Asymmetric interactions in the adenosine-binding pockets of the MS2 coat protein dimer

ABSTRACT

These experiments provide functional tests of interactions predicted from structural analyses, demonstrating the importance of certain amino acid-nucleotide contacts observed in the crystal structure, and showing that others make little or no contribution to the stability of the complex. In summary, Val29 and Lys61 form important stabilizing interactions with both A-4 and A-10. Meanwhile, Ser47 and Thr59 interact primarily with A-10. The important interactions with Thr45 are restricted to A-4.

INTRODUCTION

Introduction The interaction of MS2 coat protein with its translational operator is one of the best-understood examples of RNA-protein recognition, having been studied extensively by genetic, biochemical, and structural means. The primary and secondary structures of operator RNA are shown in Figure 1. This RNA hairpin makes contacts with both subunits of a coat protein dimer. One of the most important features of the complex of coat protein with its RNA target is the insertion of two unpaired adenosines into equivalent pockets on different subunits of the coat dimer (Figure 2). The interactions of A-4 and A-10 with coat protein involve non-identical contacts with the same five amino acid residues, namely Val29, Thr45, Ser47, Thr59 and Lys61. X-ray crystallographic analysis infers specific amino acid-nucleotide contacts, but does not by itself allow a clear definition of their individual contributions to RNA-binding and translational repression. In the experiments described here we introduced amino acid substitutions of A-pocket amino acids in single-chain coat protein heterodimers in order to determine the role of each residue in interaction with A-4 and A-10.

CONCLUSION

Conclusions The results we present here emphasize the importance of combined functional and structural approaches to understanding molecular interactions. Some of the contacts observed in the x-ray structure of the MS2 RNA-protein complex clearly make little or no contribution to its stability. Our results are summarized in Figure 4, where we schematically illustrate the important interactions at A-4 and A-10 in the context of the structure of the whole translational operator. To recapitulate, Val29 and Lys61 make important stabilizing interactions with both A-4 and A-10. On the other hand, the contributions of Thr45, Ser47 and Thr59 are highly asymmetric. Thr45 contributes to binding only through its interaction with A-4, while the effects of Ser47 and Thr59 are confined to their interactions with A-10.