

Len_Q89L

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Introduction

Unsatisfied polar side chains buried in the hydrophobic interior of a protein are known to reduce the stability of the protein. In the case of dimer formation, such a buried polar side chain would be expected to diminish the dimerization constant. We previously reported [1] that immunoglobulin light chain variable domains (V_L) dimerize in two different arrangements using the same faces of the domains: 1) The conventional form in which the V_L domains are paired in parallel as found in Fab fragments and 2) the flipped form in which the V_L domains are paired in an antiparallel orientation. In the conventional dimer, Gln89 is partially exposed to the solvent and also interacts with Tyr36 from the same monomer as well as Tyr36 and Gln89 from across the dimer interface. In the flipped form, as reported earlier in structures of Len_Q38E and Len_K30T [1], Gln89 is buried in the interface with its hydrogen bonding potential only partially satisfied. To explore the effect of removing the polar side chain of Gln89 on the dimerization of V_L Len, we have made the Q89L and Q89A mutations and determined their structures [2].

Methods and Materials

Len_Q89L 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 1.8 Å at the Structural Biology Center's (SBC-CAT) 19-ID beamline at the Advanced Photon Source (APS).

Results

Len_Q89L was crystallized from 18% PEG 8K and 0.2 M ammonium sulfate at 4°C. The crystals contained one V_L dimer per asymmetric unit with unit cell dimensions of $a = 35.3$ Å, $b = 83.6$ Å, and $c = 42.8$ Å in space group $P2_1$. The structure was determined by molecular replacement using the partially refined model of Len_Q89L solved in our laboratory (unpublished). After several cycles of refinement with CNS and intermittent model building with CHAIN, the final structure had an R-factor of 19.3% and R-free of 22.6% for 8.0–1.8 Å data with 305 water molecules included. The Ramachandran plot contained 90% of the residues in the most-favored regions. The coordinates are deposited in the Protein Data Bank code 1QAC.

The electron density for the mutated 89L residue was well defined in both monomers. The Len_Q89L mutant formed a flipped dimer as predicted. The structure of Len_Q89L is very similar to that found in Len_Q38E and Len_K30T. When α -carbons from one of the domains of Len_Q89L and

Len_Q38E are superimposed, a rotation of 7.5° and a translation of 1.2 Å were required to overlap the second domains.

Discussion

We have previously identified that excess positive potential at the interface prevents the formation of flipped dimer in case of native Len. This is demonstrated in mutants Q38E and K30T, which either add a negative charge or take away a positive charge near the interface resulting in flipped dimer formation [1]. Here we report another independent factor that prevents the flipped dimer arrangement in native Len, which is a polar residue (Gln89) that would be buried in the interface. The importance of buried polar residues on determining the energetics of dimer formation is illustrated by the property of Q89L mutant. The association constant of Q89L mutant is 500 and 172 times higher than that of the native Len and Len_Q38E respectively.

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 SBC-CAT staff with data collection.

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