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Structure of transcription factors responsible for Pseudomonas aeruginosa pathogenicity

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Introduction

In bacteria, transcription regulation factors work, for example, as many as 300 in *E. coli,* depending upon the cell growth phase and the living environment by regulating transcription of its target gene(s). The transcription factors contribute to a complicated network of regulation of transcription activity in a cell together with various RNA polymerase +-subunits.

We have tried to do a sweeping structural determination of E. coli transcription factors by x-ray crystallography, combining the recent progress in x-ray structure analysis tools and a recent collection of over 65 purified E. coli transcription factors. The study aims to reveal the over-all picture of transcription factors in structural terms. However, we encountered difficulties in this approach. The problem was their inability to crystallize. This may be probably due to their structural flexibility or unsatisfactory quality of preparations obtained by a single step Ni-NTA column chromatography.

As a move to a differerent line of study, we have recently included in the structural study transcription factors (PtxR, PtxS, PhzR, and PA3547) that are responsible for the pathogenicity of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* has nearly twice as many transcription regulation factors as *E. coli* in spite of similar genome sizes, which benefits the bacterium living in environments varying from wild water to human body. The well known opportunistic pathogenecity of *Pseudomonas aeruginosa* may come out of this background. Understanding of functional mechanisms of these pathogen-related transcription factors in terms of structure may lead to a better guard against the pathogens.

We have started cloning, expressing, and crystallization studies of these *Pseudomonas aeruginosa* transcription factors.

Results

An Over expression system was constructed by incorporating the provided expression plasmids, which were designed to have 6 His-tags at The C-terminus, into host cells (BL21 (DE3), B834 (DE3), JM109 (DE3)) and by examining which conditions, characterized by temperature (37°C, 25°C) and inducer presence (plus, minus), produce the target protein in a soluble fraction. Fortunately good conditions were found for all the proteins. Three proteins out of the four were purified by the Ni-NTA column and an extra column, of which resin was chosen empirically depending upon the protein. PhzR was an exception, ands was purified with Q-sepharose high performance, Butyl-toyopearl and hydroxyapatite columns, because the protein did not bind to a Ni-NTA column. A gel filtration analysis and the subsequent x-ray crystal analysis showed that the PhzR molecules form a tetramer, suggesting that the His-tag attached C-terminus of the molecule may be buried in the tetramer thus explaining no attachment of PhzR to Ni-NTA.

The preparations were then subjected to crystallization study. All proteins gave signs of crystals in the screening stage, however, the diffraction grade crystals were obtained only from PhzR so far. PhzR crystals were obtained from polyethylene glycol at pH 4.7, and with a typical size of 0.2 x 0.2 x 0.05 mm. The previous MAD data collected from HgCl2 derivative elsewhere were not good enough for structure determination. In the experiment at BL5A, Mosflm strategy option was successfully applied, which, we believe, allowed much better data (less prone to radiation damage) than before to be collected. MAD data were collected from HgCl2 derivative. The effective resolution of the data set was about 3.5 A resolution. The native data for PhrzR were also collected. Those data sets were processed with MOSFLM and CCP4 program suite. The unit cell is a=74.4A b=242.9 A c=129.3 A (space group C222), and its asymmetric unit contains 4 molecules of PhzR. MAD analysis using programs SOLVE and Sharp revealed a novel mode of tetramer assembly of the PhzR, and showed an electron density map which showed clear helical densities in some part.

After the diffraction experiment, we had a chance to check the origin of the expressed PhzR. This was led by a lesson from a failing expression experiment of a plant cell-death related protein SAG12 (molecular mass of about 30k) where SAG12 was not expressed but lactamase from a vector was instead expressed. C terminus amino acid sequence of PhzR (26K) expressed in our hands was examined. The sequence corresponded to that of C terminus residues of lactamase from Staphylococcus aureus, not to that of PhzR. There is a strong suspect that our PhzR crystals were not genuine. Diffraction data have been re-examined in this respect.

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