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| Experiment title: Spliceosomal U5 snRNP-specific 15 K protein | Experiment number: LS 915 | |
| Beamline: BM 14 | Date of experiment: from: 29-Nov-97 to: 01-Dec-97 | Date of report: 29-Jul-98 |
| Shifts: 8 | Local contact(s): Leonard Gordon | <i>Received at ESRF:</i> |

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Report:

The splicing of mRNA is catalyzed by the spliceosome which is formed by the ordered interaction of the U1, U2, U5, and U4/U6 snRNPs and numerous additional proteins. We had obtained single crystals of the spliceosomal U5 snRNP-specific 15 K protein. Since this protein does not display any significant sequence homology to a protein of known three-dimensional structure, we considered to solve the crystal structure by means of MAD using selenomethionine containing protein.

The crystals belong to space group $P2_12_12$ (cell constants $a=61.3 \text{ \AA}$, $b=65.2 \text{ \AA}$, $c=36.4 \text{ \AA}$) and contain one molecule in the asymmetric unit. They showed diffraction beyond 1.4 \AA resolution using the synchrotron radiation at beam line BM14. First the MAD experiment was carried out collecting data of one frozen crystal at three wavelengths (Table 1). Also a complete data set of native crystal was collected at a maximum resolution of 1.4 \AA . These diffraction data enabled us to solve and refine the novel crystal structure of the U5 snRNP-specific 15 K protein (Figure 1). The globular fold of the U5 15 K protein resembles the thioredoxin fold. However the canonical Cys-X-X-Cys motif found in thioredoxin and thioredoxin like proteins is replaced by Asp-X-X-Cys. Surprisingly, a disulfide bond is present almost in the same location formed by Cys38 and Cys79. The Cys79 is part of the loop connecting P-strands 3 and 4.

Table 1: Summary of Data Sets collected at BL19

| Data set | Resolution | No. of observations | No of unique reflections | Completeness | R _{merg} |
|-------------|------------|---------------------|--------------------------|--------------|-------------------|
| λ 1 | 30. - 1.9 | 113,639 | 11,601 | 95.0 % | 4.3 % |
| λ 2 | 30. - 1.9 | 113,617 | 11,533 | 95,9 % | 4.3 % |
| λ 3 | 30. - 1.9 | 114,335 | 11,464 | 95.5 % | 4.1 % |
| Native | 30. - 1.4 | 381,393 | 28,054 | 95,2 % | 2.9% |

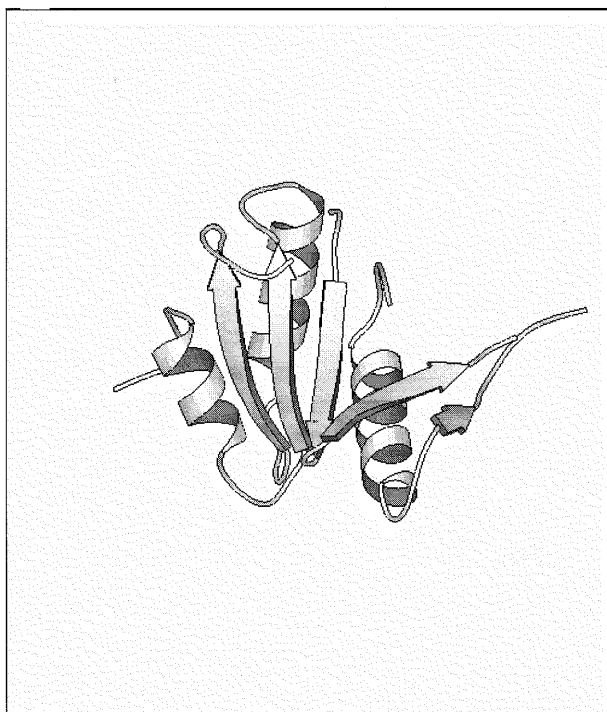


Figure 1: Structure of the U5 snRNP-specific 15 K protein