Does Fungicide “Dodine” Unfold Protein Like Kosmo-Chaotropic Agent?

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Methods and Materials

Equine skeletal muscle myoglobin (95-100%, salt free, lyophilized powder), dodine (analytical grade), n-hexylamine (99%), cyanamide (99%) and GdmCl (99.8% purity) were purchased from Sigma Aldrich and Acros Organics, respectively. Tris (hydroxymethyl) aminomethane (Tris) (99%), HCl, glacial acetic acid (99%) and iso-propanol (99.8%) were purchased from Merck and SRL (India), respectively. N-hexylguanidinium acetate (HGA) was synthesized in our laboratory. All the experiments were performed in the Tris-HCl buffer condition maintaining pH=7. All the solutions were made in the Milli-Q water (resistivity: 18.2 MΩ cm). The concentration of the myoglobin was determined by using its absorbance at 280 nm ($\varepsilon=13980 \text{ M}^{-1} \text{ cm}^{-1}$) and 409 nm ($\varepsilon=188000 \text{ M}^{-1} \text{ cm}^{-1}$). Absorption of myoglobin was measured in Thermo Fischer Scientific Evolution 201 spectrophotometer. The emission of myoglobin was measured using the fluoromax-4 (Horiba Jobin Yvon Scientific) spectrofluorometer with excitation at 280 nm. The structural change of myoglobin was explored by measuring the absorption of the Soret band as well as the emission of tryptophan of the protein in the presence of GdmCl, HGA and dodine. The slit width of the measurement was kept 2 nm. Temperature dependent fluorescence of myoglobin was measured in the presence of denaturants in order to estimate the thermodynamic parameters. The peltier thermostat cell holder was used for the temperature controlled fluorescence measurements which shows the stability with a precision of 0.1°C over the entire course of the measurement. The concentration of the protein was ~5 μM throughout the experiment. The CD spectra of myoglobin in secondary (far-UV) as well as tertiary regions (near-UV) were recorded using the JASCO J815 CD spectrometer. We have performed the docking calculation using the patch dock process whose algorithm is very efficient for the study of small molecule-protein interaction. The interactions between protein and denaturants were analyzed by Visual Molecular Dynamics (VMD) software.

Synthesis Procedure of N-hexylguanidinium acetate (HGA):

N-hexylamine (2.95 ml) was taken in a round bottom flask along with 4.5 ml of iso-propanol. The mixture was then refluxed with 1.15 ml of glacial acetic acid for 10 minutes. The solution of the mixture was cooled before the addition of 5 ml of 25% cyanamide aqueous solution. The resultant mixture was refluxed for 30 minutes. The solvent was removed using a vacuum rotor evaporator providing a colorless sticky gum which was crystallized by the addition of cold acetone. The solid was collected and recrystallized twice by dissolving in
ethanol followed by the drop wise addition of ether. Yield of the product was 1.5 g and its melting point is ~140°C. The mass peak for the cation of the product appears at m/z=144.14. 

$^1$H-NMR (DMSO-d$_6$), $\delta$ (ppm): 0.88 (t, 3H), 1.2 (s, 8H), 1.42 (d, 2H), 3.00 (t, 2H), 7.93 (s, 4H, NH$_2$’s), 9.11(s, 1H, NH) and 1.64 (s, 3H) for acetate CH$_3$. ($^1$H-NMR and $^{13}$C-NMR spectra are shown in Figures S1 and S2). The plausible reaction steps depict in Scheme S1.

Scheme S1: The procedure for the synthesis of n-hexylguanidinium acetate (HGA).

References:


Figure S1: $^1$H-NMR (DMSO-$d_6$) of N-hexylguanidinium acetate (HGA).

Figure S2: $^{13}$C-NMR (DMSO-$d_6$) of N-hexylguanidinium acetate (HGA).
Figure S3: The absorbance and emission spectra of myoglobin in presence of GdmCl (a), (d),
dodine (b), (e) and HGA (c), (f), respectively.

Figure S4: Reversibility of isothermal denaturation (at room temperature) of myoglobin with
(a) GdmCl, (b) dodine and (c) HGA.
Figure S5: The ellipticity changes of myoglobin secondary region (top) in presence of (a) GdmCl, (b) dodine and (c) HGA. Change of myoglobin heme region (below) in presence of (d) GdmCl, (e) dodine, and (f) HGA. (Inset: ellipticity plot at 293 and 409 nm).

Figure S6: The temperature dependency of the change in the intensity maxima of the emission spectra of myoglobin (~320 nm) in the presence of (a) GdmCl, (b) dodine and (c) HGA, respectively.
Figure S7: Docking results of myoglobin in (a) GdmCl, (b) dodine and (c) HGA. Probable interactions of denaturants with amino acid residues of myoglobin in (d) GdmCl, (e) dodine and (f) HGA.