggregation process.

3.3 *κ-Casein binds to SDS in a concentration-dependent manner*

We followed the SDS-induced conformational changes in κ-casein, using CD and steady-state fluorescence. Upon changing the concentration of SDS, the changes in both CD and fluorescence readouts were observed. We observed an increase in the ellipticity at 218 nm as well as 222 nm with respect to ellipticity at 205 nm, with the increase in SDS concentration (Figure 2A and B). The CD signals at 205 nm, 218 nm and 222 nm predominantly correspond to random coil, β-sheets and α-helices, respectively. Thus, our CD data indicates that in the presence of SDS, formation of secondary structure at the expense of random coil (Figure 2A and B). It is important to emphasize here that the concentrations of SDS that we have used in our experiments are non-denaturing below the critical micellar concentration of SDS. However, there could be formation of some pre-micellar aggregates under this condition.

Next, we monitored the changes in the fluorescence attributes of Trp which is present in Q/N-rich positively

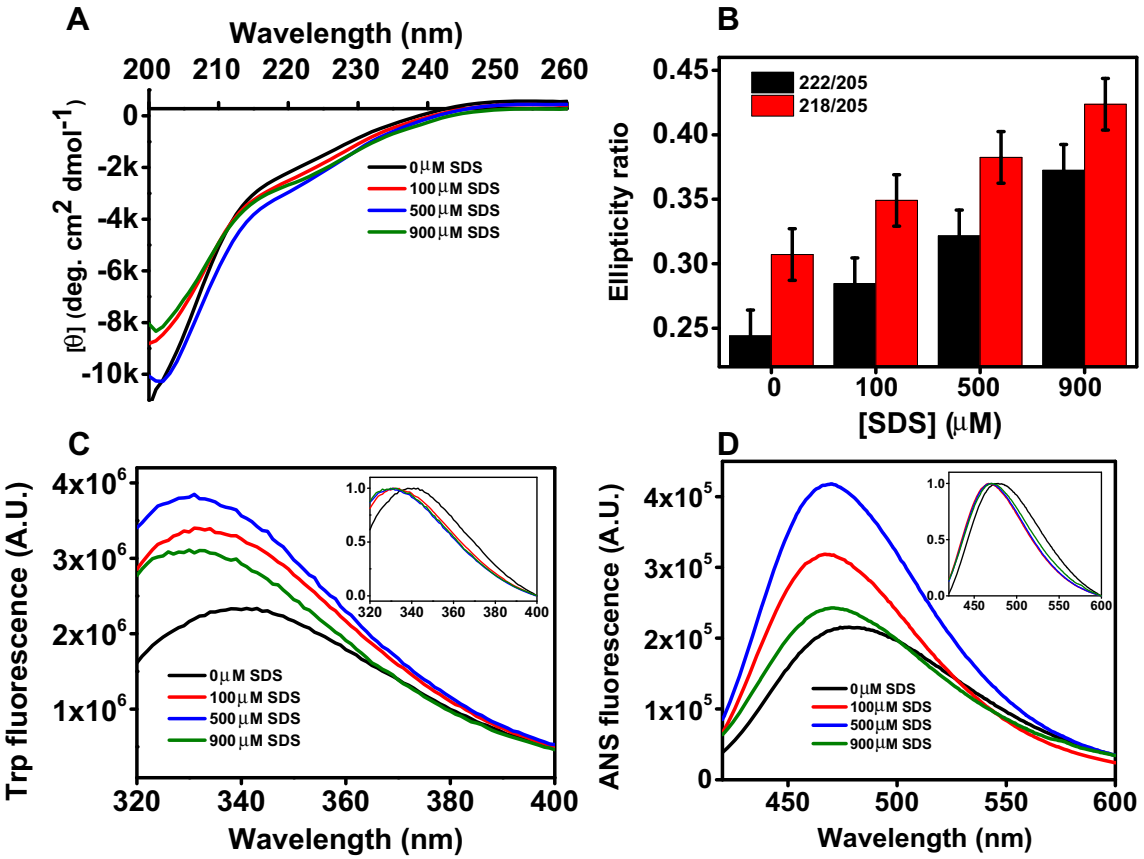

Figure 2. (A) CD spectra for κ-casein with various concentration of SDS: monomer (black), 100 μM (red), 500 μM (blue) and 900 μM (olive). (B) The ellipticity ratio from CD data are shown in panel A. (C) Trp and (D) ANS fluorescence spectra with color scheme same as in panel A. The normalized Trp and ANS fluorescence spectra are shown as insets to C and D, respectively. All the measurements were made at room temperature.

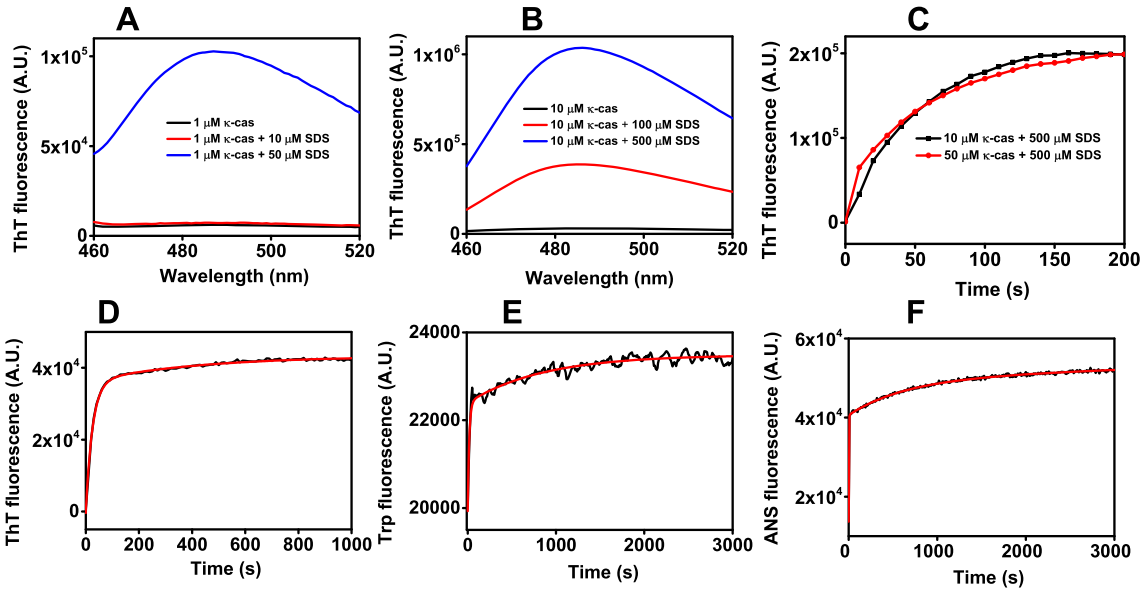

Figure 3. ThT fluorescence spectra for (A) 1 μM of κ-casein without (black) and with (i) 10 μM (red) and (ii) 50 μM (blue) of SDS. (B) 10 μM of κ-casein without (black) and with (i) 100 μM (red) and (ii) 500 μM (blue) of SDS. (C) ThT fluorescence kinetics data for (i) 10 μM (black) and (ii) 50 μM (red) of κ-casein with 500 μM of SDS. (D) ThT, (E) Trp and (F) ANS fluorescence kinetics data for 20 μM of κ-casein with 100 μM of SDS. All the measurements were made at room temperature.

charged N-terminal domain. The Q/N-rich domain of κ-casein shares some resemblance with the amyloidogenic segment of the yeast prion protein.[65,66] An increase in Trp fluorescence with a concomitant blue shift in the emission maximum was observed with increase in SDS concentration (Figure 2C). At higher concentration of SDS (> 500 μM), a drop in the fluorescence was observed. However, this drop in the fluorescence was not associated with a red shift in the emission maximum and thus, it is likely to be due to fluorescence quenching from the neighboring amino acid residues. We next monitored the changes in the hydrophobicity. An increase in the ANS fluorescence, accompanied by a blue shift in the emission maximum was observed with increase in SDS concentration and like Trp, at higher concentration of SDS (> 500 μM), a drop in ANS fluorescence was observed. This set of data suggested that the SDS-induced conformations have higher affinity towards ANS compared to the monomeric state. Since ANS gives us information about the overall hydrophobicity, we speculate that in the presence of SDS, there is formation of some hydrophobic clusters where ANS gets encapsulated. ANS molecules can potentially bind to SDS molecules. However, at the concentrations of SDS that we have used in our study, we did not observe any ANS binding (data not shown).

Taken together, our data from CD and fluorescence suggest that κ-casein undergoes appreciable conformational change in the presence of SDS. This change appears to be SDS concentration dependent. Though both CD and fluorescence clearly indicated some conformational change in κ-casein in the presence of SDS, it was not clear whether κ-casein remains its monomeric state or forms higher order aggregates. Since CD data indicated an increase in $\theta_{218}/\theta_{205}$, we suspected the possibility of aggregation leading to β-sheet rich conformations in the presence of SDS since SDS-induced fibrillation has been previously reported for some IDPs.[33,34,67] Therefore, we next carried out ThT fluorescence measurements.

3.4 *κ-Casein aggregates without a lag phase in the presence of SDS*

A high affinity for ThT was observed for SDS-induced conformations of κ-casein and this binding affinity for ThT was found to be highly concentration dependent (Figure 3). The strong dependence on the concentration of κ-casein as well as SDS was observed (Figure 3A and B). Thus, κ-casein forms ThT active aggregates in the presence of SDS. In order to decipher the mechanism of SDS-induced aggregation, we followed the kinetics of this conformational change using ThT, Trp and ANS fluorescence. All fluorescent readouts indicated the absence of a lag phase suggesting nucleation-independent or isodesmic polymerization mechanism (Figure 3D-F).[68] This is clearly in sharp contrast to the nucleation-dependent amyloid assembly kinetics observed for κ-casein fibrillation in the absence of SDS (Figure 2). Thus, our results suggested that SDS acts as

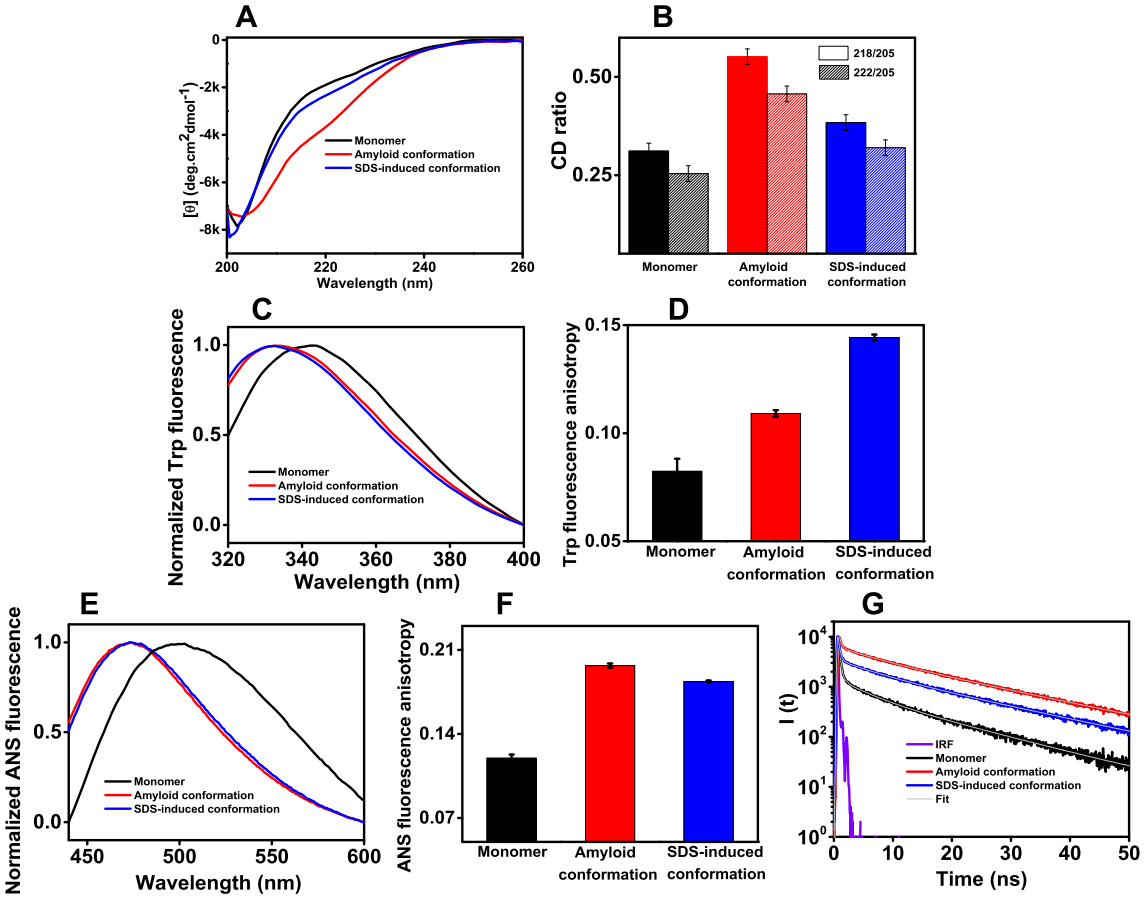

Figure 4. (A) CD spectra, (B) the ellipticity ratio obtained from CD data shown in panel A, (C) Normalized Trp fluorescence spectra, (D) Trp fluorescence anisotropy, (E) Normalized ANS fluorescence spectra, (F) ANS fluorescence anisotropy and (G) ANS fluorescence lifetime for monomer (black), Amyloid conformation (red) and SDS (100 μM)-induced conformation (blue). All the measurements were made at room temperature.

a trigger for κ-casein conformational change and aggregation. SDS switches the aggregation mechanism from a typical nucleation dependent polymerization [65,68,69] to an apparent isodesmic polymerization that does not require a high protein concentration. A similar switch in the aggregation mechanism has been reported previously in the presence of salt and chemical chaperones, upon changing the pH of the solution, etc. [70–72]

After confirming that κ-casein aggregates in the presence of SDS, we next compared the secondary structural contents and fluorescence attributes of the final conformation attained by the aggregates formed in the absence and in the presence of SDS. We refer to the aggregates formed under physiological conditions, in the absence of SDS, as amyloids as it has already been established previously by us that κ-casein forms amyloid fibrils under this condition. [61] The aggregate conformation obtained in the presence of SDS has been referred to as SDS-induced conformation. For comparison, we are only considering the aggregates formed with 100 μM SDS because the aggregates formed at higher concentration of SDS tend to precipitate as a function of time. It is interesting to note that β-sheet content is higher for aggregates formed in the absence of SDS as is evident from θ_{218} / θ_{205} (Figure 4A and B). In case of Trp fluorescence, though the extent of blue shift is similar for both type of aggregates, anisotropy is much higher for SDS-induced conformation of aggregates (Figure 4C and D). This result indicates that the local structure around Trp is much more rigid in case of aggregates formed in the presence of SDS. This plausibly suggests that SDS neutralize the positive charge in N-terminal domain that harbors Trp and is also rich in aggregation prone Q and N residues. The charge neutralization in the N-terminal domain might lead to favorable chain-chain interactions as a result of which aggregation process is accelerated. After Trp, we next compared ANS fluorescence characteristics of both the aggregates. The aggregates formed in the presence of SDS showed much lower ANS binding as is clear from the fluorescence anisotropy and lifetime data (Figure 4E-G). The lower ANS binding for the aggregates formed in the presence of SDS is probably

associated with the lower content of hydrophobic pockets compared to the aggregates formed without SDS. After gaining insights into the conformational attributes of the aggregates formed under two different solution conditions, we next performed atomic force microscopy (AFM) imaging to visualize the nanoscale morphology of these aggregates.

3.5 *Insights into the nanoscale morphology of aggregates*

AFM is used extensively for imaging the nanoscale topography of protein aggregates.[58,73–76] In the absence of SDS, we observed long thread-like amyloid fibrils of 6-7 nm in height. The height profiles of these fibrils observed in AFM images collected at different time points in the growth phase of aggregation are shown in Figure 5A-C. In the presence of SDS, AFM imaging revealed a heterogeneous co-existence of small and large-sized spherical oligomers (Figure 5D-F). Mostly, large sized oligomers ranging from 20–60 nm were observed. Though we could not detect fibrils from these oligomeric species, we do not completely rule out the possibility of fibril formation upon much longer incubation. Previous studies on κ-casein aggregation have proposed that the rate determining step in κ-casein fibrillation is the dissociation of larger oligomers into smaller species.[48] Since in the presence of SDS, mostly large oligomers were observed, it is possible that these oligomers require a much longer time to dissociate into smaller amyloidogenic species. As a result, fibril formation does not occur immediately or within few days. On the contrary, in the absence of SDS, since the oligomers formed are much smaller in size (3–6 nm) and are possibly much more amyloidogenic than the larger species, the fibril formation is much more facile.

Taken together, our studies indicate that in the absence of SDS, κ-casein aggregation occurs at a high concentration and follows a nucleation-dependent polymerization pathway. In the presence of SDS, a switch in the aggregation mechanism from nucleation-dependent polymerization to an apparent isodesmic polymerization is observed. The alteration in the aggregation mechanism also leads to a variation in the morphology of the aggregates suggesting that the morphology of the protein aggregates might be strongly linked to the

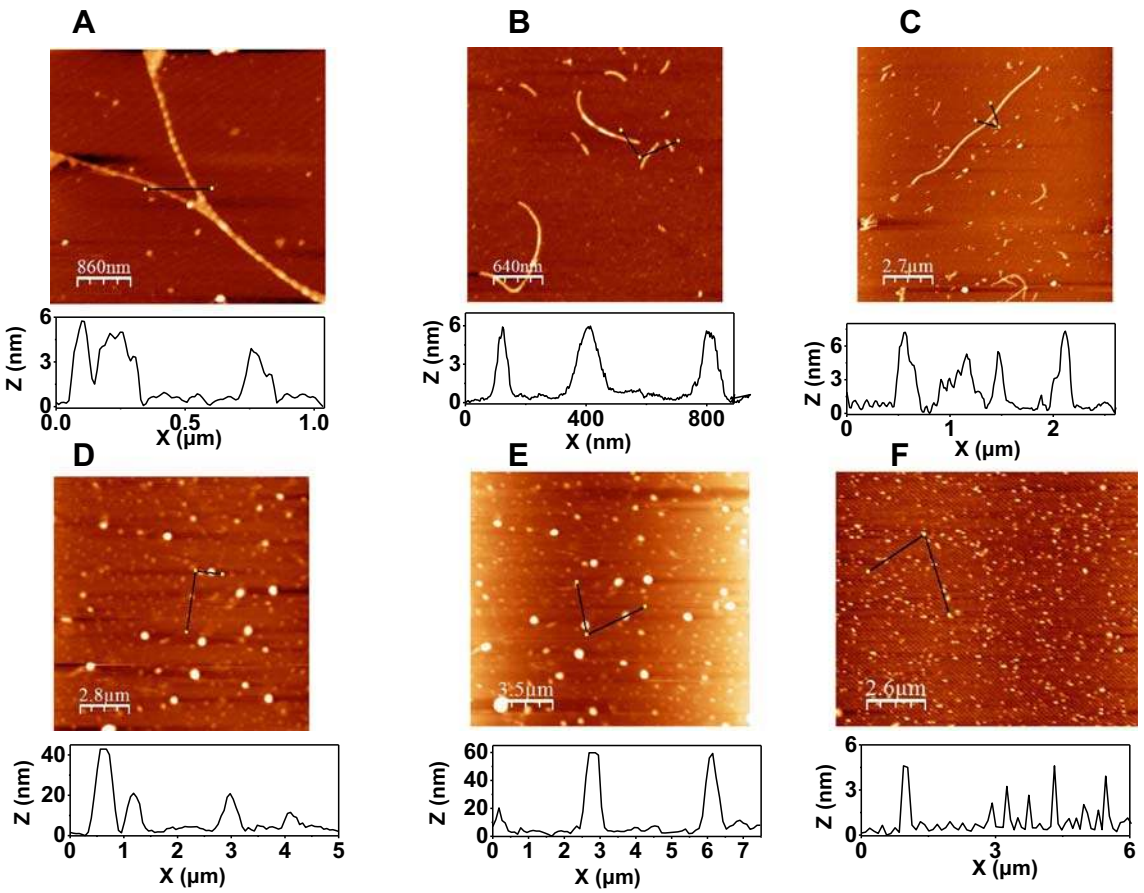

Figure 5. AFM images along with the height profiles of κ-casein (A-C) amyloid fibrils formed without SDS and (D-F) spherical oligomeric aggregates formed in the presence of SDS (100 μM).

mechanism of aggregation. We believe that SDS acts as a conformational catalyst by neutralizing the positive charge on the aggregation prone Q/N rich domain that results in the hydrophobic association and intermolecular hydrogen bonding between the polypeptide chains. This in turn facilitates the coalescence of κ-casein monomers into large sized oligomers and is in line with the charge colloid model.[77,78] As a result of facile oligomerization due to charge neutralization in the presence of anionic SDS, the lag phase is not detected during aggregation in the presence of SDS.

4. Conclusions

In this work, we showed the effect of a well-known lipid mimetic, SDS, on the mechanism of aggregation of an amyloidogenic IDP, namely, bovine κ-casein using a variety of biophysical tools. The deposition of amyloids formed by κ-casein is linked with mammary cancer. The amyloid formation from κ-casein followed a nucleation dependent polymerization in the absence of SDS, as shown previously.[61] In the presence of SDS at submicellar concentrations, a switch in the mechanism was observed. Additionally, the morphologies of the aggregates formed in the absence and presence of SDS were found to be different. Without SDS, fibril formation occurred, whereas, in the presence of SDS, mostly large spherical oligomers were observed. The fibrillar species formed without SDS have higher β-sheet content compared to the spherical oligomeric species formed in the presence of SDS. The interaction of κ-casein with SDS may have physiological relevance as SDS being a membrane mimetic is able to mimic the role of anionic lipids found in milk and milk ducts. Additionally, our studies suggest that the morphology of the protein aggregates can be modulated using detergents and can be utilized for the designing of novel protein/amyloid based nanomaterials. Since κ-casein is readily available and easy to isolate from milk, therefore, by making use of lipid mimetics/surfactants, we can control the aggregation pathway of κ-casein, modulate the morphology of the aggregates and accordingly utilize them for various bionanomaterials applications. In fact, the fibrillar κ-casein has been used in the past for the encapsulation and controlled delivery of retinoic acid, which was used to promote the differentiation of neuronal cells.[79]

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