Biochemical and spectroscopic characterization of Dap1, a novel class of heme binding proteins

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The yeast protein Dap1 belongs to a highly conserved class of putative membrane progesterone binding proteins and has recently been reported to bind heme. While primary structural analysis reveals similarities to a cytochrome b_5 motif, neither of the two axial histidines responsible for ligation to the iron center of heme are present in Dap1. We have used mutagenesis, EPR, MCD, CD, and UV-vis to characterize the nature of heme binding in Dap1. EPR confirmed a five coordinate high-spin binding, indicating only one amino acid ligand, in contrast to the six coordinate low-spin state of cytochrome b_5. The MCD spectrum suggests the axial ligand is not a histidine and argues for the presence of a tyrosine or aspartate residue. Mutagenesis was used to probe possible tyrosine and aspartate residues near the missing histidines to find alternate axial ligands to the heme iron. The spectral data of these mutants will be presented along with characterization of the axial ligand.