Interaction of *Escherichia coli* Host Factor Protein with Oligoriboadenylates†

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ABSTRACT: The interaction of *Escherichia coli* host factor 1 with oligoadenylate [oligo(A)] was studied by fluorescence and filter retention techniques. The intrinsic fluorescence of the host factor is quenched by up to 60% by the addition of oligo(A). Fluorescence titrations at high protein concentrations (6 μM) give a saturation point of 14 A residues per host factor hexamer regardless of chain length or ionic strength. Nitrocellulose filter retention experiments at much lower concentrations (1 nM) indicate equimolar complexes form between $(pA)_l$ ($12 < l < 27$) and host factor hexamers. The smallest number of contiguous A residues which allows the formation of all favorable protein–RNA contacts is 16 at both low and high salt concentrations. At 0.1 M NaCl, the molar association constants are in the range of 10^{10}–10^{11} M^{-1} ($15 < l < 27$) and decrease only slightly with ionic strength, indicating a large nonionic component in the interaction. Cyclized $(pA)_l$ was shown to have a higher affinity for host factor than its linear counterparts when l is 18 or greater but a lower relative affinity when l is 15. This suggests that the binding site on the hexamer has a circular spatial orientation.

Host factor 1 is an *Escherichia coli* protein which strongly stimulates the RNA-dependent RNA[1] synthesis activity of Qβ replicase when the plus strand of Qβ RNA is used as a template (Franze de Fernandez et al., 1972; Blumenthal & Carmichael, 1979). Host factor has little or no effect in stimulating RNA synthesis with Qβ minus strand, poly(rC), or other substrates of Qβ replicase (August et al., 1968). The protein purified from uninfected *E. coli* is a hexamer of identical subunits of 12 000–13 000 daltons each (Franze de Fernandez et al., 1972; Carmichael et al., 1975). Since maximal stimulation is observed with one to two hexamers per Qβ RNA molecule, it was thought that Qβ RNA had only a limited number of host factor binding sites. This view is supported by the observation that host factor binds Qβ RNA with a stoichiometry of two or three hexamers per RNA molecule and that two T_1 oligonucleotide fragments of Qβ RNA were found to have a high affinity for host factor (Senear & Steitz, 1976).

Our interest in the host factor–RNA interaction stems from the possibility that this system would be a good example of a simple sequence-specific RNA–protein interaction amenable to study by physical techniques. In this paper we investigate the interaction between host factor and both linear and cyclic oligoadenylylates. Carmichael (1975) has shown that poly(A) binds host factor better than other homopolymers. By varying the chain length and configuration of the oligo(A), the general nature of the RNA binding site of host factor is defined. In the following paper the interaction of host factor with homopolymers and natural RNAs is studied (de Haseth & Uhlenbeck, 1980) with the aim of determining the degree of specificity of the interaction.

Materials and Methods

Oligoadenylylates used in this work were prepared by partial alkaline hydrolysis of poly(A) with the help of Dr. T. England. Thirty-three milliliters of poly(A) (30 mg/mL) (Sigma Chemical Co.) in water was brought to 37 °C and 3.6 mL of 10 N KOH was added. Nine milliliters of the reaction mixture was removed at 4 min, brought to pH 3.0 with cold 1 M perchloric acid, and incubated at 37 °C for 60 min to hydrolyze terminal 2′,3′-phosphates. The remaining 27 mL of the base hydrolysis reaction was treated similarly after 15 min at 37 °C. The two aliquots were combined, brought to 10 mM Tris, pH 7.5, with Tris base and 1 M KOH, and centrifuged for 10 min at 5000*g* to remove the insoluble potassium perchlorate. The oligo(A) mixture was brought to 0.2 M KCl, applied to a 2 L (5 × 100 cm) RPC-5 column, and eluted with a 40-L linear gradient from 0.2 to 1.0 M KCl, 10 mM Tris, pH 7.5, at 340 mL/h. More than 50 peaks were well resolved (Walker et al., 1975; Wells et al., 1980) with the first 30 being isomeric pairs of $(Ap)_l$ having 2′- and 3′-terminal phosphates (Usher & Rosen, 1979) and the remaining 20 being mixtures of the two isomers. Pooled fractions were concentrated by rotary evaporation and desalted either by dialysis or by chromatography on Bio-Gel P2 (Bio-Rad Laboratories) columns. The chain length of several of the earlier eluting peaks was determined by comparing their mobility with standards of known length using analytical RPC-5 columns or using 20% acrylamide gel electrophoresis (Donis Keller et al., 1977). The chain length of the later eluting peaks was assigned by their elution position. In all cases at least 90% of each oligomer is of the assigned chain length; the major contaminant is one nucleotide shorter.

The $(Ap)_l$ column fractions were converted to $(Ap)_{l-1}A$ by incubation with bacterial alkaline phosphatase. The $(Ap)_{l-1}A$ were converted to $(pA)_l$ in reactions (25 μL) containing 0.2 mM $(Ap)_{l-1}A$, 4 mM ATP, 50 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 50 μg/mL bovine serum albumin, 5 mM DTT, and 40 units/mL Pset 1 polynucleotide kinase (Cameron et al., 1978). [5′-^{32}P]$(pA)_l$ was prepared in identical reactions except that 10 μM $(Ap)_{l-1}A$ and 10–20 μM [γ-^{32}P]ATP (250–500 Ci/mmol) were used. Under these conditions the reaction yields were quantitative. The reactions were incubated for 2 h at 37 °C and terminated by spotting on Whatman 3 MM

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[1] Abbreviations used: RNA, ribonucleic acid; oligo(A), oligoadenylate; oligo(C), oligocytidylate; poly(A), polyadenylate; poly(C), polycytidylate; ATP, adenosine 5′-triphosphate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DNA, deoxyribonucleic acid.

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paper. Excess ATP was removed by descending chromatography with a 70:30 (v/v) mixture of 1 M ammonium acetate and 95% ethanol. The regions of UV-absorbing material near the origins of the paper chromatograms were cut out, washed with 100% ethanol, and eluted with distilled water. Finally, the oligomer solutions were filtered through a Millipore HAWP filter. The molar residue extinction coefficient in 0.1 M NaCl and 10 mM Tris, pH 7.5, was assumed to be 9.9×10^3 L/(mol cm) at 260 nm for $l \geq 12$. Values for $l < 12$ are given in Janik (1971).

Cyclization of $(pA)_l$ was achieved in reaction mixtures (25 μL) containing 4 μM [5′-^{32}P]$(pA)_l$, 100 μM ATP, 50 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 8 mM DTT, and 400 units/mL T4 RNA ligase. Incubation was for 2 h at 37 °C. The extent of cyclization was confirmed to be quantitative by analysis on 20% acrylamide gels (Donis Keller et al., 1977). Cyclic $(pA)_l$ migrates faster than its linear counterparts for all lengths used in this study. The difference in mobility decreases with increasing chain length and is rather small for $(pA)_{27}$. For longer oligomers the situation is reversed (Kozak, 1979). No bands migrating slower than the linear form were seen, indicating that no self-addition reaction producing oligomers of greater chain lengths, occurred.

Host factor was purified in collaboration with C. Guerrier-Takada and P. Cole. Host factor activity was followed by measuring the stimulation of Qβ replicase activity on Qβ plus strand RNA using the conditions of DuBow & Blumenthal (1975) with minor modifications (I. Sawchyn, C. Guerrier-Takada, W. Szer, and P. Cole, unpublished experiments). Host factor was purified from 1 kg of frozen mid-log *E. coli* MRE 600 by the method of DuBow & Blumenthal (1975) as later modified by them (M. S. DuBow, personal communication). The heat step was omitted in order to avoid losses due to trapping of host factor in the bulk of the precipitating protein. $(NH_4)_2SO_4$ (70%) was used for concentration without significant losses of activity. The final phosphocellulose column was run in the presence of 6 M urea, a modification resulting in a sharper protein peak of higher purity. Half of the pooled host factor activity from the phosphocellulose column (1.4 mg) was concentrated on a 2.5 mL DE 52 column, dialyzed into 0.1 M NaCl, 50 mM Tris, pH 7.5, 1 mM EDTA, 0.1 mM DTT, and 5% glycerol, and stored at −20 °C.

The host factor preparation was characterized to be at least 85% pure by several criteria. (a) On a 15% polyacrylamide–$NaDodSO_4$ gel (Laemmli, 1970) more than 85% of the stained material ran as a single band slightly slower than lysozyme. The apparent molecular weight was 14 500 daltons, somewhat larger than the value of 12 000–13 000 reported previously (Franze de Fernandez et al., 1972; Carmichael et al., 1975). The most prominently stained contaminant (~10%) ran at 22 500 daltons. (b) Maximal stimulation of Qβ replicase activity on Qβ plus strand is reached at a host factor hexamer to Qβ RNA ratio of 2:1, based on the assumption of a pure protein. This value is in agreement with previous data (Franze de Fernandez et al., 1972). Heat treatment of the preparation (5′ at 95 °C) did not alter its stimulation behavior. (c) In Ouchterlony immunodiffusion tests with antiserum made against purified host factor (a gift of G. Carmichael via T. Blumenthal), a band of complete identity is found with our preparation and homogeneous host factor purified by G. Carmichael.

The concentration of host factor was determined by its ultraviolet absorption spectrum in 6 M guanidinium hydrochloride (Saxe & Revzin, 1978) where structural effects on the extinction coefficient are minimized. By use of Edelhoch's (Edelhoch, 1967) value for the molar extinction coefficient of tyrosine in 6 M guanididium hydrochloride at 275.5 nm and the finding that host factor has three tyrosines per subunit (Franze de Fernandez et al., 1972), the concentration of host factor subunits in the stock solution was found to be 29 ± 4.2 μM or 4.8 ± 0.7 μM hexamers. Measurement of the ultraviolet absorption spectrum of the native protein indicates that the absorbance at 277 nm of a 1 mg/mL solution of host factor is 0.40 ± .08. Filter binding experiments of oligo(A)'s with host factor suggest that the concentration of active host factor hexamers in the stock solution is ~3.8 μM (see Results). Either the impurities raise the apparent protein concentration or inactive host factor molecules are present in the preparation.

Fluorescence measurements were performed on a Hitachi Perkin-Elmer MPF 44A fluorometer. Due to the low signal of host factor solutions and the scarcity of material, 20-μL microcuvettes (round, 25 × 1.6 mm) were used. Typically, a 20-μL drop of host factor solution (3.0–3.8 μM) was placed on a piece of parafilm and 1-μL aliquots of RNA were added. After being mixed, the solution was transferred to the cuvette for measurement (excitation at 277 nm, emission at 303 nm). Measurements were carried out in a buffer containing 50 mM Tris, pH 7.5, 1 mM EDTA, 0.1 mM DTT, 5% glycerol, and 0.1 or 1.0 M NaCl. The intensities observed were corrected for loss of signal due to dilution. The fluorescence of the protein was not significantly affected by up to nine additions of 1 μL of buffer. A control titration of 4 μg/mL tyrosine, which gives a similar signal to our host factor solutions, with $(pA)_{15}$ showed no change in fluorescence.

The binding of host factor to oligo(A) causes a decrease in the intrinsic fluorescence of the protein. If F^0 is the fluorescence of the free protein and F is the signal when an amount of oligo(A) is added, the percent quenching, Q, is then expressed by $Q = 100(F^0 - F)/F^0$. It is assumed that the percent quenching is a linear function of the amount of protein–RNA complex in solution.

Filter binding assays (Riggs et al., 1970) were carried out in a buffer containing 3.5 mM β-mercaptoethanol, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5, 10 μg/mL bovine serum albumin, and NaCl added to the molarity indicated. The serum albumin increased the retention efficiency of the complexes without increasing the background. [5′-^{32}P]Oligo(A) (2×10^4–9×10^4 cpm) and indicated amounts of host factor were incubated in 1 mL of buffer for 15–30 min at room temperature. No time dependence of binding was observed in the range 1–30 min. The reaction mixture was transferred with a Pasteur pipet to a filtration setup containing a nitrocellulose filter (Millipore HAWP, 4.5-μm pore size, 16-mm exposed diameter) which had been soaked for 30 min in buffer without serum albumin. After rapid filtration (1 mL in 3 s) the filters were dried and counted without washing so as not to perturb the binding equilibrium. A background retention without added host factor of 2.5–5% was subtracted from all retention values.

In order to compare the binding affinity for several oligo(A)'s, it was convenient to compare their ability to compete with the same radiolabeled oligomer probe for host factor binding. In these experiments, 0.76 pmol of active host factor hexamers was added to 0.83 pmol of [15′-^{32}P]$(pA)_{27}$ and different amounts of nonradioactive oligomer. Incubation and filtration were carried out as before. The competition reaction can be described by the simultaneous occurrence of binding equilibria between a probe oligomer P and host factor hexamer HF

$$K^P = [HFP]/([HF][P])$$

and competing oligomer and host factor

$$K^R = [HFR]/([HF][R])$$

Thus the binding constant for each competitor R can be related to the binding constant for the common probe by

$$K^R/K^P = [HFR][P]/([HFP][R])$$

At $[HFP]_{0.5}$, the point where enough competitor is added to reduce [HFP] to half the value it had when no competitor is added, we can approximate that $[HFP]_{0.5} = [HFR]_{0.5}$. This approximation is valid as long as [HF] is negligible in the reaction. Thus, at the point of 50% competition, the binding constants are inversely proportional to the free oligomer concentrations

$$K^R/K^P = [P]_{0.5}/[R]_{0.5}$$

Although the free probe and competitor concentrations at 50% competition could in principle be calculated from the competition binding data, such calculations are not warranted by the accuracy of the data. Instead an apparent ratio of binding constants

$$(K^R/K^P)_{app} = [P_t]_{0.5}/[R_t]_{0.5}$$

was used where $[P_t]_{0.5}$ and $[R_t]_{0.5}$ are the total probe and competitor concentrations at 50% competition. Under the conditions of the competition experiments we calculate that $(K^R/K^P)_{app}$ should be a good approximation of K^R/K^P when the probe is $(pA)_{27}$ and at 0.1 M NaCl. With $(pA)_{27}$ probe at 1.0 M NaCl the concentration of free host factor is not negligible any more, and calculations indicate that $(K^R/K^P)_{app}$ underestimates K^R/K^P by a constant factor of ~ 2. Since a relative binding constant with respect to a constant probe is all that is required, this simplification appears to be fully justified.

The value of $[P_t]_{0.5}$ is simply $[P_t]$, the input probe concentration. The value of $[R_t]_{0.5}$ can be obtained by one of two possible methods. If $P_{f,0}$ is the amount of probe in cpm retained on the filter without competitor and P_f is the amount in the presence of competitor, then plots of $P_f/P_{f,0}$ as a function of $[R_t]$ can be interpolated to $P_f/P_{f,0} = 0.5$ to give $[R_t]_{0.5}$. Alternatively, plots of $P_{f,0}/P_f - 1$ vs. $[R_t]$ are linear and go through the origin with a slope of $[R_t]_{0.5}$. Although both methods of determining $[R_t]_{0.5}$ gave similar results, the latter method is preferable since all data points can be used to obtain the slope and data for oligomers which did not reach 50% competition could be used.

Protein–Nucleic Acid Binding Parameters. For the description of host factor interacting with oligo(A)'s or poly(A) where the potential binding sites all have an identical sequence, we follow the terminology of von Hippel and co-workers (Kelly et al., 1976; Draper & von Hippel, 1978a,b). The parameter m is defined as the minimal length of the oligonucleotide with which all possible favorable contacts with host factor can occur. The parameter n is the length of oligomer that the host factor will "cover" or make unavailable for binding another hexamer. Thus, for a given RNA, m will be less than or equal to n. We define K^l as the association constant of a single host factor hexamer with A_l, an oligo(A) l residues long. The effects of phosphate groups at the 5′ or 3′ ends are ignored. When $l < m$, K^l will be a steep function of l since a decrease in chain length will result in the loss of favorable protein–RNA contacts. When $l > m$, the number of ways a host factor hexamer can bind to an oligomer increases as a function of chain length, and K^l increases proportionally according to $K^l = (l - m + 1)K^m$. This relationship allows the determination of m from binding data obtained with a series of $(pA)_l$.

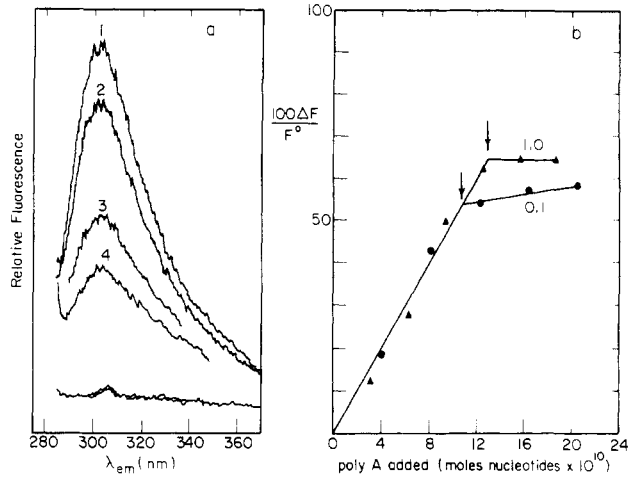

FIGURE 1: Detection of complex between poly(A) and host factor. (a) Fluorescence emission spectra (excitation at 277 nm) of 20 μL of host factor stock solution in 0.1 M NaCl buffer with no (curve 1), 0.41 nmol (curve 2), 0.82 nmol (curve 3), and 1.64 nmol (curve 4) of poly(A). (b) Fluorescence titration of host factor with poly(A). (●) 20 μL of host factor in 0.1 M NaCl buffer. (▲) 16 μL of host factor in 1.0 M NaCl buffer; the data are normalized to 20 μL of host factor to facilitate comparison. Filter binding titrations in Figure 2 indicate that 20 μL of host factor stock contains 76 pmol of hexamer.

Table I: Fluorescence Titration[a]

RNA	0.1 M NaCl: Q at saturation	0.1 M NaCl: equiv point (nmol of nucleotide)	1.0 M NaCl: Q at saturation	1.0 M NaCl: equiv point (nmol of nucleotide)
$(Ap)_6$	33 (2)[b]	0.93 (2)	48	0.95
$(Ap)_{12}$	50 (3)	1.02 (3)	37	1.05
$(Ap)_{15}$	54 (2)	0.97 (2)	62 (2)	1.28 (2)
$(Ap)_{17}A$	64	1.07	77	1.25
$(Ap)_{20}A$	56	1.00	75	1.28
$(Ap)_{27}$	59 (6)	1.02 (6)	66	0.94
poly(A)	56	1.08	64	1.29
av ± SD	54 ± 10	1.01 ± 0.05	61 ± 14	1.17 ± 0.16

[a] Titrations were carried out with 20 μL of host factor stock solution or normalized to that amount. [b] Parentheses indicate the number of titrations used to give average value.

Results

(a) Fluorescence Titration of Host Factor with Oligomers. The fluorescence emission spectrum of host factor (Figure 1a) is characteristic of a protein containing tyrosines and no tryptophan, as expected from the amino acid composition (Franze de Fernandez et al., 1972). The addition of poly(A) or oligo(A) strongly quenches the fluorescence of the protein. Plots of the percentage quenching, Q, vs. the amount of RNA added show clear break points (Figure 1b) indicating saturation of the protein with the RNA. Table I summarizes such titration experiments for a variety of oligo(A)'s and poly(A) at both 0.1 and 1.0 M NaCl. In most cases, the percentage quenching at saturation was found to be the same, providing some evidence that the type of complex formed was similar with different oligomer chain lengths. The slightly lower percent quenching observed in 0.1 M NaCl may be due to aggregation of host factor observed at the lower ionic strength (de Haseth & Uhlenbeck, 1980). $(Ap)_6$ at both salt concentrations and $(Ap)_{12}$ at 1.0 M NaCl show a significantly lower percent quenching. This may be due either to a reduced association constant for the shorter oligomers or to a slightly altered structure for the oligomer–protein complex. Attempts

Table II: Equivalence Points from Filter Binding Titrations

(A) Titration of 0.71 μL of Host Factor Stock equiv point [pmol of oligo(A)]		
chain length	0.1 M NaCl	1.0 M NaCl
15	3.0 (2)[a]	*b*
18	3.3	*b*
21	2.0	3.2
24	2.6	*b*
27	3.0	2.7

(B) Titration of 1.5 pmol of $(pA)_n$ equiv point (μL of host factor stock)		
chain length	0.1 M NaCl	1.0 M NaCl
15	0.32 (2)	*c*
18	0.26	*c*
21	0.61	0.49
24	0.67	0.43
27	0.42 (2)	0.38 (2)

[a] Parentheses indicate the number of titrations. [b] Not determined. [c] No sharp equivalence point obtained.

to demonstrate a complex between $(Ap)_3$ and host factor were not successful.

The most striking result obtained from the titration data in Table I is that for a given amount of host factor, the amount of RNA needed to reach saturation measured in *moles of A residue* is the same for all oligo(A)'s and poly(A). Thus, each host factor hexamer can bind a given number of A residues independent of the chain length of the oligomer. The precise determination of n, the size of the stretch of A residues that can be covered by a hexamer, depends upon the precise determination of active host factor concentration. By use of the concentration of host factor determined spectrophotometrically (see above), average values of $n = 10.5 \pm 0.5$ at 0.1 M NaCl and $n = 12.2 \pm 1.7$ at 1.0 M NaCl are obtained. However, as indicated in the next section, there is good evidence that the concentration of active host factor is somewhat lower so more accurate values may be $n = 13.3 \pm 0.7$ at 0.1 M NaCl and $n = 15.4 \pm 2.1$ at 1.0 M NaCl.

The fact that the value of n is the same for all oligomer chain lengths implies that more than one oligomer can bind a host factor hexamer and that more than one hexamer can bind a single oligomer. Thus, two or three molecules of $(Ap)_6$ bind each protomer, and two hexamers bind $(Ap)_{27}$. Since $(Ap)_{20}A$ also saturates at the same level, aggregates containing several hexamers and several oligomers are presumably forming under these conditions.

(*b*) *Filter Binding Titrations.* Since radiolabeled oligo(A) is only retained on nitrocellulose filters when it is bound to host factor, complex formation can be detected at very low protein and RNA concentrations. Titrations of a constant amount of host factor by oligo(A) (Figure 2a, Table II) and a constant amount of oligo(A) by host factor (Figure 2b, Table II) were carried out. As can be seen in Figure 2b, not all the available oligo(A) is bound to the nitrocellulose filter even when a large excess of protein is present. This is due to incomplete retention of the host factor–oligo(A) complex by the nitrocellulose filter. The fraction of complex retained, or retention efficiency, can be most accurately determined from the slope of the initial linear region of Figure 2a if virtually complete binding is assumed. As indicated by the binding constants for the interactions (see below), this condition is satisfied for $l \geq 15$ at 0.1 M NaCl and $l \geq 18$ at 1.0 M NaCl. From Figure 2a retention efficiencies of 53% and 40% are determined for $(pA)_{15}$ and $(pA)_{27}$, respectively. From eight such titrations, an average retention efficiency of 53 ± 10%

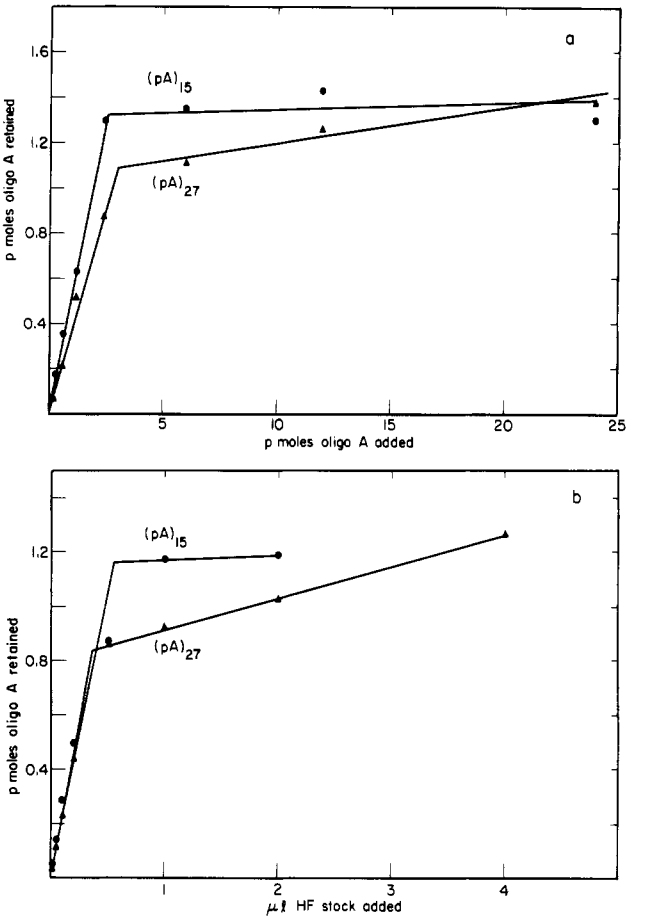

FIGURE 2: Nitrocellulose filter titrations in 0.1 M NaCl buffer of (a) 0.71 μL of host factor stock solution with $(pA)_{15}$ (●) and $(pA)_{27}$ (▲) and (b) 1.5 pmol of $(pA)_{15}$ (●) and $(pA)_{27}$ (▲) with host factor stock solution. From these titrations, 1 μL of stock solution was determined to contain 3.8 pmol of hexamer.

is obtained with no significant variation due to chain length or ionic strength.

The equivalence points obtained for the titrations in Figure 2 and in Table II indicate a similar binding stoichiometry for all $(pA)_l$ ($15 \leq l \leq 27$) when expressed in terms of moles of bound oligo(A) per mole of hexamer. Thus, it was determined that 0.71 μL of host factor stock solution binds 2.9 ± 0.4 pmol of $(pA)_l$ and 1.5 pmol of $(pA)_l$ is saturated by 0.43 ± 0.14 μL of host factor stock. By use of the spectrophotometrically determined concentration of the host factor stock solution, the stoichiometry of the interaction is calculated to be 0.83 $(pA)_l$ bound per hexamer when host factor is kept constant or 0.73 when $(pA)_l$ is kept constant. Since it is unlikely that the active host factor concentration is actually 1.5-fold higher than the spectroscopically determined value and that all $(pA)_l$ ($15 \, l \, 27$) would bind exactly two hexamers, a 1:1 stoichiometry is most likely. Thus the binding of one hexamer to an oligo(A) molecule is sufficient to cause retention of the complex. Either contaminants or inactive protein in the host factor preparation could explain the nonintegral stoichiometry. If a 1:1 stoichiometry is assumed, the concentration of active host factor hexamers in the stock solution can be calculated to be 4.1 ± 0.7 μM for oligo(A) titrations and 3.5 ± 1.1 μM for host factor titrations. We have used the average value of 3.8 μM for the concentration of host factor hexamers in the stock solution in the analysis of all our subsequent experiments.

It is interesting to contrast the results of the two types of titration experiments. The fluorescence titration experiments indicated that each host factor protomer bound a constant

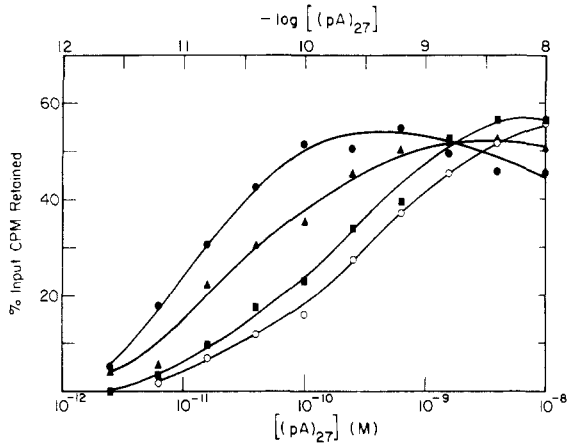

FIGURE 3: Retention on nitrocellulose filters of an equimolar mixture of host factor and $(pA)_{27}$ at indicated concentrations of the two molecular species. The solvent contained 0.1 M (●), 0.18 M (▲), 0.33 M (■), and 1.0 M (○) NaCl.

number (~14) of A *residues* independent of the oligomer chain length while the filter binding titration experiments indicated that each host factor protomer bound a single oligo(A) *molecule* independent of oligomer chain length. This striking difference is likely to be due to two factors. First, the fluorescent experiments are done at nearly 10^3 higher concentration which would allow the formation of complexes with much lower affinity constants. Second, since the binding of one protein to the oligo(A) causes its retention on the filter, the binding of subsequent proteins would not be detected. Thus, for example, $(pA)_{27}$ could have a high-affinity site for host factor, detected by filter binding, as well as a considerably lower affinity site only seen by fluorescence.

(*c*) *Binding Constants as a Function of* [*NaCl*]. Detection of complex formation by filter binding was used to obtain approximate values for K^l at various salt concentrations. For $(pA)_{15}$ and $(pA)_{27}$ this was done by measuring the retention as a function of the concentration of a nearly equimolar mixture of host factor and the $[5'\text{-}^{32}P](pA)_l$ (Spierer et al., 1978). Figure 3 shows the results of such titrations for $(pA)_{27}$. It is seen that over the range of 0.1–1.0 M NaCl the maximal retention of the complexes is not a function of ionic strength, being in all cases close to the average value of 53% determined previously. K^l was determined as the reciprocal of half the concentration of oligomer or host factor at which half-maximal retention occurred. Scatchard plots of the same data showed considerable scatter but were consistent with a 1:1 stoichiometry and yielded binding constants in agreement with those determined above. The K^l for $p(Ap)_{12}$ was determined with another, less pure host factor preparation by titration of a fixed amount of protein with the RNA (Riggs et al., 1970). The reciprocal of the free $p(Ap)_{12}$ concentration at which retention was half-maximal was then the appropriate binding constant. At 0.1 M NaCl measurements were also done with $(pA)_{12}$ and the host factor preparation used here and found to be in excellent agreement.

The data for K^l as a function of ionic strength for all three oligomers are shown in Figure 4. K^l is seen to be a sensitive function of chain length, with K^{27} being nearly 3 orders of magnitude greater than K^{12}, but the ionic strength dependence of K is nearly the same in all three cases. The chain length dependence of K^l will be examined in greater detail in the next section, and the ionic strength dependence of K^l will be discussed here.

Plots of log K vs. log [NaCl] for protein–nucleic acid binding data obtained in the absence of significant amounts of other

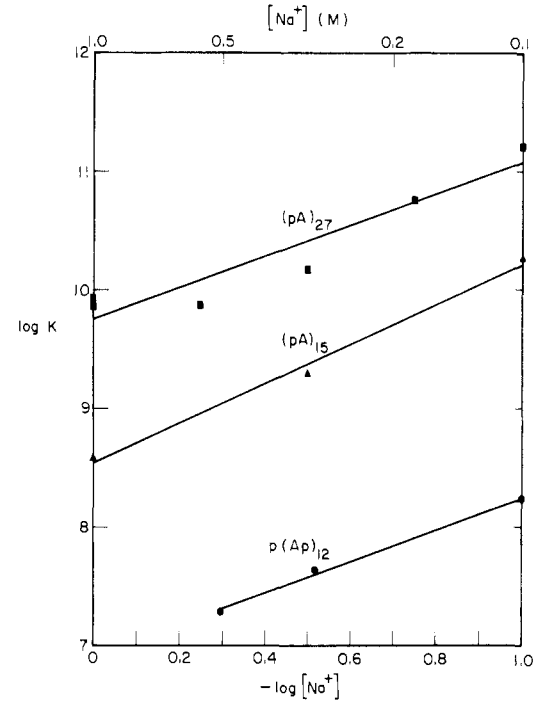

FIGURE 4: Association constants of oligo(A)'s with host factor at various NaCl concentrations.

positive ions are expected to be linear and can yield information about the involvement of charge interactions in the stabilization of the complex (Record et al., 1976, 1978). The slope is given by

$$\frac{d \log K}{d \log [NaCl]} = -Z\psi$$

where Z is the number of ionic interactions between the protein and the nucleic acid and ψ is the number of sodium ions per phosphate thermodynamically bound to the nucleic acid. The slopes in Figure 4 for $p(Ap)_{12}$, $(Ap)_{15}$, and $(Ap)_{27}$ are −1.3, −1.7, and −1.3, respectively. For poly(A), $\psi = 0.78$ (Record et al., 1976), and, although for short oligomers ψ will decrease with decreasing chain length (Record & Lohman, 1978), the effect is probably not significant for $l \geq 12$. Thus, Z is ~2 for all three oligomers. Considering that the number of A residues thought to interact with each hexamer (m is ~16 (see below), the ionic contribution to the association constant is quite small. For example, the value of Z for both RNA polymerase and lac repressor binding nonspecifically to DNA is 12; for core polymerase $Z = 24$ (Record et al., 1978). Gene 32 protein, which covers only about six nucleotides (Kelly et al., 1976) has $Z = 2$ (Record et al., 1978).

The total free energy for each oligomer binding to host factor in 1 M NaCl, obtained from the intercepts of log K^l vs. [NaCl] in Figure 4, is −9.4, −11.6, and −13.3 kcal/mol for $p(Ap)_{12}$, $(pA)_{15}$, and $(pA)_{27}$, respectively. These can be divided into electrostatic and nonelectrostatic components by assuming that the electrostatic component can be approximated by that for a dilysine binding to poly(A) in 1 M NaCl with a $Z = 2$, which is presumably a totally electrostatic interaction (Record et al., 1976). The values for the nonelectrostatic contributions to the free energy are −9.8, −12.0, and −13.7 kcal/mol of oligomer or −0.8, −0.8, and −0.5 kcal/mol of A residues, for $p(Ap)_{12}$, $(pA)_{15}$, and $(pA)_{27}$, respectively. These data are consistent with the entire oligomer filling the nucleotide binding site for $p(Ap)_{12}$ and $(pA)_{15}$ and thus showing identical values for the free energy per nucleotide. In the case of $(pA)_{27}$, only

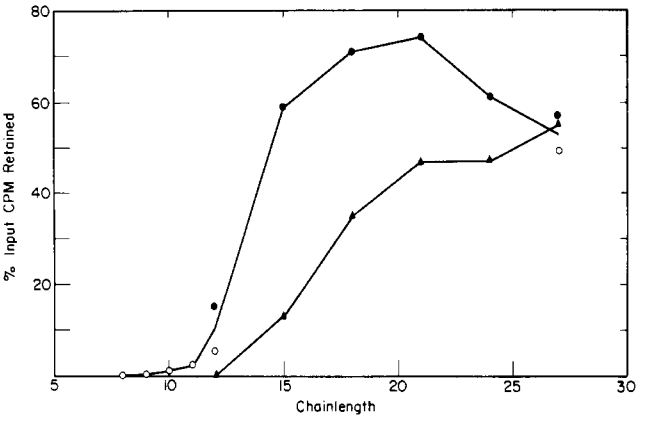

FIGURE 5: Retention of nitrocellulose filters of a mixture of 2.7 pM host factor and 1.2 pM oligo(A) in 0.1 M [(●, ○) two sets of experiments] and 1.0 M (▲) NaCl. All points are averages of duplicates, incubated and filtered separately.

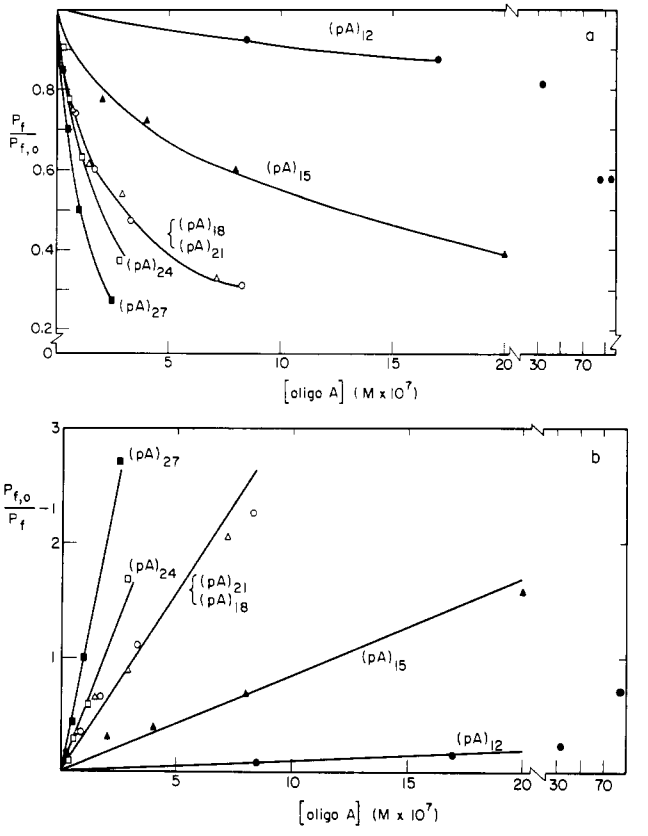

FIGURE 6: Competition between $(pA)_{27}$ and oligo(A)'s in 0.1 M NaCl. Conditions are given under Materials and Methods. All points are averages of duplicates, incubated and filtered separately. Data from the same experiments have been plotted differently in (a) and (b); see Materials and Methods.

one host factor hexamer binds, leaving many residues not in the protein binding site and a resulting lower free energy per residue. The more favorable statistical factors pertinent to the host factor–$(pA)_{27}$ interaction do not compensate for the fact that not all the nucleotide residues can take part in the complex. It is worthwhile to note that the above treatment of the ionic strength dependence of host factor–oligo(A) interaction does not take into account the salt-dependent aggregation of hexamers (de Haseth & Uhlenbeck, 1980).

(d) Chain Length Dependence of K^l. The oligomer chain length dependence of K^l observed in the previous section was examined in greater detail. In Figure 5, the retention of [5′-^{32}P]$(pA)_l$ was determined as a function of l for mixtures

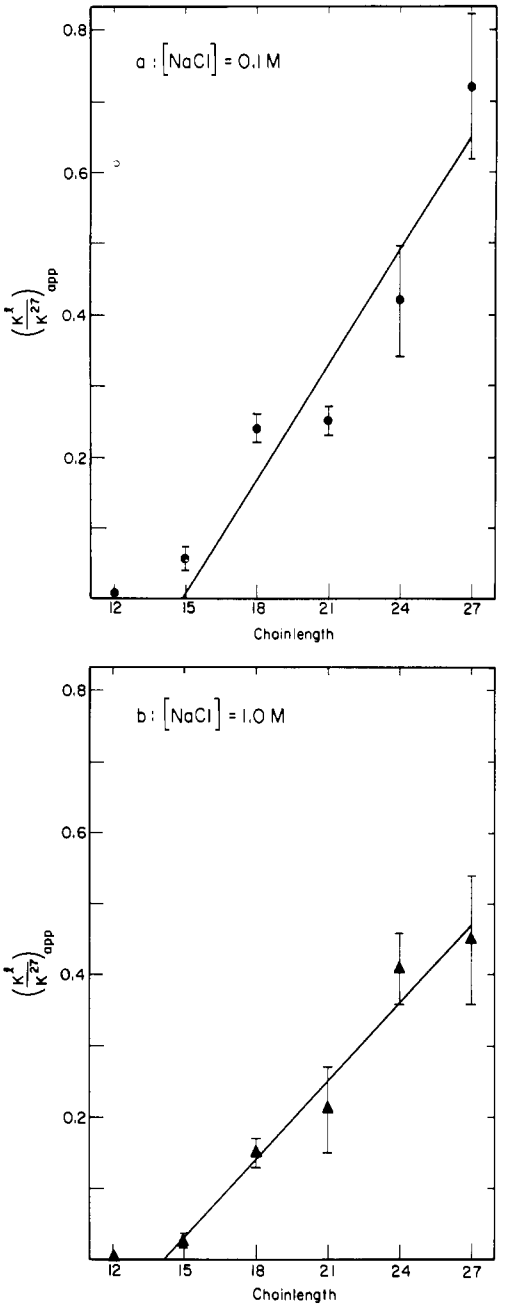

FIGURE 7: Relative binding constants as a function of chain length. Each point is the average of two to four independent experiments. Least-squares lines are based on the points with $l \geq 18$. Intercepts are 14.9 in (a) (0.1 M NaCl) 14.3 in (b) (1.0 M NaCl).

containing constant molar concentrations of host factor and oligomer. In 0.1 M NaCl, the retention is negligible for $l <$ 10, increasing sharply between $l = 12$ and $l = 15$ to a saturating value. For unknown reasons, the maximal retention for several oligomers is significantly higher than the 53% found in other experiments (see above). In 1.0 M NaCl, the steep increase is between $l = 12$ and $l = 18$. This general behavior suggests that the host factor hexamer has a binding site in the order of $m = 15$–18 residues.

In order to more quantitatively compare the stabilities of complexes between host factor and $(pA)_l$ of several chain lengths, we carried out equilibrium competition experiments between [5′-^{32}P]$(pA)_{27}$ and a variety of oligo(A)'s. The results of these competition experiments in 0.1 M NaCl are plotted in two ways in Figure 6. As the chain length decreases, increasingly higher concentrations of cold oligomer are needed

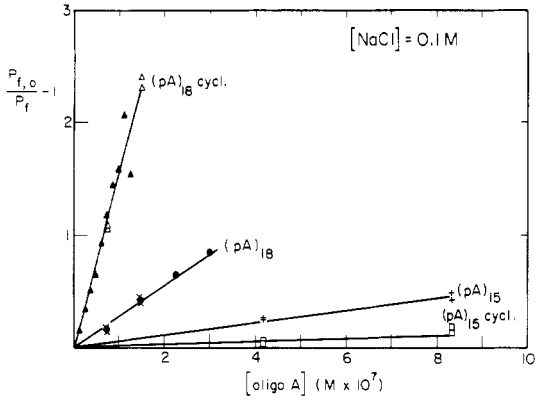

FIGURE 8: Effect of cyclization of $(pA)_{15}$ and $(pA)_{18}$ on their ability to compete with [5'-^{32}P]$(pA)_{27}$ for host factor binding. The data have been plotted as indicated under Materials and Methods.

to effectively compete with the $(pA)_{27}$ probe. Values of $[R_t]_{0.5}$ are obtained from Figure 6b and used to calculate $(K^R/K^P)_{app}$ at each chain length. These data are plotted as a function of chain length in Figure 7 for both salt concentrations. Since, as described previously, $K^l = (l - m + 1)K^m$, the ratio of binding constants of a competitor oligomer of length l to a probe oligomer of $l = 27$ is given by

$$K^l/K^{27} = (l - m + 1)/(28 - m)$$

Thus K^l/K^{27} is expected to be a linear function of l with an intercept on the abscissa equal to $m - 1$. Determination of m from the slope was not feasible, since, in contrast to the intercept, its value is dependent on the absolute values of K^l/K^{27} (see Materials and Methods). Although the error limits are quite large (about ±20%), the least-squares lines based on the points with $l \geq 18$ gave intercept values of m at the two salt concentrations which were in good agreement: m = 15.9 and 15.3 at 0.1 and 1.0 M NaCl, respectively. Inclusion of the $l = 15$ data points gives m = 15.3 and 15.3, respectively. Thus, each host factor protomer has binding sites to accomodate an oligo(A) of 15–16 residues. A simple model for the interaction would be that each monomer binds three residues.

(*e*) *Interaction of Host Factor with Cyclized Oligo(A)'s.* The interaction of cyclized oligo(A)'s with host factor might display unusual properties if the six subunits of the hexamer were arranged in such a manner that the RNA binding site would have a circular geometry. Then cyclic oligomers smaller than a certain minimal length would not be able to make as many protein–RNA contacts as their linear counterparts and thus would show markedly lower binding constants. Cyclic oligomers longer than a minimal length might show higher binding constants than the corresponding linear molecules since no binding free energy would have to be expended for bending the RNA into the binding site of the protein and end effects would be abolished. In Figure 8 precisely this behavior is seen when the binding of linear and cyclized $(pA)_{15}$ and $(pA)_{18}$ to host factor is compared by means of a competition experiment with $(pA)_{27}$. While cyclization of $(pA)_{18}$ increases its ability to compete with $(pA)_{27}$ for host factor binding, cyclization of $(pA)_{15}$ has the opposite effect. The minimal length of cyclized oligo(A) which can occupy the whole binding site of a hexamer must be between 15 and 18 nucleotides. If data for $l = 16$ and 17 were available, a more precise number could be obtained. This range of values is very similar to the values of n and m determined with linear oligomers.

From each competition experiment, a value for $(K^R/K^{27})_{app}$ can be obtained. In Table III, the ratio of the binding constants for the cyclized and linear forms is given for several chain lengths at 0.1 and 1.0 M NaCl. No significant effect of NaCl concentrations on the relative binding constants is seen. The slow decrease in the ratio of cyclic to linear binding constants as a function of l can be explained by assuming that when $l \geq m$ the only effect of cyclizing the oligomer will be to abolish end effects and thereby increase the number of ways that the host factor can bind the oligomer. Thus, for a cyclic oligomer $K_{cyc}^l = lK^m$ whereas for a linear oligomer $K^l = (l - m + 1)K^m$. Then

$$K_{cyc}^l/K^l = l/(l - m + 1)$$

The values of m obtained in Table III average to be m = 17.3 ± 1.2 which is in excellent agreement with those obtained in the previous section by a completely independent method.

Table III: Comparison of Linear and Cyclic Oligo(A)'s Bound to Host Factor

	0.1 M NaCl		1.0 M NaCl	
l	$(K_{cyc}/K_{lin})_{app}$	m^a	$(K_{cyc}/K_{lin})_{app}$	m^a
15	0.37 (2)[b]		0.38	
18	5.7 (3)	15.8	9.3 (3)	17.1
21	4.7 (2)	17.5	5.0	17.8
24	3.9 (3)	18.8	2.5 (2)	15.4
27	2.6 (2)	17.6	2.8 (2)	18.4

[a] m is the calculated minimal oligomer chain length which forms all favorable contacts with the host factor hexamer (see text). [b] Parentheses indicate the number of determinations averaged to give the indicated value.

Discussion

The simplest model for the interaction of the host factor hexamer with oligo(A)'s is that the binding site on the protein has a circular geometry and has dimensions such that it can accomodate a stretch of RNA ~16 nucleotides long. The stability of the complex is determined by contacts between the RNA and each subunit of the hexamer. This model is based on several experimental observations. The size of the binding site is given by the value of m, the minimal number of consecutive nucleotides which can form all possible favorable protein–RNA contacts. A value of $m = 16 \pm 1$ was obtained from the chain length dependence of the association constant and independently from the ratios of binding constants of cyclized oligo(A)'s to their corresponding linear counterparts. The circular geometry of the RNA binding site is inferred from the fact that cyclized $(pA)_{18}$ binds host factor better than its linear counterpart. The fact that the situation is reversed with $(pA)_{15}$ again indicates a binding site of between 15 and 18 residues. It is likely that all six subunits of host factor are involved in contacts with RNA due to the large (50–60%) extent of quenching of host factor fluorescence by added RNA. A possibility is that the tyrosine groups on the protein are stacked between the bases of the single-stranded nucleic acid (Mayer et al., 1979). If only one or two of the subunits bound RNA, a conformational change or some other mechanism would be required to cause the quenching of fluorescence in the other subunits.

The model can be made somewhat more detailed by making the reasonable assumption that each host factor subunit interacts with the RNA in an identical manner, presumably by binding an integral number of residues. The most likely possibility is that each subunit binds three A residues although this would make m = 18 which is slightly higher than we have determined. However, if only one of the three A residues is actually involved in the contacts with the protein, then $(pA)_{16}$

would be long enough to form all the favorable contacts and m would be 16 which is consistent with the experimental value. This model would require a value of $n = 18$ which is somewhat higher than the values $n = 13$–17 determined here. Since, for the calculation of n, the concentration of active protein in the stock solution needs to be known, the data are not considered to greatly disagree with the model. The observation that only two electrostatic interactions appear to occur between a host factor hexamer and an oligo(A) ($Z = 2$) is less consistent with the above model. This would require a nonintegral number of electrostatic interactions per protein subunit or two unique interactions. Since the data is well outside a value of $Z = 0$ or $Z = 6$, an explanation will have to await further refinement of the model.

It is interesting to compare the dimensions of the RNA and the protein in the host factor–oligo(A) complex. The circumference of a globular protein of molecular weight 75 000 is ~175 Å. The calculated circumference of a cyclized oligo(A) 18 nucleotides long depends on the value used for the distance between consecutive phosphates, which is a function of experimental conditions. Reasonable minimal and maximal estimates for the circumference appear to be 55 and 120 Å, respectively. Thus, the possibility that the binding site is along the perimeter of a globular hexamer can be ruled out. Among the other possibilities, one deserving mention is an arrangement of the subunits such that the RNA binds around a central hole in the hexamer.

It is shown in the next paper (de Haseth & Uhlenbeck, 1980) that at NaCl concentrations below 0.3 M, host factor hexamers aggregate to form dodecamers or larger aggregates. However, neither the stochiometry of binding nor the values of n or m of the host factor–oligo(A) interactions determined here show a significant salt dependence. Thus, it is concluded that the protein–protein contacts responsible for the aggregation do not involve the RNA binding sites. In addition, it is apparently not possible for the oligo(A)'s used in this study to simultaneously interact with two hexamers of the same aggregate. If this were the case, oligomers longer than $l = 16$ would have substantially higher association constants in 0.1 M NaCl and show the very strong salt dependences of binding seen for RNA.

In several cases, proteins which bind single-stranded nucleic acids have been shown to do so cooperatively (Alberts & Frey, 1970; Alberts et al., 1972; Draper & von Hippel, 1978b), suggesting that this might also be the case for the interaction of host factor with oligo(A) and poly(A). Unfortunately, the oligomers used in this study were too short to permit simultaneous binding of two proteins to the RNA under conditions where each protein was able to interact with $m = 16$ nucleotides. Thus the data presented here do not allow conclusions to be drawn as to the possibility of cooperative binding of host factor to oligo(A). However, experiments with poly(A) in the following paper (de Haseth & Uhlenbeck, 1980) appear to rule out strong cooperative effects.

In some respects, the RNA binding behavior of host factor resembles that of site II of *E. coli* ribosomal protein S_1 (Draper & von Hippel, 1978b). Both proteins show a strong preference for single-stranded RNA over double-stranded RNA or single- or double-stranded DNA. (Franze de Fernandez et al., 1972; Draper & von Hippel, 1978b; de Haseth & Uhlenbeck, 1980). While host factor preferentially interacts with RNA containing A residues, S_1 has a similar preference for C-containing RNA. Both interactions are stabilized by a similar, large nonelectrostatic contribution to the binding free energy. However in other respects the two proteins appear quite different. S_1 is a monomer while host factor is a hexamer. Increasing ionic strength weakens host factor–oligo(A) complexes but strengthens S_1–oligo(C) complexes (Draper & von Hippel, 1978b). The binding of S_1 to poly(C) is cooperative, but no cooperativity is seen with host factor binding to poly(A). Thus, although both host factor and S_1 appear to be composition-specific single-stranded RNA binding proteins, they interact with RNA rather differently. It is interesting to note that since S_1 is a subunit of Qβ replicase involved in the initiation of replication, the two proteins may carry out similar functions. This view is supported by the fact that Senear & Steitz (1976) find both proteins interacting with a single T_1 fragment of Qβ RNA.

Acknowledgments

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References

Alberts, B. M., & Frey, L. (1970) *Nature (London) 227*, 1313–1318.

Alberts, B. M., Frey, L., & Delius, H. (1972) *J. Mol. Biol. 68*, 139–152.

August, J. T., Banerjee, A. K., Eoyang, L., Franze de Fernandez, M. T., Hori, K., Kuo, G. H., Rensing, U., & Shapiro, L. (1968) *Cold Spring Harbor Symp. Quant. Biol. 33*, 73–81.

Blumenthal, T., & Carmichael, G. G. (1979) *Annu. Rev. Biochemistry 48*, 525–548.

Cameron, V., Soltis, D., & Uhlenbeck, O. C. (1978) *Nucleic Acids Res. 5*, 825–833.

Carmichael, G. G. (1975) *J. Biol. Chem. 250*, 6160–6167.

Carmichael, G. G., Weber, K., Niveleau, A., & Wahba, A. J. (1975) *J. Biol. Chem. 250*, 3607–3612.

de Haseth, P. L., & Uhlenbeck, O. C. (1980) *Biochemistry 19* (following paper in this issue).

Donis-Keller, H., Maxam, A. M., & Gilbert, W. (1977) *Nucleic Acids Res. 4*, 2527–2538.

Draper, D. E., & von Hippel, P. E. (1978a) *J. Mol. Biol. 122*, 321–338.

Draper, D. E., & von Hippel, P. E. (1978b) *J. Mol. Biol. 122*, 339–359.

DuBow, M. S., & Blumenthal, T. (1975) *Mol. Gen. Genet. 141*, 113–119.

Edelhoch, H. (1967) *Biochemistry 6*, 1948–1954.

Franze de Fernandez, M., Hayward, W. S., August, J. T. (1972) *J. Biol. Chem. 247*, 824–831.

Janik, B. (1971) *Physicochemical Characteristics of Oligonucleotides and Polynucleotides*, IFI/Plenum Press, New York.

Kelly, R. C., Jensen, D. E., & von Hippel, P. H. (1976) *J. Biol. Chem. 251*, 7240–7250.

Kozak, M. (1979) *Nature (London) 280*, 82–85.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Mayer, R., Toulmé, F., Montenay-Sarestier, T., & Hélène, C. (1979) *J. Biol. Chem. 254*, 75–82.

Record, M. T., & Lohman, T. M. (1978) *Biopolymers 17*, 159–166.

Record, M. T., Lohman, T. M., & de Haseth, P. L. (1976) *J. Mol. Biol. 107*, 145–158.

Record, M. T., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.

Riggs, A. D., Suzuki, H., & Bourgeois, S. (1970) *J. Mol. Biol. 48*, 67–83.
Saxe, S. A., & Revzin, A. (1979) *Biochemistry 18*, 255–263.
Senear, A. W., & Steitz, J. A. (1976) *J. Biol. Chem. 251*, 1902–1912.
Spierer, P., Bogdanov, A. A., & Zimmerman, R. A. (1978) *Biochemistry 17*, 5394–5398.
Usher, D. A., & Rosen, J. A. (1979) *Anal. Biochem. 92*, 276–279.
Walker, G. C., Uhlenbeck, O. C., Bedows, E., & Gumport, R. I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 122–126.
Wells, R. D., Hardies, S. C., Horn, G. T., Klein, B., Larson, J. E., Neuendorf, S. K., Panayotatos, N., Patient, R. K., & Selsing, E. (1980) *Methods Enzymol. 65*, 327–347.

Interaction of *Escherichia coli* Host Factor Protein with Qβ Ribonucleic Acid†

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ABSTRACT: The affinity of *Escherichia coli* host factor protein for a variety of ribonucleic acids (RNAs) is compared in an equilibrium competition assay with $(pA)_{15}$ or $(pA)_{27}$ as the common probe. Of the homopolymers tested, only polyriboadenylate [poly(rA)] binds the protein with a high affinity. At low ionic strength (0.1 M NaCl), the binding to Qβ RNA is much stronger than to the oligoadenylates, but the situation is reversed upon fragmentation of the RNA with ribonuclease T_1. Increasing the ionic strength results in a drastic reduction of the affinity of host factor for Qβ RNA over a relatively narrow salt range (0.1–0.3 M NaCl). Over the same range, added salt greatly reduces the tendency of host factor hexamers to aggregate. The tight binding of host factor to Qβ RNA is proposed to result from the binding of an aggregate, which can interact with several low affinity sites on the RNA simultaneously.

In the previous paper (de Haseth & Uhlenbeck, 1980) the RNA[1] binding site of host factor was characterized by studying the binding properties of oligo(A)'s. Each host factor hexamer can accommodate 16–18 contiguous nucleotides in a circular orientation. The interaction between host factor and $(pA)_{18}$ is extremely strong, with a binding constant of $>3 \times 10^{10}$ M^{-1} in 0.1 M NaCl. Only a small portion of the free energy is due to ionic interactions, since the association constant is only weakly dependent on the salt concentration of the binding buffer. The relationship of the function of host factor to its RNA binding properties is of considerable interest (Blumenthal & Carmichael, 1979). In its only known function, host factor greatly stimulates the synthesis of RNA by Qβ replicase in vitro, apparently by binding the Qβ RNA plus strand at a limited number of sites (Franze de Fernandez et al., 1972). In a more detailed study of the host factor–Qβ RNA interaction, Senear & Steitz (1976) were able to isolate two ribonuclease T_1 digest fragments of Qβ RNA by their retention with host factor on nitrocellulose filters. From a comparison of their sequence and the sequence of a similarly retained T_1 fragment of R_{17} RNA, Senear & Steitz (1976) suggested that host factor bound specifically to the sequence AAUAAA or a variant thereof. It appeared reasonable that host factor would bind to the same sequence in intact Qβ RNA as well. Since the position of these two sequences was internal in the Qβ RNA molecule, their relation to the function of host factor in the stimulation of the initiation of RNA synthesis by Qβ replicase remained unclear.

We report here studies on the interaction of host factor with various homopolymers and natural RNAs. A major goal is to determine the degree to which the interaction of host factor with Qβ RNA is a specific one. The relative ability of various RNAs to compete with a $[5'\text{-}^{32}P](pA)_{27}$ probe for host factor binding, as measured by millipore filter retention, is the primary assay used in this work. By use of a common, well-characterized probe to compare different RNAs, possible complications of variable backgrounds or retention efficiencies of the different RNAs are circumvented. Our results show that intact Qβ RNA binds host factor with a much higher affinity than oligo(A), but the T_1 digest of Qβ RNA binds much less well than oligo(A). Thus the T_1 fragments are not solely responsible for the tight binding of host factor to Qβ RNA. We suggest that the aggregation of host factor hexamers, which we observe at low ionic strength, is involved in the tight binding of host factor to Qβ RNA.

Materials and Methods

RNAs. Poly(A) and poly(C) were from Sigma Chemical Co., poly(U) and poly(I) were from Miles Laboratories, and poly(dA) was from P-L Biochemicals. In each case more than 80% of the material was larger than tRNA, as determined by electrophoresis on denaturing 20% acrylamide gels. Residue extinction coefficients were from Janik (1971). [8-^{3}H]Poly(A) (60 Ci/mol) was synthesized from [8-^{3}H]ADP (Amersham) by using *Micrococcus luteus* polynucleotide phosphorylase. From its migration on denaturing gels a mean chain length of 160 ± 50 nucleotides was estimated.

Qβ RNA was obtained as gifts from P. Cole, C. Guerrier-Takada and K. Campbell. The RNA was isolated from purified phage and further purified on sucrose gradients. STNV RNA was a gift from K. Browning and J. Clark, and

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[1] Abbreviations used: RNA, ribonucleic acid; poly(A) polyadenylate; poly(C), polycytidylate; poly(U), polyuridylate; poly(I), polyinosinate; poly(dA), polydeoxyadenylate; tRNA, transfer RNA; oligo(A), oligoadenylate; Tris, 2-amino-(2-hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; DNA, deoxyribonucleic acid; rRNA, ribosomal RNA.

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