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PhD topic- Protein folding: Conformational and Thermodynamic Characterization of States Occurring on the Folding ↔ Unfolding Pathway of Goat Cytochrome-*c*

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Abstract

The amino acid sequence of cyt-*c* from the goat heart (g-cyt-*c*) was determined using cDNA method. Its sequence alignment with horse cytochrome *c* (h-cyt-*c*) and bovine cytochrome *c* (b-cyt-*c*) led us to conclude that g-cyt-*c* differs in amino acid sequence from h-cyt-*c* at four positions Pro44(horse) → Ala44 (goat), Thr47(horse) → Ser47(goat), Lys60(horse) → Gly60(goat) and Thr89(horse) → Gly89(goat) and from b-cyt-*c* at one position Pro44(bovine) → Ala44(goat). Though there is single amino acid change in g-cyt-*c* from b-cyt-*c*, all the thermodynamic parameters of the goat protein are, within experimental errors, identical to those of the bovine protein. The structural coordinates of g-cyt-*c* from the known coordinates of the bovine protein is generated using the simple mutate tool from COOT package of CCP4 suite as shown in Figure I.

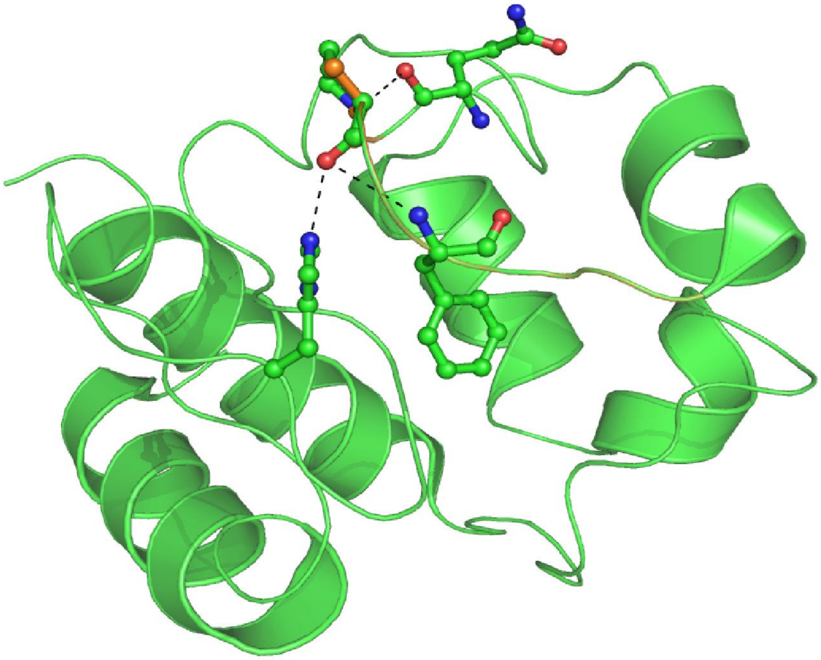

FIGURE I: 3D structural comparison of b-cyt-*c* (green) and g-cyt-*c* (orange). Residue at positions at 26, 42, 44 and 46 are represented in ball and stick, and involvements in H-bond formations are also shown.

Using PISA server, we have also estimated solvation free energy (ΔG_S) contributions to the overall stability of the goat cyt-*c* ($\Delta G_S = -87.4$ kcal mol^{-1}) and bovine cyt-*c* ($\Delta G_S = -87.3$ kcal mol^{-1}). The estimation of ΔG_S values and the structural analysis support our experimental finding that g-cyt-*c* and b-cyt-*c* have identical folding energy (ΔG_D^o). The thermodynamic and structural analysis led us to conclude that the 3D structure of g-cyt-*c* will be almost identical to that of the bovine protein.

The equilibrium denaturation of g-cyt-*c* by LiCl, $LiClO_4$ and LiBr monitored by $\Delta\varepsilon_{400}$ and $[\theta]_{409}$ shows a biphasic process ($N \leftrightarrow X \leftrightarrow D$). We characterize the X state induced by 6.0 M LiCl, 1.9 M $LiClO_4$ and 4.2 M LiBr using far- and near-UV CD, FT-IR spectroscopy, Nile red fluorescence and DLS experiments. All the structural characteristics of the intermediate state X of cyt-*c* induced by weak salts at 25 ^{0}C and pH 6.0 led us to conclude that this state has all the common characteristics of the MG state.

A comparison of Nile red and Trp-fluorescence, far- and near-UV CD, near IR-absorption and hydrodynamic radii of LiCl-, $LiClO_4$-,0.5 M NaCl- (pH 2) and LiBr-induced MG state reveals several interesting features. The $LiClO_4$-induced MG shows more expanded MG state than those induced by Cl^- in the presence of LiCl and low pH while the LiBr-induced MG state is more compact and shows the spectroscopic characteristics nearer to the native protein due to its retention of the Met80-Fe(III) axial bond and the Trp59-propionate interaction of the native state. The comparision of the thermodynamic parameters ($\Delta C_{p,I}$ and $\Delta C_{p,\,II}$) obtained from the heat-induced denaturation of cyt-*c* in the presence of weak salts also supports the heterogeneity of MG state under different solvent conditions. The structural and thermal analysis of MG state of cyt-*c* under different solvent conditions give the conclusion that the MG state of cyt-*c* is stabilized by hydrophobic interactions and secondary structure.