Structural Studies of the *H. marismortui* 50S Ribosome Complexed with Peptidyl Transferase Reaction Analogues

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Introduction

The ribosome is the large macromolecular complex that catalyzes protein synthesis under direction of mRNA. Our group is focused on determining the structure of the 50S ribosomal subunit from *Haloarcula marismortui* by using macromolecular crystallographic techniques.

Recently, our group published the complete high-resolution structure of the *H. marismortui* large ribosomal subunit [1]. We have initiated new studies on the 50S subunit in complex with various antibiotics and substrate analogues that should provide structural information on the details of protein synthesis.

In collaboration with S. Strobel's group at Yale University, we have been using small synthetic analogues of the substrates, intermediates, and products of the peptidyl transferase reaction to elucidate the mechanism of this reaction [2, 3]. Our continuing work at APS focuses on solving structures of these small analogues bound to the *H. marismortui* 50S.

Methods and Materials

Crystals are grown, soaked, and frozen and data sets are collected and processed as described in Ref. 3.

Results

Data sets were collected on various small analogues of peptidyl transferase substrates. The resolution of these data sets ranged from 3.4 to 2.9 Å. The intensity and focus of the beam at beamline 19-ID has played a key role in obtaining the highest-resolution data sets from these crystals while reducing reflection overlaps with the large area charged-coupled device (CCD).

Structure determination by molecular replacement by using the native 50S subunit as a search model allows for rapid turnaround as we test various substrate analogues. The Fo-fo, Fo-fc, and 2fo-fc maps reveal that the substrates were bound to the 50S A- and P-sites.

Discussion

Analyses of the structures of the *H. marismortui* 50S complexed with peptidyl transferase analogues continue to provide insight into the mechanism of protein synthesis. Further work to combine substrates is ongoing; the objective is to obtain a series of structures of the intermediate reactions and steps in the protein translation reaction as mediated by the 50S subunit alone.

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References

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