

Role of Guanidine and Methylguanidine Hydrochloride on the Thermodynamic Stability and Internal Dynamics of Horse Cytochrome *c*

*Thesis Submitted
In partial fulfillment of the requirement for
the degree of*

**MASTER OF SCIENCE
IN
CHEMISTRY**



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Certificate

This is certify that the thesis entitled "Role of Guanidine and Methylguanidine Hydrochloride on the Thermodynamic Stability and Internal Dynamics of Horse Ferrocycochrome c" being submitted in partial fulfillment of requirements for the award of degree of Master of Science in Chemistry, submitted in the School of Chemistry and Biochemistry, Thapar University, Patiala is a bonafide work carried out under the supervision of Dr. Rajesh Kumar,(Associate Professor),& Dr. Ranjana Prakash (Associate Professor) School of Chemistry and Biochemistry, Thapar University, Patiala and that no part of this project has been submitted for the award of any other degree.

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CANDIDATE'S DECLARATION

I hereby declare that the work presented in this thesis entitled, "Role of Guanidine and Methylguanidine Hydrochloride on the Thermodynamic Stability and Internal Dynamics of Horse Ferrocycytochrome c" in partial fulfillment of the requirement for the award of Degree of Mater of Science in Chemistry, submitted in the School of Chemistry and Biochemistry, Thapar University, Patiala, is an authentic record of my own work carried out under the supervision and guidance of Dr. Rajesh Kumar, Associate Professor & Dr. Ranjana Prakash, Associate Professor, School of Chemistry and Biochemistry, Thapar University, Patiala and refers other researcher's work which are duty listed in the reference section.

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Regards,

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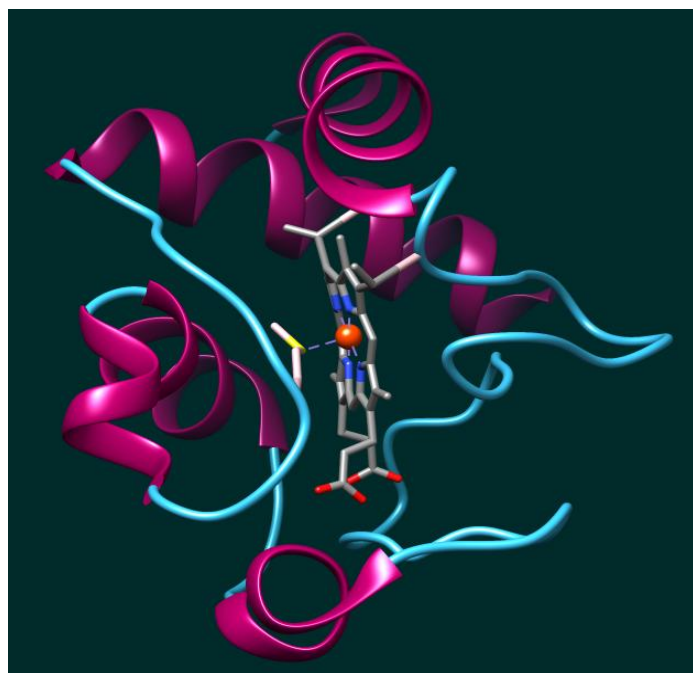
1.0 Introduction

During the past decades many experimental and theoretical studies have been performed to explore the mechanism by which protein denaturants such as guanidine hydrochloride (GdnHCl) and urea exerts their effect on protein [1-5]. However, the mechanism by which these denaturants alters the thermodynamic stability of proteins is not clearly understood. Previous studies revealed that urea and GdnHCl denature the proteins in two different fashions, (i) by the formation of direct interactions with the different groups (backbone and side chain atoms) of the protein [1,6-8] or (ii) indirectly by changing the solvent properties [9-11]. Some studies also showed that both direct and indirect mechanisms are involved in the denaturing process [12]. The detailed characterization for the effect of denaturants on the structural and functional complexity of proteins results in following important conclusions: (i) in the pretransition region the unfolding free energy for denaturant need not essentially in linear fashion [13-15] (ii) the large-amplitude conformational motion is somewhat complex [16] (iii) at low concentration, denaturant ions can stabilize the protein by electrostatic screening [15,17-20] and (iv) subdenaturing concentration of denaturant stabilize the protein via making polyfunctional interaction with certain proteins groups [5,21-24].

Many previous reports revealed that urea, alkylureas and GdnHCl decrease the thermodynamic stability of proteins [25-30]. The studies on the effect of urea and alkyl urea on structure and stability of protein suggest that by increasing the hydrophobicity on urea molecule, the denaturing efficiency of urea also increases [25-30]. The interrelationship between protein function, stability, and dynamics plays significant roles in protein engineering and biological process. In last few years, many techniques *i.e.* XRD-technique and NMR spectroscopy have been vastly used to correlate structure, conformational changes, function, and stability of proteins [31-33]. Despite the enormous progress in protein folding,

the role of denaturants on the internal dynamics of proteins at the subglobal level that includes small and large amplitude structural fluctuations are still in its infancy [34]. The structure, stability and dynamics of proteins are affected by the dynamics of the solvent [35-38]. The structural fluctuations of the proteins range from ultrafast to slow changes [39-42]. The function of the proteins could be defined by the ultrafast structural fluctuations that ultimately assist in the larger degree of protein rearrangements. Many studies have been performed to find out the role of the fast motions of the proteins that direct the conformational alteration coupled to function of the proteins [43-48]. However, the slow structural fluctuations across the folding-unfolding transitions have been less studied [22,49]. The denaturants open the subglobal structure selectively in a sequential way and thus limits the possible folding pathways [49]. The structural dynamics during protein could be studied at atomic and large scale level.

Previous reports suggest that the subdenaturing concentrations of urea and alkylureas decrease the structural fluctuations of the M-80 containing Ω -loop of ferrocyst *c* [5]. The



denaturant-mediated decrease in structural fluctuations is more pronounced for urea and least for tetramethyl substituted urea because the urea forms more denaturant-mediated intermolecular cross-linking interactions than that of tetramethyl substituted urea [5].

Fig. 1. A ribbon model for horse ferrocyst *c* (PDB: 2FRC) [50]. Heme atoms are shown in ball and stick form. The bond between Ω -loop (from 70–85 amino acids) residue M80 and Fe^{2+} of heme is shown in dashed line.

To test whether GdnHCl and methyl submitted GdnHCl also follow the same trend on thermodynamic stability and internal dynamics of ferrocyst *c*, extensive equilibrium and kinetic experiments of ferrocyst *c* were performed under varying concentrations of GdnHCl and MGdnHCl at pH 7. Kinetic and thermodynamic parameters measured for CO association reaction of ferrocyst *c* in the presence of varying concentrations GdnHCl and MGdnHCl demonstrate that the subdenaturing concentrations of these denaturant reduce the structural fluctuation of the M80-containing Ω -loop of ferrocyst *c*. Furthermore, the extent of denaturant-mediated decrease in the structural fluctuation of Ω -loop is more pronounced for MGdnHCl than that of the GdnHCl. Thermodynamic analysis of the thermal denaturation curves of ferrocyst *c* measured in the presence of varying concentrations GdnHCl and MGdnHCl suggests that the denaturant-mediated stabilization of ferrocyst *c* is not registered at global level because even low concentrations of these denaturants decrease the thermal stability of the protein.

2 Materials and Methods

Horse cytochrome *c* was purchased from Sigma-Aldrich. GdnHCl and MGdnHCl were purchased from from USB (USA) and TCI, respectively. All experiments were done in 20 mM Tris buffer at pH 7, 22 °C, under strictly anaerobic conditions.

2.1 Measurement of thermal unfolding of ferrocyt *c*

Visible absorption spectrum of ferrocyt *c* typically shows the Soret (415 nm), α (550 nm) and β (520 nm) peaks at pH 7, which correspond to native His18/Met80 axial coordination of the heme [51-52]. The intensity of these bands may vary in different chemical and physical conditions. The intensities of the α -band decrease significantly when the temperature is increased from 25 to 100 °C (Fig. 2a), so thermal denaturation of ferrocyt *c* can be studied by measuring the change in intensity or absorbance at 550 nm. To evaluate the effect of GdnHCl and MGdnHCl on the thermal stability of ferrocyt *c*, thermal denaturation of ferrocyt *c* was carried out under conditions of varying concentrations of GdnHCl and MGdnHCl at pH 7. The protein solution was heated at a rate of 1°C/min (S-1700 thermoelectric single cell holder with T_m analysis software, Shimadzu). Ferrocyt *c* was prepared under N₂ atmosphere by the addition dithionite into ferricyt *c* containing a desired concentration of GdnHCl and MGdnHCl. The final concentration of sodium dithionite and protein were ~3 mM and ~10 μ M, respectively.

2.2 Measurement of CO-association kinetics.

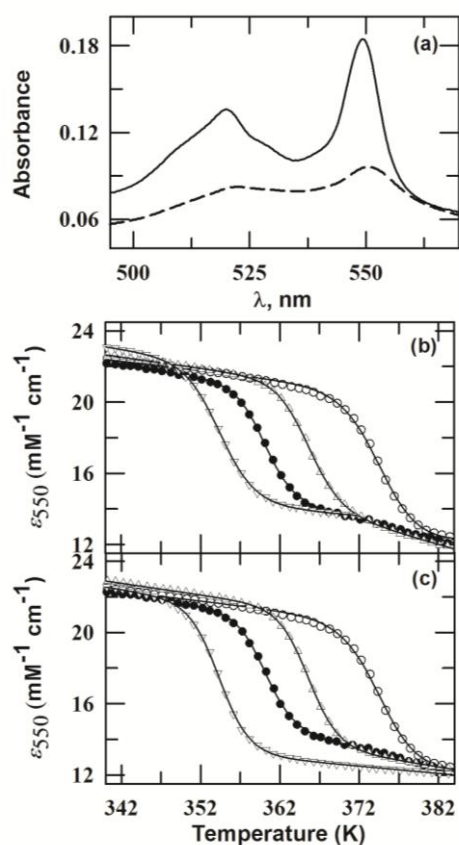
Ferrocyt *c* can be driven to bind CO when the latter is used in saturating concentration (1 mM) under neutral pH conditions [5]. Small volumes of ferrocyt *c* solution were diluted 101-fold into CO-saturated buffer containing sodium dithionite (3 mM) and varying concentrations of GdnHCl and MGdnHCl at pH 7, 22 °C. Final concentration of the protein in the reaction medium was ~10 μ M. CO association kinetics were measured by heme

absorbance at 550 nm. The CO association kinetics were recorded on Shimadzu 2450 spectrophotometer.

3. Results

3.1 Effect of guanidine hydrochloride and methylguanidine hydrochloride on the thermal denaturation of ferrocyst *c*

To evaluate the effect of GdnHCl and MGdnHCl on the thermal denaturation of ferrocyst *c*, the thermal denaturation of ferrocyst *c* was carried out in the presence of varying concentration of GdnHCl and MGdnHCl at pH 7. Fig. 2a shows the visible absorption spectrum of ferrocyst *c* recorded in the absence of additive at 25 and 100 °C. Fig. 2a clearly shows that at ~100 °C, the Fe²⁺-M80 bond of ferrocyst *c* is significantly disrupted, which



suggests that the protein is significantly denatured since ferrocyst *c* denatures via cooperative manner [5,24]. The representative thermal denaturation curves of ferrocyst *c* collected in the presence of different concentrations of GdnHCl and MGdnHCl are shown in Fig. 2b and Fig. 2c, respectively at pH 7. The thermal unfolding curves for ferrocyst *c* measured at different concentrations of GdnHCl and MGdnHCl were analyzed for thermal denaturation midpoint (T_m) and enthalpy change,

Fig. 2 Panels (a) represent the absorption spectra of ferrocyst *c* at 25 °C (solid line) and 100 °C (short dash line) recorded at pH

7.0. Panels (b) and (c) shows thermally induced unfolding of ferrocyt *c* monitored at 550 nm as the change in excitation coefficient in the absence (\circ) and presence of GdnHCl (0.5 M (Δ), 1 M (\bullet) and 1.5 M (∇)) and MGdnHCl (0.5 M (Δ), 1 M (\bullet) and 1.5 M (∇)), respectively.

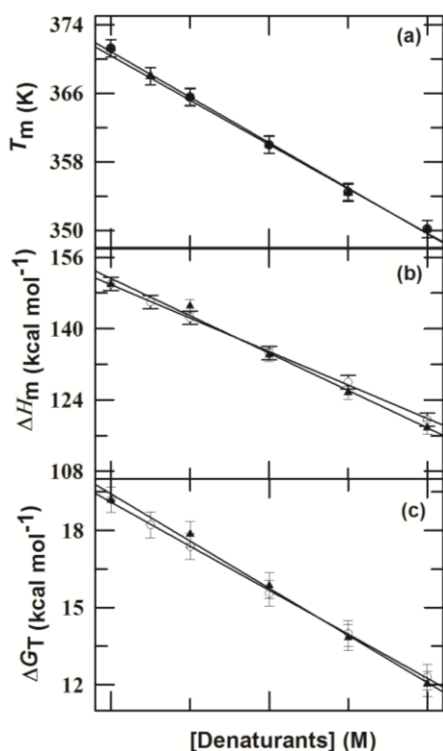
(ΔH_m) by two-state model $N \rightleftharpoons U$ using van't Hoff Equation [Eq. (1)] as described earlier [17].

$$\theta(T) = \frac{(c_{\text{pre}} + m_{\text{pre}}T) + (c_{\text{post}} + m_{\text{post}}T) \exp \left[\frac{-\Delta H_m}{R} \left(\frac{1}{T_m} - \frac{1}{T} \right) \right]}{1 + \exp \left[\frac{-\Delta H_m}{R} \left(\frac{1}{T_m} - \frac{1}{T} \right) \right]} \quad (1)$$

The resulting T_m and ΔH_m values at different pH are listed in Table 1.

3.2 Effect of guanidine hydrochloride and methylguanidine hydrochloride on the thermal denaturation midpoint, denaturation free energy, and denaturation enthalpy change of ferrocyt *c*.

Fig. 3a and Fig. 3b show the variation of T_m and ΔH_m with [denaturants]. As denaturant concentration is increased, T_m and ΔH_m decreases linearly. The linear decrease of T_m with denaturant indicating that GdnHCl and MGdnHCl decrease the thermal stability of ferrocyt *c*.



The decrease in T_m is also not greatly differs for GdnHCl and MGdnHCl. Thermodynamic stability, expressed in terms of free energy of denaturation, ΔG_T at 25 °C for various concentrations of GdnHCl and MGdnHCl was calculated from Eq. (2) using a constant value of

Fig. 3. (a) Effect of denaturants concentration (GdnHCl (\blacktriangle), and MGdnHCl (\circ)) on melting temperature (T_m) (550 nm) at pH 7. (b) Denaturants (GdnHCl (\blacktriangle), and MGdnHCl (\circ)) dependence of enthalpy change, ΔH_m for ferrocyt *c* at pH 7 (550 nm). (c) Denaturants (GdnHCl (\blacktriangle), and MGdnHCl (\circ)) dependence of denaturation free energy change (ΔG_T) for ferrocyt *c* at pH 7 (550 nm). In panels (a), (b), and (c) the solid curves represent linear least squares fitting.

heat capacity, $\Delta C_p \sim 1.34 \text{ kcal mol}^{-1} \text{ K}^{-1}$ for ferrocyt *c* [52],

$$\Delta G_T = \Delta H_m \left(1 - \frac{T}{T_m} \right) + \Delta C_p \left(T - T_m - T \ln \left(\frac{T}{T_m} \right) \right) \quad (2)$$

The resulting ΔG_T for unfolding of ferrocyt *c* under various concentrations of GdnHCl and MGdnHCl are provided in Table 1 and Fig. 3c.

Table 1. GdnHCl and MGdnHCl dependence of the T_m , ΔH_m and ΔG_T for thermal unfolding of ferrocyt *c* monitored by absorbance at 550 nm at pH 7.0.*

Additive	Concentration (M)	T_m (K)	ΔH_m (kcal mol ⁻¹)	ΔG_T (kcal mol ⁻¹)
Control	0	371.2	150.0	19.2
GdnHCl	0.5	365.5	145.1	17.9
	1.0	359.9	134.1	15.8
	1.5	354.5	125.6	13.8
	2.0	350.2	117.8	12.0
	MGdnHCl	0.25	368.0	146.0
MGdnHCl	0.5	365.6	142.4	17.4
	1.0	360.0	134.5	15.5
	1.5	354.4	128.0	14.0
	2.0	350.2	119.5	12.3

*The uncertainties of T_m , ΔH_m , and ΔG_T values reported here are $\pm 0.5 \text{ K}$, $\pm 2.0 \text{ kcal mol}^{-1}$ and $\pm 0.5 \text{ kcal mol}^{-1}$, respectively.

3.3 Effect of guanidine hydrochloride and methylguanidine hydrochloride on kinetic and thermodynamic parameters for CO-association kinetics.

To evaluate the effect of GdnHCl and MGdnHCl on the internal dynamics of ferrocyt *c*, the kinetic and thermodynamic parameters for CO association reaction of ferrocyt *c* (Ferrocyt *c*

+CO→Ferrocyt *c*-CO) were measured under varying concentrations of GdnHCl and MGdnHCl at pH 7. Fig. 4a shows the visible absorption spectrum of ferrocyt *c* recorded in the absence and presence of 1 mM CO at 22 °C, pH 7. Fig. 4a clearly shows the intensity of α -band decrease significantly in the presence of CO, indicating the CO ligation to Fe²⁺ of heme. Fig. 4b presents the representative CO association kinetic trace of ferrocyt *c* that recorded in the absence of additive at pH 7, 22 °C. CO association kinetic trace fitted well with single exponential rate expression with time constant, $\tau \approx 12.0$ min. Fig. 4c shows the logarithm of rate coefficient for CO-association reaction in the presence of various concentrations of GdnHCl and MGdnHCl at 22 °C, pH 7. Data in Fig.4c provides two important information: (i) “Chevron-like” feature of the log k_{ass} -denaturants space: As the final concentration of denaturant is increased starting from strongly native-like conditions, the value of log k_{ass} initially decreases and then increases, showing the inflections centred around 2.5 and 3.0 M for GdnHCl and MGdnHCl, respectively. (ii) The variation in magnitudes of k_{ass} with denaturants: As the denaturants concentrations are increased from 0.0 to 2.5 M GdnHCl, and 3.0 M MGdnHCl, the value of k_{ass} decreases to about 2.2, and 4.0 folds, respectively. In the subdenaturing limit of denaturants, the decrease in the log k_{ass} value is more pronounced for MGdnHCl than that of the GdnHCl (Fig.4c). At relatively higher concentrations of denaturant, the unfolding action of denaturant dominants that eventually facilitates the CO-association process, and hence the values of log k_{ass} increase sharply (Fig. 4b). The increase in log k_{ass} values are more pronounced for GdnHCl than that of MGdnHCl (Fig. 4c). To further investigate the role of denaturants on the CO dissociation reaction, the activation parameters (activation enthalpy ($\Delta H_{\text{ass}}^{\ddagger}$), activation entropy ($\Delta S_{\text{ass}}^{\ddagger}$) and activation free energy ($\Delta G_{\text{ass}}^{\ddagger}$)) for CO association reaction were determined in the absence and presence of 2.3 M GdnHCl and 3.0 M MGdnHCl at pH 7. Fig. 4d presents the Eyring plots for CO association reaction obtained from temperature-dependent kinetic data in the absence

and presence of 2.3 M GdnHCl and 3.0 M MGdnHC at pH 7. The Eyring plots in Fig 4d were analyzed by using the eq (1) [5,24].

$$\ln(k_{\text{ass}}h/k_{\text{B}}T) = (\Delta S_{\text{ass}}^{\ddagger}/R) - (\Delta H_{\text{ass}}^{\ddagger}/RT) \quad (3)$$

The estimated values of $\Delta H_{\text{ass}}^{\ddagger}$ and $\Delta S_{\text{ass}}^{\ddagger}$ are summarized in Table 2. By using the $\Delta H_{\text{ass}}^{\ddagger}$ and $\Delta S_{\text{ass}}^{\ddagger}$ values and Gibbs free energy equation ($\Delta G_{\text{ass}}^{\ddagger} = \Delta H_{\text{ass}}^{\ddagger} - T\Delta S_{\text{ass}}^{\ddagger}$), the corresponding ($\Delta G_{\text{ass}}^{\ddagger}$) and $-T\Delta S_{\text{ass}}^{\ddagger}$ values were also estimated at 25 °C (Table 2). The data in Table 2 provide two important information, (i) the subdenaturing concentrations of denaturants increases the enthalpic barrier ($\Delta H_{\text{ass}}^{\ddagger}$) for CO association reaction and which is more increased for MGdnHCl than that of the GdnHCl, and (ii) the increase of $\Delta H_{\text{ass}}^{\ddagger}$ due to denaturant is accompanied by a decrease in the entropy change $-T\Delta S_{\text{ass}}^{\ddagger}$.

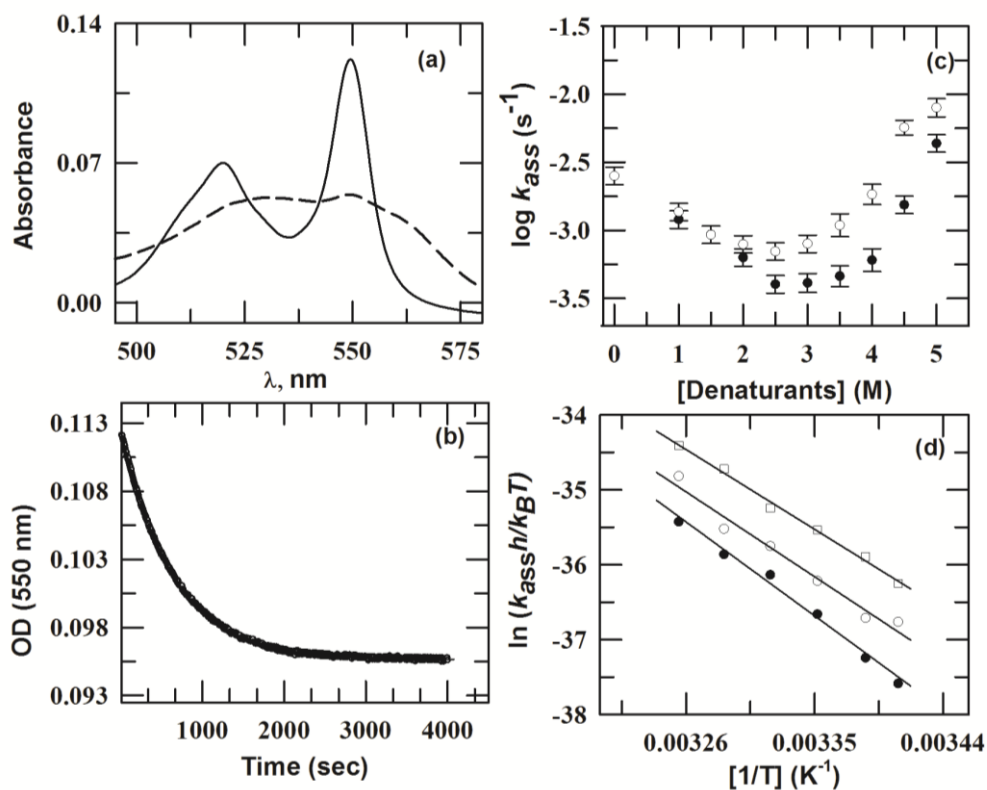


Fig.4. Panel (a) Steady-state visible absorption spectra of ferrocyanide (c) (solid line) and ferrocyanide-CO (c -CO) (short-dash line) states at pH 7.0 and 22 °C. Panel (b) represents the slow single-phase CO association reaction, ferrocyanide c +

CO→ ferrocyt *c*-CO ($\tau = 12.0$ min, 22 °C) monitored by 550-nm heme absorbance. (c) Denaturants dependence of Log k_{ass} (GdnHCl (●) and MGdnHCl (○)) at 22(±1) °C, pH 7.0. The lines through the data in panel (c) have been drawn by inspection only. Panel (d) Eyring plots for the CO-association reaction, 0.1M tris buffer, pH 7.0 with no additive (□), 2.5 M GdnHCl (○) and 3.0 M MGdnHCl (●).The solid line in panel (d) presents linear least-squares fits to the data.

Table 2. The effect of GdnHCl and Methyl-GdnHCl (MGdnHCl) on activation parameters for CO-association reaction of ferrocyt *c* at pH 7*.

Additive concentration (M)	$\Delta G_{\text{ass}}^{\ddagger}$ (kcal mol ⁻¹)	$\Delta H_{\text{ass}}^{\ddagger}$ (kcal mol ⁻¹)	$\Delta S_{\text{ass}}^{\ddagger}$ (cal mol ⁻¹ K ⁻¹)	$-T\Delta S_{\text{ass}}^{\ddagger}$ (kcal mol ⁻¹)
Control	19.0 (0.9)	23.4 (0.9)	7.4 (3.0)	-4.9 (1.8)
GdnHCl (2.5M)	18.2 (2.0)	24.9 (2.0)	11.0 (6.9)	-6.6 (4.1)
MGdnHCl (3M)	16.2 (1.5)	27.5 (1.5)	19.0 (5.1)	-11.3 (3.0)

^a Activation free energy ($\Delta G_{\text{ass}}^{\ddagger}$) and entropy changes ($-T\Delta S_{\text{ass}}^{\ddagger}$) are given at 25 °C.

*The uncertainties (standard error) in $\Delta H_{\text{ass}}^{\ddagger}$, $-T\Delta S_{\text{ass}}^{\ddagger}$ and $\Delta S_{\text{ass}}^{\ddagger}$ are indicated in parenthesis.

4. Discussions

4.1 The role of guanidine hydrochloride and methylguanidine hydrochloride on the thermodynamic stability of ferrocytochrome *c*

Fig. 4 shows the effects of GdnHCl and MGdnHCl on the T_m and ΔH_m for ferrocyt *c*. The T_m and ΔH_m decrease linearly with increasing concentrations of GdnHCl and MGdnHCl (Fig. 4). Furthermore, the decrease in T_m not greatly differs for GdnHCl and MGdnHCl. Earlier studies on urea, alkylureas, and GdnHCl denaturation of proteins have revealed that over the full range of denaturant concentration, ΔG_T decreases linearly with denaturant concentration as [5,24, 53-54],

$$\Delta G_T = \Delta G_T^\circ - m^* M \quad (4)$$

where ΔG_T° is the Gibbs free energy of denaturation in the absence of denaturant, and m^* is the rate of change of ΔG_T with concentration of denaturant, M is the molar concentration denaturant. While the physical importance of the factor m^* is not fully clear, but some earlier

reports revealed that it corresponds to the difference between the accessibility of surface areas of native and denatured states of the polypeptide chain for a given denaturant [55-57]. Fig. 4c presents the ΔG_T vs [denaturants] plot for ferrocyt *c*. Clearly, for both GdnHCl and MGdnHCl, ΔG_T decrease linearly. The parameters, m^* and ΔG_T° were calculated from linear least-squares fitting of the data (ΔG_T vs M) to equation (4). The values of m^* and ΔG_T° are summarized in Table 3. The almost equal value of m^* for GdnHCl and MGdnHCl suggests that both denaturants have almost equal denaturing efficiency against ferrocyt *c*.

Table 3 m^* and ΔG_T° -values of GdnHCl and MGdnHCl for ferrocyt *c* unfolding (eq. (4)).

Cosolute <i>Parameters</i>	GdnHCl	MGdnHCl
ΔG_T°	19.4	19.1
m^*	-3.7	-3.4

m^* and ΔG_T° (25°C) are reported as kcal mol⁻¹m⁻¹ and kcal mol⁻¹, respectively.

The uncertainties of m^* and ΔG_T° values reported here are ± 0.3 kcal mol⁻¹m⁻¹ and ± 0.5 kcal mol⁻¹, respectively.

4.2 The role of guanidine hydrochloride and methylguanidine hydrochloride on the structural fluctuations of the M80-containing Ω -loop of ferrocytochrome *c*

The M80-containing Ω -loop of cyt *c* consist the residues 70 to 85 (Fig. 1) [58], which is recognized a partially unfolded subglobal part of the protein [59-60]. The collective motions of the Ω -loop control the CO-association ($\text{Fe}^{2+}\text{-M80} + \text{CO} \rightarrow \text{Fe}^{2+}\text{-CO} + \text{M80}$) process [5,22]. Furthermore, the denaturant modulation of CO-association rate shows how the thermal motions of the Ω -loop vary in response to the denaturants in the reaction medium. In the subdenaturing region, the subdenaturing concentrations of denaturants increases the enthalpic barrier ($\Delta H_{\text{ass}}^\ddagger$) for CO association reaction, indicating that GdnH⁺ and MGdnH⁺

bound to the protein. Furthermore, the denaturant-mediated increase in $\Delta H_{\text{ass}}^{\ddagger}$ is more pronounced for MGdnHCl than that of the GdnHCl, indicating that MGdnHCl may bound to protein more efficiently than that of GdnHCl. The increase of $\Delta H_{\text{ass}}^{\ddagger}$ due to denaturant is also accompanied by a decrease in the entropy change $-T\Delta S_{\text{ass}}^{\ddagger}$, and which is found to be decreased for MGdnHCl than that of GdnHCl. Few previous reports have revealed that the subdenaturing concentrations of GdnHCl decrease the structural-fluctuations of Ω -loop of ferrocyanide *c* from both entropic effect due to intramolecular protein cross-linking action of GdnH^+ and electrostatic effect due to the interaction of Cl^- , and also possibly of GdnH^+ , with charged groups of ferrocyanide *c* [21-22]. The fact that, alkyl substituted GdnHCl molecule will have lower ability to form hydrogen bonds, therefore, they may not produce as extensive cross-linking as the GdnHCl does. So a basic question, how could the increase in hydrophobic groups on GdnHCl molecule increase the extent of denaturant-mediated constrained dynamics of Ω -loop remains unexplored?

4.3 GdnHCl and MGdnHCl-induced stabilization: local or global?

Kinetic and thermodynamic parameters measured for CO association reaction in the presence of varying concentrations of GdnHCl and MGdnHCl reveal that the subdenaturing concentrations of these denaturants stabilize the ferrocyanide *c* conformation. However, the extensive thermal unfolding experiments with ferrocyanide *c* in the presence of low concentrations of GdnHCl and MGdnHCl show that these denaturants decrease the thermal stability of protein, indicating that the denaturant-mediated stabilization of the protein is not registered at global level of the protein. The reason for this cannot be ascertained from the available data. It can be speculated that the number of GdnHCl and MGdnHCl molecules that

interact with the protein in the subdenaturing limit are insufficient to cross-link a large part of the molecule.

5. Conclusion

The effect of guanidine hydrochloride (GdnHCl) and methylguanidine hydrochloride (MGdnHCl) on the thermal stability and internal dynamics of ferrocycytochrome *c* (ferrocyt *c*) have been studied at pH 7. Kinetic and thermodynamic parameter measured for CO association reaction of ferrocyt *c* in the presence of varying concentrations of GdnHCl and MGdnHCl suggests that (i) subdenaturing concentrations of the denaturants decrease the structural fluctuations of the Ω -loop of ferrocyt *c*, (ii) the extent of decrease in structural fluctuations the Ω -loop is found to be greater for MGdnHCl than GdnHCl, (iii) both enthalpic and entropic effects are responsible for the denaturant mediated reduction the structural fluctuations the Ω -loop, and (iv) at relatively higher concentrations, the denaturing action of these denaturants dominants and result in increase of the structural fluctuations the Ω -loop. Thermodynamic analysis of thermal denaturation curves of ferrocyt *c* measured in the presence of varying concentrations of GdnHCl and MGdnHCl suggests that these denaturants decrease the thermal stability of protein. These results thus suggest that the denaturant mediated stabilization of the protein is not registered at global level of the protein.

6. References

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