Structural basis of heme-responsive sensor protein mediating the survival of hemolytic bacteria

Iron is an essential nutrient that plays a central role in many physiological processes for the survival of all living things. For example, in humans, after breathing in air, oxygen transport via the lungs to the peripheral tissues is facilitated by oxygen binding to the heme iron (iron-protoporphyrin IX) in hemoglobin in red blood cells. In some pathogens, heme is the major source of iron. These pathogens have evolved special pathways for acquiring heme from animal host blood [1] resulting in hemolysis, which is the destruction of red blood cells and the release of hemoglobin into the blood plasma (Fig. 1). Over a billion molecules of heme are released from red blood cells in animal hosts during hemolysis. These heme molecules are directly used as prosthetic groups for hemoproteins and are also catabolized by heme-degrading enzymes for pathogenic cells to obtain iron. However, excess heme accumulated in bacterial cells is highly cytotoxic owing to the reactive oxygen species that are generated. Therefore, cellular heme homeostasis is tightly controlled and as such, bacteria have evolved sophisticated regulatory systems. The failure of these systems leads to the death of pathogens, so understanding these systems may provide new leads to developing antimicrobial agents effective against globally distributed drug-resistant pathogens.

We investigated a newly discovered heme-responsive sensor protein, PefR, involved in the underlying regulatory mechanism in the hemolytic bacterium *Streptococcus agalactiae*, which causes life-threatening neonatal infections such as septicemia, pneumonia, and meningitis [2]. PefR acts as an intracellular heme sensor and a transcriptional factor to regulate the expression of the heme exporter. When only the heme required as an iron source is present, PefR is bound upstream of the heme exporter gene and heme is not exported from *S. agalactiae* cells, but during hemolysis in host blood, heme overflows into the cell, causing PefR to bind heme and dissociate from the gene. Consequently, the heme exporter is expressed, and the excess heme is exported from the cells to prevent cytotoxicity (Fig. 2). Thus, if PefR does not function, *S. agalactiae* cells are killed owing to the toxicity of the excess heme, making PefR an important protein for this bacterium. We clarified how PefR recognizes heme and how the heme binding event is translated into the signal for DNA dissociation [3].

We prepared a highly purified PefR protein and crystallized it in the apo (heme free form)-PefR–DNA complex and heme-bound PefR and collected their X-ray diffraction data at SPring-8 **BL26B2** and **BL41XU**. The structures were determined to a resolution of 2.5 Å for the DNA-bound protein and 1.7 Å for the heme-bound protein. The crystal structures reveal that the apo-PefR has a straddling structure and sandwiches the target DNA. When heme enters the apo-PefR–DNA complex, heme binds to the protein by adopting a six-coordinate structure involving the main chain nitrogen of Met1 and the side chain of His114 of each subunit in the dimer. When heme is bound, a hydrophobic core is formed by the amino acid side chains around it. The heme binding triggers an increase in the distance between the DNA-binding domains (that sandwich the DNA of PefR) between the heme-bound and heme-free PefR, preventing PefR from interacting with the target DNA. This is the structural basis by which PefR causes DNA dissociation upon heme binding (Fig. 3). Further biochemical analysis revealed that the heme-bound PefR, once dissociated from the target DNA could stably bind carbon monoxide (CO), and this CO-bound PefR could not rebind the target DNA in the cell.

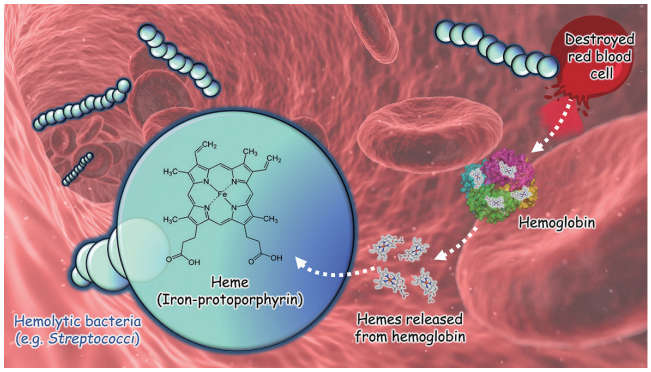

Fig. 1. Heme acquisition system in hemolytic bacteria.

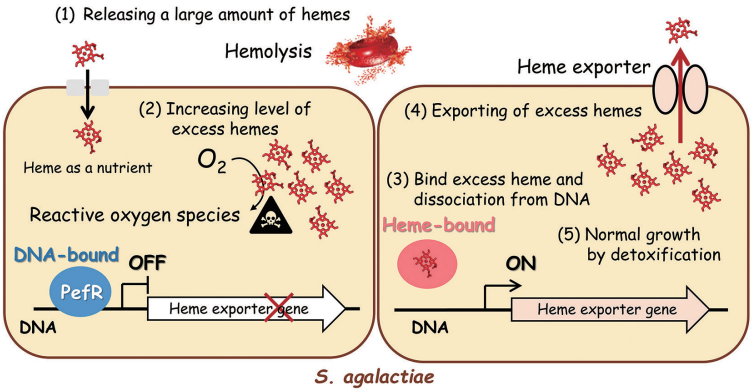

Fig. 2. Physiological function of the heme-responsive sensor protein PefR in *S. agalactiae*.

We have discovered that the heme-bound PefR have the ability to capture intracellular CO. The CO is released from host cells as a product of the heme degradation reaction, but when it flows into pathogenic cells, it shows cytotoxicity, causing cell death or dormancy [4]. The results of this study indicated that PefR is a multifunctional protein that acts as a transcriptional factor, a heme sensor, and possibly a CO scavenger involved in iron acquisition by hemolytic bacteria from animal hosts (Fig. 3) [3]. Inhibiting these detoxification mechanisms of hemolytic bacteria by using these PefR functions could be an important strategy for developing antimicrobial agents to address the global problem caused by drug-resistant pathogens.

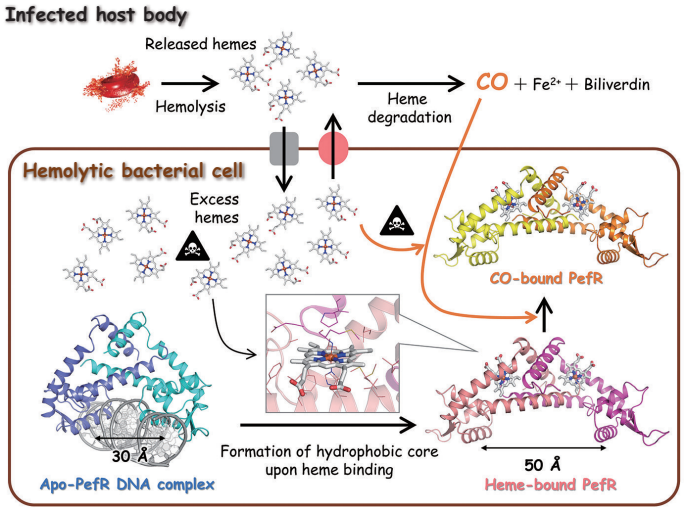

Fig. 3. Schematic diagram of the structure and function of PefR in *S. agalactiae*. A cell of the hemolytic bacterium is represented by the brown square. The area outside the square is the body of the infected host. Hemolytic bacterial cells survive by acquiring heme molecules from hemoglobin in red blood cells from their animal hosts. To avoid the cytotoxicity of excess heme during hemolysis, PefR acts as a transcriptional factor to regulate the heme efflux system in response to the cellular heme concentration. The result of crystallographic, spectroscopic, and biochemical studies indicate that the heme coordination to DNA-bound PefR controls the structural rearrangement of the DNA-binding domains to dissociate PefR from the target DNA. After dissociating from the target DNA, the heme of holo-PefR can stably bind CO, which is a by-product of heme degradation by heme oxygenase.

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