Single-Wavelength Anomalous Diffraction Phasing to Determine the Structure of the *E. coli* DNA Polymerase II

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Introduction

The timely and accurate repair and replication of genomic DNA is essential for any organism. To carry out these processes, an organism typically has a number of different DNA polymerase enzymes that play different roles. The bacterium *E. coli* has five DNA polymerases. One of them, DNA polymerase II is a class B polymerase related to the human α, δ and ε polymerases. This polymerase is a multidomain protein with two active sites. One catalyzing exonucleolytic degradation of DNA and the other polymerization.

Materials and Methods

The *E. coli* DNA polymerase II I428V mutant was prepared and crystallized as previously described for the wild type enzyme.[1] The mercury derivative was prepared by soaking a crystal in mercury-bis-2-mercaptoacetate for 25 minutes prior to mounting. The xenon derivative was prepared by incubating a mounted crystal for 10 minutes at 230 psi xenon gas using a MSC Cryo-Xe-siter. Diffraction data were collected at 100°K using radiation with a wavelength of 1.00 Å for the mercury derivative and a wavelength of 1.77 Å for the xenon derivative on the 5-ID-B station of DND-CAT at the Advanced Photon Source using a Mar165 CCD detector. The data were integrated and merged with DENZO/SCALEPACK.

Results and Discussion

Because it was previously observed that different variants (site-directed mutants) of Pol II crystallize in the same $P2_12_12$ lattice with similar cell dimensions but with large variation in crystalline order and diffraction limit, another variant, a mutant having isoleucine 428 replaced by a valine, was examined. Data were collected at the Advanced Photon Source in the DuPont-Northwestern-Dow sector using a MarCCD detector. Single wavelength anomalous diffraction data were collected for the Hg (R_{merge}=0.070 to 2.2 Å resolution) and Xe (R_{merge}=0.079 to 2.3 Å resolution) derivatives. These derivatives for the I428V variant diffracted better than any earlier derivatives and allowed collection of higher resolution data. Because anomalous data were used for phasing and isomorphous differences were only used at low resolution, the phases that were obtained were not degraded by nonisomorphism and yielded a vastly improved electron density map.[2]

The greatly improved resolution and quality of the experimental phases have aided interpretation of the electron density maps.

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References

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