Crystallographic studies of *Thermus aquaticus* RNA polymerase

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Introduction

Transcription is the major control point of gene expression, and RNA polymerase (RNAP) is the central enzyme of transcription. The RNAP in all cellular organisms is a complex molecular machine. In its simplest bacterial form, the enzyme comprises at least four subunits with a total molecular mass of about 400 kDa. The eukaryotic enzymes comprise up to a dozen subunits with a total molecular mass of about 500 kDa. The essential core component of the bacterial RNAP (subunits $\alpha_2\beta\beta'$) is evolutionarily conserved from bacteria to man, making the bacterial RNAPs excellent model systems for human studies.

Recently, we made a major breakthrough in our structural and functional studies of RNAP. Namely, we solved the x-ray crystal structure of *Thermus aquaticus* (*taq*) core RNAP to a resolution of 3.3 Å [1]. Our studies at the Structural Biology Center (SBC) are aimed towards adding to our understanding of the enzyme's function and its regulation. Specifically, we performed experiments to accomplish the following:

1. *Collect better native data sets to complete the refinement of the* taq *core RNAP model.* The current model of *taq* core RNAP has been refined to an R factor of 33% (Rfree = 39%) using data from 8–3.3 Å resolution [1]. We will further refine the model of the 380 kDa asymmetric unit through cycles of model building, refinement, and phase combination.

2. *Determine the crystal structure of* taq *core RNAP complexed with nucleotide substrates.* We found that soaking the *taq* core RNAP crystals with ATP noticeably improved the diffraction limit of the crystals. The structure should reveal the details of substrate binding in the RNAP, and may result in a higher resolution structure of the RNAP.

3. *Determine the structure of* taq *core RNAP complexed with antibiotic inhibitors.* We collected data from *taq* core RNAP crystals soaked with the antibiotic inhibitors rifampicin (3.2 Å resolution), streptolydigin (3.6 Å resolution), and tagetitoxin (3.2 Å resolution) to reveal the binding sites of the inhibitors on the RNAP, which will shed light on their inhibitory mechanisms, provide insight into the origins of antibiotic resistance, as well as provide a possible basis for designing more effective inhibitors.

Methods and Materials

Methods and Materials were as described in [1]; RNAP inhibitors were obtained from commercial sources.

Results

1. *Collect better native data sets to complete the refinement of the* taq *core RNAP model.* We collected several native data sets in the hopes of obtaining higher resolution diffraction due to the brightness of the beam at SBC. Unfortunately, the data we obtained are only slightly better than data we collected elsewhere, and the resolution limit is still around 3.2 Å, indicating the limitation is imposed by crystal. We also collected several data sets near the Zn edge (RNAP contains two bound Zn^{2+} ions), with the hope of locating the two Zn^{2+} ions using anomalous difference Fouriers.

2. *Determine the crystal structure of* taq *core RNAP complexed with nucleotide substrates.* We collected several very good data sets with improved diffraction (3.0 Å instead of 3.2 Å). The data from the core RNAP:ATP crystals are not isomorphous with the native core RNAP crystals, and difference Fouriers have not yielded interpretable results.

3. *Determine the structure of taq core RNAP complexed with antibiotic inhibitors.* In terms of each inhibitor:

i) Rifampicin – We collected 3.2 Å data sets from co-crystals of *taq* core RNAP with rifampicin. Difference Fouriers clearly reveal the rifampicin molecule with good detail, as well as interacting protein side chains. We are currently refining this co-crystal structure.

ii) Streptolydigin – We collected 3.6 Å data sets from co-crystals of *taq* core RNAP with streptolydigin. Difference Fouriers clearly reveal the streptolydigin molecule, but the difference density is very 'blobby' and we are unable to fit in the streptolydigin molecule into the density.

iv) Tagetitoxin – We collected 3.2 Å data sets from co-crystals of *taq* core RNAP with tagetitoxin but difference Fouriers suggest binding did not occur under the crystallization conditions (2 M ammonium sulfate).

Discussion

In the near future, we will solve the core RNAP:ATP structure using rigid body refinement, molecular replacement, or multiple isomorphous replacement, whichever is necessary. The rifampicin co-crystal structure is well on its way to being refined and will be tremendously informative from both a scientific and medical point of view. For streptolydigin, we will attempt to attach heavy-metal 'tags' to specific sites of streptolydigin and collect co-crystal data from the tagged streptolydigin derivatives. The tags, combined with difference Fouriers, will allow positioning of the streptolydigin molecule in the difference density. For tagetitoxin (as well as other ligands that bind in a salt-sensitive manner), we are working on a procedure to 'desalt' the crystals into low ionic strength.

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Reference

[1] G. Zhang, E.A. Campbell, L. Minakhin, C. Richter, K. Severinov, and S.A. Darst, *Cell* **98**, 811–824 (1999).