Crystal structures of MS2 and Qβ RNA stemloop operators complexed with a bacteriophage MS2 coat protein mutant.

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Introduction

MS2 and Qβ are evolutionarily related T=3 icosahedral bacteriophages with single stranded RNA genomes that infect E. coli. Although the protein subunits of the MS2 and Qβ capsids share less than 25% sequence identity, the structures of the Qβ subunits are very similar to those of MS2. Subunits of both the MS2 and Qβ capsid shells exist as three distinct conformers (A, B and C) that associate to form AB and CC dimers which comprise the basic building blocks of both capsid shells. The structures of the MS2 and Qβ capsids have been determined via X-ray crystallography by our collaborators in Uppsala, Sweden.

The two bacteriophages both utilise a similar mechanism of translational repression. In vivo, a small RNA stemloop within the viral genomes binds to a specific site on a coat protein dimer, acting to inhibit viral replicase gene translation. The translational complex of MS2 (Fig. 1) has, for many years, been the paradigm for studying RNA/protein interactions at the atomic level.

The RNA stemloop operator binding site is located on a 10 stranded β sheet formed by AB and CC dimers within the capsid shells of both MS2 and Qβ. Many of the amino acid residues that have previously been shown to be important for high affinity binding of MS2 and Qβ stemloops are conserved between the two bacteriophages (Fig. 1). Although the protein surfaces of the stemloop binding site of the two bacteriophages display considerable similarity, profound differences exist in the sequence and secondary structures of the two stemloop operators (Fig. 2).
In vivo, each bacteriophage preferentially discriminates against binding the stemloop operator of the other. Affinity binding studies have, however, identified specific coat protein mutants of MS2 that overcome this discrimination mechanism, some of the mutations allowing the binding of the Qβ RNA operator to MS2 mutant capsids with an affinity comparable to that of the wild type MS2 operator. In order to gain new insight into this discrimination mechanism, Qβ RNA stemloop operators were soaked into pre-crystallised MS2 mutant capsids and the structure of the capsid/RNA complexes determined via X-ray crystallography.

Results

Thus far, crystallographic studies have concentrated on a single mutation (N87S) within the MS2 coat protein, a mutation that decreases the capsids affinity for the MS2 stemloop operator whilst increasing affinity for the Qβ operator. Data were collected for the RNA free MS2 mutant capsid and MS2 mutant capsids soaked with wild type MS2 and Qβ stemloop operators at the SRS, Daresbury, UK.

Electron density maps demonstrated that there was little change in the mode of binding of the MS2 operator with the exception of two novel water mediated hydrogen bonds between the N87S mutation and the RNA operator. Electron density for the Qβ operator was weak in the N87S mutant/Qβ RNA complex, however, a tentative model for Qβ binding has been proposed. Further structural determinations using other MS2 mutant capsids are underway to test the validity of this hypothesis.

Collaborator

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