ESRF	**Experiment title:** RUNT domain crystal structure determination by MAD phasing	**Experiment number:** LS-1341
Beamline: ID14-H4	**Date of experiment:** from: June 4 to: June 6, 1999 (Block allocation: LS-1341, LS-1342, LS-1343, LS-1344)	**Date of report:** Aug. 27,1999
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Report:

Scientific background

Acute myeloid leukemia (AML) is still associated with a mortality of 60 to 80%[1]. Mutations of the 'acute myeloid leukemia related gene product 1', (**AML1**) or its heterodimerization partner 'Core Binding Factor β' (**CBFβ**) are the most common cause for acute human leukemias[2]. Leukemia causing point mutations of AML1 all cluster within the evolutionary conserved **Runt Domain** (RD or RUNT)[3,4], located within the 190 N-terminal residues of AML1. The RD is sufficient for both DNA binding and hetero-dimerization. CBFβ does not bind to DNA on its own but enhances the DNA-binding affinity of the RD[5,6,7,8,9]. The RD is observed to co-operate with other transcriptions factors on the DNA, e.g. Ets1, Myb, C/EBP and ALY. Thus acting as a complex genetic switch in a variety of differentiation events[1].

Aims of the experiment

To determining the structure of a Se-Methionine labelled apo-RD construct from MAD data collected around the Se K-edge in order to be able to analyze the effects of the reported point mutations on DNA binding as well as possibly interactions with other transcription factors.

Preliminary Results

We determined the crystal structure of the **apo-AML1 Runt domain to 1.15 Å resolution**. The initial phases to 2.2 Å were obtained from a Se-Met MAD experiment. Surprisingly the RD displays an S-type immunoglobulin fold similar to the core DNA-binding domains of STAT3, NF-kB and p53, which could not be inferred from sequence data only.

The RD construct used comprises residues 46 to 185 of the AML1 N-terminus. The protein crystallized in space group C2 with cell dimensions a = 91.3 Å, b = 46.2 Å, c = 63.0 Å and β = 92.3°. There are two molecules in the asymmetric unit which leads to solvent content of 35%. Data was collected from a sinlge single flash cooled crystal at 100K. First we collected three wavelength around the Se K-edge for MAD phasing to 2.2 Å resolution and than a high resolution data set to 1.15 Å. The overall completeness is 98% with about 40% in the highest resolution shell (1.18-1.15Å). Data was collected at ID14-H4, June 4-5, 1999, with the help of Sean McSweeney. The data was processed using the HKL2000 package. Sove was used to find the Se sites. The first electron density map could be inspected on site, 6 hours after data collection was completed.
We have now build residues 48 to 179 into a clear electron density map. The termini of the protein seem to be very flexible and can not be traced. The refinement of the structure is still going on. At present the R-factor is about 15% and the R-free about 18% after anisotropic B-factor refinement using Refmac.
The article describing the results is in progress.

References

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