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The Oligomeric Assembly of the Novel Haem-Degrading Protein HbpS Is Essential for Interaction with Its Cognate Two-Component Sensor Kinase

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HbpS, a novel protein of previously unknown function from *Streptomyces reticuli*, is up-regulated in response to haemin- and peroxide-based oxidative stress and interacts with the SenS/SenR two-component signal transduction system. In this study, we report the high-resolution crystal structures (2.2 and 1.6 Å) of octomeric HbpS crystallized in the presence and in the absence of haem and demonstrate that iron binds to surface-exposed lysine residues of an octomeric assembly. Based on an analysis of the crystal structures, we propose that the iron atom originates from the haem group and report subsequent biochemical experiments that demonstrate that HbpS possesses haem-degrading activity *in vitro*. Further examination of the crystal structures has identified amino acids that are essential for assembly of the octomer. The role of these residues is confirmed by biophysical experiments. Additionally, we show that while the octomeric assembly state of HbpS is not essential for haem-degrading activity, the assembly of HbpS is required for its interaction with the cognate sensor kinase, SenS. Homologs of HbpS and SenS/SenR have been identified in a number of medically and ecologically relevant bacterial species (including *Vibrio cholerae*, *Klebsiella pneumoniae*, *Corynebacterium diphtheriae*, *Arthrobacter aurescens* and *Pseudomonas putida*), suggesting the existence of a previously undescribed bacterial oxidative stress-response pathway common to Gram-negative and Gram-positive bacteria. Thus, the data presented provide the first insight into the function of a novel protein family and an example of an iron-mediated interaction between an accessory protein and its cognate two-component sensor kinase.

Keywords: coupled oxidation; haem; oxidative stress response; static light scattering; X-ray crystallography

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Introduction

Streptomycetes are highly differentiated soil bacteria that produce a wide repertoire of medically relevant antibiotic, antifungal and cytostatic compounds, as well as many extracellular hydrolytic enzymes and enzyme inhibitors.[1,2] The cellulose degrader *Streptomyces reticuli* was recently shown to secrete a novel haem-binding protein, HbpS,[3] and a comparative sequence analysis[4] demonstrated that HbpS shares a high level of sequence homology with a number of proteins of unknown function from the Gram-positive bacteria *Corynebacterium diphtheriae*,[5] *Streptomyces coelicolor* A3(2),[6] *Rhodococcus* sp. RHA1,[7] *Arthrobacter aurescens* TC1[8] as well as proteins from the pathogenic Gram-negative bacteria *Vibrio cholerae*,[9] *Pseudomonas putida*[10] and *Klebsiella pneumoniae*.[11]

The production of HbpS is highly enhanced *in vivo* during the cultivation of *S. reticuli* and *S. lividans* transformants in the presence of large amounts of haemin (the Fe^{3+} oxidized form of haem), and HbpS has been shown to directly bind haemin.[3] Haemin, a

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Abbreviations used: SLS, static light scattering; TCS, two-component system; NCS, non-crystallographic symmetry; WT, wild type.

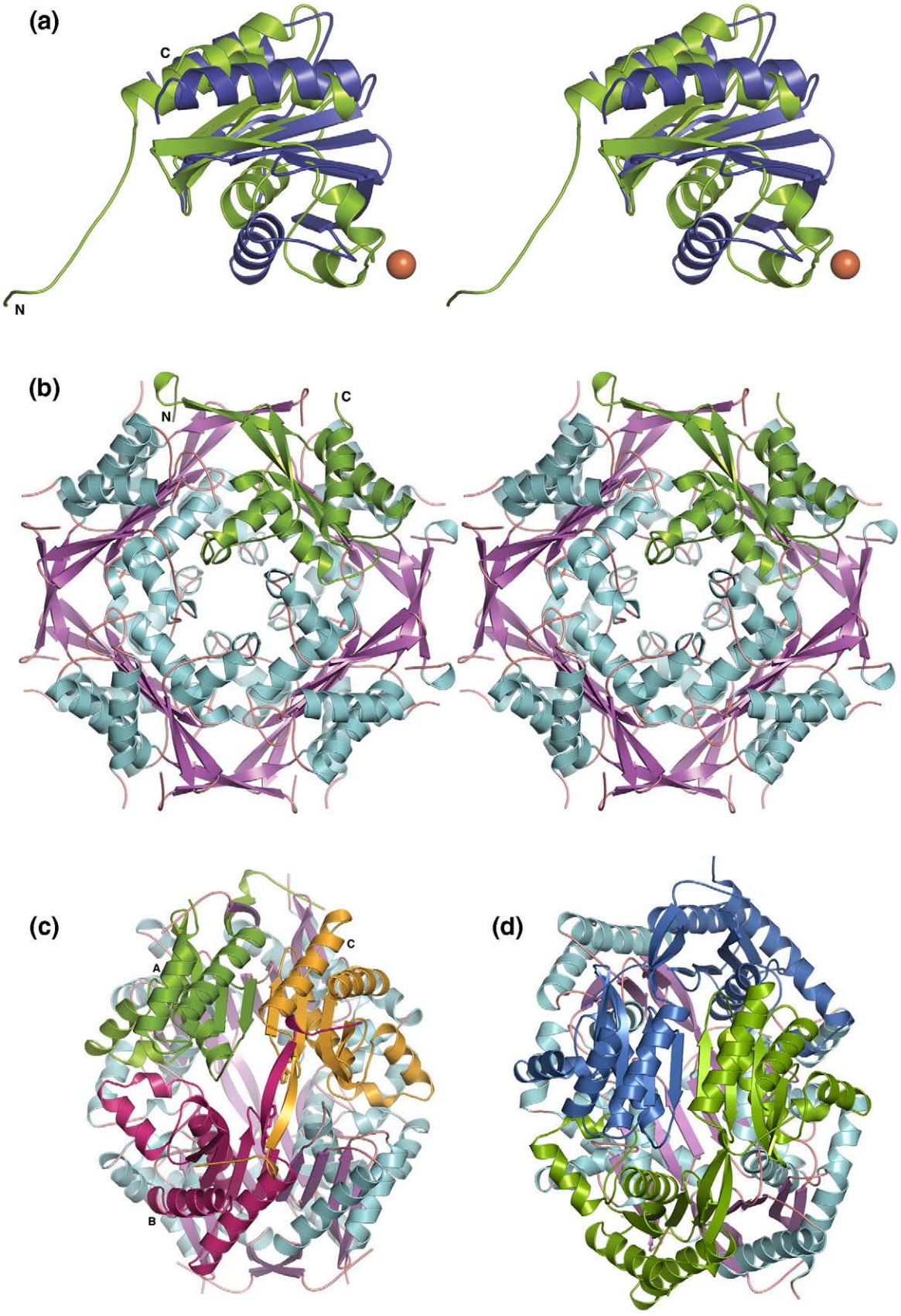

Fig. 1 (*legend on next page*)