

Structural Studies of the Stress Response Regulator PerR from *Streptococcus pyogenes* by X-ray Crystallography

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Streptococcus pyogenes is a significant human pathogen that causes versatile diseases. Stress response plays an important role in *Streptococcus pyogenes* because this bacterium does not produce catalase, an oxidoreductase, which can repair damage to bacteria when grown in an aerobic environment. Instead, *S. pyogenes* depends upon an iron-binding protein, Dpr, to confer the resistance to multiple stresses.

The expression of Dpr is induced when the concentrations of iron, zinc, nickel, and hydrogen peroxide increase. It has been shown that the expression of Dpr is negatively regulated by PerR. Increased concentrations of iron and hydrogen peroxide decrease PerR binding to promoter region of *dpr*. This observation suggests that the regulation of Dpr by environmental signals is mediated by PerR directly. Our hypothesis is that PerR may undergo conformational change upon the iron-binding or other metals that leads to the loss of its DNA-binding ability and released from *dpr* promoter. To test this hypothesis, we intend to determine the structures of both iron-free and iron-bound PerR proteins, by comparing and contrasting them, to understand the molecular mechanism how PerR regulates the expression of Dpr.

We are the new users at the NSRRC and have only collected data at beamline 13B1 once up to date when this report is written. The structure of the iron-free PerR protein has been previously determined to 2 Å resolution before the beamtime is allocated at NSRRC. In this beamtime cycle (2009-3) allocated at 13B1 at NSRRC, we have collected a complete native dataset of PerR crystal to 1.5 Å resolution (Fig. 1), which is the highest resolution of the diffraction data of PerR we obtained so far. We have used this data for the final structural refinement and it is still in progress. We were also intending to collect the diffraction data of the iron-bound PerR crystals and to solve the structure by Zn-MAD because the PerR protein contains intrinsic zinc ions. We prepared 22 iron-bound PerR crystals by soaking the native PerR crystals in various concentrations of ferrous sulfate solution. Unfortunately, none of the PerR crystals prepared this way diffracts well. In the future, we plan to solve the iron-bound PerR structure as well as the structure of PerR-DNA complex. We have been able to prepare the PerR-DNA protein complex and now the sample is screening for crystallization.

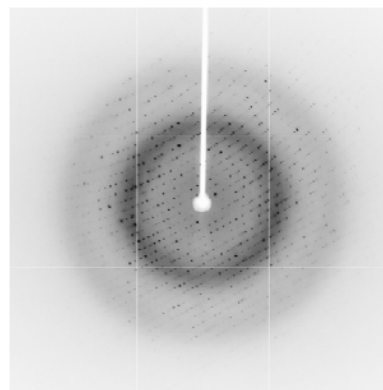


Fig. 1: The diffraction pattern of the native PerR crystal to 1.5 Å resolution.