

Len_M4L/Y27dD/Q89D/T94H

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Introduction

Aspartic acid, being a polar residue, is expected in general to be acceptable anywhere on a protein surface. However, statistical analysis of the distribution of Asp residues in protein structures has shown that they are substantially under represented in β -strand regions both in the middle and ends of the β -strand segments [1]. We have observed that the stability of immunoglobulin light chain variable domain (V_L), Len, decreases significantly when glutamines 38 and 89 are replaced by aspartic acid residues. The Q89D mutant of Len is so destabilizing that no protein expression was observed. Thus, the Q89D mutation was made in the background of a stable triple mutant [2] of Len (Len_M4L/Y27dD/T94H).

Methods and Materials

Len_M4L/Y27dD/Q89D/T94H was prepared as reported earlier [3] and crystallized by the hanging-drop vapor-diffusion method. X-ray diffraction data were collected to a resolution of 1.6 Å at the Structural Biology Center's (SBC-CAT) 19-ID beamline at the Advanced Photon Source (APS).

Results

The Len_M4L/Y27dD/Q89D/T94H was crystallized from 20% PEG4000, 20% 2-propanol, and 0.1 M HEPES (pH = 7.5). The crystals contained one V_L dimer per asymmetric unit with unit cell dimensions of $a = 62.9$ Å, $b = 104.8$ Å, and $c = 42.4$ Å in space group $P2_12_12$. This crystal form was isomorphous to that of the triple-mutant Len_M4L/Y27dD/T94H determined in our laboratory [2]. The structure was determined by rigid-body refinement of the triple-mutant structure (with atoms beyond CB removed for residue 89 of both monomers) against the data of Len_M4L/Y27dD/Q89D/T94H. After further refinement with CNS and intermittent model building with CHAIN, the final structure had an R-factor of 19.5% and R-free of 22.2% for 8.0–1.6 Å data with 336 water molecules included. The Ramachandran plot contained 90% of the residues in most-favored regions. The coordinates are deposited in Protein Data Bank (PDB) under code 1EEU.

The electron density for the Asp89 residue is well defined in both monomers.

Len_M4L/Y27dD/Q89D/T94H has a slightly different dimer structure compared to that of Len_M4L/Y27dD/T94H. When α -carbons from one of the domains of the two structures were superimposed, a rotation

of 0.7° and a translation of 0.5 Å were required to superimpose the second domains. The major change caused by the Asp89 mutation was a rotation in the phenyl ring of residue 398 across the dimer interface compared to the same in the triple mutant. The solvent structure is also different at the site of Q89D mutation where two 2-propanol molecules were located in the dimer interface.

Discussion

The residues 89 and 90 in a V_L form last two residues of β -strand F, which is part of the C''C'CFG β -sheet. The Q89D mutation when modeled in native Len structure (PDB code 1LVE) using the program CHAIN was found to have severe short contacts with other main-chain or side-chain atoms in any of the three standard rotomers of the Asp side chain. It was thought that an Asp residue at this position would not be accommodated without some significant structural changes in the neighborhood of this residue, which would explain the apparent loss of stability. However, in the structure of Len_M4L/Y27dD/Q89D/T94H, very small differences in the atomic positions were observed compared to Len_M4L/Y27dD/T94H. The main chain has slightly different ψ, ϕ angles for residues 89, 90, 97, and 98, and the side-chain conformation of the Phe98 residue in the interface has changed. Thus, at present it is not clear why the Q89D mutation is destabilizing by about 5 kcal/mole.

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References

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