| ESRF | **Experiment title:**<br>**Block Allocation Group (Portugal)** | **Experiment number**:<br>LS-1667 |
|---|---|---|
| **Beamline**:<br>ID14-3 | **Date of experiment**:<br>from: 07-07-00 to: 08-07-00 | **Date of report**:<br>21-08-2000 |
| **Shifts:**<br>3 | **Local contact(s)**:<br>Dr. Steffi ARZT | *Received at ESRF:* |

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## Report:

**Overview: drugs affecting transthyretin stability - preliminary X-ray studies of TTR with sulphite and tiosulphite complexes**

Transthyretin associated amyloidosis are a conformational disturbance that, like otheramyloidoses, represents a life threat. 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. Drugs designed to impair protein aggregation should increase TTR stability. Such is the case of sulphite, which leads to more stable monomers and an increase in the tetramer/monomer ratio of the protein in circulation (Altland and Winter, 1999).

**Wild type transthyretin soaked with sulphite:**

X-ray data to 1.42 Å resolution were collected at 100 K, using 15% glycerol in the mother liquor as the cryoprotectant. The overall statistics are R-merge = 3.6%, completeness = 99.8%, $I/\sigma(I)$ = 36.1, redundancy = 4. The highest resolution shell statistics (1.47-1.42 Å) are R-merge = 18.1%, completeness= 99.7%.

**Wild type transthyretin soaked with thiosulphite:**

The overall statistics for the data collected at 100 K (20-1.52 Å are: R-merge = 2.8%, completeness = 100%, $I/\sigma(I)$ = 35.7, redundancy = 8. The highest resolution shell statistics (1.57-1.52 Å) are R-merge = 10.1%, completeness = 100%.

In order to compare the binding affinities of sulphite and tiosulphite to TTR amyloidogenic variants, we have also performed soaking experiments with the most frequent human amyloidogenic variant TTR Val30Met.

**Val30Met transthyretin variant soaked with thiosulphite:**
One data set to 1.60 Å resolution was collected at 100 K. The overall statistics are R-merge = 2.8%, completeness = 99.6%, I/σ(I) = 39.9, redundancy = 12. The highest resolution shell statistics (1.66-1.60 Å) are R-merge = 21.9%, completeness = 97.2%. This data set was a great improvement in resolution and quality over the data previously measured on ID14-2 under LS-1523.

The three crystal structures are currently being refined.

**Tyr78Phe transthyretin variant:**
A 99.4% complete data set were collected at 100 K to a resolution of 2.05 Å with R-merge = 4.9%. Crystallographic refinement, structure analysis and comparison with other known 3-D structures of TTR are underway, in order to design putative inhibitors for TTR amyloidosis, by increasing the protein resistance to dissociation.