

## $\lambda$ -6 light chain, Wil

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### Introduction

The human  $\lambda$ -6 immunoglobulin light chains have been found, with rare exception, to be associated with AL amyloidosis. Yet there has been no three-dimensional structure described for this important subclass of immunoglobulin light chains. In this study we have crystallized and determined the structure of a recombinant version of protein Wil (rV  $\lambda$  6) found in amyloid deposits of a patient [1].

### Methods and Materials

Recombinant V<sub>L</sub>, Wil, was prepared as reported in Pokkuluri *et. al.* [1] and crystallized by the hanging-drop vapor-diffusion method. X-ray diffraction data were collected first on an R-Axis IIC detector and then later at Structural Biology Center's (SBC-CAT) 19-ID beamline at the Advanced Photon Source (APS).

### Results

Recombinant V <sub>$\lambda$</sub>  Wil was crystallized from 1.8 M ammonium sulfate, 0.01 M cadmium chloride, and 0.1 M sodium citrate (pH = 5.6). The crystals belonged to space group R32 with unit cell dimensions of a = b = 147.9 Å and c = 46.6 Å in the hexagonal lattice. The crystals contained one V<sub>L</sub> dimer per asymmetric unit. Structure determination was carried out by molecular replacement method with x-ray diffraction data collected on R-Axis IIC detector. The coordinates of  $\lambda$ 2 protein Mcg [Protein Data Bank (PDB) code 1dcl; constant domains were removed] were used as the search model. The refinement of this model did not converge and hence a higher resolution data set (to 1.7 Å) was collected at the APS. The model was then further refined with APS data and the final structure had an R-factor of 29% and R-free 35% for 8.0–1.8 Å data with 112 water molecules included. Though the structure appears to be correct, the R-factors are still high. The high R-factor and R-free can be accounted for by poorly defined regions on only one of the V<sub>L</sub> units in the asymmetric unit. The structure of first V<sub>L</sub> is well defined except for poor electron density for residues 1, 39–43, and 91–95. The second V<sub>L</sub> is relatively disordered with weak electron density for residues 1, 24–34, 41–43, 49–57, 65–70, and 92–95. Only 85% of the residues were in the most-favored region of the Ramachandran plot. The coordinates are deposited in the PDB (code 2CD0).

### Discussion

The three-dimensional structure of Wil is compared with another  $\lambda$ -6 protein, Jto, whose structure was determined in our laboratory. Jto was found to be nonamyloidogenic where as Wil was amyloidogenic. However, the three-dimensional structures of the two proteins were found to be very similar. The rms deviation between  $\alpha$ -carbons for residues 2 to 110 is 0.6 Å. With the exception of N- and C-terminal residues, complementarity determining regions (CDRs) and the insertion-in-frame work region 3 (FR3) the structure of  $\lambda$ -6 Wil was found to be very similar to other known  $\lambda$ -type light chain structures [1].

The V <sub>$\lambda$</sub>  6 germline gene uniquely encodes a bulky hydrophobic Phe at position 2 and a large ionic Arg at position 25 (a position more commonly occupied in other types of  $\lambda$  chains by Gly). The structures of Wil and Jto revealed unexpectedly that the side chains of these residues were in close proximity resulting in interaction between the face of the phenyl ring of Phe and the guanidinium group of Arg. This tertiary structural feature distinguishes  $\lambda$ -6 light chains from protein products of other V <sub>$\lambda$</sub>  gene families and it results in an alteration of the CDR1 conformation.

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### Reference

- [1] P.R. Pokkuluri, A. Solomon, D.T. Weiss, F.J. Stevens, and M. Schiffer, *Amyloid: Int. J. Exp. Clin. Invest.* **6**, 165–171 (1999).