Determination of Stabilities of Native, Molten Globule and Partial Unfolded States of Bovine Cytochrome-C.

Auther
Shabir Hussain Qureshi

Supervisor:
Prof. Faizan Ahmad
Department of
Biosciences
Jamia Millia Islamia

How an unstructured polypeptide chain condenses to form a biologically functional protein, the protein folding problem, has attracted great interests in recent years. The number of conformations of the protein in unfolded state is astronomically so large that, at first glance, one would hardly expect that proteins might fold into the unique native conformation in physiological time scale. Obviously many efficient folding pathways and mechanisms have evolved. The involvement of folding intermediates during protein folding has been strongly argued, and their existence in small monomeric proteins has been experimentally confirmed. Molten globule is one such intermediate, which has been proposed as a general intermediate in protein folding.

In this study cytochrome-c from bovine heart has been chosen as a model protein. Cytochrome-c is the evolutionary divergent protein with several homologues providing a natural mutant to study the effect of amino acid substitution in different homologues. The equilibrium studies provide valuable information about the thermodynamic stability of cytochrome-c and the role of amino acid substitution to the globular stability. Various chemical denaturants, namely, lithium chloride (LiCl), calcium chloride ($CaCl_2$), lithium perchlorate ($LiClO_4$), lithium bromide (LiBr), guanidine hydrochloride (GdnHCl) and urea at pH 6.0 were used to carry an equilibrium denaturation study of cytochrome-c by observing change in molar absorption coefficient in visible and Soret region and by measuring mean residue ellipticity in Soret, near-UV and far-UV regions. Heat-induced denaturation of bovine cytochrome was also carried in the presence and absence of these denaturants using two different optical probes, namely, De (difference in molar absorption coefficient) and [q] (mean residue ellipticity).

The denaturation of bovine cytochrome-c by weak salt denaturants (LiCl, $CaCl_2$, $LiClO_4$ and LiBr) was measured by observing changes in De_{400} and circular dichroism (CD) at 222 nm and 409 nm at pH 6.0 and 25 ^{o}C Measurements of De_{400} and mean residue ellipticity at 409nm ($[q]_{409}$) gave the biphasic transition for both modes of denaturation of cytochrome-c. It has been observed that the first denaturation phase, N (native) conformation « X (intermediate) conformation and the second denaturation phase, X conformation « D (denatured) conformation are reversible. Conformational characterization of X state was carried by far-UV CD, near-UV CD, 8-anilino-1-napthelene sulfonic acid (ANS) binding, tryptophan florescence and intrinsic viscosity measurements were carried out at pH 6.0 and 25 ^{o}C. The conformational characteristics

of X state obtained from these measurements reveal that X state has all the common structural characteristics of the molten globule: (i) it possesses all the native secondary structure, (ii) most of the characteristics native tertiary interactions are absent, (iii) most of the hydrophobic groups are accessible to solvent, (iv) hydrophobic clusters are present on the surface of the protein, and (v) radius of gyration (R_g) is increased by 8-10%.

Analysis of the denaturation transition curves for the stability of different states in terms of Gibbs energy change at pH 6.0 and 25 ^oC led us to conclude that the N state is more stable than X state by 10 $\pm$ 0.5 kcal mol^{-1}, whereas X state is more stable than D state by only 1.2 $\pm$ 0.3 kcal mol^{-1}.

We have also studied the effect of temperature on the equilibria, N conformation « X conformation and X conformation « D conformation in the presence of different denaturant concentrations using two different optical probes, namely De_{400} and $[q]_{222.}$ These measurements yeilded T_m (mid point of denaturation) and DH_m (enthalpy change) at T_m as a function of denaturant concentration. A plot of DH_m versus corresponding T_m was used to determine the constant pressure heat capacity change DC_p $(=DH_m/T_m)_{p.}$ value of DC_p for N conformation « X conformation and X conformation « D conformation is 0.94 $\pm$ 0.02 kcal mol^{-1} K^{-1} and 0.42 $\pm$ 0.01 kcal mol^{-1} K^{-1}, respectively. These measurements suggest that about 30% of hydrophobic groups in the molten globule state are not accessible to the water. The coincidence of thermodynamic parameters like DH_m and DC_p obtained from different optical probes led us to conclude that the transition N conformation « X conformation and X « D conformation are two state mechanism.

The equilibrium studies on the folding « unfolding process of bovine cytochrome-c in the presence of GdnHCl and urea at pH 6.0 and 25 ^oC unlike weak salts induce a cooperative single state transition curve, N « D. The coincidence of normalized transition curves monitored by measurements of absorbance at 405, 530, and 695 nm and circular dichroism (CD) at 222, 416, and 405 nm reveal that denaturation follows a two-state mechanism. The heat-induced denaturation in the presence of increasing concentration of GdnHCl show that the denaturation of bovine cytochrome-c is a two-state process, and the enthalpy change strongly depend upon GdnHCl concentration. A value of 10.0 $\pm$ 0.5 kcal mol^{-1} for Gibbs free energy in the absence of GdnHCl estimated using Gibbs Helmoltz equation is in agreement with the values obtained from the isothermal denaturation of bovine cytochrome-c in absence of GdnHCl and urea. The value of Gibbs free energy change for N « D process obtained from GdnHCl and urea denaturation is in agreement with that of total Gibbs free energy change for N « X and X « D processes, validating the thermodynamic rule that the overall change in the thermodynamic properties of the process should be same whatever the path is followed.