Insights into the programmed cell death: molecular mechanism of the cytochrome c-cardiolipin interaction.

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Cytochrome c (**Cyt c**), a six-coordinated low-spin (**6cLS**) heme iron with **His18** and **Met80** as the axial ligands (His18-Fe-Met80), has an important role in programmed cell death, apoptosis. In fact it has been demonstrated that upon binding to cardiolipin (CL, a phospholipid of the inner mitochondrial membrane), Cyt c acquires peroxidase activity (1) that leads to peroxidation of CL, detachment of Cyt c from the inner mitochondrial membrane, release into the cytosol and subsequent induction of apoptosis through caspase activation. The appearance of the peroxidase activity is attributed to the rupture of the bond between the heme iron and its axial ligand Met80 (2) and the consequent partial unfolding of the protein (3), but there is still no overall consensus regarding the conformational rearrangements that follow the protein-lipid interaction, since many different results have been reported (4, 5, 6).

Recently a possible trigger for the CL-induced rearrangements has been identified in the rupture of the hydrogen bond between His26 and the backbone carbonyl of Pro44 (7). This H-bond has an important role because it bridges the 20s and the 40s Ω-loops, enhancing the overall rigidity of the protein and stabilizing the heme crevice (8). Its rupture initiates rapid formation of β-sheet structure that leads to the displacement of the heme and the disruption of Met80 ligation (7).

In the present study, via UV-Vis and RR spectroscopies, we have investigated the formation of the CL-complex of the ferric horse heart protein (WT) and selected variants in which residues considered to play a key role in the interaction with CL (His26, Hiss33, Lys72, Lys73, Lys79) (9, 5, 10) have been mutated.

The spectroscopic data allowed us to conclude that:

1) CL binding enhances the flexibility of the Met80-containing loop and leads to a partial rupture of the Met80 ligation to the heme iron. At the Cyt c-CL molar ratio (R) of 1:5, a global rearrangement of the protein and the formation of a 6cLS bis-His species (His18-Fe-His) is observed. This form increases with the increase of the Cyt c-CL molar ratio.

2) Based on the spectroscopic features of the mutants, the His26 is the residue which replaces the native Met80 ligand forming the misligated 6cLS bis-His species (His18-Fe-His26).

3) the 6cHS (His18-Fe-H_2O, aquo) and the 5cHS (His18-Fe) species appear at a Cyt c-CL R 1:15, indicating that the increase of the protein-lipid molar ratio increases the flexibility of the Met80-containing loop. This finding is supported also by the RR spectrum of the CO-adduct, which shows that the distal cavity is open.

4) On the contrary, the fairly high ν(Fe-Im_{His18}) stretching mode at 229 cm^{-1} suggests that the proximal cavity is closed due to the formation of a strong hydrogen bond between the His18 N_δ hydrogen and an accepting group.

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