ESRF	**Experiment title:** Block Allocation Group: Portugal	**Experiment number:** LS-1523
Beamline: ID14-2	**Date of experiment:** from: 22/11/99 at 08:00 to: 23/11/99 at 07:00	**Date of report:** Feb. 28, 2000
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Report:

HSP-3 purified from Horse (*equus caballus*) seminal fluid:
A Native data collection on a frozen crystal of this protein, gave a complete data set to 1.5 Å resolution. The structure is being solved by MIR methods and the search for good heavy atom derivatives (two are already available) is in progress using data collected in-house on a rotating anode generator.

Tyr78Phe TTR variant (Human, expressed in *E.coli*):
The fibrillar structure resulting from the self-association of an abnormal conformation of transthyretin (TTR) is thought to be the causative agent in Familial Amyloidotic Polyneuropathy (FAP). Dissociation of the TTR tetramer is a prerequisite for amyloid formation in vitro and involvement of monomers in fibril formation has been suggested by structural studies [1,2,3]. In particular, the crystallographic structure of the highly aggressive amyloidogenic Leu55Pro TTR variant revealed putative residues involved in amyloid formation [2]. These results lead us to design TTR mutants with weakened dimer/tetramer interactions, in order to determine the quaternary structures of the amyloidogenic intermediates in the fibrillogenesis cascade. The constructed mutant, Tyr78Phe TTR is predicted to exhibit an unstable tetrameric fold.

Corroborating this fact, the Tyr78Phe mutant is recognised by a monoclonal antibody, previously reported to react only with highly amyloidogenic mutants lacking the tetrameric native fold and with amyloid fibrils. Other biochemical properties indicate that this mutant might resemble an early intermediate in the fibrillogenesis pathway [4].

During the Tyr78Phe TTR crystallisation screening, we obtained needles with dimensions of 150x20x? μm3, with 30% PEG 8000, 100 mM HEPES pH 6.0, 50 mM potassium phosphate. X-ray data to 3.8 Å were collected from one of these needles at the ESRF beam line ID14-2, at 100 K, using 15% glycerol in the mother liquor as the cryoprotectant. Crystals belong to the monoclinic space group $P2_1$, with pseudo-orthorhombic unit cell dimensions a= 78.56 Å, b= 100.78 Å, c= 193.42 Å, β= 90.7° which give a Matthews coefficient of 3.0 $Å^3$/Da for 18 TTR monomers in the asymmetric unit. Due to crystal deterioration and beam time schedule, we obtained X-ray data that were only 67.5% complete and with R_{merge} =5.8%. Also, Preliminary attempts to solve the structure by molecular replacement failed, very likely because there are 18-20 monomers in the asymmetric unit and the data is not complete. Future work includes the improvement of the X-ray data, in order to solve this unique TTR structure, which opens new perspectives for modelling the assembly of TTR molecules in amyloid fibrils.

REFERENCES

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