Crystallographic Study of the Proteins Related to DNA Repair

Naoki KUNISHIMA (0003650), Kazuhiro YAMADA (0003657), *Kosuke MORIKAWA (0003651)

Biomolecular Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan

Homologous recombination is an essential process in all organisms for the repair of damaged DNA and for the generation of genetic diversity. It is thought to be initiated by a single-stranded DNA (ssDNA) molecule invading a homologous region of double-stranded DNA (dsDNA). In this process, RecA protein, which binds to ssDNA, promotes homologous pairing and strand exchange. When the strand exchange reaction proceeds into a double-stranded region, the two duplex DNA molecules are linked at a single-stranded crossover, which is called a Holliday junction. In *Escherichia coli*, RuvA, RuvB, and RuvC proteins process these Holliday intermediates into mature recombination products. RuvA and RuvB proteins act together to provide a junction-specific DNA helicase activity that promotes branch migration of the Holliday junction. In electron microscopic images, *E. coli* RuvB was observed as a hexameric ring, like several other DNA helicases, including *E. coli* DnaB protein, SV40 large T-antigen, T4 phage gp41 and T7 phage gp4 helicases. The hexameric ring of RuvB encircles dsDNA molecules. RuvB possesses a Mg^{2+} dependent ATPase activity that is greatly enhanced by RuvA and dsDNA, and its intrinsic DNA helicase activity is also enhanced by RuvA.

The native crystals were obtained by the hanging-drop vapor diffusion method using sodium chloride as a precipitant. The crystals belong to the space group $P4_32_12$ with a cell dimension of $a = b$ = 84.9 Å, c = 355.2 Å. The two molecules were present with a pseudo non-crystallographic dyad axis in an asymmetric unit. A selenomethionine derivative crystals were obtained by the same procedure as the native crystals except for using selenomethionyl RuvB. The mercury derivative crystals were prepared by co-crystallization method with 0.5 mM thimerosal for 5 days. The platinum derivative crystals were prepared by soaking the native crystals in a mother liquor containing 10 mM K_2PtCl_4 for 30 min. The data collection was performed at 100 K using the R-AXIS IV detector. A typical size of the crystals used for data collection was 300 μm × 300 μm × 600 μm. The raw data were digitized and merged using the programs *DENZO* and *SCALEPACK*. The initial phase was obtained according to the method of multiple isomorphous replacement (MIR), through a procedure including a determination of heavy atom sites with the program RSPS and the subsequent refinement with the program of *MLPHARE* and *SHARP*. The overall figure-of-merit for all reflection between the resolution of 12 Å and 3.4 Å was 0.305. The MIR phase was then improved by several density modification techniques with the program *DM*; which contained solvent flattening, histogram matching and 2-fold molecular averaging. The model building is now in progress using the density-modified map.