Time-efficient data collection on crystals of the large ribosomal subunit from *Haloarcula marimortui*

Poul Nissen, Nenad Ban, Jeffrey Hansen, Peter B. Moore, and Thomas A. Steitz
Yale University, Mol. Biophys. Biochem. P.O. Box 208114, New Haven, CT 06520-8114 USA

Introduction

The ribosome consists of two subunits called the small and the large ribosomal subunits. In eubacteria and archaebacteria, the subunits sediment as 30S and 50S particles, respectively. The large subunit contains the two important catalytic centers of the ribosome, namely the peptidyl transferase center and the GTPase center. The 50S subunit from *H. marismortui* (Hma 50S) contains 36 proteins and two ribosomal RNA molecules (5S and 23S rRNA).

Crystals of Hma 50S that diffract to 3 Å resolution were reported in 1991 [1]. Yet only recently, low-resolution phases were obtained by the use of large heavy atom cluster compounds for MIRAS phasing [2]. The low-resolution phasing was further improved by a combination of multiple isomorphous replacement with anomalous diffraction (MIRAS) and single wavelength anomalous diffraction (SAD) phasing, by density modification using multicrystal averaging, and by solvent flipping to 5 Å resolution [3]. To obtain a complete and precise structure of the ribosome and to understand the chemistry in the catalytic centers, high-resolution electron density maps (better than 3.5 Å) are required.

We have developed new protocols for reproducibly growing high-quality crystals of Hma 50S (Table 1). These crystals diffract to high resolution (< 2.5 Å) and exhibit low mosaic spread (0.2–0.4°). Using these crystals, reliable MIRAS and SAD phases at 3.3 Å resolution have been obtained using data collected at the beamlines X25 [National Synchrotron Light Source (NSLS), Brookhaven National Laboratory] and 19-ID [SBC-CAT, Advanced Photon Source (APS), Argonne National Laboratory] and using various heavy-atom derivatives that had a large number of single heavy-atom sites in the crystals (20–130 sites per asu) (Table 2). The phases have been extended to even higher resolution (2.7 Å) by solvent flipping. The resulting electron density map is of excellent quality and allows for an almost complete and unambiguous tracing of all the ribosomal RNA and the proteins. The complete structure of the particle is currently being built and crystallographic refinement will soon be initiated. The focus in data collection on this project is accordingly shifted from experimental phasing to high-resolution native data collection for refinement and to collecting data on complexes of the 50S subunit with ligands.

Methods and Materials

We are currently limited by the availability of large detectors with high pixel resolution to resolve the reflections at high angle from our large unit cell (Table 1). Thus, we use several means to minimize the reflection spot dimensions and to maximize spot separation on the detector, such as: a) using only excellent crystals, b) aligning the crystals with the longest cell dimension approximately along the spindle, c) using a very fine beam (≤100 μ) with minimal crossfire, d) putting the detector at a maximal distance from the crystal, and e) collecting data in thin oscillation frames (0.2–0.4°).

Results

In only three days of scheduled beam time, complete data was collected on four native, six heavy-atom derivatized, and five antibiotic soaked crystals (Table 2). A 100 μ beam proved to be sufficiently intense and stable for the data collection. The crystals were stable in the beam and 180° data was routinely collected from single crystals of both native and derivatized crystals in 0.2–0.4° frames without observing any severe decay (with one exception). Total data collection time on a crystal was in the order 90 to 120 minutes. Using the 100 μ beam and the full pixel resolution of the 3 x 3 CCD developed at 19-ID, we were able to collect 3.0–3.2 Å data without applying any 2θ offset of the detector. This was from crystals diffracting to a maximum of 2.9 Å. The strategy of no 2θ offset was chosen to maintain the highest possible redundancy and completeness in the data collection with a minimum expenditure of data collection time; yet, it had the cost of a suboptimal spot separation (Table 1).

Discussion

Data collection with the detector centered on the beam has reached a practical limit in resolution. For effective collection of 2.5 Å data or better, 2θ of the detector at 19-ID will be offset. The resulting decrease in redundancy and completeness can be compensated for by large total rotation of the crystal and by merging data from several crystals. The feasibility of this approach was demonstrated on a 2.7 Å data set, which was collected on a MAR 345 at X25 (NSLS, Brookhaven). The ultrafast readout of the 3 x 3 CCD detector at 19-ID is a great advantage. It offers the possiblity

to collect very thin oscillation frames to minimize overlaps with little cost in readout time. This makes data collection within a reasonable period of time practical and minimizes the decay of the crystal, which will also occur while the shutter is closed during readout.

Moving to higher resolution, we find in our experience that a 100 μ beam or smaller is required. The 19-ID beamline has proved that it can maintain such a fine beam in a very stable manner. For that reason, combined with the advantages of the fast data acquisition time at the 19-ID beamline, we believe that a high-resolution data collection (< 2.5 Å) can be performed at that beamline.

Acknowledgements

Use of the Argonne National Laboratory Structural Biology Center beamlines at the APS was supported by the U. S. Department of Energy, Office of Energy Research, under Contract No. W-31-109-ENMG 38. We are very grateful for the assistance of Ruslan Sanishvili and Andrzej Joachimiak at beamline 19-ID at APS. We are also grateful to Joseph Ippolito for help with data collection.

References

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Table 1: Characteristics of Hma 50S crystals and data collection at 19-ID.

Space group	C2221
Unit cell	a=212 Å, b=300Å, c=575Å, α, β, γ=90°
ASU content	50S particle MW ~1,600 kDa
	23S rRNA, 5S rRNA MW ~1,000 kDa
	36 ribosomal proteins (L1 - L36) MW ~600 kDa
Detector distance	300 mm (native data)
Wavelength	1.00 Å (native data)
Beam size	100 μ
Spot size	~0.5 mm
Spot separation	~0.5 mm
Orders resolved along l	~420 (excl. corners of square detector)
Unique reflections	~360,000 at 3.0 Å resolution (720,000 anomalous)
Exposure time	5 sec/0.2° or 10 sec/0.4°
Attenuation	2 - 4 fold

Table 2: Data collection and phasing from 19-ID (best crystals).

						Phasing (30 - 3.3 Å)				
Crystal	Reso	Compl (%)	Mult	I/σI	Rsym (%)	Rcross (%)	no. sites	Phasing power	Rcullis (centrics)	fom
Native	60 - 3.0 Å	100	~7	17.6	10.1					
	3.1 - 3.0 Å 99.9	~5	2.3	84.0						
Os83 (ano)	30 - 3.3 Å	99.9	~4	11.3	12.7	17.0	40	0.49	0.69	0.15
	3.4 - 3.3 Å 99.8	~4	3.2	40.3	26.0		0.43	0.69	0.11	