Structural studies of the SOUL/HBP family of heme binding proteins

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The study of biomolecules that interact or contain metals or metal containing cofactors is of great importance, as the understanding of the factors that stabilize these interactions can be used in areas such as tailored biomaterials or in functionalised nanomaterials. To this end we are studying a group of proteins that contain metal centres and proteins related to heme biosynthesis.

Both members of the SOUL/HBP family of heme-binding proteins were discovered around 1999. The first member, p22HBP, was found to be a monomeric 22 kDa cytoplasmic heme or porphyrin binding protein isolated from mouse liver cell extracts. The solution structure has been determined and chemical shift mapping located the tetrapyrrole-binding site and binding constants were found to be in the nM range [1]. p22HBP is thought to be involved in heme regulation or heme synthesis.

The second member of the family, SOUL, was isolated from retinal and pineal gland tissues [2], and is a cytoplasmic 23kDa monomeric protein that upon heme binding becomes hexameric [3]. No structural or functional information is available although SOUL has been found to induce necrotic cell death via mitochondrial membrane permeability in the presence of calcium.

No structure of the bound form of HBP exists therefore modeling has been used to probe the interaction between HBP and various tetrapyrroles. A flexible docking protocol was carried out using Autodock4 centered on the binding site identified by NMR. Subsequent molecular dynamics and comparison with calculated and experimental ring current shifts indicated that both hemin and protoporphyrin-IX are stabilized by strong electrostatic interactions via their propionate groups to K64, K177, and R56 located at the edges of the binding pocket. Subtle differences between calculated and experimental ring current shifts suggest that the protein, especially around the D173-G180 loop, changes conformation on binding.

Initial structural studies of hSOUL by NMR and X-ray diffraction in conjunction with fluorescence quenching studies suggest that hSOUL binds tetrapyrroles but with a μM Kd suggesting a different binding mode to HBP. Also, the single His residue, thought to bind heme, probably plays no part as Kds were found to be similar for hemin and PPIX. No evidence for hexamer formation was found. An initial low resolution X-ray structure solved by molecular replacement using the HBP structure has been obtained.

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