The structure of zinc (II)-substituted cytochrome c (Zn-Cytc), recently resolved by NMR, suggested that Zn is in an unusual coordination with six ligands (Anni et al., Biochemistry 1995, 34, 5744-5753). Furthermore, fluorescence line narrowing spectroscopy has shown that Zn becomes penta-coordinated upon protein unfolding. An hexa-coordinated state of Zn in Cytc is unique since there are no examples of any hexa-coordinated Zn-proteins, and there are no hexa-coordinated Zn-porphyrins in solution. Moreover, the particular coordination of Zn with five nitrogens and one sulfur ligand in Cytc is surprising, because even among the Zn organic compounds that are hexa-coordinated (27% of the 428 crystal structures) none is of the type (N) 5-Zn-(S) 1. Taken together these findings point to a tight protein modulation of the binding preference and strength of Zn to its ligands in Cytc. The Zn-edge extended x-ray absorption fine structure (EXAFS) studies of Zn-Cytc were undertaken in order to define the central metal environment, specifically the distances of the Zn metal to its ligands and their nature. Both native and guanidine-denatured Zn-Cytc at neutral pH were measured, alongside with the corresponding Fe(III)-Cytc references. Sets of experimental EXAFS data were fit to theoretical curves (FEFF 6.0) using a novel refinement procedure were obtained. This data analysis establishes the coordination of the Zn site in Cytc: an hexa-coordinated Zn in the native protein environment (Zn-Np=2.052Å, Zn-Met80 (SD) =2.34Å, Zn-His(NE)=2.371Å) and a mixture of penta-coordinated Zn states in the denatured sample (Zn-Np=2.10Å, Zn-His(NE) =2.10-2.20Å). Fe-cyt-c data showed close agreement with crystallographic data. Analysis of denatured cytochrome-c shows ligand exchange as expected.