Structural analysis of dehydroascorbate reductase from spinach chloroplast

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Dehydroascorbate reductase (DHAR: EC 1.8.5.1) catalyzes the reduction of DHA to ascorbate using GSH as the electron donor. Recently, we isolated the most specific DHAR in the history of its study from spinach chloroplasts. It is considered to play a pivotal role to regenerate ascorbate which was oxidized in large quantities to scavenge active oxygen species generated in the process of photosynthesis. The specificity constants for DHA and GSH of the DHAR were approximately 40 and 35-fold higher than those of pig liver thioltransferase which is the most studied protein having DHAR activity, respectively. It is very intriguing how spinach chloroplast DHAR establishes its high specificities. The three-dimensional structure of the DHAR will answer the question through comparing its active site structure with pig liver thioltransferase's one.

First, for the phasing and the structure determination, multiwavelength anomalous diffraction data (peak, edge, remote 1 and remote 2) were collected for Hg-derivative crystal. The diffraction beyond 2.6 Å resolution was observed. However, the spots was not so clear. This may result from that the defects of the crystal which could not have found in our laboratory system was disclosed by the very low divergency beam of SPring-8. Anyway, the data reduction is now under way.

Secondly, a native crystal was used for the high-resolution data collection. Although the X-ray diffraction of the crystal had been only to 2.7 Å resolution in our laboratory system, it was dramatically improved up to 2.15 Å resolution using BL41XU in the present study (Table 1).

Table 1. Data collection statistics for native crystal of spinach chloroplast DHAR.

Beam source	BL41XU, SPring-8
Wavelength	0.7085 Å
Space group	*C*2
Cell dimensions	a = 98.25, b = 39.96, c = 106.81 Å, β = 110.29°
Resolution range	50 – 2.15 Å
Measured reflections	145,921
Unique reflections	19,715
I / σ	11.1
Completeness (outer shell)	97.3 (90.5)%
R_{merge} (outer shell)	6.4 (25.5)%

CRYSTALLOGRAPHIC STUDY OF THE PROTEIN COMPLEX OF HUMAN FEN-1 AND PCNA

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Introduction

DNA replication in eukaryotes is a highly coordinated process, and it is well known that many proteins work cooperatively to ensure accurate and efficient DNA replication. In such a system, flap endonuclease-1 (FEN-1) plays a crucial role in the removal of RNA primers during Okazaki fragment maturation in lagging strand DNA synthesis, and proliferating cell nuclear antigen (PCNA) stimulates FEN-1 nuclease activity by 10-50 fold through direct interaction. The human FEN-1 is a structure specific-nuclease with 5' flap DNA endonuclease, and also has double-stranded DNA 5'-exonuclease activities. The human PCNA, the processivity factor required by eukaryotic DNA polymerase δ, is otherwise known as the 'sliding clamp'. The crystal structures of both of FEN-1 and PCNA have been individually determined, and described apropos of the folding motif and the molecular assembly, but several important points, such as a detailed view of the interactive sites between FEN-1 and PCNA, remain unclear. Therefore, the three-dimensional structure of the protein complex is needed to clarify the mechanisms of the intermolecular interactions and to understand the DNA replication process at an atomic level.

To this end, we established a modified purification protocol of the recombinant human FEN-1 and PCNA, and crystallized the FEN-1-PCNA complex.

Crystallization and Data collection

Extensive crystallization screening was carried out by the hanging-drop vapor-diffusion method using commercial crystallization screening solutions at 283 K. The protein solution was mixed in a 1:1 ratio with the reservoir solution. The crystals obtained were dissolved and analysed by 12.5%(*w/v*) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Clusters of plates appeared in a week with maximum dimensions of 400 x 400 x 20 mm. X-ray diffraction data for the FEN-1-PCNA complex crystal were collected at SPring-8, using a CCD detector system. Before data collection, the crystals were flash-cooled at 100 K and mounted in a rayon-loop. The crystals diffracted X-rays to at least 3.0 Å resolution, and belonged to the space group $P2_12_12_1$, with unit-cell parameters a = 82.1 (1), b = 142.7 (2), c = 247.2 (2) A. A complete data set was collected to 3.5 Å resolution from a single crystal. Structure determination by the molecular replacement method is in progress.