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Crystallization and preliminary X-ray diffraction studies of Leu55Pro variant transthyretin

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Abstract

The amyloidogenic Leu55Pro variant of transthyretin has been expressed, purified and crystallized in space group $C2$. The cell constants are $a = 149.99$, $b = 78.74$, $c = 98.95$ Å, $\beta = 100.5°$ and the crystals diffract to 2.7 Å resolution. There are eight monomers in the asymmetric unit giving a $V_M = 2.6$ Å^3 Da^{-1} and 53% solvent content. In the wild-type protein, the crystals are orthorhombic with two monomers in the asymmetric unit. The wild-type protein is a tetramer composed of four identical subunits [Blake, Geisow, Oatley, Rerat & Rerat (1978). *J. Mol. Biol.* **121**, 339–356.] and a molecular-replacement solution for the Leu55Pro variant was obtained using one monomer of the wild-type protein as a model. Rigid-body refinement of the eight monomers in the asymmetric unit and subsequent refinement using molecular dynamics were performed with *X-PLOR*, leading to a current R factor of 20.3% for all the data. The crystallographic packing of the molecules is different from the one presented by the wild-type protein, opening new perspectives for understanding how this protein aggregates to form amyloid fibrils.

1. Introduction

Human transthyretin (TTR) is a tetrameric plasma protein composed of equivalent 127-residue monomers. It is encoded by a single copy gene on chromosome 18 and is synthesized by the liver and the choroid plexuses of the brain and the eye. The protein plays a major role in the transport of thyroxine and retinol-binding protein.

The three-dimensional structure of the wild-type protein was reported by Blake *et al.* (1978) at 1.8 Å resolution. It is a tetrameric protein, composed of identical subunits, with a high percentage of β-sheet conformation.

It is now known that more than 40 point mutations in TTR are responsible for the deposition of amyloid extracellularly in tissues, leading to different pathologies; the major protein component of amyloid deposits is the mutated protein. Depending on the mutation, the carriers exhibit polyneuropathies, cardiac failure, vitreous problems or carpal tunnel syndrome (Saraiva, 1995).

In Leu55Pro variant transthyretin (TTR Pro55), residue Leu55 is replaced by Pro. This is the most pathological mutation found to date; patients with this variant have an early age of onset of the disease, with rapidly progressive symptoms, and present diffuse amyloidosis with cardiac and neurologic involvement (Jacobson, McFarlin, Kane & Buxbaum, 1992). Studies by Kelly & Lasbury (1994) show that the mutation affects TTR denaturation and it was hypothesized that an amyloidogenic conformation is favoured by this mutation at pH's where wild-type TTR remains stable and non-amyloidogenic.

The mechanisms involved in amyloidogenesis are not known; however, it is possible that a conformational change, due to the mutation, will induce the protein deposition. How the protein assembles to form the fibrils is highly unknown and we expect that the crystal structure of this variant will provide key information to understand the molecular basis of amyloid formation.

2. Experimental

TTR Pro55 was expressed in *Escherichia coli* and isolated according to the procedure described by Furuya *et al.* (1991).

The initial screening of crystallization conditions was performed according to the procedures reported for other variant TTR's (Terry *et al.*, 1993; Damas, *et al.*, 1993), but they always resulted in amorphous precipitates. Therefore, the Hampton Research crystallization kit protocol was used for exploring new conditions. Crystals suitable for X-ray diffrac-

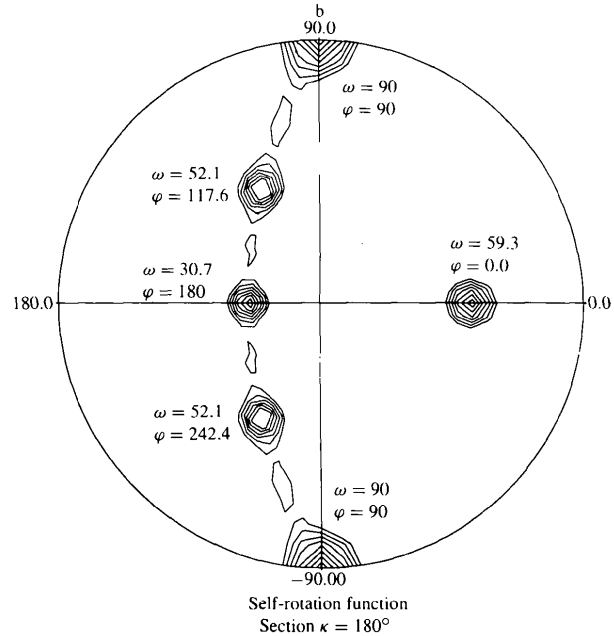

Fig. 1. Stereographic projection of the section $\kappa = 180°$ of the self-rotation function of TTR Pro55. Integration radius in Patterson space is 17 Å and data are included from 15 to 3 Å. Two peaks at $\omega = 90°$ and $\varphi = \pm 90°$ (with the relative height of 100) correspond to the crystallographic twofold b axis and four peaks (with the relative height of 94) at $\omega = 59.3°$, $\varphi = 0.0°$; $\omega = 30.7°$, $\varphi = 180°$; $\omega = 52.1°$, $\varphi = 117.6°$; $\omega = 52.1°$, $\varphi = 242.4°$ correspond to the non-crystallographic twofold axes.

tion analysis were obtained in hanging drops containing 50:50 mixture of 12 mg ml^{-1} TTR Pro55 in water and the reservoir solutions. The reservoir solution contained 45% ammonium sulfate, 7% PEG 400 and 100 m*M* HEPES buffer, pH 7.5.

X-ray diffraction data were collected to 2.7 Å, using synchrotron beamline X31 (EMBL, Hamburg) equipped with an MAR Research imaging-plate scanner. The crystal was mounted with the a^* axis approximately parallel to the spindle axis and c^* initially perpendicular to the direction of the X-ray beam. In this orientation the complete data set could be obtained after 90° rotation. The crystals were monoclinic, space group $C2$ with cell dimensions $a = 149.99$, $b = 78.74$, $c = 98.95$ Å and $\beta = 100.5°$.

The determination of the crystal orientation and integration, merging and scaling of the reflections were performed with the *DENZO* and *SCALEPACK* programs (Otwinowski, 1993). A total of 107 226 independent measurements were merged to obtain 31 394 unique reflections with $R_{merge} = 5.5\%$, representing 99% of the theoretically possible data.

The self-rotation function was calculated using the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) in order to check for non-crystallographic symmetry. The rotation and translation functions were calculated using the program *AMoRe* (Navaza, 1994) and the coordinates of one wild-type TTR monomer obtained from the Protein Data Bank at Brookhaven (Hamilton *et al.*, 1993). Initial refinement was carried out with the program *X-PLOR* (Brünger, 1992).

3. Results and discussion

The self-rotation functions was calculated using data from 15 to 3 Å; it revealed four strong peaks representing non-crystallographic twofold axes (Fig. 1). This confirmed the presence of four dimers in the asymmetric unit.

The structure was solved with the molecular-replacement method and the *AMoRe* package. The final model had a correlation coeffient of 70.7% and *R* factor (*AMoRe*) of 34.5%.

The structure was then refined using *X-PLOR*. The initial *R* value was 38% and after one session of map fitting using the program *TURBO-FRODO* (Roussel & Cambillau, 1989), followed by 20 cycles of positional refinement, the *R* value was down to 29%. The current *R* factor for all the data with $I > 2\sigma(I)$ is 20.3%.

The asymmetric unit contains eight monomers, represented in Fig. 2(*a*) in bold, which assemble into one tetramer in a general position and two dimers near the twofold axes; in effect there are three non-equivalent tetramers within the structure of which two lie on crystallographic twofold axes. In the case of wild-type TTR, the space group is orthorhombic and there is

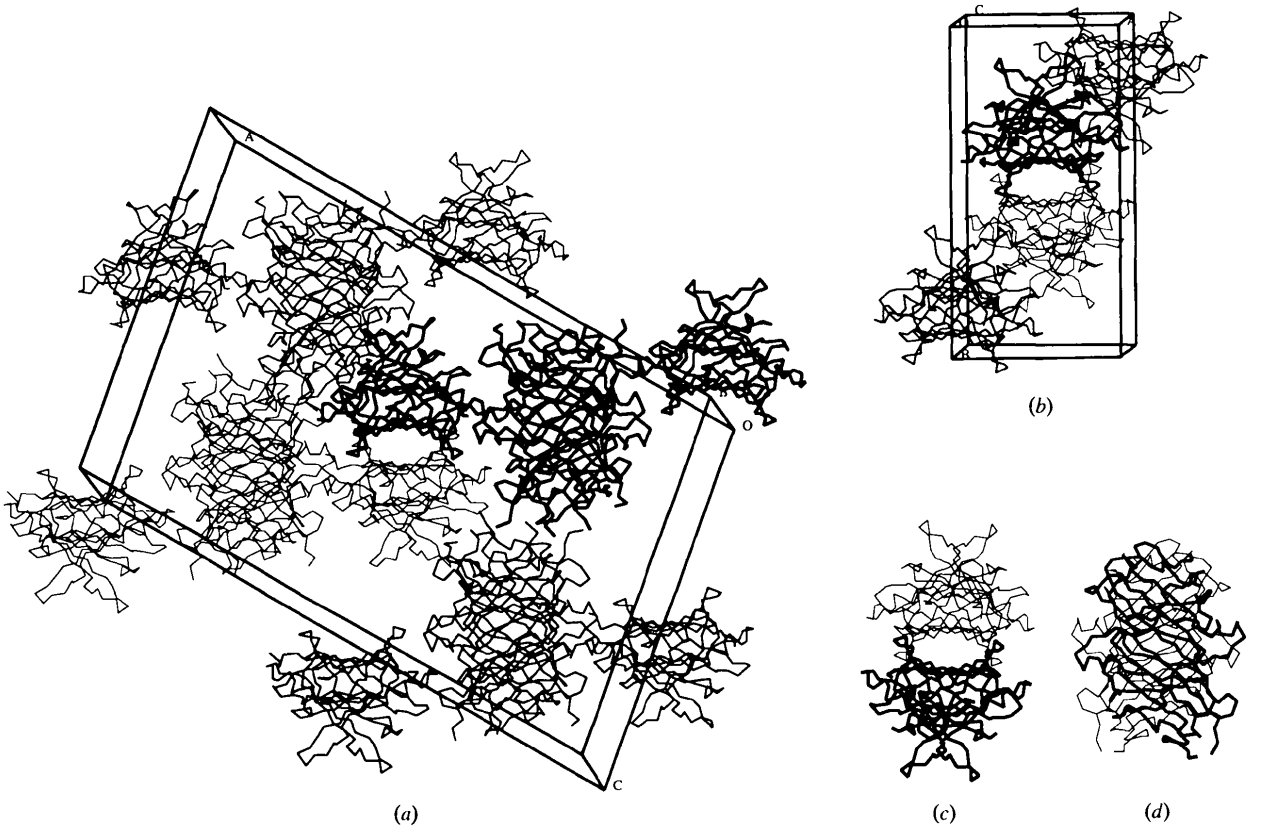

Fig. 2. The packing of (*a*) TTR Pro55 for the $C2$ cell ($a = 149.99$, $b = 78.74$, $c = 98.95$ Å, $\beta = 100.5°$) and (*b*) wild-type TTR for the $P2_12_12$ cell ($a = 43.80$, $b = 86.22$, $c = 65.78$ Å). The asymmetric unit is represented in bold and the four asymmetric units are represented for each cell. In this figure, the monoclinic *b* and orthohombic *c* axes are represented parallel to each other. (*c*) and (*d*) show the tetrameric protein viewed down the orthorhombic *c* and *b* axes, respectively.

only one dimer in the asymmetric unit (Fig. 2*b*). We observed that the crystallographic packing in the case of TTR Pro55 is different from the one presented by the wild-type protein (Fig. 2). Normally, in the wild-type protein, two dimers associate through hydrogen bonds forming a channel that runs through the molecule and the tetramers assemble in the unit cell having their channels parallel to each other (Fig. 2*b*). Interestingly, with the TTR Pro55, the dimers associate in the same way but the tetramers assemble with their channels perpendicular to each other (Fig. 2*a*).

Improvement of the crystal quality is in progress and higher resolution data will be collected. A detailed comparison between the structures of this variant and wild-type TTR will enable the identification of conformationally flexible regions responsible for the aggregation of the protein. These results open new perspectives for modelling the assembly of TTR molecules in amyloid fibrils.

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