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Ordered Water within the Collapsed Globules of an Amyloidogenic Intrinsically Disordered Protein

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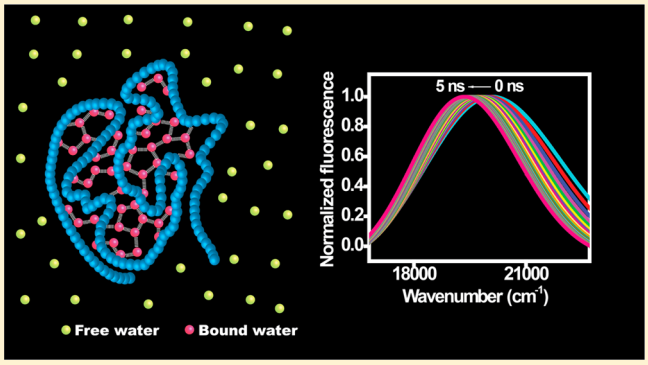
Supporting Information

ABSTRACT: Intrinsically disordered proteins (IDPs) confront the traditional sequence−structure−function paradigm and are associated with important functions and amyloid disorders. Water molecules residing in the vicinity of the polypeptide chain play potentially important roles in directing the course of binding-induced folding and amyloid aggregation of IDP. Here we characterized the nature of water molecules entrapped within the collapsed globules of an amyloidogenic IDP, namely, κ-casein. These globules can undergo further compaction in the presence of an anionic detergent that is capable of diminishing the intrachain repulsion from the positively charged glutamine/asparagine-rich amyloidogenic N-terminal domain comprising 100 residues. Using time-resolved fluorescence spectroscopy, we estimated the longer component of the solvation time to be ∼1.4 ns, which is 3 orders of magnitude slower than that in bulk water and more than an order of magnitude slower than the "biological water" present at the protein surface. Profoundly restrained water within the collapsed IDP globules resembles the ordered water cluster found under nanoconfinement. We suggest that the association of these globules would result in the release of ordered water molecules into the bulk milieu causing an entropic gain that would eventually drive the formation of the key (obligatory) oligomeric intermediates on the pathway to amyloids via nucleation-dependent polymerization.



1. INTRODUCTION

The traditional sequence−structure−function paradigm cannot account for the existence of a large number of proteins that lack a well-defined 3D structure under physiological conditions. Such proteins possessing high net charge and low hydrophobicity belong to a distinct class of proteins commonly known as natively unfolded or intrinsically disordered proteins (IDPs).[1−11] Unlike the folded (globular) proteins, IDPs lack the ability to undergo autonomous folding[1] and usually exist as dynamic ensembles having rapidly fluctuating backbone Ramachandran dihedral angles.[4] Many IDPs upon binding to their respective biological partners either undergo disorder-to-order transition or form "fuzzy complexes",[6,7] thereby maintaining their chameleon state to perform various specialized functions like signaling, molecular recognition, regulation of transcription,and translation, etc.[9−11] However, the astonishing conformational plasticity of IDPs can also be detrimental because it allows the polypeptide chain to adopt aberrant toxic amyloidogenic conformations that are associated with a range of neurodegenerative disorders like Alzheimer's, Parkinson's, and Huntington's diseases.[12,13] Many theoretical and experimental approaches have been successfully applied to gain insight into the complex conformational behavior of IDPs.[14−27] These studies have revealed that most IDPs have a tendency to form collapsed globules in water,[14,17,19] especially the IDPs that are rich in polar, uncharged amino acid residues albeit devoid of hydrophobic amino acid residues. These IDPs include polyglutamine (polyQ),[19−21] N-terminal domain of the yeast prion protein Sup35,[14] and glycine-serine block copolymers.[22,27]

An important factor that can control the intrinsic preference of the polypeptide chain to undergo collapse is net charge per residue.[15] Many IDP sequences have been found to have a high net charge per residue,[23] and it has been postulated that an increase in net charge per residue makes the globule-to-coil transition more favorable.[15,24,25] Furthermore, it has been observed that the polypeptide chain devoid of either nonpolar or polar side chains, such as polyglycine, also prefers an ensemble of collapsed structures in aqueous milieus, suggesting that the collapse of IDPs might originate, at least partially, from the conformational preferences of amide backbones in water.[22] Thus, water is believed to act as a poor solvent for polypeptide chains and favors the intrachain interactions over the chain−solvent interactions leading to the formation of conformationally labile collapsed globules that are not capable of undergoing autonomous folding.[15,26] The collapsed globule is at the

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crossroad of several biologically significant processes and can either undergo binding-induced folding in the presence of a binding partner or associate to form oligomers that can eventually mature into ordered amyloid fibrils.[26] Therefore, to understand how the intricate balance between the extended, collapsed, folded, and misfolded conformations of the IDPs is maintained, it is important to assess the conformational propensities of IDPs in the presence of different solutes that can modulate the conformational preferences of the polypeptide chain in water. A novel approach for elucidating the conformational attributes of IDPs would be to monitor the distribution and dynamics of water molecules present in the vicinity of the polypeptide chain.

Bovine κ-casein is an amyloidogenic isoform of caseins that belongs to the class of functional IDPs known as scavengers.[11] Aggregation of κ-casein results in the formation of amyloid fibrils that get deposited in the mammary glands.[28−31] We have previously shown that κ-casein under native monomeric condition undergoes a chain collapse in water to form unordered globules.[17] In this work, we characterize the dynamics of water molecules entrapped within the globules. These water molecules exhibit profoundly restrained relaxation dynamics indicating the formation of highly structured water cluster resembling nanoconfined water.

2. EXPERIMENTAL METHODS

2.1. Materials. κ-casein (from bovine milk), dithiothreitol (DTT), iodoacetic acid, guanidinium chloride (GdmCl), Tris HCl, sodium chloride (NaCl), sodium dodecyl sulfate (SDS) and sodium hydrogen phosphate (monobasic) were procured from Sigma (St. Louis, MO) and used as received. 6-Acryloyl-2-(dimethylamino)naphthalene (acrylodan) was obtained from Molecular Probes, Invitrogen Inc. All solutions were prepared in Milli-Q water. The pH of the buffers used was adjusted using a Metrohm pH meter at ∼25 °C.

2.2. Methods. *Circular Dichroism (CD) Measurements.* The CD spectra were recorded in Chirascan Spectrophotometer (Applied Photophysics, UK) using a 1 mm path length quartz cell, and the spectra so obtained were corrected for buffer background signal. The buffer corrected spectra were then smoothened using Pro Data software.

Steady-State Fluorescence Measurements. Bovine κ-casein was reduced and carboxymethylated (RCM) using DTT and iodoacetic acid as described previously.[17] RCM 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 studying the effect of detergent, a fresh stock of SDS was prepared in Milli-Q water. For acrylodan fluorescence measurements, reduced and denatured κ-casein was labeled with a 10 M excess of acrylodan 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 ϵ_{365} = 12 800 M^{-1} cm^{-1} for acrylodan.[32] The acrylodan-labeled κ-casein was stored under denatured condition (6 M GdmCl in pH 7, 50 mM phosphate buffer). For steady-state fluorescence experiments, acrylodan-labeled protein was diluted into the native buffer (pH 7.2, 50 mM phosphate buffer) and denaturant (6 M GdmCl in pH 7, 50 mM phosphate buffer) to obtain a final concentration of 10 μM.

All the steady-state fluorescence measurements were made on Fluoromax-4 (Horiba Jobin Yvon, NJ). The samples were excited at 295 nm (Trp) and 375 nm (acrylodan). The steady-state fluorescence anisotropies were measured at 350 nm (Trp) and 500 nm (acrylodan). The steady-state fluorescence anisotropy (r_{ss}) is given by the following relationship:

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

where $I_{\parallel}$ and $I_{\perp}$ are fluorescence intensities collected using parallel and perpendicular geometry, respectively. The perpendicular components were always corrected using a *G*-factor. For the red-edge excitation shift (REES) experiments, the excitation wavelength for tryptophan was varied from 280 to 305 nm.

Stopped-flow Fluorescence Measurements. The fluorescence kinetics data were collected using a stopped-flow apparatus (Applied Photophysics, UK). The deadtime of mixing was ∼5 ms. A 320LP (Trp) optical filter was used for collecting the total fluorescence,. The mixing ratio was 10:1. The final concentrations of κ-casein and SDS were 10 and 100 μM, respectively. Native κ-casein was mixed with phosphate buffer (pH 7.2, 50 mM) to obtain the baseline signal. The stopped-flow data were acquired for 0.5 s with 10 000 samples per point. The kinetic traces were collected 10 times in triplicate.

Time-resolved Fluorescence Measurements. All time-resolved measurements were made using a time-correlated single photon counting (TCSPC) setup (Fluorocube, Horiba Jobin Yvon, NJ). The position of excitation and emission polarizers was fixed at 54.7° (magic angle) and the peak count at 10,000 for fluorescence lifetime measurements. A 375 nm laser diode was used as excitation source for acrylodan. Ludox (colloidal silica) solution was used for collecting the instrument response function (IRF). The IRF had a full width at half maxima of ∼265 ps. For time-resolved fluorescence depolarization measurements, the peak difference was set as 20,000 counts and the orientation of emission polarizer was toggled between 0° and 90° with respect to the fixed orientation of excitation polarizer for parallel ($I_{\parallel}$) and perpendicular fluorescence intensities ($I_{\perp}$), respectively. The emission wavelength was fixed at 500 nm with a bandpass of 6 nm. The perpendicular fluorescence intensities were corrected for *G*-factor. The anisotropy decays were analyzed using DAS6 software by globally fitting $r(t)$ using the following relationship:

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)} \quad (1b)$$

The anisotropy decays were analyzed by biexponential decay function that describes the fast and slow rotational motion.[17,33,34]

$$r(t) = r_0[\beta_1 \exp(-t/\phi_1) + \beta_2 \exp(-t/\phi_2)] \quad (2)$$

where r_0 is the intrinsic fluorescence anisotropy, ϕ_1 and ϕ_2 are the short and long rotational correlation times and β_1 and β_2 are the amplitudes associated with short and long rotational correlation times.

The concentration of acrylodan-labeled κ-casein was 10 μM, and the concentrations of SDS used were 100, 250, and 500 μM. To construct the time-resolved emission spectra (TRES), the fluorescence decays were collected at different emission

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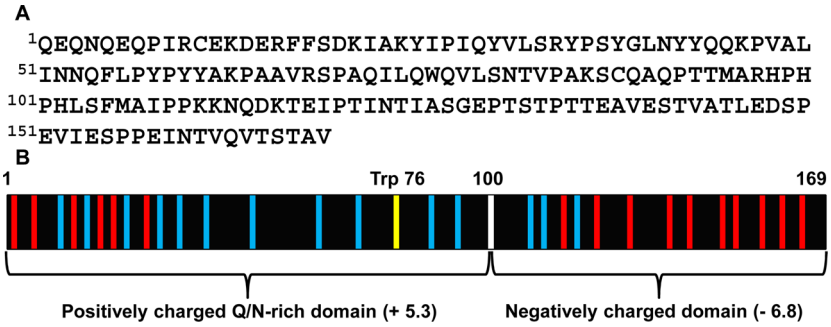

Figure 1. (A) Amino acid sequence of κ-casein. (B) Polypeptide divided into two distinct domains: positively charged Q/N-rich N-terminal segment (1−100) and negatively charged C-terminal segment (101−169). Red and blue lines represent negatively and positively charged residues, respectively. Trp 76 is shown by a yellow line.

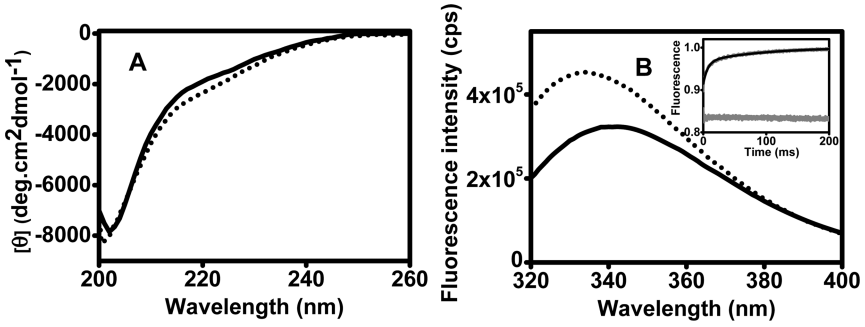

Figure 2. (A) CD and (B) Trp fluorescence spectra (λ_{ex} 295 nm) of κ-casein in the absence of SDS (solid line) and in the presence of 100 μM SDS (dotted line). The inset in (B) shows the stopped-flow kinetics of Trp fluorescence to monitor the structural changes from the native to SDS-induced state.

wavelengths from 440 to 600 nm after every 10 nm using an emission bandpass of 6 nm. The decays obtained at different emission wavelengths were then analyzed and fitted taking into account the IRF using DAS6 software provided with the TCSPC system. The fitted parameters were then used to simulate the fluorescence decays at different emission wavelength in Origin 8.5 software using $I_\lambda(t) = \Sigma\alpha_i e^{-t/\tau_i}$, where α_i and τ_i represent the contributions and lifetime of the different lifetime components, respectively. The time-resolved emission spectra (TRES) were constructed using

$$I(\lambda,t) = \frac{I_\lambda^{SS} I_\lambda(t)}{\sum \alpha_i \tau_i} \quad (3)$$

where I_λ^{SS} represents the steady-state fluorescence intensity at a fixed emission wavelength λ and $I_\lambda(t)$ as mentioned above is given by $I_\lambda(t) = \Sigma\alpha_i e^{-t/\tau_i}$. The spectra obtained at different time points were then normalized and fitted using log-normal function to extract the peak frequency $\nu(t)$ as a function of time. The solvation correlation function is given by

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \quad (4)$$

where $\nu(0)$, $\nu(t)$, and $\nu(\infty)$ are the emission peak frequencies at time 0, t, and ∞, respectively. The correlation function was then fitted using a sum of exponentials as the following:

$$C(t) = \sum \beta_i e^{-t/\tau_{si}} \quad (5)$$

where β_i and τ_{si} represents the contributions and solvation time of the different solvation components, respectively.

3. RESULTS

3.1. Water Accessibility within the Compact Disordered Globules. Our previous bioinformatics analysis of bovine κ-casein has indicated that the amino acid sequence describes an intrinsically disordered state of the protein.[17] A closer look at the amino acid sequence of κ-casein revealed that the protein comprises at least two distinct segments having opposite net charge (Figure 1). The positively charged N-terminal segment comprising residues 1−100 contains fairly high occurrence of glutamine (Q) and asparagine (N) that contain amide side-chain (Q + N = 18). This segment also contains 9 tyrosines (Y), 6 lysines (K), and 5 arginines (R). The Q/N-rich segment of κ-casein is reminiscent of amyloidogenic segment of yeast prion proteins.[35,36] Therefore, this N-segment of κ-casein could potentially act as a prion-like amyloidogenic domain albeit the occurrence of Q/N/Y is much lower compared to that in yeast prion proteins. On the contrary, the C-terminal segment (101−169) is largely negatively charged and has a lower content of Q/N. The N-terminal segment adopts a collapsed premolten globule like structure under the physiological condition.[17] We hypothesized that this N-terminal segment will undergo further collapse in the presence of anionic detergent molecules. Upon binding to negatively charged detergent molecules, the charge repulsion in the N-terminal segment arising from K and R will be diminished and therefore the polypeptide chain is likely to undergo a transition from a labile collapsed globule to a more compact globule. We also conjectured that as a result of compaction of the globule, the water molecules within the globule will become more ordered.

First, to monitor the secondary structural changes, we carried out circular dichroism (CD) experiments. The CD spectrum of

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κ-casein under the physiological condition represents a disordered state.[17] Next we monitored the CD spectrum in the presence of an anionic detergent, SDS, at submicellar concentration. Although a little increase in the ellipticity at 222 nm was observed upon addition of 100 μM SDS (Figure 2A), the polypeptide did not undergo any large scale structural rearrangement and retained the premolten globule-like state as assessed by the CD double wavelength plot analysis [$\theta_{222}/\theta_{200}$].[10] Next, to further structurally characterize the SDS-induced state, we recorded the fluorescence of the single tryptophan (Trp 76) that is present in the Q/N-rich positively charged domain. In the absence of SDS, the Trp emission spectrum exhibited a maximum ∼342 nm, suggesting that Trp experiences partial protection from the bulk water (Figure 2B). Upon addition of SDS, the fluorescence intensity increased with a concomitant blue shift to ∼335 nm, suggesting further compaction of the unstructured globule in the presence of SDS (Figure 2B). The stopped-flow fluorescence experiments revealed that the compaction occurs on the 100 ms time scale with some amplitude of milliseconds and unresolved submillisecond kinetic phases (Figure 2B inset). We point out that this compaction of the disordered polypeptide chain does not cause Trp to experience a significant protection from water because the emission maxima do not exhibit a substantial blue shift (<330 nm). Therefore, the compact globules in both the absence and the presence of SDS represent wet globules containing water molecules within the collapsed yet disordered polypeptide chain. This interpretation is further corroborated by the acrylamide quenching experiments that demonstrated only a marginal protection of Trp from water in the compact disordered state (bimolecular quenching constant, $k_q \sim 4 \times 10^9$ M^{-1} s^{-1}). Taken together, this set of results reveals that unordered yet collapsed globule of κ-casein undergoes a further compaction in the presence an anionic detergent that diminishes charge repulsion within the polypeptide chain in a poor solvent such as water. Our Trp fluorescence readouts provide a support in favor of the presence of inner aqueous environment within the collapsed globules. We next performed experiments aiming at characterizing the nature of water molecules within the globules.

3.2. Slow Dipolar Relaxation of Trp Observed by Red-Edge Excitation Shift. To delineate the characteristics of entrapped water molecules, we took advantage of a fluorescence readout, namely the red-edge excitation shift (REES) that represents a unique and sensitive approach to monitor the dynamics of restricted water molecules.[37−41] In bulk (nonviscous) media, the fluorescence emission peak maxima is independent of the excitation wavelength because the time scale of water reorientation (solvation dynamics in response to a transiently created excitation dipole) is orders of magnitude faster (picoseconds) compared to the fluorescence emission time scale (nanoseconds). In a highly ordered microenvironment, the water reorientation time gets impeded and often compares with the time scale of fluorescence emission (fluorescence lifetime). Under such conditions, the emission maxima exhibit a progressive red shift when the excitation wavelength is shifted to the red edge of the absorption spectrum.[37] Because a variety of spectral signatures suggested that the single tryptophan (Trp 76) of κ-casein resides in the wet globule, we hypothesized that the REES measurements on Trp 76 will report the dynamics of otherwise optically silent water molecules present within the globule. Under native condition in the absence of salt, Trp demonstrated ∼6 nm REES upon changing the excitation wavelength from 290 to 305 nm (Figure 3A,B). In the presence

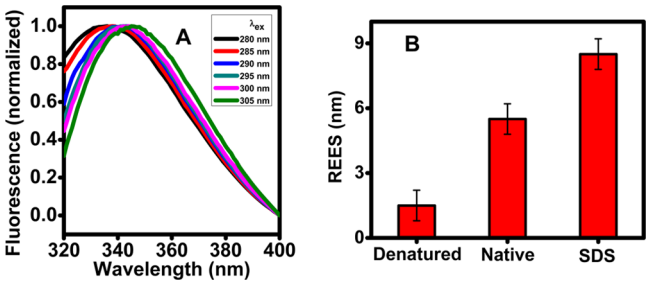

Figure 3. Red-edge excitation shift (REES) of Trp 76 in κ-casein. (A) The normalized fluorescence spectra obtained with different excitation wavelength from 280 to 305 nm. (B) The extent of REES (in nm) obtained using λ_{ex} from 290 to 305 nm plotted for different conformational states of the protein. Data are shown as mean ± SEM (n = 3).

of SDS, the extent of REES increased to ∼9 nm (Figure 3B), suggesting that the water molecules become progressively ordered as the chain undergoes further compaction. On the contrary, the GdmCl denatured state showed a much lower extent of REES (Figure 3B). Although the REES measurement is a reliable and sensitive indicator of slow solvent relaxation, it does not allow us to estimate the time scale of water relaxation. Therefore, to directly monitor the water dynamics, we next embarked on the study of the solvation dynamics using time-resolved emission spectra (TRES) that has previously been successfully employed in understanding the organization and dynamics around a fluorophore in complex chemical and biological systems.[42−52]

3.3. Dynamics of Water within the Globules. To measure the dynamics of water molecules within the unstructured globules, we covalently attached an environmentally sensitive dye, such as acrylodan, which reacts with the free thiol groups of Cys present in the protein. Acrylodan was chosen among the other thiol-active fluorescent dyes because it is a small environment-sensitive fluorophore, creates a highly stable thioether bond with Cys, demonstrates high fluorescence even at low (micromolar) protein concentrations and has been previously used for the solvation dynamics studies of proteins.[51,52] As a prelude to our solvation dynamics experiments, we first recorded the fluorescence spectrum of acrylodan-labeled κ-casein under denatured and native conditions. The emission peak shifted from ∼530 to ∼510 nm upon changing the condition from denatured to native suggesting partial burial of the fluorophore in the collapsed state under the native condition (Figure 4A). However, the blue shift was much lower compared to that seen when acrylodan is well protected from the aqueous environment (≲480 nm).[53] Additionally, the time-resolved fluorescence depolarization measurements indicated considerable local flexibility of the fluorophore along with the global tumbling of the globules with hydrodynamic radii of ∼2 nm (Figure 4B).[17] These findings corroborate the intrinsic Trp fluorescence results, indicating that the polypeptide chain adopts a wet-globule state under the native condition.

We next embarked upon TRES experiments to estimate the time scale of water reorientation around the fluorophore within the globules under native condition by monitoring the time-dependent Stokes shift (TDSS). Figure 5A shows the time-

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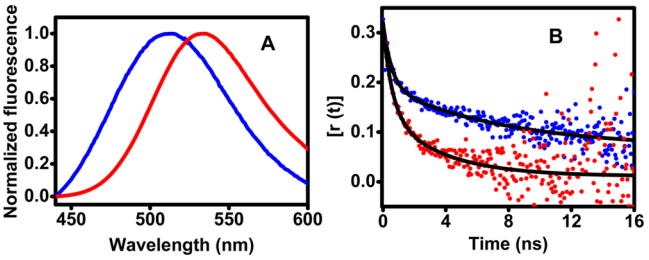

Figure 4. (A) Steady-state fluorescence spectra and (B) time-resolved fluorescence anisotropy decays of acrylodan-labeled κ-casein under denatured condition (red line) and native condition (blue line). Anisotropy fits have been represented with a black line. The recovered rotational correlation times are ϕ_1 = 0.4 ± 0.2 ns, ϕ_2 := 2.7 ± 1.0 ns (denatured) and ϕ_1 = 0.4 ± 0.2 ns, ϕ_2 := 7.2 ± 1.0 ns (native). The intrinsic time-zero anisotropy of acrylodan was 0.30.

resolved decays of acrylodan monitored at different emission wavelengths ranging from 440 to 600 nm. The fluorescence decays become progressively longer with an increase in the average fluorescence lifetime as a function of emission wavelength and thus provide a clear indication of slow nanosecond solvent relaxation. The time-resolved spectra constructed from these decays exhibit gradual red shift in frequency, total shift of ∼740 cm^{-1} (Figure 5B) (for details, see Experimental Methods). The hydration correlation function $[C(t)]$ was then plotted as a function of time. The fitting of $C(t)$ using a sum exponentials yielded two well-separated solvation time components: τ_{s1} ∼ 0.4 ns and τ_{s2} ∼ 1.4 ns (Figure 5C and Figure S2, Supporting Information). The longer relaxation time is 3 orders of magnitude slower than the slowest component (picoseconds) found in bulk water.[45] In the presence of SDS, the longer component of solvation time progressively increased from ∼1.4 to ∼2 ns as a function of SDS concentration (Figure 6, Figure S3, Supporting Information). Taken together, the nanosecond reorientation of water molecules suggests that the compact yet unordered globules of κ-casein contain motionally restrained water molecules.

4. DISCUSSION

The N-terminal segment (1−100) of κ-casein possesses an amino acid composition that is reminiscent of a Q/N/Y-rich intrinsically disordered prion determinant of the yeast prion protein, Sup35. In this segment, the composition (Q + N + Y) is 27%, which is much higher compared to the average natural occurrence (∼11%) in proteins but much lower compared to the composition found in the N-domain (1−121) of Sup 35 (61%). We have previously shown that under native condition this N-region of κ-casein undergoes a chain collapse, a phenomenon that is also observed for Sup35, polyQ, and many other amyloidogenic IDPs.[17] The N-segment of κ-casein has a net charge of +5.3 at neutral pH. In the presence of low nondenaturing concentration of an anionic detergent, possibly because of the charge neutralization, the N-terminal segment demonstrates a transition to a more compact yet disordered wet globule.

The coil-to-globule transition as a result of chain collapse of an IDP in a poor solvent, such as water, has important implications in amyloid formation. However, the nature of water present within an unstructured globule remained unexplored and elusive. Our results demonstrate that the water molecules within the globules are highly restrained. Many previous theoretical and experimental investigations on the relaxation of bulk water revealed that the solvation time ranges between 100s of femtoseconds to picoseconds.[45] On the contrary, water molecules present on the protein surface, also termed as "biological water", has much slower relaxation time ranging between 10 and 100 ps.[45,46] The mobility of water molecules at the protein surface has been implicated in various important events like protein folding, molecular recognition, and enzyme catalysis.[45,47−49] In our case, the water relaxation occurs on the nanosecond time scale and is 3 orders of magnitude slower than that of bulk water. Therefore, the water within the disordered globule is significantly more ordered than the "biological water" at the protein surface and resembles the constrained water pool found in nanoconfined systems such as reverse micelles and molten-globule intermediates.[42,49] Taken together, our results show the presence of a highly restrained water molecules within the IDP globule (Figure 7). A recent study has also indicated the restricted water motions on the surface of an amyloidogenic IDP, namely tau.[54]

What is the origin of highly ordered water within the compact disordered globule? The chain collapse leads to the formation of globules in which backbone amides participate in a hydrogen bonding network with the entrapped water molecules. The amide side chains from glutamines (Q) and asparagines (N) as well as polar hydroxyl groups from tyrosines can also enrich the hydrogen-bonded network of water molecules in the collapsed globule. These water molecules become even more strongly networked and highly ordered when the globules undergo more compaction by decreasing the internal charge per residue. Additionally, the chain fluctuations can also contribute to the solvation dynamics.[47]

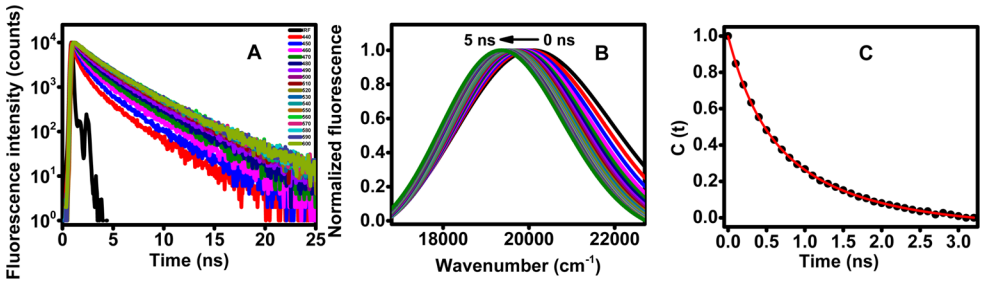

Figure 5. (A) Time-resolved fluorescence decays of acrylodan in native state of κ-casein obtained at different emission wavelengths from 440 to 600 nm. (B) Time-resolved emission spectra (TRES) constructed from the time-resolved fluorescence decays at different wavelengths. (C) Solvation correlation function (filled circle) and the biexponential fit (solid red line) to obtain the solvation time. From independent experiments, the total frequency shift was estimated to be 739 ± 60 cm^{-1} and the recovered solvation times are τ_{s1} = 0.38 ± 0.10 ns and τ_{s2} = 1.39 ± 0.15 ns.

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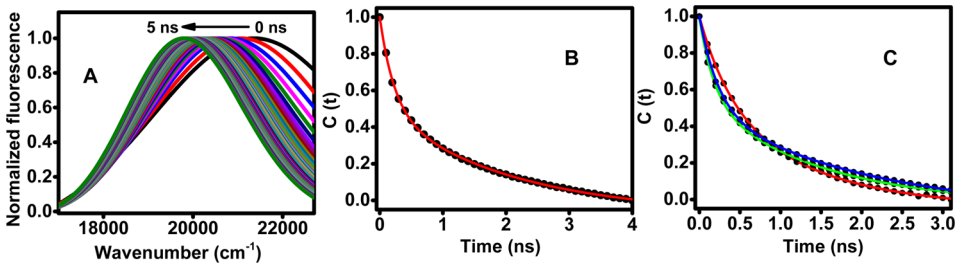

Figure 6. (A) Time-resolved emission spectra (TRES) constructed from the time-resolved fluorescence decays at different wavelengths for acrylodan in the SDS-induced state of κ-casein ([SDS] = 500 μM). The total frequency shift was 1565 cm^{-1}. (B) The solvation correlation function and the biexponential fit (solid red line). The longer component of solvation time (τ_{s2}) was 2.1 ns (see Figure S3, Supporting Information, for details). (C) Comparison of the solvation correlation functions showing dampening of solvent relaxation as a function of SDS concentration. Red, green, and blue lines represent the fits for the correlation functions in the absence of SDS, in the presence of 100 and 500 μM, respectively.

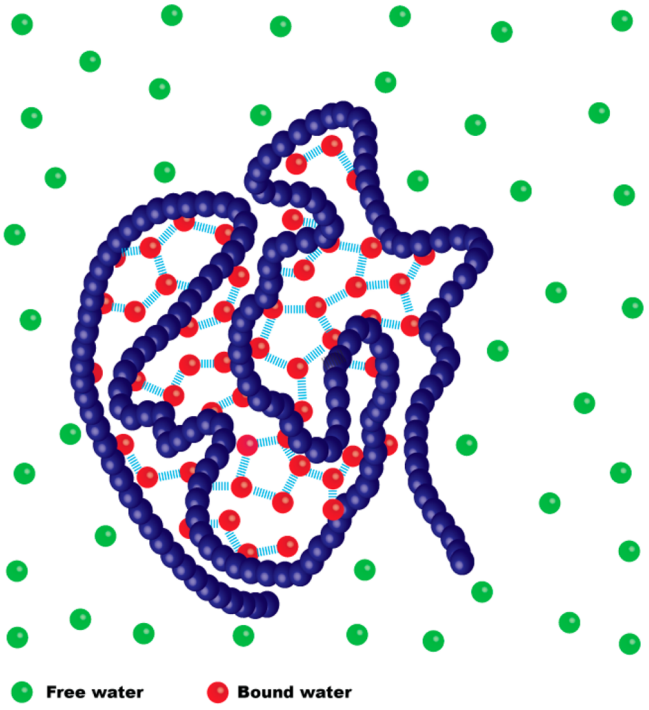

Figure 7. Schematic model showing ordered water cluster within the collapsed disordered state of the globule.

What is the implication of ordered water in the collapsed globules of an amyloidogenic protein? Experiments and simulations on amyloidogenic IDP consisting of Q/N-rich sequence have indicated that the formation of key oligomeric intermediates is crucial for amyloid assembly.[14,19,26,36] These obligatory oligomers are formed by coalescence of collapsed globules. We speculate that the process of oligomer formation will be favored by an entropic gain arising out of the release of ordered water molecules from the interior of the globule to the bulk milieu.[55] The conformational maturation of the polypeptide within the oligomers is likely to lead to the formation of ordered amyloid fibrils via the nucleated conformational conversion mechanism.[35,36,56]

■ ASSOCIATED CONTENT

Supporting Information

Time-resolved spectra and corresponding fits with log-normal function, assessment of single exponential and biexponential fits for solvation correlation function, and time-resolved emission spectra and solvation correlation functions in the presence of SDS (Figure S1–S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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