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Dodine as a Kosmo-Chaotropic Agent

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Abstract

Denaturants such as the guanidinium cation unfold proteins at molar concentration, which interferes with ultraviolet- and infrared-based spectroscopy measurements. Dodine denatures some proteins cooperatively at a thousand-fold lower concentration, allowing for spectroscopy measurements. Nonetheless, dodine's microscopic mechanism of interaction with proteins is not understood. We probe the effect of dodine on α -helices and tertiary structure by investigating the stability of the small helical protein B. Experiments show that dodine promotes formation of helical structure (a kosmotropic effect), while inducing the loss of tertiary structure (a chaotropic effect). Although dodine destabilizes native protein structure, it does not lower the thermal denaturation midpoint temperature of protein B. All-atom simulations reveal the cause for both observations: The denaturant action of dodine's guanidyl headgroup is counteracted by its aliphatic tail, which stabilizes amphipathic helices and associates with an expanded protein core. The Janus-like behavior of headgroup and tail make dodine a simultaneous stabilizer-destabilizer or 'kosmo-chaotrope.'

Keywords

hydrophobicity; hydrogen bonding; protein folding; secondary structure

Dodine, commonly used as a fungicide,^{1,2} contains a guanidyl headgroup and a dodecyl tail, giving it both denaturant and detergent properties. Earlier studies with dodine as a protein denaturant showed that dodine acts at millimolar concentrations.^{3,4} Unlike urea and guanidinium chloride, which both act at molar concentrations,^{5–7} dodine's millimolar range of action is especially useful when high denaturant concentration interferes with spectroscopic probes such as circular dichroism (CD), ultraviolet (UV) and infrared (IR) absorption.⁸ Detergents like sodium dodecyl sulfate (SDS) also unfold proteins at millimolar

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concentration, but dodine acts much more cooperatively:⁹ susceptible proteins unfold fairly suddenly, not gradually, when dodine is added.

Dodine exhibits some unusual behavior as a denaturant. For example, it denatures the α -helical protein λ -repressor fragment and the mixed α , β protein phosphoglycerate kinase, but it has no discernible effect on the β -sheet protein Fip35 WW domain.^{3,4} Thus the question remains how dodine interacts microscopically with proteins, sometimes acting to destabilize, sometimes not. It seems plausible that the Janus-like denaturant headgroup and long aliphatic tail of dodine could interact very differently with the polypeptide backbone and hydrophobic amino acid sidechains of the core thereby facilitating this unusual behavior.

Here we combine experimental spectroscopy and all-atom molecular dynamics (MD) simulation to dissect how different parts of dodine interact with a protein. We probe the interaction of dodine with the small three α -helix bundle protein B (PrB). PrB is the albumin-binding domain from the bacterial surface protein PAB.¹⁰ PrB was chosen for two main reasons. Firstly, its small size facilitates the characterization of the dodine-protein interaction by experiment and MD simulation. Secondly, previous studies have shown that dodine denatures α -helical proteins more readily than β -sheet proteins.⁴ Since PrB is predominantly α -helical, it serves as a good model to probe the specific interaction of dodine with helices and a small hydrophobic core.

We use intrinsic tryptophan fluorescence¹¹ and far-UV CD^{12,13} to probe the integrity of local tertiary structure and global secondary structure. Since PrB lacks any intrinsic tryptophan residues that report on protein unfolding, we engineered a mutant PrB21 with a tryptophan (W) in helix 1 and a tyrosine (Y) in helix 2 (Figure 1A inset; sequence in Supplementary Methods). In the folded state, longer-wavelength W fluorescence is quenched by Y, which fluoresces at shorter wavelength.¹⁴ When the two residues move apart during unfolding, W fluorescence is restored.

PrB21 denatures cooperatively as a function of dodine concentration, with a midpoint concentration $C_m = 0.26 \pm 0.02$ mM (Figure 1A) that is over 10^4 times smaller than for guanidinium chloride (Figure S1A). We stay at 1 mM dodine to avoid micelle formation (see SI for further Methods details). Dodine addition increases the fluorescence wavelength, indicative of reduced quenching of W by Y and increased exposure of the W sidechain to a more polar solvent environment (W undergoes a Stokes shift in aqueous solution).¹⁵ Either of these events indicates loss of local tertiary structure of PrB21. However, λ_{peak} reaches only 331 ± 1 nm in dodine, vs. a Stokes shift to 350 nm when PrB is denatured with guanidinium chloride (Figure S1A).^{3,4} Thus the endpoint in Figure 1A is not a fully unfolded state. Denaturation of PrB21 in both guanidinium chloride and dodine at 20 °C is largely independent of protein concentration (Figure S2) and reversible (Figure S3) when monitored by fluorescence peak wavelength (λ_{peak}) shift in tris(hydroxymethyl)aminomethane (Tris) buffer.

Is further unfolding possible? Thermal denaturation in Figure 1B answers this question. PrB21 unfolds cooperatively with a melting temperature (T_m) of 79 °C in the absence of any denaturant to $\lambda_{peak} \approx 350$ nm (see SI for fit details). As expected, addition of guanidinium

chloride reduces T_m of PrB21 (Figure S3B). Dodine has no such effect: up to 0.4 mM dodine, T_m remains at 79 °C. At the highest concentration of dodine (1 mM), we even observe stabilization against thermal denaturation ($T_m \approx 84$ °C).

Instead of reducing T_m , addition of dodine raises the native state fluorescence baseline in Figure 1B to a longer wavelength, in accord with Figure 1A. Thus, dodine denaturation in Figure 1A and thermal denaturation in Figure 1B are distinct events. Dodine cooperatively attacks the tertiary structure of PrB21 without affecting the thermal stability of the residual tertiary structure. A similar effect was also observed with SDS (Figures S4 and S5).

In contrast to denaturing local tertiary structure, dodine increases global helical content when probed by CD (Figure 1C). In addition, dodine stabilizes helical structure against thermal denaturation (Figure 1D). We hypothesize that the gain in helical content when dodine interacts with the amphiphilic helices of PrB21 leads to the formation of a ‘super-helical’ state which is responsible for thermal stabilization of PrB21 in Figure 1B.

Thus, dodine denaturation differs from guanidinium chloride denaturation in four basic aspects: (1) dodine acts at 10,000-fold less concentration; (2) although dodine disrupts local tertiary structure around our W-Y probe pair, it has no effect on thermal denaturation of residual tertiary structure; (3) dodine increases helical secondary structure; and (4) dodine stabilizes secondary structure against thermal denaturation. We further investigate the reason behind this behavior by using MD simulations to provide atomic-level structural insight (see SI Figures S6 to S24 for details).

We simulated a sequence with wild-type lysine 39 (Figures 2–5, Figures S6–16) as well as the K39V mutant used in experiments (Figures S18–24, sequences in SI Methods), with similar results. We first examined the effect of temperature on the non-native state by conducting molecular dynamics simulations with a dodine concentration of 0.087 M (10 dodine molecules and one protein in the simulation box) at three different temperatures, 320, 340 and 360 K starting from folded PrB21. The chosen dodine concentration keeps the simulated dodine:protein ratio similar to experiment (Figure S2) and micelle formation is not an issue with 10 dodine molecules on the time scale of the simulation. The probability distributions of radius of gyration, W-Y distance and helical content at each temperature were calculated from five replicas (Figures S6–S8). While R_g is informative of the global shape of the protein, W-Y distance is a proxy for the experimentally observed fluorescence, and helical content is a proxy for the experimental CD spectrum.

The distribution of radius of gyration (R_g) in dodine (Figure 2A) is bimodal, where both ensembles are expanded compared to the native state ($R_g \sim 1.2$ to 1.4 nm, Figures 2A and S6B). Like most materials, proteins usually expand when temperature is increased, as is observed when PrB21 R_g increases from 1.2 nm at 340 K to 1.4 nm at 360 K.^{16,17} Interestingly, at 340 K a significant fraction of the population adopts a more compact state than at 320 K or 360 K in four out of five replicas. Compaction of PrB21 as the temperature increases from 320 K to 340 K is counterintuitive. This is also seen in the W-Y distance, since the state when these residues are farther apart (~ 0.75 nm) is less populated at 340 K as compared to 320 K and 360 K (Figures S7B and S9).

It is well-known that the strength of hydrophobic interactions increases with temperature because it becomes more favorable to exclude water between hydrophobic surfaces, and thus increase solvent entropy.¹⁸ It is possible that dodine shifts the balance of protein configurational entropy and hydrophobicity in favor of the latter for PrB21 at 340 K. However, as temperature is increased to 360 K, protein configurational entropy dominates hydrophobic collapse. Therefore, the interaction of the PrB21 hydrophobic core with dodine hydrophobic tails could overcome complete thermal unfolding at an optimal temperature.

To test the ‘super-helical state’ hypothesis proposed based on CD experiments, we examined helical content in the simulations. Helical content of PrB21 increases in dodine at all three simulation temperatures (Figures 2B and S10A) for helix 1 and 2. Helical content for helix 3 increases up to 340 K and then decreases to the native level at 360 K (Figure S10B). At 360 K the helical content distribution is significantly broadened as compared to 320 and 340 K for both helix 1 and 2. This broadening indicates the formation of multiple low-helical states. These low-helical states and the decrease in helicity for helix 3 are consequences of increasing temperature because helical structure is temperature sensitive and even stable helices are denatured at sufficiently high temperature.¹⁹

We compared the dodine-induced super-helical state to the folded and unfolded PrB21 conformations in absence of dodine. Simulations of PrB21 at 300 K starting with the folded structure yielded a native R_g of ≈ 1 nm (Figure 2C). Relaxation of the unfolded structure yielded an R_g of ≈ 2 nm, but still had high helical content in the simulation (Figures 2E and S11). In the presence of dodine, PrB21 forms an expanded but super-helical state (Figure 2D and S6) which is far from either the native state or from an unfolded coil. In addition to increased helical content, this expanded state has an increased W-Y distance and dodine tails are intercalated into the protein core, unzipping the helices and expanding the PrB21 tertiary structure (Figure 2D). We propose that these three simulated states (native, expanded and unfolded) are analogous to the ‘320 nm fluorescence’, ‘330 nm fluorescence’ and ‘350 nm fluorescence’ states in Figure 1 as they match the trends in W-Y distance and helical content as a function of dodine concentration and temperature inferred from experiment.

We also compared simulations at a constant temperature (360 K) but at two different dodine concentrations. We compared 10 and 20 dodine molecules, corresponding to ~ 0.087 M and ~ 0.175 M to study the effect of higher dodine concentration on PrB21 (maintaining a similar protein:dodine ratio as in experiment). The rate of formation of the non-native super-helical state is dodine concentration-dependent in our simulations. In all five replicas, we observed formation of the super-helical state in < 5 ns with 0.175 M dodine vs. ~ 10 to 20 ns with 0.087 M dodine (Figures 3A and S12). As expected, R_g increases with increasing dodine concentration (Figure S13).

The probability distribution of helical content for each replica (Figure S14) was combined to estimate the overall probability distribution for helix 1, 2 and 3 as a function of dodine concentration. As previously discussed for 0.087 M dodine at 360 K, the helical content increased for helix 1 and 2 and remained unchanged for helix 3 in 0.175 M dodine (Figure 3B and S15). In Figure 2B, we also observed the formation of low-helical states due to thermal denaturation of helices at 360 K with 0.087 M dodine. The population of this low-

helical state decreases when the concentration of dodine is increased to 0.175 M (Figures 3B and S15A). With 0.175 M dodine the helical content at 360 K mimics that at 320 K with 10 dodine molecules (Figure 2B). This means that the addition of dodine stabilizes helical content against thermal denaturation. These simulations help explain why experimentally PrB21 is not destabilized against thermal denaturation by dodine.

Next, we turned to a more detailed structural analysis of how dodine interacts with the solvent- exposed protein surface. Different amino acid side chains may interact differently with the denaturant head or detergent tail²⁰ of dodine to stabilize the super-helical but expanded state. Figure 4 shows the contact-coefficient_{head-tail} (CC_{HT}), for each residue with either the tail or head of the dodine molecule. Positive values (up to +1) imply that the residue prefers interaction with the guanidinium chloride head group and negative values (up to -1) imply preferred interaction with the hydrophobic dodecyl tail. Values close to zero show no preference for either the head or the tail.

The interaction of residue sidechains and backbones with dodine is shown for 0.087 M and 0.175 M dodine at 360 K as a function of residue hydropathy (Figure 4B), and as a function of protein sequence (Figure 4C). About a quarter of all residues interact preferentially with either the head ($CC_{HT} > 0.25$) or the tail ($CC_{HT} < -0.25$, SI Table S1). Moreover, these interaction values correlate well with the amino acid hydropathy. More than ~90% of all amino acids that preferentially interact with the head are polar and those that interact with the tail are hydrophobic (SI Table 1). Interactions with the head group increased at higher dodine concentration, while those with the tail remained mostly unchanged with both dodine concentration and temperature. A similar result was obtained when only residue backbones were considered (Figure S16C).

Thus, the dodine tail preferentially interacts with hydrophobic sidechains in helices, stabilizing amphipathic helices in PrB21. The charged head group preferentially interacts with negatively charged sidechains and with the backbone, leading to tertiary structure denaturation. Polar residues such as threonine (T) or serine (S) show little propensity either way. The increased dodine head interaction with protein backbone-only at higher dodine concentration (Figure S16C) signals an increase in guanidinium chloride-like denaturant properties at higher dodine concentrations. In contrast, no significant concentration- (Figure 4C) or temperature-dependence (Figure S16B) of CC_{HT} for backbone+sidechains was observed.

The exploration of novel denaturants such as dodine has recently gained more interest because traditional denaturants interfere with common spectroscopic techniques. Here, we propose a possible mode of interaction between dodine and a model helical protein, PrB21. Dodine interacts with the protein via both the guanidinium chloride head group (attacks backbone and charged side chains) and the tail (attacks non-polar side chains). At low temperature, this interaction induces an expanded super-helical state of PrB21. Increasing the dodine concentration does not affect the thermal stability of this non-native PrB21 state because the aliphatic tail counteracts the denaturant effect of the guanidinium chloride headgroup. Only at high temperature does the protein finally unfold completely. A control

with SDS supports this model because SDS, which lacks the denaturing guanidinium chloride group, stabilizes PrB21 even more against thermal denaturation (Figure S4B).

Dodine has shown quite a range of denaturation behaviors with different proteins, consistent with our explanation in terms of dodine's stabilization of secondary structure and insertion into the hydrophobic core. The small β -sheet protein Fip35 WW domain (34 residues) is not denatured by dodine. Unlike PrB21, WW has no significant hydrophobic core for dodine to insert into; likely dodine would have to be used above its critical micelle concentration (6–8 mM) to be effective.⁴ The larger α -helical protein λ_{6-85} is denatured by dodine, but no super-helical state forms. As the hydrophobic core gets larger, the decrease in stability due to loss of tertiary structure may not be sufficiently overcome by stabilizing secondary structure elements. In this case dodine would act more like a traditional denaturant. The guanidinium chloride head group is imperative for the destabilizing denaturant-like properties of dodine, but is able to act at much lower concentration thanks to assistance from the hydrophobic tail interacting with the larger core. This is indeed reinforced by dodine's ability to effectively denature phosphoglycerate kinase, a much larger (415 residue) protein with two large hydrophobic cores. The Janus-like behavior of headgroup and tail make dodine a simultaneous secondary structure stabilizer and tertiary structure destabilizer or a 'kosmo-chaotrope,' dependent on protein size.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The effect of dodine on PrB21 stability. Solid markers show experimental data and solid lines show model fits in A, B and D. Solid lines in C show experimental data. Unfolding was monitored by change in λ_{peak} as a function of denaturant concentration. Secondary structure was monitored by mean residue ellipticity of PrB21 at various dodine concentrations. Errors bars are reported by the standard error of the mean for two repeats. (A) Denaturation of PrB21 with dodine at 20 $^{\circ}\text{C}$. The inset shows a ribbon structure of PrB21 with helix 1 (residues 4–15), helix 2 (19–27) and helix 3 (31–45) color-coded. The location of the tryptophan residue on helix 1 is highlighted by an orange disk, and the tyrosine location on helix 2 by a red disc. (B) Dodine-assisted thermal denaturation of PrB21. (C) Circular dichroism spectra of native PrB21 and PrB21 in dodine. (D) Thermal denaturation of PrB21 without denaturant (black) and 0.5 mM dodine (red) monitored by mean residue ellipticity change as a function of temperature.

Figure 2.

The structural effect of temperature and dodine on PrB21. Probability distribution for (A) radius of gyration and (B) helical content of helix 1 of PrB21 in simulations with dodine (0.087 M dodine, 10:1 dodine:PrB21 ratio) at 320 K (yellow), 340 K (orange), and 360 K (red). The black dashed line indicates the crystal structure (native state) values. (C) An overlay of ~500 structures (one every ns) obtained from a 500 ns simulation of PrB21 without dodine at 300 K. Residues 1 to 47 are colored red to blue (see also inset in Figure 1A) and shown in a line representation. The first frame of the simulation can be seen partly as a dark gray ribbon representation. (D) A representative structure of the dodine-expanded form of PrB21, highlighting four dodine molecules in or near the protein core. Protein residues are colored according to hydrophobicity (green) and charge (blue/red), whereas dodine molecules' carbon atoms are shown in cyan and nitrogen atoms in blue. (E) An overlay of ~50 structures obtained from the first 5 ns of a PrB21 refolding simulation. The first frame is shown in gray as a ribbon representation and the remaining frames as a line representation where residues 1 to 47 are colored red to blue.

Figure 3. MD simulations of PrB21 with 0.087 M and 0.175 M dodine at 360 K. (A) Radius of gyration of the protein as a function of time. The dark traces show the data averaged over a 5 ns window, and the lighter envelope shows the full range of the data recorded at 100 ps timesteps in our simulations. (B) Probability distribution for the helical content of helix 1 with 0.087 M dodine (red) and 0.175 M dodine (green) at 360 K. The black dashed line indicates the crystal structure (native state) value.

Figure 4. Contact-coefficient_{head-tail} values for each residue of PrB21. (A) Highlighted atoms show atoms included to calculate head group interactions (pink) and tail interactions (orange). (B) Contact-coefficient_{head-tail} (CC_{HT}) as a function of residue hydrophathy for all residue (backbone+sidechain) of PrB21 in 0.087 M dodine (red) and 0.175 M dodine (green) at 360 K. Positive values indicate preference for guanidinium chloride head while negative values indicate preference for the hydrophobic tail. (C) Same plot as a function of sequence. The background colors represent residue hydrophobicity with blue colors being more polar and redder more hydrophobic.