Use of Sulfur Anomalous Diffraction to Locate the Cysteines and Methionines of E. coli DNA Polymerase II

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Introduction

The E. coli DNA polymerase II is a class B polymerase related to the human α, δ, and ε polymerases. Interpretation of initial electron density maps was problematic due to the poor quality of the initial phase estimates. Because the registration of the initial polyalanine model with the 783 amino acid residue sequence was questionable in some regions, an independent experimental check was desired. Anomalous diffraction data were collected from the native enzyme to locate the sulfurs of cysteine and methionine residues.

Materials and Methods

The E. coli DNA polymerase II I428V mutant was prepared and crystallized as previously described.1 Diffraction data were collected by using radiation with a wavelength of 1.77 Å at 100°K to 2.35 Å resolution on the 5-ID-B station of the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) at the Advanced Photon Source with a Mar165 CCD detector. The data were integrated and merged with DENZO/SCALEPACK.

Results and Discussion

In combination with a new, higher resolution, experimentally phased electron density map, model building was aided by analysis of the native anomalous scattering due to the sulfur atoms contained in the 7 cysteine and 17 methionine residues in the 783 residue protein. Although the sulfur anomalous signal is less than 0.5 electron per sulfur at this wavelength, the anomalous difference electron density map revealed the positions of many of the sulfurs in the enzyme and allowed definition of the correct registration of the amino acid sequence with the structure. The present model has a free R of 29% to 2.2 Å resolution and includes 743 of the 783 residues and 198 water molecules.

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References