A unique zinc-binding site revealed by the high-resolution x-ray structure of homotrimeric Apo2L/TRAIL

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Introduction

Apoptosis-inducing ligand 2 (Apo2L, also called TRAIL), a member of the tumor necrosis factor (TNF) family, induces apoptosis in a variety of human tumor cell lines but not in normal cells [1,2]. Apo2L/TRAIL is known to interact with two signaling or "death" receptors (DR4, DR5) [3-5] and three nonsignaling or "decoy" receptors (DcR1, DcR2, OPG) [3-6]. The resistance of normal cells to the apoptosis-inducing effects of Apo2L/TRAIL may be explained in part by expression of the decoy receptors on normal cells [7]. Administration of soluble Apo2L/TRAIL to mice bearing human tumors reduces tumor size with no discernible toxicity to normal tissues [8,9]. Thus, Apo2L/TRAIL has potential as an anticancer agent in humans. Here we describe the structure of Apo2L/TRAIL at 1.3 Å resolution using data measured at Structural Biology Center beamline 19-ID at the Advanced Photon Source (APS).

Methods and Materials

Crystals of Apo2L/TRAIL mutant Asp 218 Ala (residues 91-281 with Asp 218 mutated to alanine) grew at 4°C in 14 µL sitting drops containing 4 µL of 4% MPD and 10 µL protein (1.7 mg/mL in 20 mM Tris HCl, pH 7.5) over a well solution of 32% MPD. The space group was R32 with one monomer per asymmetric unit and cell parameters of a = 66.4 Å, c = 197.7 Å, and the crystals diffracted to a resolution of 1.3 Å at -180°C with synchrotron radiation. A 1.3 Å data set was measured at the Structural Biology Center at Argonne National Labs and processed with HKL, giving R_{merge} = 6.4% (34% in the 1.35–1.30 Å shell), 100% completeness, 12-fold redundancy, and $\langle I/\sigma I \rangle$ = 12.4. The wild-type Apo2L/TRAIL structure was solved using a model of TNF (based on Protein Database entry 1TNF) and was initially refined against 3.9 Å resolution data collected in-house. The Asp 218 Ala Apo2L/TRAIL mutant was partially refined against an initial 1.9 Å data set and subsequently against the 1.3 Å data set using REFMAC [10] and SHELXL [11]. During refinement, a 28σ peak of difference electron density was observed on the three-fold axis near Cys 230 and modeled as a zinc ion; its B-factor refined to 11.8 $Å^2$. A fourth zinc ligand was identified on the trimer axis and refined as chloride (B = 14.3 $Å^2$). The final R and R-free are 14.1% and 19.7%, respectively, and the model has good stereochemistry, with rms differences in bond lengths and angles of 0.013 Å and 2.4°. The model consists of residues 120–130, 142–194, and 197–281 with 226 water molecules, one zinc ion, and one chloride ion. The coordinates for the Apo2L/TRAIL Asp 218 Ala structure have been deposited with the Protein Data Bank (access code 1DG6).

Results and Discussion

The high-resolution structure of Apo2L/TRAIL mutant Asp 218 Ala was solved by molecular replacement using a 3.9 Å structure of the wild-type protein [12]. Overall, the 1.3 Å structure of Apo2L/TRAIL reveals that, as expected from a low resolution structure [13] and from sequence alignments, the core β strands are well conserved with respect to TNF [14]. A novel and unexpected finding was the presence of a zinc binding site at the tip of the trimer (Figure 1). Further mutational and biophysical studies demonstrated that this zinc binding site is required for maintaining native Apo2L/TRAIL structure and optimal bioactivity [12]. The mutagenesis studies, in conjunction with the crystal structure, also predicted that, like TNF, the receptor binding site is located at the interface between monomers and appears to be composed of two discrete patches of residues. The larger patch is centered on Gln 205 and the smaller patch on Tyr 216. The 1.3 Å crystal structure of Apo2L/TRAIL was subsequently used to phase data of a complex between Apo2L/TRAIL and the extracellular portion of one of its signaling receptors, death receptor-5 (DR-5) [15]. This structure, in conjunction with an independently determined structure of Apo2L/TRAIL and DR5, confirmed the location and nature of the receptor binding site [15,16].

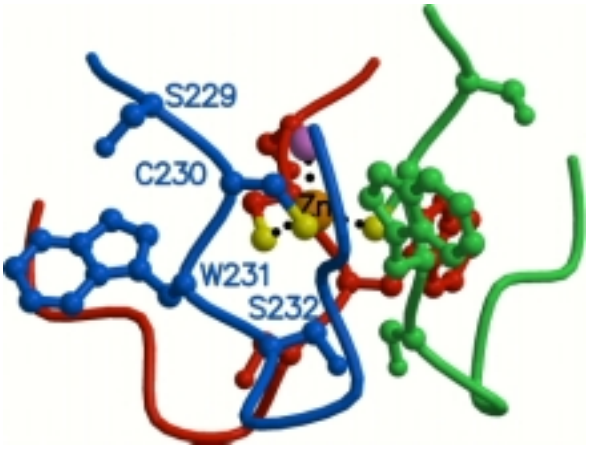

Figure 1: The Apo2L/TRAIL zinc binding site. The three Apo2L/TRAIL monomers are colored blue, red, and green. The side chains are shown in ball-and-stick rendering. The sulfur atoms are colored yellow, the zinc is orange, and the chloride is pink.

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