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Interplay between desolvation and secondary structure in mediating cosolvent and temperature induced alpha-synuclein aggregation

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Abstract

Both increased temperature and moderate concentrations of fluorinated alcohols enhance aggregation of the Parkinson's disease-associated protein α -synuclein (α S). Here, we investigate the secondary structural rearrangements induced by heating and trifluoroethanol (TFE). At low TFE concentrations, CD spectra feature a negative peak characteristic of disordered polypeptides near 200 nm and a slight shoulder around 220 nm suggesting some polyproline-II content. Upon heating, these peaks weaken, while a weak negative signal develops at 222 nm. At high TFE concentrations, the spectra show distinct minima at 208 and 222 nm, indicative of considerable α -helical structure, which diminish upon heating. We observe a crossover between the low-TFE and high-TFE behavior near 15% TFE, where we previously showed that a partially helical intermediate is populated. We postulate that the protein is well solvated by water at low TFE concentrations and by TFE at high TFE concentrations, but may become desolvated at the crossover point. We discuss the potential roles and interplay of desolvation and helical secondary structure in driving α S aggregation.

Keywords

Circular dichroism; Parkinson's disease; amyloid; protein aggregation; protein-solvent interactions; chemical physics and physical chemistry; biological physics; soft matter; liquids and polymers

1. Introduction

Parkinson's disease (PD) is characterized by dense Lewy body inclusions, which are primarily composed of amyloid fibrils formed from the protein α -synuclein (α S). The precise role of α S aggregation in PD remains unclear, but recent evidence suggests that amyloid fibrils may be protective, while smaller oligomers or alternate aggregate structures may be responsible for dopaminergic cell death [1]. Solution conditions, including pH,

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PACS Classification Codes

87.14.E Proteins

82.35.Pq Biopolymers, biopolymerization

87.14.em Amyloids

87.15.kr Protein-solvent interactions

87.15.B Structure of biomolecules

87.15.N Properties of solutions of macromolecules

87.64.Ee Electron microscopy in biophysics

temperature, or the presence of detergents, lipids, or alcohols, affect both the conformation of monomeric α S and the amount and type of aggregates that are produced [2-4]. Observations of α S secondary structural changes under aggregation-promoting conditions led to the hypothesis that “folding intermediates”, or specific partially structured monomer conformations, initiate aggregation reactions [5].

We recently showed that the N terminal lipid-binding domain of α S adopts a partially helical intermediate conformation in the presence of moderate amounts of the fluorinated alcohol 2,2,2-trifluoroethanol (TFE), and that population of the intermediate state is correlated with the formation of annular and fibrillar aggregates [2]. Partially helical conformations are also detected when α S is incubated in the presence of detergents [6], and flexible aggregates that may be similar to TFE-induced species can be grown in such solutions [3]. However, a causal relationship between a particular “intermediate” state and an aggregation pathway is difficult to establish—solution conditions that promote aggregation may produce coincidental changes in protein structure, or a conformational state may be a true intermediate in a fibrillization pathway.

Here, we use circular dichroism (CD) spectroscopy to investigate the combined effects of temperature and TFE on α S secondary structure. We examine structural changes affecting the N terminal portion of the protein by examining the 1-102 C terminal truncation mutant (α S102). We observe qualitative similarities between α S conformational changes induced by heating and by low [TFE], which are consistent with loss of polyproline-II (PPII) and a gain in helical secondary structure, possibly as a result of weakened water-protein interactions [7]. Interestingly, CD spectra from α S102 solutions containing ~15% TFE appear to be invariant with respect to temperature, which suggests that the TFE-induced intermediate conformation is similar to the high-temperature state. Moreover, we observe a distinct crossover at ~15% TFE, below which the CD spectra feature a negative peak near 200 nm that diminishes with increased temperature, and above which the spectra reflect α -helical structure that is disrupted by heating. Therefore, the local environment near α S102 molecules seems to be “water-like” at low TFE and “TFE-like” at high TFE.

Hydration is a protective factor that helps to stabilize disordered proteins [8]. We postulate that the addition of small amounts (<~15%) of TFE to aqueous solution reduces protein hydration, resulting in increased secondary structure formation and reduction of monomer solubility, while high TFE conditions inhibit aggregation as a result of stable secondary structure that appears to be well solvated by TFE. Thus, both secondary structure formation and desolvation may contribute to initiating α S aggregation.

2. Materials and Methods

2.1. Reagents and solutions

All chemicals were reagent grade and all solutions were prepared using MilliQ (18.2 M Ω cm) or HPLC grade water. Acros Organics brand 99.8% pure 2,2,2-Trifluoroethanol (TFE) was purchased from Fisher Scientific.

2.2. Protein expression and purification

Recombinant WT and mutant α S were produced and purified as previously described [9]. Lyophilized α S variant protein was dissolved at 1-2 mg/mL in pH 7.5 buffer. Insoluble material was removed by filtering each stock solution through a 100 kDa (Microcon YM-100, Millipore) centrifugal spin filter.

2.3. Circular dichroism spectroscopy

An Aviv 400 Circular Dichroism Spectrometer (Aviv Biomedical) was used to obtain far-UV CD data. All samples were measured using a 1 cm path length, a strain free quartz cuvette, and a bandwidth of 1 nm. A noise-reducing option in the instrument software was used to smooth the data. Three scans with a speed of 1 sec per nm were averaged to obtain each curve.

For variable-temperature experiments at pH 7.5, the solutions contained 10 mM sodium phosphate buffer (Sigma), while the pH 2.4 samples contained 10 mM phosphoric acid (Mallinckrodt Baker). The pH values we report refer to the pH of solutions in the absence of TFE; TFE-induced pH shifts for buffer and water ionization constants are expected to be minimal at low to neutral pH; therefore, we ignore these effects [10]. Each sample was prepared by mixing the protein, water, and buffer salts or acid, chilling these solutions to $\sim 4^{\circ}\text{C}$, and then adding room-temperature TFE to the aqueous protein solutions on ice. Then, these samples were placed in the CD spectrophotometer and cooled to 2°C . CD spectra were obtained starting at 2°C and heating to the maximum temperature. After the heating cycle, the solutions were cooled and a final measurement was performed at 2°C to quantify hysteresis. The baselining procedure averaged over temperature-related drifts but accounted for some solvent expansion and contraction due to temperature changes (see the supplementary data). Errors in the measurements were estimated from the standard deviations of three measurements and from uncertainties due to temperature drifts in the baseline signals.

2.4. Transmission electron microscopy imaging

50 μM αS variant solutions in 10 mM pH 7.5 sodium phosphate buffer were incubated at 70°C for three days in quiescent conditions prior to examination. 0.02% sodium azide (Sigma) was added to these solutions as a preservative. TEM images of fibrils were obtained as described previously [2].

3. Results

3.1. Variable-temperature CD spectra of 0.5 μM αS102 in 0–60% TFE

We previously showed that secondary structural changes induced by TFE involve the N-terminal lipid-binding domain of αS [2]. We find that the heating-induced secondary structure changes reported by Uversky et al. [5] are also largely restricted to the N-terminal lipid-binding domain of the protein (see below) and accordingly, in this paper, we focus on structural changes in this same region and perform most of our experiments on αS102 . We find that the truncated protein is significantly less aggregation-prone upon heating at pH 2.4 compared to pH 7.5 (figure S1), and because this facilitates studies of changes in monomer structure, we collect much of our data at pH 2.4 and compare these to data collected at pH 7.5 when possible. We confirm (figure S2) previous reports showing that the effects of low pH on the secondary structure of synuclein are confined to the C-terminal tail of the protein [11–14] and note that protonation of the C-terminal region is also associated with increased αS aggregation [5].

Figure 1 shows variable-temperature (T) CD spectra for αS102 at pH 2.4 in the presence of various amounts of TFE. We verified that temperature-dependent changes in the structures were reversible by measuring the spectra at $T = 2^{\circ}\text{C}$ before and after heating, and we assume that the proteins remain monomeric in these dilute (0.5 μM) solutions when no significant hysteresis is observed (see the supplementary data, figure S3(a-o), and table S1). For some TFE concentrations, we must measure in a restricted temperature range to avoid hysteresis. The 0% TFE, variable-temperature spectra in figure 1(a) resembles the variable-temperature

data obtained by Uversky et al. [5] for the full-length protein, suggesting that heat-induced structural changes involve primarily the N terminus of α S. Indeed, CD spectra for the full-length WT protein at 0 % and 60 % TFE behave similarly upon heating to those obtained for α S102, both qualitatively, and when the signal at $[\theta]_{222}$ is quantified and plotted (figure S4). Thus, the effects of temperature on α S structure, as reported on by CD, are largely restricted to the N-terminal domain of the protein.

Inspection of the α S102 spectra reveals two general categories of behavior. Below ~12% TFE (figure 1(a-e)), the curves resemble the 0% TFE case and feature a negative peak near 200 nm, which diminishes with heating, while the ellipticity at 222 nm ($[\theta]_{222}$) becomes more negative at high T . Above ~20% TFE (figure 1(k-o)), the spectra show the two negative peaks near 208 and 222 nm that are suggestive of α -helical structure; heating these samples leads to a decrease in the amplitude of $[\theta]_{222}$. Near 15% TFE (figure 1(f-i)), the curves do not change much with heating, although we must measure a restricted temperature range because these samples are aggregation-prone. Data for pH 7.5 samples show qualitatively similar behavior at low and high TFE (figure S5).

There appear to be two distinct types of isodichroic points for spectra in each of the two described categories (table 1). Below ~10% TFE, the points are located near 207 nm and $-9 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$, while above ~20% TFE, they occur near 204 nm and $-20 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$. The locations of these points are similar to the isodichroics observed upon increasing TFE concentrations at constant-temperature in Anderson et al. [2], although there is a possible trend toward larger negative values at high TFE.

Figure 2(a) shows $[\theta]_{222}$ vs. T curves for the samples from figure 1. This plot again demonstrates distinct categories of behavior. At low TFE, the curves show a negative slope, while above ~25% TFE, the slopes are positive. Near 10–15% TFE, the signals change very little with temperature. Distinct high and low TFE behavior is also observed for pH 7.5 $[\theta]_{222}$ vs. T plots (figure S6).

We note that at 17–20% TFE, the curves are non-monotonic, with a slight negative slope changing to a positive slope between 10 and 20°C (figure 2(a)). These curves appear analogous to low-HFIP curves for model peptides that were reported previously [15] and may reflect a slight degree of cold denaturation. For both peptides in HFIP and α S in TFE, non-monotonic helix induction curves are observed for a limited range of alcohol concentrations, which may reflect enhanced “solvophobicity” at moderate alcohol concentrations, combined with a high heat capacity for disordered states relative to helical conformations [15]. Alternatively, three-state coexistence, which may occur at moderate alcohol concentrations where α -helical structure is marginally stable, could lead to non-monotonic helix induction curves [16].

When plotted as a function of [TFE], the $[\theta]_{222}$ curves appear sigmoidal (figure 2(b)). However, the data for all temperatures appear to overlap or approach similar values in the ~12–16% TFE range. A comparison of $[\theta]_{222}$ vs. [TFE] plots for pH 2.4 and pH 7.5 samples at 25°C (figure 2(c)) reveals that the curves overlap for mostly aqueous solutions, but diverge above ~20% TFE. However, when the data is rescaled so that the maximum and minimum values coincide, the curves are similar at both pH values (figure 2(c) inset). When we compare 2.4 and pH 7.5 samples at various temperatures, we find that these trends persist; low (~7%) TFE spectra are similar for both pH values, while above ~30% TFE, the amplitude of $[\theta]_{222}$ is significantly larger for pH 2.4 samples compared to pH 7.5 samples (figure S7). Apparently, the protein conformations at high TFE vary slightly with pH, with ~5-10 more residues per protein molecule adopting helical structure at low pH (table S2). The origin of this small effect is unclear at present.

In figure 3(a), we plot points derived from the spectra in figure 1 on a transition diagram [17]. Figure 3(b) shows a similar plot for the pH 7.5 samples from figure S5. Points on the pH 2.4 transition diagram appear slightly offset from the constant-temperature lines from Anderson et al. [2], particularly at low [TFE]. The differences are mostly due to reduced $[\theta]_{200}$ for pH 2.4 samples compared to pH 7.5 samples. It is unclear whether this difference is due to increased signal from the pH 7.5 baseline buffer at low wavelengths or whether it reflects a slight pH-dependent shift in the disordered conformation.

Figure 3 shows that, for both pH 2.4 and pH 7.5 samples, heating appears to shift the conformation toward the point of intersection of the constant-temperature lines, which we previously associated with the TFE-induced intermediate conformation [2]. Moreover, the existence of two distinct isodichroics at different wavelengths (table 1, table S3), along with the qualitative differences in the behavior of the high- and low-TFE samples, confirms that the protein is sampling at least three conformations at each pH value.

We previously observed that TFE-dependent changes in the CD signal of α S appear to saturate at ~40% TFE for pH 7.5 samples [2]. We verify that higher [TFE] does not lead to significant changes in the secondary structure of α S102 by investigating the CD spectra at ~80–99% TFE; these high-TFE CD spectra are nearly identical to the pH 7.5, 60% TFE α S102 curve (see the supplementary data and figure S8).

3.2. Ultrastructure of α S and α S102 aggregates produced by elevated temperatures

Figure 4 shows transmission electron microscope (TEM) images of WT α S and α S102 fibrils grown in pH 7.5 buffer containing 0% and 15% TFE after three days incubation at 70°C under quiescent conditions. Note that TEM imaging may not uniformly sample all species present in solution, and so we cannot draw definite conclusions about the typical fibrils formed in each sample. In particular, rare species and fibrils that do not adhere to the TEM grids may not be represented in our images.

In the absence of TFE, we observe rigid, linear fibrils for both WT α S and α S102, although α S102 samples appear to contain more and thicker fibrils, which often clump together. We observe fibril diameters ranging from 6–14 nm for WT α S in 0% TFE; the median width is 9 nm. For the α S102 images, the median fibril diameter is 14 nm, and a range of 8–18 nm widths is observed. Moreover, the fibril lengths for the WT α S samples range from 35 nm to over 600 nm, whereas fibrils 25–195 nm long are found in the α S102 sample.

For WT α S in 15% TFE, large quantities of “TFE fibrils” similar to those observed previously [2] are produced and no classic amyloid is observed. However, α S102 samples in 15% TFE tend to contain linear fibrils, while a few flexible, helical TFE fibrils are observed as a minor fraction. The median width of TFE fibrils found in the WT α S sample is 13 nm, and the range of diameters is 9–21 nm. The WT α S TFE fibril lengths range from 57 nm to over 600 nm. For the α S102 samples, the TFE fibril diameter range is 10–16 nm, with a median of 11 nm. The α S102 TFE fibril lengths range from 133 nm to over 600 nm. Rigid, amyloid-like fibrils grown from α S102 appear to be 88 nm to over 600 nm long. These rigid α S102 fibrils are 8–15 nm wide, with a median diameter of 12 nm.

4. Discussion

We have examined the effects of temperature and TFE on α S secondary structure in order to determine the relationships among conformational rearrangements induced by various aggregation-promoting conditions. We previously found that aggregation was enhanced near ~15% TFE, a condition coinciding with the formation of a helical intermediate [2]. Here, we demonstrate that the CD spectra show distinct temperature-dependent behavior above and

below this threshold. We postulate that the α S is hydrated at low TFE, while at high TFE the protein experiences a TFE-rich local environment. Heating favors desolvation under both conditions. Aggregation is maximally enhanced at intermediate [TFE] where the spectra are neither fully TFE-like nor water-like. Desolvation may thus play a role in the formation of both the TFE-induced and the high-temperature intermediate states and their aggregation.

4.1 Variable-temperature CD spectra of α S show distinct behavior above and below ~15% TFE

Inspection of our variable-temperature CD curves (figure 1) reveals two types of spectra. Below ~12% TFE, the curves show the large negative peak near 200 nm that is characteristic of disordered polypeptides, as well as a small shoulder near 220 nm that suggests the presence of some PPII structure. Above ~20% TFE, the spectra are distinctly α -helical, with minima at 208 and 222 nm. Isodichroic points for the variable-temperature CD spectra occur near 207 nm for the low-TFE samples, and near 204 nm for the high-TFE samples (table 1, table S3). Crossover behavior is also apparent in the temperature dependence of $[\theta]_{222}$ at different TFE concentrations (figure 2(a)); below ~12% TFE, plots of $[\theta]_{222}$ vs. temperature exhibit negative slopes, while above ~20% TFE, the slopes are positive. Interestingly, the crossover point (~15% TFE) coincides with conditions in which the TFE-induced intermediate is expected to be highly populated and aggregation enhancement occurs [2].

4.2. Temperature-induced conformational changes in α S affect the N terminal region and are consistent with loss of PPII and gain of helical structure

Uversky et al. first reported temperature-induced changes in the CD signal of WT α S in aqueous solution [5]. We demonstrate that the structural changes induced by heating are similar for α S102 and full-length WT α S (figure 1, figure S4, figure S5). The α S102 results indicate that the N terminal portion of α S is predominantly affected by heating, while the C terminus is not, or only slightly, affected (figure S4(e-f)).

Heat-induced changes in the CD spectra of disordered model peptides were initially observed decades ago [18]. Similar spectral changes, characterized by a decrease in ellipticity near 220 nm and concurrent weakening of the ~200 nm negative peak, were subsequently observed for additional proteins and peptides at elevated temperature (reference [19] and references therein). These changes are generally thought to reflect loss of PPII structure from the ensemble of disordered conformations [7]. The spectral changes we observe for the α S variants in water or low TFE concentrations suggest similar disruption of PPII structure at high temperatures. In addition, heating such samples promotes the appearance of a negative signal at 222 nm, suggesting the formation of some degree of helical structure.

4.3. The secondary structural changes induced in α S by low TFE also suggest loss of PPII and gain of helical structure

The variable-temperature, 0% TFE CD spectra (figure 1(a)) are qualitatively similar to previously observed changes induced by addition of small amounts of TFE [2]. The 5–10% TFE, variable-temperature CD spectra also are similar to the 0% TFE case, and show weakening of the 200 nm peak and the appearance of negative signal at 222 nm as the solutions are heated (figure 1(b-d)). In addition, both TFE- and heat-induced transitions involve the N terminal portion of the proteins [2]. These similarities suggest that loss of PPII and gain of helical structure may be induced by either heating or by incubation of α S in solutions containing <~15% TFE.

4.4. TFE and increased temperature may drive similar structural changes in α S

Our spectra suggest that TFE and heating induce intermediate conformations in α S that are similar and possibly related to one another. Plots of $[\theta]_{222}$ as a function of [TFE] (figure 2(b)) demonstrate that the curves approach similar values near 15% TFE for all T , which suggests that the TFE-induced intermediate structure may be invariant with respect to temperature. The $[\theta]_{222}$ vs. T curves (figure 2(a)) also appear to approach intermediate-TFE values at high temperatures, which is consistent with heating leading to increased population of a conformation similar to the TFE-induced intermediate.

The transition diagram representations of our CD data also reveal significant overlap between temperature- and TFE-induced transitions. At pH 7.5, points derived from CD spectra for various [TFE] and temperatures collapse onto the two straight lines that characterize the constant-temperature, TFE-induced structural transitions we identified previously (figure 3(b)). Increasing the temperature causes a shift toward the region of the diagram that corresponds to the TFE-induced intermediate conformation. Transition diagrams for samples at lower pH are qualitatively similar (figure 3(a)).

For constant-temperature (25°C), variable-TFE samples, we previously detected three factors via PCA, which were readily identifiable as reflecting the disordered state, the intermediate conformation, and a highly α -helical state [2]. However, the combined temperature- and TFE-dependent PCA results are more complex. The total number of significant factors is estimated to be at least four for our pH 2.4 data, and at least three for our pH 7.5 data (see the supplementary data and figure S9(a-b)). This precludes a direct comparison of any temperature-induced intermediates with the previously characterized TFE-induced intermediate. We note that some of the variations appear to be due to temperature-induced differences in the relative magnitudes of the CD signal at 208 vs. 222 nm for the highly helical conformation (figure S9(c-h) and figure S10), which may be expected for helical proteins and peptides [20]. Ultimately, while PCA clearly reveals components that are associated with temperature-induced spectral changes, it is unclear whether these factors are indicative of distinct states or result from temperature-dependent variations in the signals from the three previously-identified conformations.

We emphasize that CD spectroscopy alone cannot provide proof that the protein is sampling the same structures in different solution conditions. Nevertheless, our data are consistent with this possibility.

4.5. TFE- or heat-induced desolvation may contribute to helical structure formation

The precise mechanisms by which TFE promotes helical or other secondary structures have been much studied, yet remain controversial. It is likely that part of the effect of TFE can be ascribed to a favoring of intra-protein hydrogen bonds over protein-solvent hydrogen bonds. This may be due either to a chaotropic effect on protein-water interactions [21] or to an indirect kosmotropic effect on bulk water [22]. The latter effect may disfavor non hydrogen-bond mediated protein-water interactions, promoting desolvation, and also potentially contributing somewhat to helix formation since this process involves a significant degree of backbone desolvation [23].

Increased temperatures may also be expected to lead to protein desolvation by increasing the strength of the hydrophobic effect. This increase is accompanied by a shift from a largely entropic mechanism to an enthalpic one at higher temperatures [24], but in either case it favors removal of water from the protein surface. For a disordered protein like α S, an increase in the hydrophobic effect may drive the formation of more compact states, including those containing helical structure, which as described above can bury a significant

degree of hydrophobic backbone character while simultaneously satisfying backbone hydrogen-bonds.

4.6. TFE- or heat-induced desolvation may promote α S aggregation

We find that α S adopts “water-like” secondary structure below ~12% TFE; the CD spectra for these samples contain a peak near 200 nm that weakens with heating, likely reflecting the loss of some PPII structure, and a weak signal at 222 nm, suggestive of helicity, that increases with heating. Above ~20% TFE, “TFE-like”, highly α -helical structures are implied by the spectra. Aggregation is enhanced near 15% TFE, where a partially helical intermediate conformation is highly populated [2].

PPII structure may be a signature of water-protein interactions, particularly water-protein hydrogen bonding, while stable highly α -helical structure may reflect preferential TFE solvation of proteins. The fact that aggregation enhancement occurs where both PPII is disrupted and helical structure is only marginally stabilized suggests that loss of protective solvent interactions may contribute significantly to initiating aggregation in TFE. This may occur in concert with secondary structure formation, because desolvation of amphipathic helical structure would strongly favor inter-molecular protein-protein interactions.

Recent studies have implicated dehydration in protein aggregation processes. Zhang and Yan demonstrated aggregation coupled to dehydration for proteins in the presence of ethanol, and they suggested that similar effects should occur in TFE [25]. In addition, aggregation enhancement was observed when reverse micelles were used to limit water availability [26]. Furthermore, structural studies of amyloid fibrils suggest that the fibril cores are dehydrated, implying that removal of water from the protein backbone is a necessary step in the aggregation reaction [27].

4.7. Both desolvation and secondary structure may contribute generally to TFE-induced aggregation

The structural properties of a flexible, disordered protein may be tightly coupled to solvent properties. Therefore, structural changes and solvation variations are likely to occur in tandem, and it may be very difficult to separate causation from correlation in the aggregation process. In the case of α S, the only data suggesting that the helical secondary structure formed in the TFE-induced (and possibly also in the heat-induced) intermediate state contributes to aggregation is the observation that the A30P mutant decreases the helical content of the intermediate, and at the same time slows down the conversion of the intermediate to β -sheet rich oligomeric structures [2]. This observation is hardly conclusive, however, and the matter will require further investigation.

More generally, increased aggregation in intermediate [TFE] occurs for many proteins and peptides [28]. Helical structures, such as the intermediate we observe for α S [2], are often detected prior to aggregation [29-32]. However, β -sheet-rich intermediates have also been reported [33-35], and one study suggested that multiple partially structured intermediate conformations are correlated with aggregation [36]. Even very short (5 or 6 amino-acid-long) peptides, which are unlikely to form stable α -helical structure, experience enhanced aggregation in ~7–10% TFE [37], and TFE has also been reported to induce the formation of aggregates from globular proteins in the absence of significant tertiary structure disruption [38,39].

Given the variety of structures potentially associated with TFE-induced aggregation, it seems possible that different structural intermediates might promote aggregation via different mechanisms. For helical intermediates, such as the one we observe for α S, amphipathic helix-helix interactions are often thought to align neighboring disordered

regions, enabling their association [40]. In contrast, β -structured intermediates may aggregate in order to bury “sticky” unpaired β -sheet edges [34,35]. In all cases, however, it is likely that destabilization of protective solvating interactions also contributes significantly to aggregation enhancement.

4.8. Preferential solvation and/or TFE-protein interactions may promote α -helical structure at high TFE

The CD spectral changes for both our pH 2.4 and our pH 7.5 samples level off above ~30–40% TFE (figure 2(b-c)). In fact, the CD spectra of our ~40–60% TFE, pH 7.5 α S102 samples are very similar to spectra measured in ~80–100% TFE (figure S8). These observations indicate that the protein experiences a TFE-rich local environment above ~40% TFE [41,42]. Preferential solvation at this relatively low TFE concentration is consistent with the multiple reports of TFE coating or binding proteins at concentrations above ~30% TFE [43–45]. In the TFE-rich environment, α S may adopt α -helical structure as a result of the low polarity of TFE or as a consequence of TFE-protein interactions [46]. Heating of high-TFE α S102 samples, including ~80–100% TFE samples, results in CD spectra that show reduced helicity. Therefore, the conformation of a protein in the TFE-rich environment depends on temperature. Heating may shift the ensemble of conformations toward entropically favored disordered states, or elevated temperatures might disrupt enthalpic protein-solvent interactions.

5. Conclusion

We have measured temperature-dependent changes of the secondary structure of a truncated form of α S, α S102, as well as of the WT protein, in various [TFE]. We demonstrate a distinct crossover at ~15% TFE between water-like and TFE-like behavior in the variable-temperature CD spectra. We hypothesize that, as TFE is titrated into an aqueous solution containing α S, water-protein interactions are weakened, leading to the population of a dehydrated partially helical intermediate state. At high TFE, preferential TFE solvation of protein molecules leads to the formation of stable and extensive α -helical structure.

Aggregation is enhanced at moderate [TFE] and high temperatures, where the CD spectra show minimal amounts of PPII and only marginally stable α -helical structure. Because PPII structure is likely a signature of protein-water hydrogen bonding, while α -helical conformations reflect intra-protein hydrogen bonding, we propose that aggregation occurs where protective protein-solvent interactions with both water and TFE are minimized, resulting in both secondary structure formation and inter-molecular protein-protein interactions. It remains to be determined whether secondary structure directly contributes to enhanced aggregation under such conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Variable-temperature CD spectra for ~monomeric, 0.5 μM αS102 samples at various [TFE] and pH 2.4. The arrows show the general direction of increasing temperature when trends are apparent (see also figure 2(a)). The spectra were obtained for (a) 0% TFE, 2–70°C, (b) 5% TFE, 2–70°C, (c) 7% TFE, 2–50°C, (d) 10% TFE, 2–25°C, (e) 12% TFE, 2–25°C, (f) 14% TFE, 2–25°C, (g) 15% TFE, 2–25°C, (h) 16% TFE, 2–25°C, (i) 17% TFE, 2–40°C, (j) 18% TFE, 2–40°C, (k) 20% TFE, 2–50°C, (l) 22% TFE, 2–60°C, (m) 25% TFE, 2–70°C, (n) 30% TFE, 2–70°C, and (o) 60% TFE, 2–70°C.

Figure 2.

The ellipticity at 222 nm vs. (a) temperature and (b) TFE concentration for the α S102 spectra from figure 1. The [TFE] and temperatures for the curves are noted in the figure legends. (c) A comparison of $[\theta]_{222}$ vs. TFE curves measured at 25 $^{\circ}\text{C}$ for pH 2.4 samples in figure 1 (black circles), pH 7.5 data from Anderson et al. [2] (white triangles), and additional pH 7.5 data (gray squares, see figure S5). The inset shows the main plot curves normalized by subtracting the lowest-magnitude point and dividing by the absolute value of the ellipticity of the 60% TFE sample. The inset x-axis units are the same as the main plot.

Figure 3.

Transition diagrams derived from variable-temperature α S102 CD spectra for (a) pH 2.4 samples (figure 1) and (b) pH 7.5 samples (figure S5). The arrows show the general direction of increasing temperature, and the points are color coded to show [TFE] (see the figure legends). The lower right (upper left) solid line shows a linear fit of the 0–13% (17–60%) TFE data from Anderson et al. [2].

Figure 4.

TEM images of aggregates grown from 50 μ M WT α S (left column) and α S102 (right column) incubated at 70°C for 3 days in pH 7.5 buffer in the presence of 0% (top row) and 15% (bottom row) TFE. The scale bar is 200 nm, and all images are shown at the same magnification.

Table 1

Isodichroic wavelengths (λ_{iso} in nm) and the CD signal at the isodichroics ($[\theta]_{\text{iso}}$ in units of $10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$) for the variable-temperature α S102 spectra in figure 1. The uncertainties in $[\theta]_{\text{iso}}$ are due to experimental error, and the uncertainties in the wavelength measurements result from the CD spectrometer bandwidth and experimental error.

[TFE]	λ_{iso}	$[\theta]_{\text{iso}}$
0%	207 ± 1	-10.2 ± 1.8
5%	207 ± 1	-9.9 ± 1.5
7%	208 ± 1	-9.5 ± 1.5
18%	204 ± 1	-15.7 ± 1.8
20%	204 ± 1	-16.2 ± 2.9
22%	204 ± 1	-18.1 ± 3.8
25%	204 ± 1	-17.5 ± 4.9
30%	204 ± 1	-18.3 ± 5.8
60%	204 ± 1	-20.6 ± 6.9