Conformational Transition of Cytochrome c

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Abstract: Conformational transitions of oxidized and reduced cytochrome c at various solvent conditions are summarized. Sorbitol stabilizes and NaCl destabilizes native cytochrome c structure against the acid denaturation. In the process of heating, NaCl more strongly stabilizes molten globular cytochtome c state than sorbitol in secondary structure region, but in heme region, sorbitol is stronger stabilizer of cytochrome c molten globular state than NaCl. In the presence of strong inorganic acids H_2SO_4 and $HClO_4$ and their salts two low spin and one high-spin heme conformers occur. The unstable pentacoordinated conformer is formed in 8M H_2SO_4. Cytochrome c creates complexes with polyanions poly(vynilsulfate) and poly(4-styrene-sulfonate), which are able to mimic the effect of cytochrome c natural redox partner on its structure, as well as to prevent its the aggregation in aggregation prone conditions. Cytochrome c forms complexes with gold nanoparticles covered by glutation, which enhance stability in heme region at room temperature.

Keywords: Conformational transition, stability, heme coordination, complexation, nanoparticle.

Cytochrome c (cyt c) is one of the most studied proteins with respect to its conformational transitions. This protein is an essential redox biomacromolecule found in the mitochondria. It functions as an electron transporter in the energy-yielding respiratory chain and participates in a process of apoptosis. Its prosthetic group (heme) is covalently bound to the polypeptide through thioether linkages with two cysteine residues. The iron in the heme coordinates two axial ligands, histidine (His18) and methionine (Met80), so that it adopts a low-spin configuration in both its ferrous and ferric forms at physiological conditions (Fig. **1**).

Acidification of a salt-free solution of ferricyt c to pH 2.0 leads to the unfolding of the protein associated with a conversion of the low to the high-spin state. The structure of unfolded protein is an extended coil having a dimension greater than that of a random coil owing to electrostatic repulsions among the positively charged lysine and arginine residues. Upon addition of salts to the acid unfolded cytochrome c the protein cooperatively folds to a compact structure with a molten globule (MG) character. Molten globular conformation of cyt c has the secondary structure content similar to that of the native conformation, but less packed tertiary structure.

Conformational transition from the unfolded to the molten globule is also promoted by variety of uncharged molecules, ions and polyanions. NaCl and sorbitol exhibited antagonistic effect on the acid-induced transition of the protein. Sorbitol enhanced the stability of native conformation (Fig. **2**), while NaCl destabilized this state. The midpoints of acid-induced transitions in the axial coordination of heme as well as in the secondary structure occurred nearly at the same pH

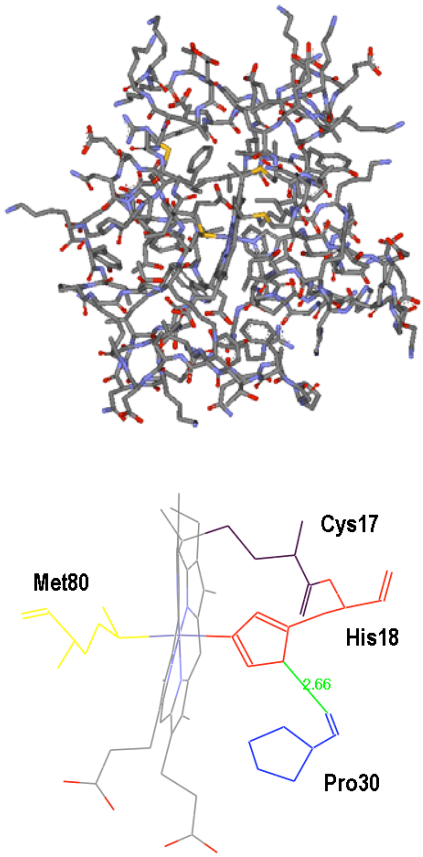

Fig. (1). 3 D structure of horse heart ferricytochrome c, 1HRC PDB.

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values. However, temperature-induced transitions in the unfolding of the secondary structure were almost coincidental with the cleavage of Met80–Fe bond only in the sorbitol solutions. In the salt solution, the Met80–Fe bond was markedly more labile than the secondary structure [1].

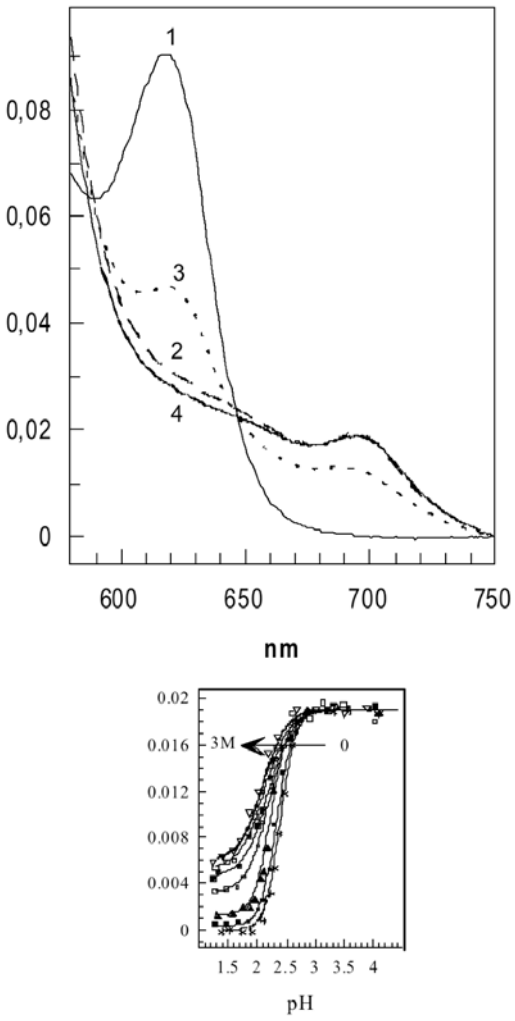

Fig. (2). Upper: absorption spectra of cytochrome c in the presence of 3 Msorbitol. Cyt c concentration, 27 μM. Temperature, 25 °C.

(1) cyt c, pH 3.2; (2) cyt c, pH 3.2+sorbitol; (3) cyt c, pH 2+sorbitol; (4) cyt c, pH 2. Lower: The pH dependencies of absorption band at 695 nm at various concentrations of sorbitol (0.5, 1, 1.5, 2, 2.5 and 3 M).

Transition from low-spin to high-spin heme coordination of ferricyt *c* in the water solution is complete in 1 M $HClO_4$ or 3 M H_2SO_4. High-spin pentacoordinated ferricyt *c* with the heme ligand of His18-Fe is formed in 8 M H_2SO_4. This state is unstable at higher concentration of H_2SO_4 and porphyrin ferricyt *c* is formed [2].

The reduced form (ferrocytc) is far more stable toward unfolding than the oxidized form. An apparent p*K* value for denaturation was found to be 0.86 at 25 °C. Visible absorption spectra indicate that the dominant population is a high-spin, five-coordinate form under acidic conditions. Our data also indicate that even at a pH below 2 the iron-sulfur bond in ferrocyt *c* is present [3]. The reduced form is very stable also to the thermal denaturation. Transition temperature of ferrocytochrome c is above 100 °C at neutral pH. Our results from heat denaturation of ferrocyt c in the presence GdnHCl lead us to describe two steps process of transition. The first phase of the thermally induced unfolding is characterized by a transition from the native low-spin (Met80–Fe–His18) to a non-native, low-spin state (X–Fe–His18). In the second phase, an increase of flexibility of the chain caused by the increase in backbone rotational freedom at increasing temperature induces dissociation of the non-native ligand from the heme and a transition to a five-coordinate, high-spin form (Fe–His18) [4].

It is well-known that cyt c alkaline transition occurs with an apparent pK_a of about 8.9–9.3 depending on ionic strength (at room temperature) [5]. Formation of cyt c– cytochrome c oxidase complex is accompanied by a significant increase of the apparent pK_a constant of the alkaline transition of cyt c [6]. Experimental data show that cytochrome bc_1 causes a similar shift of the apparent pK_a value of cyt c from 9.0 to 10.3 [7].

Cytochrome c as a component of respiratory chain forms complexes with other redox enzymes such as cytochrome c oxidase, or cytochrome bc_1. plays Especially electrostatic interactions play the important role in complex formation. It has been shown by us and other authors [8, 9], that polyanions are able to mimic the negatively charged surface of redox proteins and induce similar effects on cytochrome c structural properties. We have also found, that polyanions poly(vynilsulfate) (PVS) and poly(4-styrene-sulfonate) (PSS) enhance thermal transition reversibility of cytochrome c in conditions near isoelectric point (pI = 10.1) which favor protein aggregation [10]. Whereas PVS decreases transition temperature of cytochrome c detected by calorimetry, PSS causes total diminishing of thermal transition at saturation concentration (Fig. **3**). Both polyanions have comparable charge density, but PSS contains additional hydrophobic phenyloxy group, which is probably responsible for the sig-

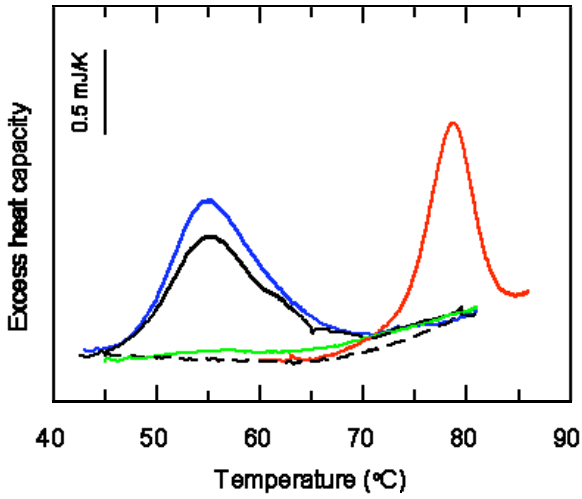

Fig. (3). DSC thermograms of cytochrome c – red line and its complexes with PVS – blue line and PSS – green line in 2 mM glycine buffer, pH 10.0. Black full line – 2. heating scan of cytochrome c – PVS complex; black dashed line - 2. heating scan of free cytochrome c.

Protein concentration 73μM, 1mg/ml PVS, 0.5 mg/ml PSS.

nificant conformational change of hydrophobic cytochrome c core and following lack of thermal transition. Presence of PVS leads to recovery of thermal denaturation reversibility of cytochrome c. The loss of thermal transition in presence of PSS disables to test the reversibility of this transition. From absorption and viscometric measurement it follows that also PSS as well as PVS prevent the aggregation of denatured protein molecules [10].

Cytochrome c created stabile complex with gold nanoparticles covered with tripeptide glutathione. Our results show that the structural stability around the heme of complexed cyt c was increased. Glutathione layered gold nanoparticles caused a significant increase of the apparent pK values of the cyt c alkaline transition. Similarly, the heme crevice became more stable to heat after assembly of cyt c with gold nanoparticles. In contrast, gold nanoparticles weaken the overall thermal stability of the cyt c by decreasing the denaturation temperature estimated from far-UV CD measurements [11].

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