Len_K30T

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Len_K30T was designed to study the effect of removing a positively charged residue in the dimer interface of the protein Len. It was predicted to form a different type of dimer compared to the native protein. Len_K30T was crystallized from 1.8 M ammonium sulfate and 0.1 M HEPES (pH = 7.5). X-ray diffraction data to 2.3 Å were collected on a R-Axis IIc. The unit cell dimensions were a = 46.2 Å, b = 54.6Å, and c = 91.4 Å and space group $P2_12_12_1$ (V_M = 2.3). The structure was solved and refined to an R-factor of 19.0% and R-free of 26.7%. Len_K30T forms a flipped dimer as predicted [1].

The crystals of Len_K30T were sensitive to the method of introduction of sucrose, the cryoprotectant used for flash cooling the crystals for data collection. The unit cell dimensions varied depending upon whether the crystals were soaked in mother liquor containing sucrose prior to flash cooling (and if so, on the length of soaking time) or whether they were just dunked in the cryoprotectant solution for just a few seconds before flash cooling. The unit cell of the crystals differed by a few angstroms in each cell length although the space group did not seem to change. Higher resolution data to 1.6 Å were collected at the Structural Biology Center's (SBC-CAT) 19-ID beamline on a crystal with unit cell dimensions of a = 41.9 Å, b = 49.9 Å, and c = 83.5 Å and space group $P2_12_12_1$. Preliminary attempts to solve the structure of this crystal form were not successful, probably due to the tight packing as reflected in the V_M value of 1.7; the data has not yet been further analyzed.

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Reference

[1] Pokkuluri, Huang, Raffen, Cai, Johnson, Wilkins Stevens, Stevens, and Schiffer, *Structure* **6**, 1067–1073 (1998).