Len_K103A

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Introduction

Len, an immunoglobulin light chain (V_L), forms a conventional dimer as seen in Fab fragements with the V_L domains arranged parallel to each other. In Len_Q38E and Len_K30T mutants, a new dimer was observed in which the domains are arranged antiparallel to each other [1]. We have demonstrated that in native Len, there is excess positive potential at the interface that prevents the flipped dimer formation. In mutants Q38E and K30T, the excess positive potential at the interface was reduced by either adding a negative charge or by removing a positive charge at the interface. This study reports on another mutant, K103A, designed to study the effect of removing a positively charged residue close to the interface on the type of dimer formed.

Methods and Materials

Len_K103A 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 3.5 Å at the Structural Biology Center's (SBC-CAT) 19-ID beamline at the Advanced Photon Source (APS).

Results

Len_K103A was crystallized from 2% PEG 8K and 1.0 M lithium sulfate. The crystals were too small (0.075 x 0.05 x 0.05 mm^3) for data collection on a conventional laboratory source. The crystals contained one V_L monomer per asymmetric unit with unit cell dimensions of a = b = 106 Å and c = 42.8 Å in space group $P6_122$. This crystal is isomorphous to that of the native Len. Rigid-body refinement of the Len model (Protein Data Bank code 2LVE) using the K103A data (8.0–4.0 Å) gave an R-factor of 23% and R-free of 28%.

Len_K103A formed a conventional dimer as the native Len. The electron density clearly indicated the presence of Ala at position 103.

Discussion

The removal of a positive charge at position 103 does not seem to affect the type of dimer formed by Len. This may be because, as we reported [3], there are two independent factors in controlling what type of dimer is formed: 1) excess positive potential at the interface and 2) presence of a polar residue (Gln89) that would be buried in the flipped dimer. Thus in the K103A mutant, it may be speculated that the reduction in positive potential at the interface was not sufficient enough to overcome the negative barrier of burying a polar residue.

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