s1.m3.p10 **Towards Crystal Structure of XEndoU, a novel Endoribonuclease from *X.laevis.*** F.Renzi[a], P.Laneve[b], U.Gioia§, L.Leone§, F.Altieri[a], M.Arceci§, I.Bozzoni§, M.Brunori[a], D.Tsernoglou[a], E.Caffarelli[b], B.Vallone[a], [a]*Dept. Biocemical Sciences "A.Rossi Fanelli" and* [b]*Dept. Genetic and Moleular Biology "C.Darwin" Univ.of Rome "La Sapienza", P.le A.Moro, 5-00185-Rome (Italy)*.
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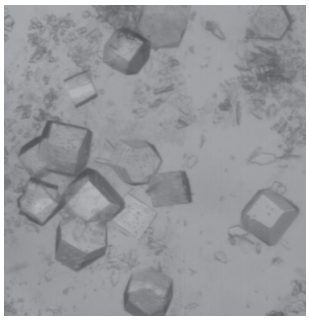
Keywords: XEndoU; *Xenopus laevis*; snoRNA

XendoU is a novel endoribonuclease from X.laevis (XEndoU) which is implied in the splicing independent processing of intron-encoded small nucleolar RNAs (snoRNAs) precursors and doesn't share homology with other known ribonucleases. Its cleavage mode, producing 2'-3'cyclic phosphate termini and Mn^{2+}-dependent activity, resembles ribozyme activity. Gaining insight into the crystallographic structure of such ribonuclease bound to its substrate would aid in the dissection of the molecular mechanism of its enzymatic activity and to better understand how XendoU is involved in the pathway which leads to the syntheses of snoRNAs through a mechanism independent from and alternative to splicing; it would also help to understand the role played by divalent metal ions, whether they are involved in the stabilization of the folded structure or directly involved in the catalytic reaction. The atomic structure of XendoU could also shed light on unexpected similarities with ancient RNA based enzymatic systems, which could have evolved into a more versatile and variegated protein based system.



XendoU, cloned with an N-terminal 6His-tag, expressed in *E.coli*, affinity purified, has been crystallized in 40% NH_4SO_4, in the presence of different additives, with and without an oligoribonucleotide mimicking the substrate.

Native data have been collected from two different crystal's forms at DASY and ELETTRA; one crystal form belonging to p3121 space group (unit cell a=b=83.410 c=313.764) was collected at 3.5 Å resolution, with 92.0 % completeness (linear R factor 0.067, Chi^2 0.95, I/sigma 11.1), with 4 molecules per AU; a second crystal form belonging to c2 space group (unit cell a=168.920 b=53.485 c=137.492 beta=119.241) was collected at 3.0 Å resolution, with 99.2 % completeness (linear R factor 0.103, Chi^2 0.91, I/sigma 10.4) and 3 molecules per AU.

Hg-derivative of both crystal forms were obtained and data were collected at ELETTRA: the crystal form belonging to space group p3121 was fairly isomorphous (unit cell a=82.757 b=82.757 c=312.808) and was collected at 3.5 Å resolution with 99.2% completeness (linear R factor 0.055, Chi^2 1.50, I/sigma16.4). Crystal form belonging to space group c2 was more isomorphous (unit cell a=168.337 b=53.409 c=136.929 beta=119.331) and was collected at 3.0 Å resolution with 88.0 % completeness (linear R factor 0.149 Chi^2 1.08 I/sigma 5.5). Scaled data were used in SOLVE-RESOLVE to calculate phases which however were not good enough to allow model building. Phase improvement is going to be attempted using Se-Met derived protein wich has been synthesized and employed for a crystallization screening to hopefully allow crystals for a MAD experiment.

s1.m3.p11 **Happy experiences with SAD phasing using Cu radiation.** Bram Schierbeek,[a] Anita Coetzee,[a] Cary Bauer[b] and Matt Benning[b], [a]*Bruker Nonius BV, Oostsingel 209, P.O. Box 811, 2600AV Delft, The Netherlands, and* [b]*Bruker AXS Inc., 5465 East Cheryl Parkway, Madison, Wisconsin 53711-5373, USA. E-mail: bram.schierbeek@bruker-nonius.com*

Keywords: SAD phasing; SHELX; Anomalous scattering

Rotating anode sources in combination with graded multilayer optics, a 3- or 4-circle goniostat and sensitive CCD detectors have made it possible to solve protein structures from Cu-K-alpha native data only using the SAD (single-wavelength anomalous diffraction) method [1]. With the increased brilliance of a micro-focus rotating anode generator X-ray source it is now possible to collect redundant data in a much shorter period of time, not only because of the higher intensity, but also because of the smaller X-ray beam, which makes it possible to resolve the diffraction spots at a much closer crystal to detector distance.

In this study we will show how a number of crystal structures representing different space groups have been solved from redundant native data collected at 100 K. The weak anomalous scattering signal (mostly from sulfur) was sufficient to locate all the anomalous scatterers using the integrated direct and Patterson methods in *SHELXD*. These positions and occupancies were used without further refinement to estimate phases. These were extended to native (in-house) resolution by the *sphere of influence* algorithm in *SHELXE*.

The use of Cu-K-alpha radiation for sulfur-SAD phasing on medium- to well-diffracting crystals makes it possible to do structure solution and refinement on the same dataset collected in-house on a single X-ray diffraction system.

[1] J.E.Debreczeni *et al. Acta Cryst.* (2003). D**59**, 686-696