Metal Ion Coordination and Conformational Flexibility in a Viral RNA Pseudoknot at Atomic Resolution

M. Egli,[1] G. Minasov,[2] L. Su,[3] A. Rich[4]
[1]Department of Biochemistry, Vanderbilt University, Nashville, TN, U.S.A.
[2]Department of Molecular Pharmacology and Biological Chemistry, Northwestern University, Chicago, IL, U.S.A.
[3]Fish & Neave, New York, NY, U.S.A.
[4]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, U.S.A.

Introduction

Many pathogenic viruses use programmed –1 ribosomal frameshifting to regulate the translation of their structural and enzymatic proteins from polycistronic mRNAs. Frameshifting is commonly stimulated by a pseudoknot located downstream from a slippery sequence, the latter positioned at the ribosomal A- and P-sites. We report here on the structures of two crystal forms of the frameshifting RNA pseudoknot from the beet western yellows virus at resolutions of 1.25 and 2.85 Å. Because of the very high resolution of 1.25 Å, 10 mono- and divalent metal ions per asymmetric unit could be identified, giving insight into the potential roles of metal ions in stabilizing the pseudoknot. A magnesium ion located at the junction of the two pseudoknot stems appears to play a crucial role in stabilizing the structure. Because the two crystal forms exhibit mostly unrelated packing interactions and because the local crystallographic disorder in the high-resolution form was resolvable, the two structures offer the most detailed view yet of the conformational preference and flexibility of an RNA pseudoknot.

Methods and Materials

Crystallization and Data Collection

Crystallization conditions of the trigonal form were previously described. A crystal was frozen without further cryoprotection and used for data collection. Separate low-, medium-, and high-resolution data sets were collected for a single crystal on the DND-CAT insertion device beamline at sector 5 of the APS. Cubic crystals were grown by using the sitting-drop vapor-diffusion technique. Droplets containing 2.7 mg/mL of RNA, 1 M ammonium sulfate, and 33 mM sodium citrate (pH 5.0) were equilibrated over 2 M ammonium sulfate at 4°C for 1 to 2 weeks. Crystals were cryo-protected in 2.4 M lithium sulfate, 100 mM sodium citrate, and 20% glycerol and frozen in liquid nitrogen. A complete data set from a single crystal was collected on the BL1-5 line at the Stanford Synchrotron Radiation Laboratory (SSRL), Stanford, CA. All data were integrated and scaled with DENZO and SCALEPACK.

Structure Refinement

The structure of the trigonal crystal form was isotropically refined with the program CNS. The 1.6-Å resolution structure served as a starting model. Regions displaying disorder were built into the so-called "omit electron density" maps by using the program TURBO FRODO. Anisotropic refinement was carried out with the program SHELX97. The structure of the cubic crystal form was determined by the molecular replacement technique with the program AmoRe, by using the refined trigonal structure minus the looped-out and capping residues as a starting model. Several regions were manually rebuilt, and refinement was carried out subsequently with the program CNS.

Metal Ion Binding

To potentially identify alkali metal ions (Na^+ or K^+) in the trigonal crystal form, a variety of native data sets were collected at low-energy wavelengths of >1.5 Å to maximize the anomalous contribution of potassium. In addition, trigonal crystals were soaked in solutions of potassium chloride to enhance the occupancy of metal ions putatively bound to the RNA. In an alternative approach, thallium acetate was added to droplets containing trigonal crystals so that the final concentration of Tl^+ was between 25 and 50 mM. Diffraction data for soaked crystals were collected at a wavelength of 0.97626 Å (12.700 keV; the theoretical value for the L-III edge of Tl is 12.658 keV or 0.97950 Å). These data were then used to generate anomalous difference maps ($[F_{ano} - F_{calc}] \times \phi_{calc}$) in order to determine if thallium ions had potentially replaced potassium ions.

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