**Len_Y27dD**

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**Introduction**

The mutation Y27dD in Len was found to be stabilizing by about 2.7 kcal/mole. This mutation also introduces a negatively charged residue close to the dimer interface. Thus, the structure is of interest from two perspectives: 1) to understand the structural basis for stability and 2) to see if this protein forms a flipped dimer.

**Methods and Materials**

Len_Y27dD was crystallized from 30% PEG 4K and 0.2 M ammonium sulfate. X-ray diffraction data were collected to a resolution of 1.3 Å at the Structural Biology Center's (SBC-CAT) beamline 19-ID at the Advanced Photon Source (APS).

**Results**

The crystal belonged to space group P6_1 2_2 with unit cell dimensions of a = b = 91.7 Å and c = 66.2 Å. Based on one monomer per asymmetric unit V_M = 3.2. The structure was solved by the molecular replacement method using the native Len structure [Protein Data Bank (PDB) code 1LVE] as the search model. After refinement with CNS and model rebuilding with CHAIN, the current R-factor is 29.5% and R-free is 32.8% for 8.0–1.6 Å data.

Len_Y27dD forms a conventional dimer like the native Len. In the crystal, the dimer's two-fold axis coincides with a crystallographic two-fold axis. The electron density for the monomer is of very good quality. However, the refinement appears to be stuck at high R-factors. Although the space group and structure seem to be correct, the high R-factors may be due to unidentified electron density present in a large channel along the six-fold axis created by crystal packing. This cavity is not large enough for another monomer (if two monomers are present then the V_M would be too low at 1.6). At this time it is not clear whether this density could represent frozen solvent (PEG and water) or something else. A similar channel was found in the native Len structure (PDB code 2LVE), which was refined at a relatively low resolution of 2.7 Å. In the case of Len, there was no electron density in the channel.

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