Global and Local Structural Changes of Cytochrome-c Induced by Urea-Mediated Unfolding in Aqueous Solutions

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Using small angle X-ray scattering (SAXS), X-ray absorption (XAS), and Resonance Raman scattering, we have studied global and local structural changes of cytochrome c mediated by urea and GuHCl denaturants in aqueous solutions (21 mg/ml). The SAXS data demonstrate the unfolding of the cytochrome-c from a globular shape of a radius of gyration R_g = 12.8 Å to an elongated rod-like shape of R_g = 29.7Å, with a change in the aspect ratio up to ~ three folds, when the urea concentration increased from 0 to 10 M or the GuHCl concentration from 0 - 3M. SAXS data fitting using the dummy residuals gives the envelopes of cytochrome c during the unfolding process. For the local structure observation by XAS, both the folded and unfolded states of cytochrome c show the same pre-edge features at Fe K-edge, indicating that the Fe(III) of the heme of the molecule is still in a six-coordinated environment in the unfolded state. Furthermore, the discernable differences in the XANES features of the two states are mainly attributed to a spin transition of the iron from low spin to high spin. The consequent changes of the spin transition on the vibration modes of the porphyrin that surrounds the heme group, are complementarily observed by the Resonance Raman scattering, which shows a suppression of the ~1584 and ~1566 cm^{-1} peaks in spectrum of the unfolded state. For the folded state, the extracted bond distances of Fe-S (Methionine), Fe-N (Porphyrin), and Fe-N (Histidine) from the EXAFS data are compatible with previous NMR results. For the unfolded state, the concurrent spin transition and perseverance of six coordination of the iron of the heme group is explained on the basis of a ligand exchanged model, with urea replacing the Met80 ligation of the iron. Based on the model, we fit the EXAFS data of the unfolded state with the distances of Fe-O(urea) = 2.24(2)Å, Fe-C(urea)=2.59(5)Å and Fe-O(water) = 2.30(2)Å. The expanded first coordination shell around the Fe site, compared to the folded state, is a consequence of the six coordinated environment of the ligand exchange model. Based on the local and global structural changes observed, we have proposed sequential, local structural changes initiated by the denaturant near the heme group, which lead to the partial unfolding of the molecule.