doi:10.1016/j.jmb.2005.11.046 *J. Mol. Biol.* (2006) **356**, 613–624

JMB

Available online at www.sciencedirect.com
SCIENCE DIRECT

ELSEVIER

Alanine Scanning of MS2 Coat Protein Reveals Protein–Phosphate Contacts Involved in Thermodynamic Hot Spots

Dagmar Hobson and Olke C. Uhlenbeck*

Department of Biochemistry Molecular Biology, Cell Biology Northwestern University 2205 Tech Drive, Hogan 2-100 Evanston, IL 60208, USA

*Corresponding author

The co-crystal structure of the MS2 coat protein dimer with its RNA operator reveals eight amino acid side-chains contacting seven of the RNA phosphates. These eight amino acids and five nearby control positions were individually changed to an alanine residue and the binding affinities of the mutant proteins to the RNA were determined. In general, the data agreed well with the crystal structure and previous RNA modification data. Interestingly, amino acid residues that are energetically most important for complex formation cluster in the middle of the RNA binding interface, forming thermodynamic hot spots, and are surrounded by energetically less relevant amino acids. In order to evaluate whether or not a given alanine mutation causes a global change in the RNA–protein interface, the affinities of the mutant proteins to RNAs containing one of 14 backbone modifications spanning the entire interface were determined. In three of six protein mutations tested, thermodynamic coupling between the site of the mutation and RNA groups that can be even more than 16 Å away was detected. This suggests that, in some cases, the mutation may subtly alter the entire protein–RNA interface.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: alanine scanning; backbone modifications; energetic coupling; equilibrium binding constant; RNA–protein interaction

Introduction

RNA binding proteins often achieve part of their affinity and specificity by making contacts with the RNA backbone phosphates, which are presented in a specific spatial orientation defined by the RNA secondary and tertiary structure.[1–6] Several of such protein–phosphate contacts are observed in the co-crystal structure of the MS2 coat protein with its RNA hairpin operator, where eight amino acids form charged hydrogen bonds or ion pairs with seven of the RNA phosphates.[7] Furthermore, the strong ionic strength dependence of RNA binding to the MS2 coat protein suggests a substantial thermodynamic contribution of this electrostatic component to the free energy of binding.[8,9]

In order to understand if a particular protein–phosphate contact makes an energetic contribution to the overall binding affinity, one of the interacting residues can be modified and the change in complex stability can be determined. Indeed, the affinity of the MS2 coat protein to RNA hairpins containing single phosphorothioate or methylphosphonate substitutions indicated that all of the protein–phosphate contacts contribute to the overall binding affinity.[10,11] However, the effect of the two types of phosphate modifications on the free energy of complex formation was often not the same at a given site, as the two modifications differ significantly in their properties. Substitution of a sulfur for one of the non-bridging oxygen atoms in the phosphorothioate linkage modifies the hydrogen bonding capability of the phosphate and redistributes the negative charge with the majority of the charge being on the sulfur. In contrast, the methylphosphonate linkage lacks the negative charge and eliminates hydrogen-bonding capability, but may form new hydrophobic interactions and/or change the structure and flexibility of the RNA. Therefore, neither of these substitutions provided a clear estimate of the thermodynamic contribution of a given phosphate contact.

Single-site alanine mutagenesis has been very useful for studying the thermodynamics of protein–

E-mail address of the corresponding author: o-uhlenbeck@northwestern.edu

0022-2836/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.

protein interfaces and protein–ligand interactions on the molecular level.[12–21] Assuming that an alanine substitution removes all interactions of the side-chain beyond the C^{β}-atom without introducing new properties, changes in the free energy of complex formation due to the mutation should provide a reasonable estimate of the energetic content of the disrupted contacts. Here, the eight amino acids of the MS2 coat protein that contact seven phosphates of the RNA operator as well as five control amino acids were individually replaced by alanine residues. Comparison of the RNA affinity of these mutant proteins with that of the wild-type protein identified thermodynamically relevant protein–phosphate contacts.

In order to test whether an observed change in the RNA binding affinity due to an alanine mutation reflected a local loss of a contact or a more global rearrangement of the RNA–protein interface, six of the alanine mutations were assayed for binding to a panel of 14 RNA hairpins containing single backbone modifications. In all cases, modification of the RNA at the site that contacts the mutated amino acid is expected to leave the binding affinity unchanged compared to that observed for the unmodified RNA. However, if the mutation only locally disrupts the interface, all the other RNA modifications should have the same deleterious effect on binding to the mutant protein as they do to the wild-type protein. In contrast, if the mutation causes a more global rearrangement of the interface, modification of one or more of the distal RNA backbone groups could differentially affect binding to the mutant protein compared to the wild-type protein. Such cases of thermodynamic coupling indicate that these mutations cause an overall change in how the protein interacts with the RNA.

Results and Discussion

Effect of alanine substitutions on complex stability

In the co-crystal structure of the MS2 coat protein dimer with its RNA hairpin, 90 coat protein homodimers assemble into a phage-like capsid with each dimer binding a single RNA hairpin.[7] The assembly induces an asymmetry in the structure of 60 of the dimers (called the AB dimers). As a consequence, binding of the RNA hairpin to these AB dimers is observed in only one of the two possible orientations (related by the pseudo 2-fold symmetry axis), resulting in a unique set of interactions with amino acids of both the A and the B-subunits (Figure 1). In contrast, the remaining 30 CC dimers are fully symmetric, so that the RNA hairpin binds in either orientation and therefore does not yield a well-resolved structure. When free in solution, the MS2 coat protein forms a symmetrical dimer which binds a single RNA hairpin in two possible orientations. Thus, in order to perform interpretable site-directed mutagenesis experiments, it was necessary to mimic the asymmetry of the viral AB subunits in the crystal. This was done using the method described by Peabody and Lim to link the two monomers into a single polypeptide chain[22] and then introducing a mutation of Tyr85 into one of the two monomers. This highly deleterious Tyr85Ala mutation[9] weakens RNA binding 700-fold, when present in both subunits (Table 1) and only decreases binding twofold, when mutated in a single subunit (L. S. Behlen & O.C.U., unpublished data). Therefore, a Tyr85Ala in just one subunit of the linked dimer should (in more than 99% of the molecules) fix binding of the RNA hairpin in a single orientation, with the RNA loop interacting with Tyr85 in the unmodified subunit and the RNA stem interacting with the subunit containing the Tyr85Ala mutation. Binding experiments with various phosphorothioate containing RNAs supported the idea that RNA binding to the linked dimer containing the single Tyr85Ala mutation closely resembles that observed with the unlinked wild-type dimer, suggesting that the presence of the linker and the Tyr85Ala mutation do not alter the overall thermodynamics of the RNA-protein interface in solution (L. S. Behlen & O.C.U., unpublished data). However, it remains possible that one or more of the RNA–protein interactions formed in solution could differ from those observed for the unlinked AB dimer in the crystal structure.

In order to probe the protein–phosphate contacts formed in solution, the eight amino acids (LysA43, ArgA49, TyrA85, ArgB49, SerB52, AsnB55, LysB57 and LysB61) shown to interact with the RNA phosphates in the co-crystal structure, as well as five amino acids (LysB43, SerA52, AsnA55, LysA57 and LysA61) that do not, were individually replaced by alanine residues in the linked dimer protein containing the single Tyr85Ala mutation. As explained in Materials and Methods, the subunit of this linked protein dimer that does not contain the Tyr85Ala mutation corresponds to the crystallographic A-subunit regardless of whether it is in the N-terminal or C-terminal half. Most of the mutations are not expected to disrupt the structure of the free protein, because nearly all of the replaced side-chains are exposed on the surface of the extended β-sheet that binds the RNA. Thus, for most of the mutations, any decrease in binding affinity probably reflects the disruption of interactions made in the complex. Possible exceptions are mutations of Lys57, Lys43 and Arg49. In the free protein, the side-chain of Lys57 is partly buried under the side-chain of Arg49 and all three residues form ionic interactions with negatively charged amino acids in both the free protein and the complex. Replacing these amino acids with an alanine will therefore not only remove all direct interactions of that amino acid with the RNA, but will also disrupt the stacking interaction and ion pairs within the protein. Consequently, if the loss of the surface ion pair does not destabilize the free protein and the complex to the same extent, part of

Download English Version:

https://daneshyari.com/en/article/2190076

Download Persian Version:

https://daneshyari.com/article/2190076

Daneshyari.com