Minireview

On the structural basis of peptide-bond formation and antibiotic resistance from atomic structures of the large ribosomal subunit

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Received 16 November 2004; revised 16 November 2004; accepted 22 November 2004

Available online 30 November 2004

Edited by Gunnar von Heijne and Anders Liljas

Abstract The atomic structures of the large ribosomal subunit from Haloarcula marismortui and its complexes with substrates and antibiotics have provided insights into the way the 3000 nucleotide 23S rRNA folds into a compact, specific structure and interacts with 27 ribosomal proteins as well as the structural basis of the peptidyl transferase reaction and its inhibition by antibiotics. The structure shows that the ribosome is indeed a ribozyme.

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Keywords: Ribosome structure; Antibiotics; RNA structure; Ribozyme; Peptidyl transferase mechanism

1. Introduction

The complete atomic structure of the large ribosomal subunit from Haloarcula marismortui has not only enabled insights into how this large ribonuclear protein machine forms a compact specific structure out of RNA and protein components, but has also provided many insights into its functions as the site of peptide-bond formation in protein synthesis and as a major target of antibacterial antibiotics [1,2]. Although the experimental tools required to establish the atomic structure of the large ribosomal subunit were largely in place by the late 1980s, the strategies that enabled the determination of its structure were only successfully employed 10 years later [3]. An 18 tungsten heavy atom cluster compound, which was used as a heavy atom derivative, was studied at very low (20 Å) resolution where it produced a large and measurable signal in the diffraction pattern from the subunit crystals. The extra large signal at this very low resolution results from the fact that the scatter produced by the cluster compound at low resolution approaches that of a single heavy atom containing 1332 electrons, which produces X-ray scatter that approaches 300 times the scatter of a single bound tungsten heavy atom. The correct positioning of this heavy atom was confirmed by difference Fourier using phases produced by molecular replacement with an cryo-EM reconstruction at 20 Å resolution. This led to the first electron density map, which was at 9 Å resolution, that showed recognizable features of RNA rods [3]. Structural studies of the small ribosomal subunit and of the whole 70S ribosome quickly followed similar strategies [4,5]. From the 2.4 Å resolution atomic structure, the RNA structural database was increased by a factor of 5- to 6-fold as was the structural database on RNA–protein interactions. A new RNA secondary structure element termed the “kink-turn” was found [6] as well as a major tertiary structure element in RNA folding termed the “A-minor motif” [7]. The observation of polypeptide tails extending into the interior of the RNA to help stabilize its structure was partly anticipated earlier [8] but visualized in detail in this first structure of a ribosomal subunit.

1.1. The role of RNA in peptide-bond formation

The major functional conclusion from these first structural studies was that the large ribosomal subunit functions as a ribozyme in catalyzing peptide-bond formation. No protein component was observed to lie within 18 Å of the peptidyl transferase center and the CCA ends of the A-site and P-site tRNAs were held in place and positioned entirely by 23S RNA and related to each other by a 180° rotation [9]. Although the initial observation that the N3 of A2486 (A2451, Escherichia coli) interacts with the attacking z-amino group of the A-site aminoacyl-tRNA might suggest that it might function as a general base to activate the nucleophilic attack of the z-amino group on the carbonyl carbon of the peptidyl-tRNA, subsequent genetic studies have not supported this hypothesis [10]. However, the more recent finding that the 2’ OH of A76 in the P-site substrate was also hydrogen bonded to the z-amino group of the aminoacyl-tRNA might suggest that it might function as a general base to activate the nucleophilic attack of the z-amino group on the carbonyl carbon of the peptidyl-tRNA (Fig. 1) implied that it might also be functioning to properly orient the z-amino group for attack [11]. Furthermore, it might be assisting catalysis as a general base to abstract a proton from the attacking z-amino group and a general acid to donate a proton to the deacylated 3’ OH group [11]. Following on earlier biochemical experiments that implicated the 2’ OH of the P-site A76 [12], recent kinetic experiments from the Strobel laboratory have shown that aminoacyl-tRNA containing a deoxy-A76 react about 106-fold slower than the ribo-A76 counterpart [13], consistent with the possibility of an important role for this 2’ OH in the catalysis of peptide-bond formation. However, the alternative possibility that P-site bound peptidyl-tRNA containing dA76 manifests an altered structure.

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or interactions that are reducing the catalytic rate has not yet been eliminated.

More recently, additional structures of substrate and intermediate analog complexes with the peptidyl transferase center have been determined at resolutions ranging from 2.2 to 2.5 Å [T.M. Schmeing, K. Huang, S. Strobel, T.A. Steitz, in preparation, 2004]. The crystal structure of the large subunit bound simultaneously to two substrates, C-hydroxy puromycin at the A-site and CCA-phenylalanine-caproic acid-biotin at the P-site, shows an essentially similar ribosome structure and orientation of the substrates to those structures observed in the complexes with the individual substrates. Again, the 2′ OH of A76 of the P-site peptide-CCA interacts with the hydroxyl group ω-amino group mimic of the C-hydroxy puromycin so that it could assist in catalysis as both a general acid and a general base.

Attempts thus far to demonstrate the presence of either a divalent or monovalent cation in the immediate vicinity of peptide-bond formation that could be involved in assisting with catalysis have not supported the possibility of metal ion involvement in catalysis [T.M. Schmeing et al., in preparation]. Crystals of the H. marismortui large subunit were soaked in the manganese and anomalous difference Fouriers showed no peak in the peptidyl transferase center (PTC) that would be relevant to catalysis. Furthermore, substrate containing crystals soaked in 1.7 M potassium also showed no peak in the PTC. Thus far, neither manganese nor potassium appear to bind in positions that would be catalytically important for peptide-bond formation.

The first complex of the 50S subunit with a phosphoryl analog of the tetrahedral carbon intermediate of the peptidyltransferase reaction was determined at 3.2 Å resolution and appeared to show an interaction of a non-bridging phosphate oxygen with A2456 (A2451) [9]. However, a modeling of the tetrahedral intermediate from the substrate complexes suggested that the oxyanion points away from A2251 [11].

Complexes with many additional analogs of the transition state in peptide-bond formation have been determined at 2.2–2.5 Å resolution [T.M. Schmeing, K. Huang, S. Strobel, T.A. Steitz, in preparation]. These studies confirm the hypothesis derived from model building [11] that the mimic of the oxyanion of the tetrahedral carbon intermediate points away from A2486 (2451) and is interacting with what is likely to be a water molecule. The extent to which this bound water molecule can enhance the ribosome’s catalytic power by stabilizing the transition state remains to be determined.

2. Insights into the mechanisms of antibiotic function and resistance

In order to provide the structural basis of antibiotic inhibition of protein synthesis, the structures of H. marismortui

Fig. 1 Structural insights into peptide-bond formation. (A) A space-filling representation of the complex between the H. marismortui large subunit and three intact tRNAs added in the positions that the tRNAs assume when bound to the A, P and E sites of the 70S ribosome. rRNA is white and ribosomal proteins are yellow. The subunit, which is oriented in the crown view, has been cut in half along a plane that passes through the peptide exit tunnel, and the front of the structure has been removed to expose the polypeptide exit tunnel, which is 100 Å long and 12–20 Å wide. The active-site area is boxed. (B) A close-up of the active site showing the peptidyl product CC-puromycin-phenylalanine-caproic acid-biotin (CC-pmm-pcb; green) bound to the A-loop (tan) and the deacylated product (CCA; violet) bound to the P-loop (blue). The N3 of A2486 (A2451 in E. coli; light blue) is close to the 3′ OH of the CCA, and the base of U2620 (U2585 in E. coli; red) has moved close to the new peptide bond and the 3′ OH of A76. (C) A model of the peptidyltransferase center of the large ribosomal subunit from H. marismortui with substrates bound to both the A- and P-site. This model was obtained by superimposing the structure of an A-site substrate complex on the structure of a P-site substrate complex. The ω-amino group of the A-site substrate (purpose) is positioned for a pro-S attack on the carbonyl carbon of the ester linking the peptide moiety of the P-site substrate (green). Possible hydrogen-bonding interactions involving the ω-amino group and the N3 of A2486 (A2451 in E. coli) and the 2′OH of A76 are indicated. The 2′OH of A2486 (A2451 in E. coli) is also close enough so that it might interact.
50S ribosomal subunit complexed with nine antibiotics were determined [14,15]. Additional complexes with the D. radiodurans 50S subunit were established by the Yonath group [16]. The complexes of the H. marismortui large subunit with 15- and 16-membered macrolides showed that they block the egress of the nascent polypeptide down the tunnel and that the 16-membered macrolides form a covalent link between an ethylenaldehyde substituent at its C6 position and the N6 of A2103 (A2062) (Fig. 2). In these complexes, the N2 of G2099 (A2058, E. coli) lies buried under the hydrophobic side of the macrolide ring and it was hypothesized that the desolvation of the N2 required upon macrolide binding reduced their affinity for ribosomes containing a G at this position rather than the A that is common to eubacteria [14]. Five other classes of antibiotics were observed (Fig. 2) to bind at sites that overlap with the binding sites for the CCA ends of the tRNA substrates [15].

In order to provide a structural understanding of the sources of antibiotic resistance mutations in the 50S ribosomal subunit, a genetic system was developed for H. marismortui that has enabled a production of both RNA and protein mutations in the 50S subunit isolated from H. marismortui [D. Tu, G. Blaha, P.B. Moore, T.A. Steitz, submitted, 2004]. Structural studies of these mutated subunits bound to antibiotics have provided structural insights into the sources of resistance that obviate the usefulness of specific antibiotics. Mutation of A2058 in E. coli (G2099 in H. marismortui) from an A to a G reduces the affinity of E. coli ribosomes for erythromycin by about 10^4-fold. H. marismortui wild-type ribosomes contain a G at this position and are thus not able to bind erythromycin in their crystals even at a concentration of 3 mM [14]. However, when the 23S rRNA of H. marismortui was mutated at this position from a G to an A, erythromycin binds to the macrolide binding site at a concentration of 1 micromolar, at least 10^8 times more tightly [D. Tu, G. Blaha, P.B. Moore, T.A. Steitz, submitted, 2004]. Thus, this single mutation appears to reverse the effect of A to G mutation observed in eubacteria. The orientation of the 14-membered macrolide ring of erythromycin bound to the H. marismortui 50S subunit is identical to that of telithromycin, essentially identical to that observed for the 15-membered macrolide azithromycin [14] and extremely similar to the rings of the 16-membered macrolide family which includes tylosin [Tu et al., submitted, 2004]. However, the conformations and orientations of erythromycin and telithromycin differ significantly from those derived from the crystalline complexes with the D. radiodurans 50S subunit [16].

![Diagram of ribosome structure](image)

Fig. 2. The positions of seven antibiotics and A-site (red) plus P-site (yellow) substrates bound to the peptidyl transferase center. The ribosome has been split open to reveal the lumen of the exit tunnel and adjacent regions of the peptidyl transferase site. Ribosomal components are depicted as a continuous surface that is colored green at two positions where splayed out bases provide hydrophobic binding sites for small molecules. Seven independently determined co-crystal structures have been aligned by superimposing the 23S rRNA in each complex. The positions of sparsomycin (green), puromycin (red), blasticidin S (pink), chloramphenicol (light blue), carbomycin (dark blue), anisomycin (yellow) and virginiamycin M (light blue) are shown. The sites to which each of these antibiotics bind are all different, but there is extensive overlap.

2.1. New antibiotics from structure-based drug design

Microbial resistance to antibiotics is growing and spreading rapidly, particularly in hospitals [17,18]. About two million people will acquire an infection in a US hospital this year and 90 000 of them will die of it. Thus, new approaches to developing novel antibiotics are clearly needed, and since about one half of the currently used antibiotics target the ribosome, mostly the large subunit, structure-based drug design using the large subunit structure should be useful. Indeed, these structures of the H. marismortui large ribosomal subunit complexed with several families of antibiotics that inhibit peptide-bond formation by binding in the vicinity of the peptidyl transferase center have been highly enabling for Rib-X Pharmaceuticals, Inc. to design and synthesize novel compounds that have the property of being active against bacterial strains that are resistant to presently used antibiotics such as azithromax. Use of the structural information has greatly increased the speed with which new potential drug candidates can be developed. Thus, it is anticipated that this ribosome structure based design effort will lead to new and powerful antibiotics that will be capable of replacing those antibiotics that become ineffective due to bacterial resistance mutations.

Acknowledgments: I thank my colleagues and collaborators who participated in the ribosome structural work at Yale that is reported here. These include: Nenad Ban, Poul Nissen, J. Hansen, T.M. Schmeing, D.J. Klein, D. Tu, G. Blaha, P.B. Moore, B. Freeborn and L. Vasilenko.

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