Specific RNA Binding by Qβ Coat Protein†

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ABSTRACT: The interaction between the bacteriophage Qβ coat protein and its specific binding site on Qβ genomic RNA was characterized by using a nitrocellulose filter binding assay. Qβ coat protein bound to a synthetic 29-nucleotide RNA hairpin with an association constant of 400 μM^{-1} at 4 °C, 0.2 M ionic strength, pH 6.0. Complex formation had a broad pH optimum centered around pH 6.0 and was favored by both enthalpy and entropy. The salt dependence of K_a revealed that four to five ion pairs may be formed in the complex although approximately 80% of the free energy of complex formation is contributed by nonelectrostatic interactions. Truncation experiments revealed that coat protein binding required only the presence of a hairpin with an eight base pair stem and a three-base loop. Analysis of the binding properties of hairpin variants showed that the sequence of the stem was not important for coat protein recognition and only one of the three loop residues was essential. A bulged adenosine present in the coat protein binding site was not required for coat protein binding. Qβ coat protein binding specificity is therefore primarily achieved by the structure and not by the sequence of the operator.

As in other *Escherichia coli* RNA bacteriophage, the coat protein of Qβ acts as a translational repressor by binding to a site on the Qβ RNA that contains the initiation codon of the replicase gene (Weber, 1976). Nuclease protection experiments have established that the site includes a small hairpin containing an eight base pair stem, a bulged A, and a three-base loop (Figure 1). Similar hairpins are present in the replicase translational operators of the related RNA bacteriophage R17, fr, and GA (Uhlenbeck, 1986). Extensive characterization of the interaction between the R17 coat protein and its translational operator has shown that binding specificity is achieved by the structure of the hairpin and the presence of four essential single-stranded residues, including a bulged purine (Romaniuk et al., 1987; Wu & Uhlenbeck, 1987).

In this paper, the interaction between the Qβ coat protein and its translational operator is studied in detail. A filter binding assay was established to examine the solution properties of the interaction. Variants of the Qβ operator were synthesized to determine the size of the binding site and the sequence and structural requirements of binding. Many similarities to the R17 system were found as well as several remarkable differences.

Materials and Methods

Coat Protein. Bacteriophage Qβ was propagated on *E. coli* Q13 and purified according to Carey et al. (1983a). Purified phage was stored at approximately 10^{16} pfu/mL in 150 mM NaCl/15 mM sodium citrate, pH 6.7, at 4 °C. Qβ coat protein was prepared by a modification of the cold acetic acid method of Sugiyama et al. (1967). Approximately 10^{15} pfu of Qβ phage in 2.2 mL of cold 66% acetic acid was vortexed for 30 s every 10 min for 1 h. Qβ RNA was precipitated by centrifugation at 10000 rpm for 20 min at 4 °C. The supernatant was removed to within 0.5 cm of the pellet and dialyzed against 3 × 1 L changes of 1 mM acetic acid/10 mM dithiothreitol, pH 3.2, overnight at 4 °C. The dialysis tubing had a molecular weight cutoff of approximately 12000 and was EDTA treated before use. The coat protein was stored at a concentration of 50 μM in 1 mM acetic acid/20 mM dithiothreitol, pH 3.2 at 4 °C. Stored protein maintained its activity for greater than 5 months with bimonthly additions of dithiothreitol. Protein concentrations were determined by the Bio-Rad protein assay using R17 coat protein as a standard.

RNAs. Qβ and R17 genomic RNAs were purified from bacteriophage by phenol extraction (Kolakofsky, 1971) followed by EtOH precipitation. Yeast $tRNA^{Phe}$ was purchased from Boehringer Mannheim.

Sixteen different RNA fragments were prepared by in vitro transcription from synthetic DNA templates by T7 RNA polymerase (Milligan et al., 1987). Transcription reactions contained 40 mM Tris-HCl (pH 8.1 at 37 °C), 1 mM spermidine, 5 mM dithiothreitol, 50 μg/mL bovine serum albumin, 0.01% (v/v) Triton X-100, and 80 mg/mL poly(ethylene glycol) (M_r 8000). Labeled RNAs were prepared in a 40-μL transcription reaction with 1 mM each of three NTPs, 0.25 mM [α-^{32}P]NTP (5 μCi), 6 mM $MgCl_2$, 100–400 nM template, and 0.037–0.11 mg/mL T7 RNA polymerase. Unlabeled RNAs were prepared in a 10-mL reaction with 4 mM of each NTP, 24 mM $MgCl_2$, 400 nM template, and 0.037–0.11 mg/mL T7 RNA polymerase. RNA fragments were gel purified by electrophoresis on 20% denaturing polyacrylamide sequencing gels, cut out, soaked in 0.5 M NaOAc (pH 5.2), and ethanol precipitated (Carey et al., 1983a). The sequence of each fragment was confirmed by complete T1 digestion patterns.

Nested sets of 3′ and 5′ truncated RNA fragments were obtained by partial alkaline hydrolysis of fragment **1**. The 5′-triphosphate of unlabeled fragment **1** was removed by digestion with calf intestine phosphatase. The RNA was then gel purified and either 5′-^{32}P labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP or 3′-^{32}P labeled with T4 RNA ligase and [5′-^{32}P]pCp (England & Uhlenbeck, 1978); 12 μCi of gel-purified 5′-^{32}P-labeled RNA and 6 μCi of gel-purified 3′-^{32}P-labeled RNA were incubated in 120 mM $NaHCO_3$/Na_2CO_3 and 3 mM EDTA, pH 9.0, for 10 min at 95 °C. The reactions were terminated by freezing in a dry ice/ethanol bath and partial digestion products separated by electrophoresis on

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20% denaturing polyacrylamide gels. Each band was cut out, soaked in 0.5 M NaOAc (pH 5.2), and ethanol precipitated. The length of each RNA was determined by comparison to partial T1 digestion patterns of the 5′- and 3′-^{32}P-labeled full-length RNA.

All RNAs were stored in 10 mM Tris/0.1 mM EDTA, pH 7.0 at −20 °C.

Filter Binding Assays. Qβ coat protein was diluted to appropriate concentrations in coat protein storage buffer, added to ^{32}P-labeled RNA in 500 μL of MMK buffer [0.1 M MES, pH 6.0, 80 mM KOAc, 10 mM $Mg(OAc)_2$, and 80 μg/mL BSA], and incubated at 4 °C for 1 h. All RNAs were heated in RNA storage buffer to 90 °C for 3 min and quick-cooled on ice before use; 450 μL of each reaction was filtered through 24-mm, 0.45-μm pore size, nitrocellulose filters (MSI, Westborough, MA) which had been presoaked in MMK buffer at 4 °C for 1 h. Filters were dried in scintillation vials at 180 °C for 10 min and counted in 0.4% 2,5 diphenyloxazole in toluene. Backgrounds obtained in the absence of protein were less than 10% of the input radioactivity and subtracted in all cases. Protein excess assays contained ^{32}P-labeled RNA (~10 pM) and coat protein dimer concentrations from 0.1 nM to 0.5 μM. Retention efficiencies of the RNA ranged from 40% to 60%. RNA excess assays contained 50 nM coat protein dimer and 10 nM to 0.15 μM ^{32}P-labeled RNA.

Dissociation rates for coat protein–RNA complexes were determined by incubating ^{32}P-labeled RNA (~10 pM) with 15 nM active coat protein dimer in 4.2 mL of MMK buffer under standard assay conditions. Dissociation was observed by the addition of unlabeled Qβ operator RNA to 1 μM, and 400-μL aliquots were filtered at 0.5-min intervals. The half-life of the complex ($t_{1/2}$) was obtained from a plot of log (complex remaining) versus time and K_{off} calculated from $K_{off} = (\ln 2)/t_{1/2}$.

Competition assays were performed by incubating 10 nM ^{32}P-labeled fragment **1** and 30 nM active coat protein dimer in MMK buffer at 4 °C for 1 h. Unlabeled competitor RNA at concentrations up to 0.5 μM was added and the reaction incubated an additional hour before filtration. The data were analyzed by the method of Lin and Riggs (1972), and a K_a was estimated for each competitor RNA.

Computer Search. Nucleic acid sequences were searched for putative Qβ coat protein binding sites by using the program SEARCH from the Delila system (Schneider et al., 1982). Two sets of sequences were used to search for coat protein binding sites: N1-N2-N3-N4-N5-N6-N7-X1-X2-A-N7^c-N6^c-N5^c-N4^c-N2^c-N1^c and N1-N2-N3-N4-N5-N6-X1-X2-A-N6^c-N5^c-N4^c-N3^c-N2^c-N1^c. Xn is any nucleotide, and Nn^c is any nucleotide complimentary to Nn. The searches located any six base pair stem with a three-base loop containing an A at the +8-position. The first search located hairpins with a nucleotide bulge at the +1-position, and the second search located bulgeless hairpins.

Results

Binding of the Qβ Operator. The relevant region of the Qβ genome near the start site of the replicase gene is shown in Figure 1. The minimum RNA fragment protected from T1 digestion by the Qβ coat protein (indicated by arrows) can be drawn as an eight base pair hairpin with additional 5′- and 3′-nucleotides (Weber, 1976). The synthetic Qβ operator used for filter binding studies (fragment **1**, Figure 1) was identical with the minimal genomic coat protein binding site except for the removal of one 3′-nucleotide and the addition of two 5′-terminal guanosines. These changes were made in order to increase the yield of T7 transcription (Milligan et al., 1987).

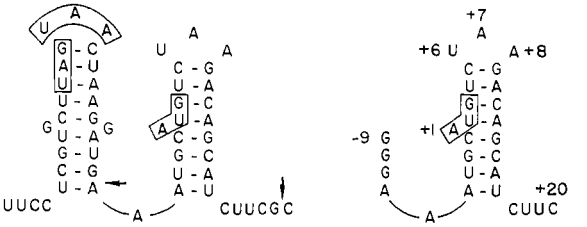

FIGURE 1: (Left) Possible secondary structure of the Qβ genomic RNA near the translational start site of the replicase gene. The A1 protein (Hofstetter et al., 1974) stop sites and the replicase start site are boxed. Arrows indicate the minimum size fragment protected from T1 digestion by the Qβ coat protein (Weber, 1976). (Right) RNA hairpin transcribed from synthetic DNA used for coat protein binding assays (fragment **1**). Numbers indicate the nucleotide position relative to the A(+1) of the replicase start codon.

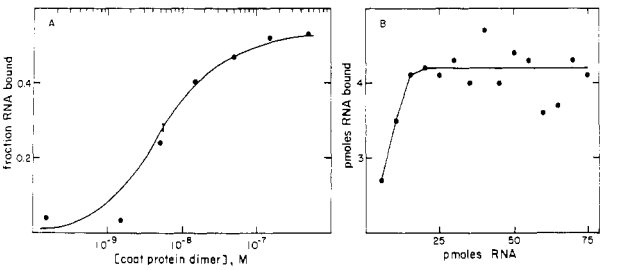

FIGURE 2: (A) Coat protein excess binding curve for ^{32}P-labeled fragment **1** assayed in MMK buffer, pH 6.0, 4 °C. The solid line is a theoretical binding curve calculated by using a retention efficiency of 54% and a half-saturation of 5.5 nM. (B) RNA excess binding curve. 25 pmol of Qβ coat protein dimer (50 nM) was incubated at 4 °C with 5–75 pmol of RNA in MMK buffer, pH 6.0, 4 °C. 4.2 pmol of RNA was bound at saturation.

A two-step procedure was used to determine the binding affinity of the coat protein for the synthetic binding site. Protein–RNA complexes were formed by incubating coat protein and RNA together in MMK buffer at 4 °C for 1 h and detected by retention on a nitrocellulose filter. Figure 2A shows a typical protein excess assay in which a constant, low concentration (~10 pM) of ^{32}P-labeled RNA is incubated with varying concentrations of coat protein. The protein concentration at half-saturation is equal to the inverse of the equilibrium constant for the reaction if the fraction of protein active in RNA binding is known. A RNA excess assay was used to calculate the percentage of active coat protein. In this assay, the coat protein concentration is held constant well above half-saturation, and the labeled RNA concentration is varied in the neighborhood of the coat protein concentration. As shown in Figure 2B, the amount of RNA bound increases with the RNA concentration until the protein becomes saturated. By correcting for the 54% retention efficiency in Figure 2A, 7.8 pmol of RNA is calculated to be bound at saturation. This corresponds to approximately 16% of the moles of coat protein present. Early studies on the R17 coat protein–RNA interaction suggested a stoichiometry of one protein per RNA (Gralla et al., 1973; Carey et al., 1983a), but a more complete investigation (Beckett & Uhlenbeck, 1988) using fluorescence quenching, filter binding, and gel filtration chromatography showed that each dimer of R17 coat protein binds one operator. If we assume that like R17 and other T = 3 phage (Rossman, 1984) each Qβ coat protein dimer binds a single hairpin, we conclude that 32% of the protein is active. Thus, the K_a in Figure 2A is 570 μM^{-1}. This method was used for all subsequent K_a determinations. The percentage of active protein varied from between 29% and 35% from one coat protein preparation to another but did not vary with buffer conditions

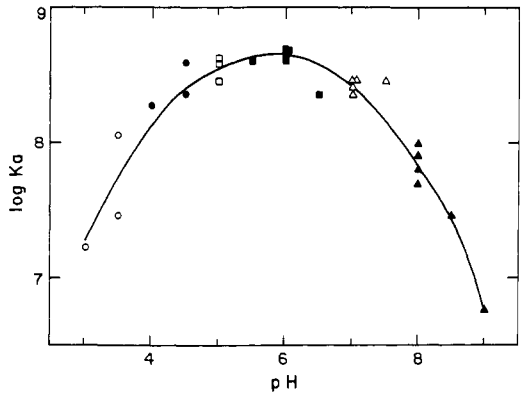

FIGURE 3: pH dependence of K_a. Filtration assays were performed with fragment **1** at 4 °C, 0.2 M ionic strength, and either sodium citrate (○), NaOAc (●), Tris–acetate (□), MES (■), MOPS (△), or Tris (▲) adjusted to the indicated pH.

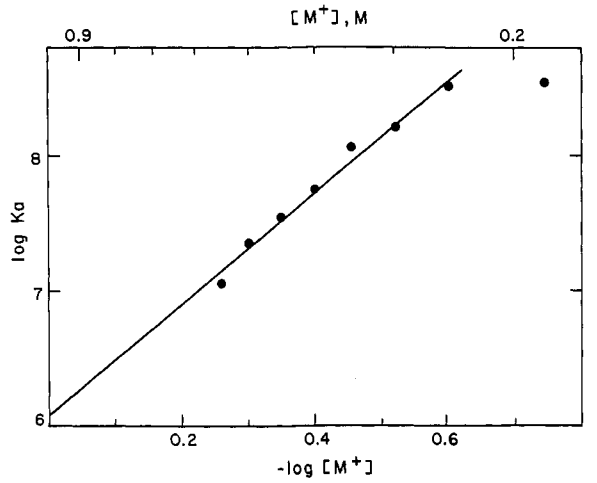

FIGURE 4: Salt dependence of K_a. Filtration assays were performed in MMK buffer, pH 6.0, 4 °C, with the KOAc concentration adjusted to give the total cation concentration, [M+], indicated. The slope of the line corresponds to 4.8 ion pairs formed between the protein and the RNA when Ψ is assumed to be 0.85, as for the R17 synthetic operator (Carey & Uhlenbeck, 1983).

or temperature. The precision in determining K_a by this method was within a factor of 2 for independent replicates.

Filter binding assays were obtained as a function of pH by substituting a buffer with an appropriate pK_a for the standard MES buffer. The Qβ coat protein–RNA interaction showed a broad pH optimum from pH 5.5 to pH 6.5 (Figure 3). The moderate pH dependence indicates that only a small number of titratable groups are involved in the interaction.

The ionic strength dependence of K_a was determined by performing standard filter binding assays in which KOAc was added to give the indicated cation concentration. Figure 4 shows that K_a decreases with increasing ionic strength, indicating that there are ionic interactions involved in complex formation. Record and co-workers have developed a quantitative analysis of the salt dependence of K_a for DNA–protein interactions based on ion displacement (Record et al., 1976). Because application of this analysis to RNA–protein interactions requires several untested assumptions, the number of ion pairs determined is considered to be an upper limit. From the slope of Figure 4, approximately 4.8 nucleotide phosphates on the RNA are involved in ion pairs with cationic groups on the protein. It has been estimated that at 1 M salt each lysine phosphate type ion pair contributes about +0.2 kcal/mol (Lohman et al., 1980). According to this estimate and a ΔG = −7.7 kcal/mol of complex at 1 M salt, the nonelectrostatic contribution to ΔG is −8.7 kcal/mol of complex, approximately

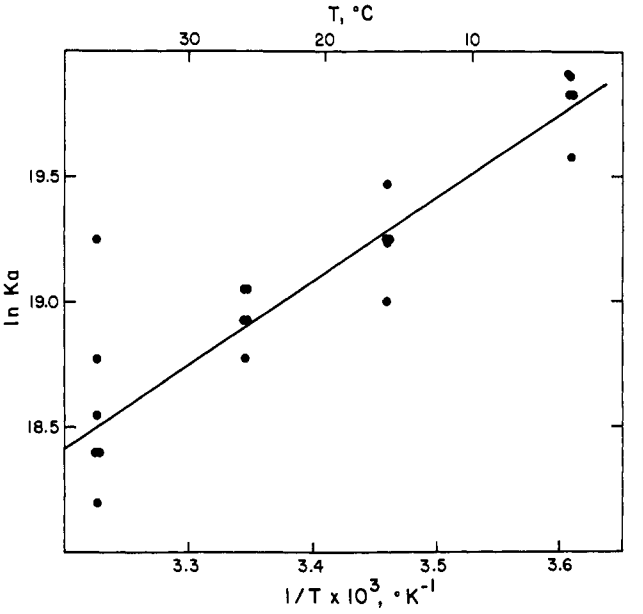

FIGURE 5: Temperature dependence of K_a for fragment **1**. Filtration assays were performed in MMK buffer, pH 6.0, with the temperature adjusted as indicated. The slope of the line corresponds to ΔH = −6.7 kcal/mol of complex.

80% of the total free energy of the interaction at standard assay conditions.

The temperature dependence of K_a was measured by performing standard filter binding assays at various temperatures. A van't Hoff plot of the data is shown in Figure 5. The ΔH obtained from the slope of the line is −6.7 kcal/mol of complex, and ΔS is calculated to be +15.2 cal K^{-1} (mol of complex)$^{-1}$ at 4 °C. Complex formation is therefore favored by both enthalpy and entropy.

Specificity of the Interaction. The specificity of the interaction was examined by measuring the competition for coat protein binding between ^{32}P-labeled fragment **1** and a variety of unlabeled RNAs. Figure 6A shows that intact unlabeled Qβ genomic RNA competes for coat protein binding with a K_a = 156 μM^{-1}. Thus, the two RNAs bind Qβ coat protein with approximately the same affinity. Identical results were obtained using unlabeled fragment **1** as competitor (Figure 6A). These results suggest that fragment **1** contains all the nucleotides required for specific binding.

Relatively weak competition was observed between fragment **1** and either a synthetic R17 translational operator or R17 genomic RNA (Figure 6B). Direct binding of labeled R17 translational operator to Qβ coat protein was not detected (data not shown). No competition was observed with yeast $tRNA^{Phe}$. It is therefore clear that the Qβ coat protein binds quite specifically to the Qβ translational operator site.

Size of the Binding Site. The size of the Qβ coat protein binding site was determined by performing standard filtration assays on oligomers truncated from either the 5′ or the 3′ terminus of fragment **1** (Table I). Only two nucleotides (−9, −8) could be removed from the 5′ terminus without affecting K_a. Removal of the next five nucleotides (−7, −6, −5, −4, −3) showed a small gradual reduction in K_a, and further removal of nucleotides from the 5′ terminus reduced K_a below the detection limit of the assay. Four nucleotides (+20, +19, +18, +17) could be removed from the 3′ terminus of fragment **1** without affecting K_a. Removal of the next two nucleotides (+16, +15) reduced K_a 1.7-fold and 4.8-fold, respectively. Further removal of nucleotides from the 3′ terminus reduced K_a below the detection limit of the assay.

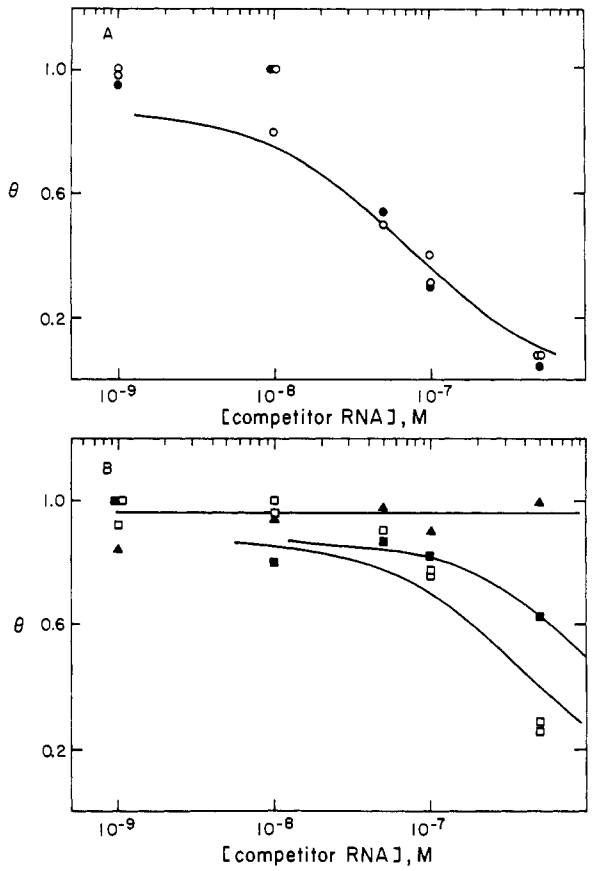

FIGURE 6: Competition for coat protein binding between 10 nM ^{32}P-labeled fragment **1** and unlabeled RNAs. (A) Qβ genomic RNA (○); fragment **1** (●). (B) R17 genomic RNA (□); R17 synthetic operator (■); yeast tRNA^{Phe} (▲). Solid lines are theoretical curves calculated for K_a = 400 μM^{-1} for ^{32}P-labeled fragment **1** and K_a values of 156, 156, 25, and 7.7 μM^{-1} for Qβ genomic RNA, fragment **1**, R17 genomic RNA, and the R17 synthetic operator, respectively. θ is defined as the ratio of radioactivity bound to the filter in the presence of competitor to the radioactivity bound in the absence of competitor.

Table I: Coat Protein Binding to Fragment **1** Truncated on the 5′ or 3′ End[a]

5′-nucleotide		3′-nucleotide	
removed	K_a (μM^{-1})	removed	K_a (μM^{-1})
	400		400
−9	440	+20	400
−8	440	+19	560
−7	240	+18	560
−6	330	+17	400
−5	240	+16	240
−4	150	+15	83
−3	150	+14	<30
−2	<30	+13	<30
−1	<30	+12	<30
+1	<30	+11	<30
+2	<30	+10	<30
+3	<30		

[a] Numbers of the nucleotides removed refer to the nucleotide position relative to A(+1) of the AUG start codon shown in Figure 1.

These results suggest that full binding requires the eight base pair hairpin from nucleotides −4 to +16 while a detectable level of binding can be achieved with nucleotides −2 to +14, encompassing only the upper six base pair hairpin. The much lower K_a values of fragments truncated further may be due either to the loss of specific coat protein contacts or to a decrease in the stability of the hairpins with shorter stems.

Sequence and Structural Requirements of the Qβ Coat Protein Binding Site. The sequence and possible secondary

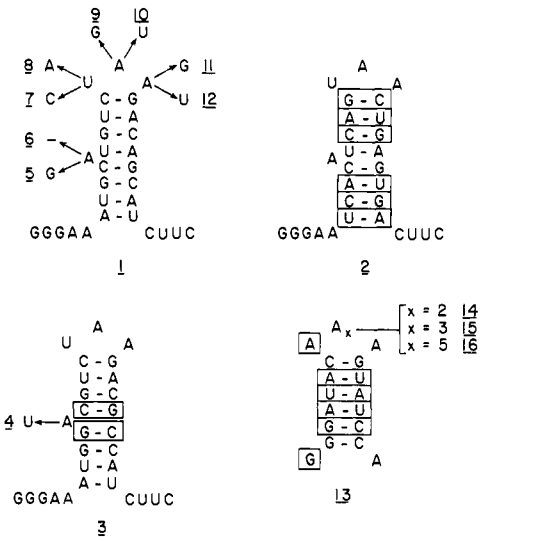

FIGURE 7: Sequences and possible secondary structures of RNA fragments. Nucleotides which differ from the Qβ operator are boxed. K_a values are given in Table II.

Table II: Coat Protein Binding to Qβ Operator Variants Shown in Figure 7

type of variant	fragment	K_a (μM^{-1})
	1	400
stem sequence	**2**	800
	3	480
bulge nucleotide	**4**	330
	5	310
	6	250
loop sequence	**7**	310
	8	330
	9	310
	10	220
	11	<4
	12	<4
loop size	**13**	82
	14	12
	15	<4
	16	<4

structures of a series of Qβ operator variants are shown in Figure 7. K_a values were measured under standard assay conditions and are shown in Table II. Dissociation rates measured for variants **1**, **2**, **5**, **7**, and **9** were consistent with K_a values measured by the equilibrium assay (data not shown).

The sequence of the stem is not important for coat protein binding. Variants **2** and **3** have altered stem sequences but retain the same number of base pairs as fragment **1**. Only a slight increase in K_a was measured for either variant.

The bulged A residue could be substituted or removed without affecting K_a. Placing a bulged U (variant **4**) or a bulged G (variant **5**) at the +1-position lowered K_a only slightly. The bulged U was placed in the stem sequence of variant **3** to avoid the possible formation of alternative secondary structures. Removing the bulged A entirely (variant **6**) gave only a slightly lower K_a than fragment **1**. This result was checked under several assay conditions which showed that the bulgeless variant had the same salt and temperature dependence as fragment **1** (data not shown).

The three-base loop contains only one essential residue for coat protein binding. Substituting a C (variant **7**) or an A (variant **8**) for the U at +6 had little effect on K_a. Similar results were obtained when the A at +7 was substituted with a G (variant **9**) or a U (variant **10**). When the A at +8 was substituted by a G (variant **11**) or a U (variant **12**), K_a was reduced by more than 100-fold to below the detection limit

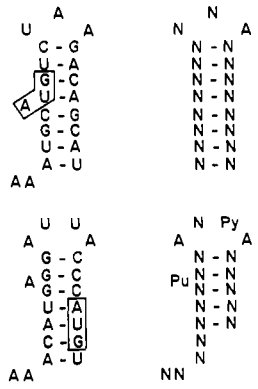

FIGURE 8: Binding sites (left) and models for binding sites (right) of the Qβ (top) and R17 (bottom) coat proteins. The start codon of the replicase gene is boxed. N represents G, A, C, or U, and N-N represents any Watson–Crick base pair.

of the assay. These results suggest that the A at +8 is essential for coat protein binding while the sequence of the other two loop nucleotides is unimportant.

Combination of the above results suggested that variant **13**, which has a 6 base pair stem, no bulged A, and a sequence which differs from fragment **1** at 10 of 17 positions, should bind the coat protein reasonably well. As shown in Table II, this variant bound the Qβ coat protein with an affinity only 5-fold lower than fragment **1**. Most if not all of this decrease in the binding affinity can be attributed to the loss of the two lower base pairs in the stem since as seen in Table I, removal of 5′-nucleotides to position −3 reduces K_a 3-fold and removal of 3′-nucleotides to position +15 reduces K_a 5-fold.

A series of hairpins, related to variant **13** and made previously for a separate study (Groebe & Uhlenbeck, 1988), were used to determine the size dependence of the loop for coat protein binding. When the loop size was increased to four bases (variant **14**), a large decrease in K_a was seen. Further increases in loop size to five bases (variant **15**) or to seven bases (variant **16**) lowered K_a below the detection limit of the assay. A three-base loop is therefore required for efficient coat protein binding.

Model of the Qβ Coat Protein Binding Site. The truncation experiment results and the binding properties of the hairpin variants suggest a model for the binding site of the Qβ coat protein. As shown in Figure 8, the site requires 19 nucleotides folded into a hairpin with an 8 base pair stem and a 3-base loop. The only sequence requirement is that the +8-position must be an A. As noted previously, the absence or presence of a bulged nucleotide at the +1-position has no effect on the binding affinity of the protein.

The model Qβ coat protein binding site contains 17 bits of binding information according to the criteria of Schneider et al. (1986). This method assigns an information value to the binding site based on the sequence variability of each nucleotide position in the model. The bulged nucleotide was assigned a value of −1 bit since neither its presence nor its absence was required. The information content of the Qβ binding site corresponds to a probability of finding 1 full-affinity Qβ binding site in 130 000 random nucleotides.

A search program was used to locate possible binding sites in the Qβ plus and minus strands and the MS2 plus strand. The search was designed to locate potential low-affinity binding sites by requiring only 6 base pairs in the stem and allowing for G-U base pairs, thereby increasing the probability of finding a binding site to 1 in 700 random nucleotides. A search of the Qβ plus strand revealed 9 possible binding sites, including the Qβ operator, and a search of the Qβ minus strand revealed 10 possible binding sites. Only 4 of the 18 nonoperator sites (positions 708 and 4132 of the plus strand and positions 71 and 3495 of the minus strand) had a high enough G-C content to form reasonably stable hairpin structures. The highly structured Qβ genome may prevent several of these sites from forming stable hairpins in vivo. Since the two plus strand sites lie within genes, it is unclear whether they have a role in regulating phage RNA or protein synthesis. Multiple coat protein binding sites could aid, however, in the initiation of capsid assembly (Beckett et al., 1988).

A search of the MS2 plus strand revealed six possible binding sites, two of which (positions 1455 and 2248) could form stable hairpin structures. One of these hairpins has been shown to exist in the secondary structure of the MS2 genome by structure mapping (Min Jou et al., 1972). This may explain the moderate competition observed between the R17 genomic RNA and fragment **1** for Qβ coat protein binding.

Discussion

Establishment of a filter binding assay for the Qβ coat protein and its translational operator permits comparison with the well-characterized R17 system (Carey & Uhlenbeck, 1983; Romaniuk et al., 1987; Wu & Uhlenbeck, 1987). In a general sense, the Qβ and R17 coat proteins recognize rather similar RNA structures (Figure 8). Both coat protein binding sites are small hairpins containing 19 nucleotides. The hairpins are approximately 30 × 20 × 20 Å in size which corresponds closely to the estimated size of the 28 000-dalton Qβ and R17 coat protein dimers. The length dependence of K_a suggests that contacts may be made between the surface of the protein and the entire hairpin in both systems. The Qβ and R17 binding sites also contain approximately the same amount of binding information. The greater number of base pairs required for binding the Qβ operator compensates for the fewer number of sequence-specific recognition sites. Thus, the probability of finding a Qβ coat protein binding site in a random sequence is approximately the same as the probability of finding a R17 coat protein binding site.

The solution properties of the two interactions are similar but not identical. Complex formation in both the Qβ and R17 systems involves only a small number of titratable groups. The pH optimum of the Qβ system is 2.5 pH units lower than the pH optimum of the R17 system, suggesting that the two systems utilize different sets of titratable groups for binding. Ionic interactions contribute to binding with four to five ion pairs formed in each homologous complex. In both cases, approximately 80% of the binding free energy is the result of nonelectrostatic interactions. The Qβ and R17 interactions have nearly the same ΔG under optimal in vitro binding conditions. The Qβ interaction, however, has a small favorable ΔH and a small favorable ΔS while the R17 system has a large favorable ΔH and a large unfavorable ΔS. The unfavorable ΔS is believed to be due to configurational constraints on the protein, RNA, or solvent upon binding that are not entirely compensated for by ion displacement. Any unfavorable configurational constraints in the Qβ system are apparently not of the magnitude found in the R17 system.

The sequence-specific recognition sites on the Qβ operator are quite different from those of the R17 operator (Figure 8). Four single-stranded residues are essential for coat protein binding of the R17 operator, including a bulged purine. In the Qβ operator, only one single-stranded residue is essential for binding. It is very surprising that the bulged A residue of the Qβ operator is not important for binding. Bulged A residues have been found in the binding sites of several phage coat proteins (Uhlenbeck, 1986) and several ribosomal proteins

(Garret et al., 1984), leading to the proposal that bulged A residues are a general feature of protein recognition (Peattie et al., 1981; Wickens & Dahlberg, 1987). Recognition could be specific for the bulged nucleotide or specific for the structure of the stem which may be bent as much as 20° by the bulge (Woodson & Crothers, 1988). The Qβ coat protein may be flexible enough to bend with the helix so that it can recognize a hairpin with or without a bulged nucleotide. The Qβ system illustrates the fact that the bulged A residue, often found in protein binding sites, may not always be essential and each case must be examined experimentally.

A Michael adduct has been proposed to occur between a cysteine of the R17 coat protein and a single-stranded U residue of the R17 translational operator (Romaniuk & Uhlenbeck, 1985). While the Qβ coat protein contains two cysteine residues located at similar positions to those in R17 coat protein and the Qβ operator contains a single-stranded U residue in the hairpin loop, no evidence of a Michael adduct was found. Substitution of the U at +6 by either a C or an A had no effect on K_a. In the R17 system, the U to C change stabilizes the covalent interaction and increases K_a approximately 50-fold (Lowary & Uhlenbeck, 1987). Michael adducts do not form with purines, and thus the U to A change decreases K_a approximately 10-fold in the R17 system (Carey et al., 1983b). The very different behavior of the two systems suggests that the Qβ system does not utilize the formation of a Michael adduct for binding.

Thus, we find that while the overall secondary structures of the Qβ and R17 translational operators are quite similar, the sequence-specific features required for recognition are idiosyncratic.

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