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In-cell enzymology in heme oxygenase 1

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1 Introduction

Measurement of the intrinsic properties of enzymes *in vivo* may sometimes contrast with those measured *in vitro*. In living cells several factors may contribute to support enzyme catalysis. Heme oxygenase (HO), a ubiquitous enzyme that extracts iron from heme to produce biliverdin, coordinates heme iron through a conserved histidine residue. In this study we use an in-cell bacterial model system to test whether residues other than histidine can support enzymatic activity and we compared these results with structural data.

2 Experiment

To examine the dependence of heme oxygenase activity on the His-heme interaction a live-cell model system was used. This system permits the co-expression of heme oxygenase together with a fluorescent biliverdin biosensor (IFP), a reporter of biliverdin expression in *Escherichia coli*. A broad panel of histidine mutants showing no activity in previous *in vitro* assays [1] was tested with our *in-cell* methodology.

In a second experimental approach we assessed the structural consequences of the histidine mutations on the enzyme's active site by solving the X-ray crystal structure of the arginine substituted proximal histidine of human heme oxygenase 1 bound to either heme or biliverdin.

3 Results and Discussion

Co-expression of human heme oxygenase mutant H25R with the IFP produced a fluorescent signal indicative of the formation of an IFP-biliverdin complex. This result indicated that the enzyme retained activity in living cells in the absence of a proximal His-heme ligand. Similar results were obtained with the cyanobacterial heme oxygenase 1, but not those with *Arabidopsis thaliana* heme oxygenase 1. Mutations on the proximal histidine residues did not affect different heme oxygenases the same way, probably because they different redox partners assisting the enzyme during heme catalysis or distinct active site residues in the enzymes from different organisms [2].

The crystal structure for mutant H25R in complex with heme was determined (Table 1). The electron density map revealed that the side chain of Arg25 is oriented away from the original His25, not allowing ligation with heme. In comparison with the wild-type protein, several rearrangements affecting the bound heme, the proximal backbone helix, and the orientation of the Glu29 side-chain (moving close to heme) were observed. The orientation of Glu29 suggested its involvement in the *in-cell* activity. However, the double mutant H25R/E29A discarded this idea as it displayed fluorescence from the IFP-biliverdin complex.

Table 1: Resolution of crystal structures.

H25R + biliverdin	2.08 Å
H25R + heme	2.95 Å

The crystal structure of the protein bound to biliverdin was also determined (Table 1, Fig. 1). The orientation of biliverdin was very similar to that of bound heme although somewhat distorted. It was placed within hydrogen bond distance to the side-chain of Arg25, which approached the tetrapyrrole ring.

The question as to why proximal histidine mutants show activity in bacterial cells but no activity *in vitro* still remains unclear, but may be related to the existence of specific adaptor proteins in bacteria and how they enhance electron transfer between heme oxygenases and their redox partners [3].

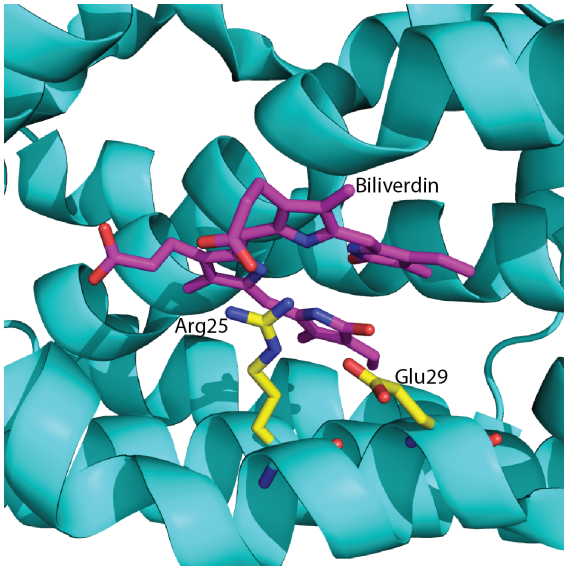

Fig. 1: X-ray structure of the mutant H25R of human heme oxygenase 1 bound to biliverdin.

References

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