Inventory of Supplemental Information

Supplemental Experimental Methods

Expanded methods.

Supplementary Information

Structures of minimal catalytic fragments of topoisomerase V reveals conformational changes relevant for DNA binding

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Supplemental Experimental Methods

Crystallization

Topo-31 crystals were grown by sitting drop vapor diffusion method by mixing 2 µl of protein (4.5 mg/ml) and 2 µl of reservoir solution (23% PEG 6000, 0.1 M Na citrate pH 5.5) at 22°C. For data collection, the Topo-31 crystals were cryo-protected by adding glycerol to the mother liquor to a final 20% concentration. Topo-44 was crystallized by hanging drop vapor diffusion method under three different crystallization conditions (Forms I, II, and III). Crystal Form I was obtained by mixing 3 µl of protein (4.5 mg/ml) with 3 µl of reservoir solution (0.1 M phosphate citrate pH 5, 0.2 M NaCl, 15% PEG 3350 and 8% dioxane). Form II crystals grew by mixing 2 µl of protein (4 mg/ml) with 2 µl of reservoir solution (0.1 M phosphate citrate pH 5, 0.2 M NaCl, 16% PEG 8000 and 1M guanidium hydrochloride). Form III crystals were grown by mixing 2 µl of protein (4 mg/ml) with 2 µl of reservoir solution (0.1 M phosphate citrate pH 5.5, 0.15 M sodium sulfate, 0.01 M $MgCl_2$, 1 M guanidium hydrochloride, and 28 % PEG 3350). Streak seeding (Stura and Wilson, 1991) was employed to assist the growth of Form II and III crystals. Form II crystals grew as long thin plates which show multiple lattices in the diffraction pattern. The crystals were improved by screening different crystal growing temperatures, (4°C, 10°C, and 30°C). The best diffracting crystals were obtained when the crystal tray was left at 4°C for a day and then transferred to 10°C. The crystals appeared slowly and grew thicker over 5-7 days (0.3 x 0.2 x 0.05 mm^3). A number of cryo-conditions were screened to improve the diffraction pattern and the best cryo-protectants turned out to be 20% 2, 3 butanediol and 20% DMSO. For data collection for the Form I crystals, PEG 3350 concentration in the mother liquor was increased to 30% in incremental steps of 5% with 30 seconds incubations between each transfer. Form II crystals were transferred to a solution with 1.5X reservoir solution and 20% 2,3

butanediol or 20% DMSO for 10 seconds and immediately flash frozen under liquid nitrogen. Form III crystals were grown at 30°C and were frozen using 40% PEG 3350 as cryo-protectant.

Data collection and structure determination

Diffraction data were collected at the Dupont Northwestern Dow and Life Science Collaborative Access Team stations (DND and LS CAT) at the Advanced Photon Source in Argonne National Laboratory. Data collection and refinement statistics are shown in Table I. Topo-31 crystals are orthorhombic with one molecule in the asymmetric unit. Topo-44 Form I crystals are monoclinic with one molecule in the asymmetric unit. Topo-44 Form II crystals are tetragonal and Topo-44 Form III crystals are orthorhombic, and both forms II and III have two monomers per asymmetric unit. All data were processed and integrated using XDS (Kabsch, 1993) and scaled with SCALA (Collaborative-Computational-Project-4, 1994).

Data on the Topo-31 crystals were collected to 2.4 Å resolution. The structure was solved by Molecular Replacement (McCoy et al., 2007) using the topoisomerase domain from the Topo-61 structure (residues 1-266) (Taneja et al., 2006) as the search model. It was refined with refmac5 (Murshudov et al., 1997) and Phenix (Afonine et al., 2005) to a final R_{work} of 20.0 % and R_{free} of 24.8%. Topo-44 Form I crystals diffract to 1.8 Å. The structure of Form I crystals was solved by Molecular Replacement (McCoy et al., 2007) using the topoisomerase domain from the Topo-61 structure as the search model. The $(HhH)_2$ domains were placed manually into the density, followed by model rebuilding with coot (Emsley and Cowtan, 2004), and refinement using refmac5 (Murshudov et al., 1997). There is very clear electron density for the C-terminus of the molecule, including the C-terminal oxygen atom.

Molecular Replacement did not produce a solution for Topo-44 Form II and Form III crystals and hence seleno-methionine derivatized crystals were used for single-wavelength

anomalous dispersion (SAD) phasing. For SAD, data were collected at the peak energy of the absorption spectrum of selenium (12.662 keV). The native crystals diffract to 2.6 Å and have one long axis (349.6 Å) and hence data were collected using a 0.1º rotation of the spindle axis in order to avoid spot overlap. AutoSharp (Vonrhein et al., 2007) located eight of the twelve selenium atoms corresponding to residues 40, 155, 166, and 286 in each monomer. The topoisomerase domain from the Topo-61 structure was placed on the experimental electron density using the positions of the selenium atoms as a guide. After refining the topoisomerase domain for both subunits, the $(HhH)_2$ domains from the Topo-61 structure were placed manually into the density. The structure was improved by several rounds of model building in coot (Emsley and Cowtan, 2004) and restrained refinement using refmac5 (Murshudov et al., 1997) using medium NCS restraints between the two monomers. Towards the later stages, TLS refinement (Winn et al., 2003) was included using two separate domains per monomer, the topoisomerase domain and the $(HhH)_2$ domains. Three phosphate ions were noticed in the Form II structure; two are present in the topoisomerase active site and are separated by a distance of ~7.5 Å, while the third phosphate is at the C-terminus near the disulfide bond between Cys314 and Cys338. Topo-44 Form III crystals diffracted to 1.4 Å. AutoSharp (Vonrhein et al., 2007) located all five selenium sites (except the N-terminal methionine) for each monomer. The Sharp/Solomon (Abrahams and Leslie, 1996) solvent flattened map was of high quality and the program arp/warp (Perrakis et al., 1999) was able to trace the A monomer. The B monomer was built by applying the NCS operator relating the two monomers to the A subunit. Model building was done using coot (Emsley and Cowtan, 2004) and refinement was carried out with refmac5 (Murshudov et al., 1997) using medium NCS restraints between the two monomers. TLS (Winn et al., 2003) restrained refinement of the topoisomerase domain and $(HhH)_2$ domains was included during the later stages of the refinement process. An interesting observation is the presence of both phosphate ions and guanidium ions in the Form III Topo-44

structure. There are three phosphate ions each in the A and B monomers and one in between both of the monomers. There are putative Mg^{2+} and Cl^- ions in the Form III structure and they were modeled based on the location of large positive density peaks. The placement of water molecules instead of the ions gave extra positive density. The linker helix and part of the first HhH motif of the B monomer show alternate conformations and were built as two separate chains with occupancy of 0.5 each. All figures were made with Pymol (DeLano, 2002) and the electrostatic surfaces were calculated with APBS (Baker et al., 2001).

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