Crystal structures of four chemically modified RNA translational operators complexed with bacteriophage MS2 coat protein.

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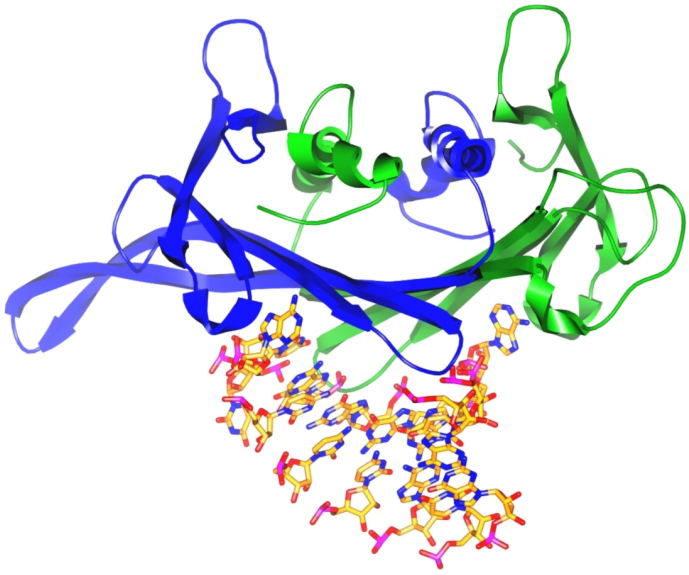
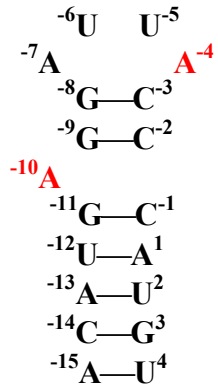
Introduction

MS2 is a *T=3* icosahedral bacteriophage with a single stranded RNA genome that infects *E. coli*. The coat protein subunit exists as three conformers (A, B and C), with the same amino acid sequence but with distinct tertiary structures. Two types of protein dimer (AB and CC) are present within the capsid shell. As a consequence of conformer folding and packing, pores connecting the capsid interior with the external environment are present at both the 3-fold and 5-fold symmetry axes. The pores are large enough to allow the passage of single stranded nucleic acid molecules in to and out of the capsid. *In vivo,* a 19nt RNA stemloop within the viral genome binds to a specific site on a coat protein dimer, acting both to initiate phage self assembly and to inhibit viral replicase gene translation (Figure 1). The translational complex formed has, for many years, been the paradigm for studying RNA/protein interactions at the atomic level.

The adenines at the –10 and the –4 positions in the RNA stemloop (Figure 1) have been shown to be important determinants of binding affinity, forming three and four hydrogen bonds with amino acids of the capsid respectively. In order to investigate the effects of removing these specific interactions RNA stemloops with 4-methyl-indole and purine moieties (Figure 1) substituted at both –10 and –4 positions were synthesised. The modified





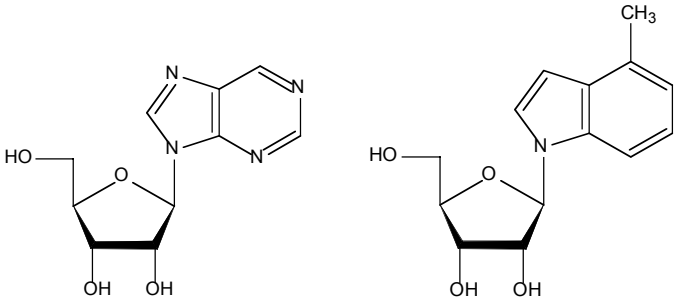


Figure 1;
(Top) Structure of WT MS2 RNA stemloop-coat protein dimer complex, protein subunit A (blue) and subunit B (green).
(Middle) Secondary structure of WT MS2 RNA stemloop. Adenines at –10 and –4 positions are indicated in red.
(Bottom) Structure of purine (left) and 4-methyl indole (right) moieties incorporated into stemloops.

stemloops were soaked into pre-crystallised MS2 capsids, the RNA entering via the capsid pores, and the structure of the capsid/RNA complex determined via X-ray crystallography.

Results

Data were collected for all four modified stemloops at the SRS, Daresbury, UK. Preliminary electron density maps demonstrated that purine moieties incorporated at either the –4 or –10 positions led to no gross changes in the RNA/protein interface. The loss of the exocyclic amino group from the base at these positions however, did lead to the loss of one hydrogen bond interaction in the case of the –10 and two hydrogen bonds in the case of the –4 substitution. In the case of the 4-methyl indole substitutions, which removed all hydrogen bonding potential from the base, only weak electron density for the RNA was observed. Further structural refinement and analysis is currently underway.

Funding

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