High-Resolution Ribosomal Crystallography

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Introduction

Ribosomes are the universal cell organelles facilitating the translation of the genetic code. These nucleoprotein assemblies contain two subunits that associate at the beginning of the process. The small ribosomal subunit from bacteria is of 0.85 mDa, contains 20-21 proteins and one rRNA chain (of about 1500 nucleotides). It organizes the decoding of genetic information, initiates mRNA engagement, discriminates against aminoacyl tRNA molecules and ensures translational fidelity. It plays a central role in the initiation step and is inhibited by various antibiotics that act by blocking protein synthesis in different ways.

Methods and Materials

Crystals of the small subunits from *Thermus thermophilus* (T30S) belonging to the tetragonal space-group $P4_12_12$ (a=b=406.3 Å; c=173.1 Å) were treated with a multi W cluster as described.[1] Data were collected at 85K from shock-frozen crystals at SBC-CAT beamline ID-19 at the Advanced Photon Source (APS) and recorded on the APS CCD detector. The bright synchrotron radiation necessary for the collection of the higher resolution x-ray diffraction data introduces severe decay even at cryotemperatures. To maximize the amount of data collected from individual crystals, a beam with a cross section smaller than the crystals was used. Once decay was observed, the crystals were translated to new positions for further data collection.

The anomalous signal W was used for phasing. The 3.2 Å structure of the 30S subunit so obtained was refined against the structure factor amplitudes of each of the three ligand-30S complexes, using rigid body refinement as implemented in CNS. Unweighted, as well as sigmaA weighted, difference maps were used for the initial manual placement of the ligands.

Results and Discussion

Our current 3.2 Å map[2] enabled tracing of 1503 nucleotides (out of 1518) and all 20 proteins (Fig. 1, Table 1). It shows essentially the same RNA fold as was determined independently, but several RNA bulges, hairpin loops and protein extensions have slightly different conformations. This model served as a reference for the determination of the structures of three functionally relevant complexes (with antibiotics and the C-terminal domain of IF3 (IF3C).

We identified six tetracycline-binding sites on the 30S subunit (Fig. 2), with relative occupancies 0.42-1. That of the highest occupancy is located at the A-site and carries the inhibitory effect: preventing the binding A-site tRNA. The five additional sites may have a secondary contribution: limiting the mobility needed to switch between error-prone and the restrictive conformations[3] and disturbance of the early assembly steps of 30S particles. The observation of multiple tetracycline binding sites is consistent with biochemical results.

The complex of the 30S subunit with edeine revealed one binding site and the formation of a new RNA base pair. Edeine binds so that its one side reaches the P-site and the other in the vicinity of the E-site (Fig. 2), connecting universally conserved bases involved in the initiation process, consistent with the biochemical data. In addition to the direct interactions of edeine with 16S RNA, the new base pair may impose constraints on the mobility of the platform.

IF3C was placed unambiguously in the difference map, at the upper end of the platform on the solvent side of the particle, in a position similar to that revealed by EM for a eukaryotic system.

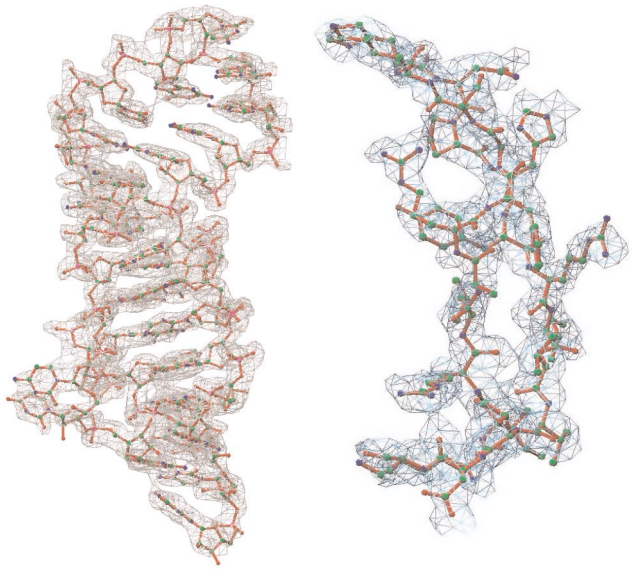

FIG. 1. Portions of the 3.2 Å map of T30S. Left: RNA (H27). Right: protein S16.

Table I. Crystallographic statistics and refinement (in brackets = the highest resolution bin).

Compound	Resolution [Å]	Number Observed	Unique Reflections	Completeness [%]	<I>/<(i)>	Rsym [%]	R/Rfree [%]
Native	40-3.2	2485385	228166	86.8 (83.2)	19.8 (2.0)	13.6 (38.1)	20.3/24.5
etracycline	35-4.5	365437	79036	89.7 (79.8)	9.1 (3.4)	9.9 (31.0)	22.3/25.4
Edeine	35-4.5	268539	66468	76.1 (68.2)	9.8 (2.5)	8.8 (41.8)	23.7/24.4
IF3C	35-4.2	417631	83090	81.5 (69.6)	9.2 (2.4)	9.9 (35.7)	20.5/26.4

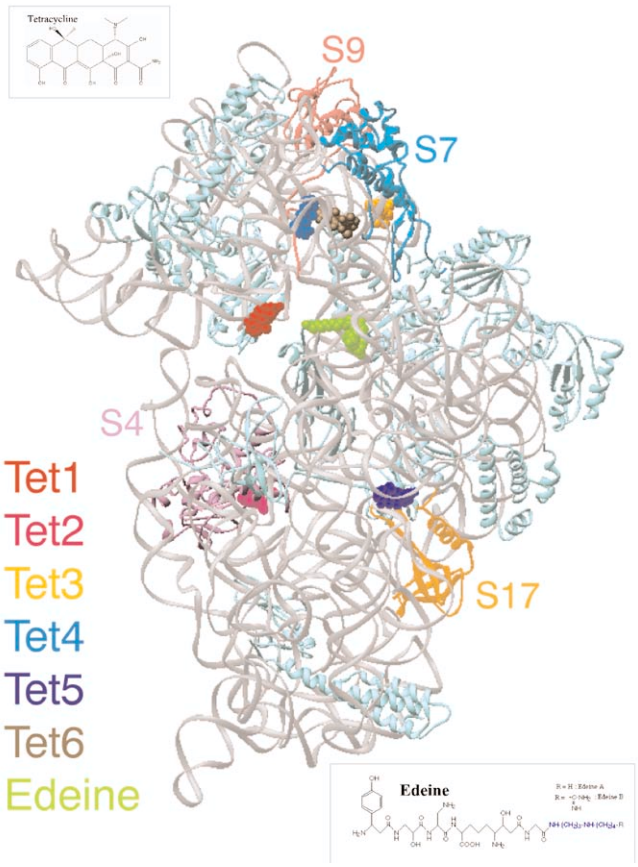

FIG. 2. The binding sites of tetracycline and edeine are indicated. Ribosomal proteins interacting with tetracycline have been colored and labeled.

This indicates that certain mechanisms underlying the initiation process have been evolutionarily conserved. Our structural results are consistent with all biochemical observations and support the hypothesis that IF3C acts by changing the conformational dynamics of the subunit. The docked N-terminal domain (IF3N) leaves only a limited space for P-site tRNA and requires small conformational changes for simultaneous binding of both components, suggesting that P-site selection is based on space-exclusion principles. Owing to its flexibility and ability to alter its fold, the linker may act as a strap that transmits information between the two IF3 ends.

Conclusions

The atomic structures of the ribosomal subunits have already contributed tremendously to the understanding of the mechanism of the translation. Apart from the immense value of this basic information, their applicability for therapeutic aspects, such as the understanding of antibiotic action, has been proven.

Acknowledgments

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