Protein Folding: Significance of Residue 72 in Human Cytochrome c

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The folding of proteins into their native states is a requirement for proper function. Proteins that aggregate or fail to fold correctly can lead to many proteopathic diseases, including but not limited to, Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and prion diseases.\(^1\) Christian Anfinsen famously postulated that the amino acid sequence was the determinate of the native fold in small globular proteins. Here we investigate the lysine residue found at position 72 in an important conserved region of cytochrome \(c\) called the heme-crevice loop. The heme-crevice loop plays an important part in regulating cytochrome \(c\)’s ability to transfer electrons in the electron transport chain helping to make the energy molecule ATP, and to oxidize the mitochondrial membrane lipid cardiolipin during programmed cell death.

For this investigation we made a mutation at position 72 from a large, basic lysine (K72) residue to a small, non-polar alanine (K72A) residue. This allows us to measure changes that the protein undergoes in its overall stability, local movement of the heme-crevice loop, and changes in the functions discussed above. Cytochrome \(c\) has a covalently bound heme group. This is the part of the protein that allows the chemistry of electron transfer and peroxidase activity (catalyzing the \(\text{H}_2\text{O}_2\) to \(\text{H}_2\text{O}\) reaction) to take place. Other lysine residues such as those at positions 79 and to a lesser extent 73 have been shown to have the ability to swing in and displace the natively bound residue (methionine 80, M80) once they lose a proton at alkaline/basic pH. While investigating residue 72, we discovered that this lysine may in fact also bind to the heme during the alkaline conformational transition, which was previously thought not to take place based on studies with horse heart cytochrome \(c\).\(^2\) (The replacement of the natively bound heme ligand, M80, with alternative ligands is called the alkaline conformational transition).

When K72 is replaced with an alanine we see an increase in the pKa (the pH where half of the protein is in the native conformer and half is in the alkaline conformer) compared to the native (WT) protein (9.54±0.03 for WT to 10.00±0.13 for K72A). This means that the native M80 bound state is being stabilized. We pursued this interesting observation with stopped-flow pH Jumps. This experiment allows us to quickly change the pH of our protein and monitor the heme-crevice loop adjusting to the rapid change in pH. Fitting these data, we can determine the number of protons (n) that are involved in each binding reaction. When we fit for WT we get \(n = 2.32±0.18\) and for K72A we get \(n=1.08±0.016\). This shows that one more proton is involved in the WT jump compared to the K72A jump. Lastly, we examined the peroxidase activity of cytochrome \(c\). Here we saw a sharp separation of WT and K72A peroxidase activity with increasing pH. As M80 is weakly bound, we see increased peroxidase activity. This observation indicates that K72 is important for controlling peroxidase activity.