Holo-ZitRMG structure determination by x-ray crystallography

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MATERIALS TEXT

Holo-ZitR_{MG} protein was subjected to crystallization assays using spare matrix screens in a robotic system (Nano-Robot Cartesian) (crystallography platform, LEBS/IMAGIF). In preliminary experiments crystals were first obtained using ZitR_{MG} tetrameric form by vapor diffusion in 0.2 M ammonium acetate, 0.1 M sodium citrate tribasic dihydrate (pH 5.6) and 30 % polyethyleneglycol 4,000. They diffracted to a resolution of less than 6 Å. These crystals belong to $P4_1$ space group with cell parameters of $a = b = 129$ Å; $c = 88$ Å; a = b = g = 90º. Crystallization conditions were then optimized using the dimeric form. New X-ray data were collected at the zinc peak absorption edge up to 3.7 Å resolution. Because molecular replacement with different MarR protein coordinates did not work, after data processing using XDS. we used Zn anomalous signal to solve the structure by SAD method using PHASER. Eight molecules were found in the asymmetric unit, with a solvent content of 60 %, and two zinc atoms were found per protomer. Phases were improved and extended by NCS averaging and solvent flattening using PARROT. Refinement was done using BUSTER-TNT. To help building this low-resolution model, *B. subtilus* OhrR structure was used. From a poly-Ala chain, we could identify the extended wHTH DNA-binding domain, but most of the lateral chains were not visible. New data collection recorded on a Pilatus 6M detector on the PROXIMA1 beamline at SOLEIL synchrotron went up to 2.8 Å resolution. An anisotropic correction factor was applied STARANISO server (http://staraniso.globalphasing.org/) to sharpen the data in the weaker-diffracting direction. Structure was determined by molecular replacement using AdcR (3TGN) as model. The high values of average B-factors of the final structure can be explained by the crystal anisotropy. All structure figures are created using The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.