Len_Q38D

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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. In this study, we report the structure of Q38D mutant of Len.

Methods and Materials

Len_Q38D was prepared as reported earlier [2] 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

Len_Q38D was crystallized from 25% PEG monomethyl ether 550, 0.1 M MES (pH = 6.5), and 0.01 M zinc sulfate in presence of 1 mM uranyl acetate. The crystals contained one V_L monomer per asymmetric unit with unit cell dimensions of a = b = 65.8 Å and c = 48.1 Å in space group $P6_3$. The structure was determined by molecular replacement using the native Len model [Protein Data Bank (PDB) code 1LVE; atoms beyond CB were removed for residue 38]. After several cycles of refinement with X-Plor and intermittent model building with CHAIN, the final structure had an R-factor of 23.0% and R-free of 28.9% for 8.0–1.6 Å data with 103 water molecules, one uranyl ion, and one zinc ion included. The Ramachandran plot contained 90% of the residues in the most-favored regions. The coordinates are deposited in the PDB (code 1EFQ).

Len_Q38D mutant appears to be a monomer in the crystal. The electron density for Asp38 residue was well defined. A uranyl ion was found to be coordinated by the Asp38 side chain, the carbonyl oxygen of Gln42, and the hydroxyl group of Tyr27d residue from a crystallographic-symmetry-related molecule. A zinc ion was also located in a different crystal contact.

Due to the presence of a uranium atom in the crystal, there was significant anomalous contribution to the diffraction data. When Friedel pairs were not averaged, the anomalous difference Patterson map calculated clearly showed the position of the uranium atom.

Discussion

The residue Gln38 in a V_L forms an important contact point in dimer formation. The side chain of Gln38 forms hydrogen bonds with the side chain of another Gln38 across the dimer interface in a typical V_L dimer. Residue 38 is the last residue of β-strand C, which is part of the C"C'CFG β-sheet. The Q38D 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. In one orientation, the carboxyl oxygen of Asp38 would be very close (2.5 Å) to carbonyl oxygen of residue 42. Thus, 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.

In the structure of Len_Q38D with a uranyl ion, the turn-connecting strands C and C' (residues 39–44) are wider than in the native Len and a uranyl ion is bound by Asp38 side chain and carbonyl oxygen of residue 42. Thus, the uranyl ion may stabilize the local structure as the presence of uranyl acetate was found to be essential for crystallization.

The decrease in stability was less when Asp was introduced at the equivalent residue 38 in another V_L (Rei) than in Len. A possible reason for the difference may be that in protein Rei, residue 43 is Ala while in Len it is Pro. With the more flexible Ala residue, a wider loop is possible, which can accommodate the Asp side chain better at position 38. The structure of the "40" loop in Len_Q38D is very similar to that found in V_L Rei.

Acknowledgments

This work was supported by the U. S. Department of Energy, the Office of Biological and Environmental Research under contract No. W-31-109-Eng-38 and by the U.S. Public Health Service Grant DK43757. Use of the Argonne National Laboratory SBC-CAT beamline at the APS was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Energy Research, under contract No. W-31-109-Eng-38. We appreciate the help of Dr. Norma Duke with data collection at the SBC-CAT beamline 19-ID.

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