NUCLEOTIDE SEQUENCE SPECIFIC INTERACTION OF HOST FACTOR I WITH BACTERIOPHAGE Qβ RNA

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1. Introduction

An RNA-dependent RNA polymerase induced in *Escherichia coli* during infection of bacteriophage Qβ (Qβ replicase) requires two additional host proteins, HFI and HFII, in the replication of Qβ RNA [1,2]. These proteins were shown to be involved in the synthesis of Qβ complementary strand from Qβ RNA, but not to be required in the synthesis of progeny Qβ RNA from the complementary strand [3]. Whereas HFII can be substituted for by several proteins such as protamine, histones or ribosomal proteins [4], HFI is absolutely required for the reaction [3]. The factor is also found to interact with single stranded RNA, but not with double stranded RNA, single and double stranded DNA [5].

In this communication we will describe the specific interaction of HFI with Qβ RNA. Interaction of HF1 with Qβ RNA was studied using synthetic homopolymers, poly (A), poly (C), poly (I) and poly (U) as competitor. Among polymers examined, poly (U) specifically inhibited the interaction. Further experiments indicated that Qβ RNA annealed with poly (A) completely abolished to interact with HFI but the RNA annealed with polymers other than poly (A) still sustained the ability. These results strongly suggest that HFI preferentially binds to the uridine nucleotide-rich region(s) on Qβ RNA molecule.

2. Materials and methods

Qβ replicase was purified as previously described [6]. HFI was prepared from *E. coli* Q13 by the method of Franze de Fernandez et al. [5]. Synthetic homopolymers, poly (A), poly (C), poly (I) and poly (U) were obtained from Miles Laboratories and dissolved in 0.01 M Tris–HCl (pH 7.6) –0.1 M NaCl–5 × 10^{-4} M EDTA. Concentrations of polymers were calculated on the basis of molar extinction coefficient as described [6]. Unlabeled and ^{3}H-Qβ RNA were prepared as previously described [7]. The binding of HFI to ^{3}H-Qβ RNA was determined by retention of the complex on Sartorius membrane filter (MF 50, 12 mm), essentially as previously described [8].

3. Results and discussion

3.1. *Inhibition by synthetic polymers of the interaction of HFI with ^{3}H-Qβ RNA*

Interaction of HFI with ^{3}H-Qβ RNA, using synthetic homopolymers as competitor, was examined in order to explore whether the interaction is of nucleotide sequence specificity or not.

As can be seen in fig. 1, at the concentrations of 4–8 nmoles/ml, about 90% of the interaction was inhibited by poly (U). Less strong inhibition occurred at the same concentrations of poly (I). Poly (A) and poly (C), however, did not inhibit at all. Similar results were obtained with the experiments in which HFI was preincubated with these polymers and was followed by the incubation with ^{3}H-Qβ RNA. Thus, it appears that the interaction of HFI with the RNA is specifically inhibited by poly (U). These results would imply that the factor preferentially binds to poly (U) and consequently the binding of the factor to ^{3}H-Qβ RNA diminishes as a function of poly (U) concentration. Although it remains another implication that poly (U) interacts directly with ^{3}H-Qβ RNA which causes the decreased

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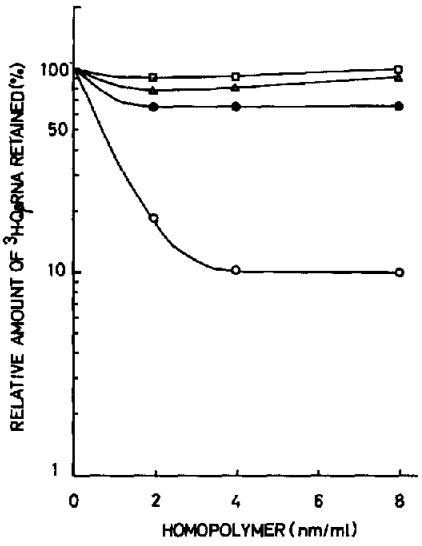

Fig. 1. Effect of synthetic polymers on the interaction of HFI with ^{3}H-Qβ RNA. Incubation mixture contained in 0.25 ml; 25 μmoles of Tris–HCl (pH 7.6), 2.5 μmoles of $MgCl_2$, 1μmole of 2-mercaptoethanol, 0.22 μg of HFI and 2 μg of ^{3}H-Qβ RNA (1.4×10^4 cmp/μg). Preincubation was carried out for 10 min at 30°C. Incubation continued for additional 10 min at 30°C after adding various quantities of polymer. ^{3}H-Qβ RNA–protein complex was determined as described in Materials and methods. The amount of ^{3}H-Qβ RNA retained on membrane filter was given as the percentage of that of control, in which synthetic polymer was omitted and ^{3}H-radioactivity retained on a filter was 1.62×10^4 cpm.

Table 1
Binding of HFI to ^{3}H-Qβ RNA annealed with poly (A)

RNA samples		NaCl (M) added at annealing	^{3}H-Qβ RNA retained (cpm)
(a)	^{3}H-Qβ RNA	0	10 300
		0.05	11 504
		0.10	7894
(b)	^{3}H-Qβ RNA annealed with poly (A)	0	180
		0.05	22
		0.10	95
(c)	^{3}H-Qβ RNA poly (A) mixture	0	7621
		0.05	7796
		0.10	6801

(a) one μg of ^{3}H-Qβ RNA (1.4×10^4 cpm/μg) was incubated, in a 0.1 ml incubation mixture, for 20 min at 80°C in the presence of NaCl as indicated and then cooled slowly to room temperature for 4 hr. (b) 1 μg of the ^{3}H-Qβ RNA was annealed, in a 0.1 ml incubation mixture, with 2 nmoles of poly (A) and cooled as described in (a). One μg of the ^{3}H-Qβ RNA and 2 nmoles of poly (A) were incubated separately in a 0.05 ml each of incubation mixture and cooled as described in (a). These samples were mixed together at 30°C just prior to the binding assay. Binding was carried out for 10 min at 30°C in a 0.25 ml of binding mixture which was prepared by adding 25 μmoles of Tris–HCl (pH 7.6), 2.5 μmoles of $MgCl_2$, 1 μmole of 2-mercaptoethanol, 0.22 μg of HFI and 0.1 ml of preincubation mixture (a), (b) or (c).

binding of the RNA to the factor, the alternative seems less likely since there was no detectable association between poly (U) and Qβ RNA under the conditions used.

3.2. *Interaction of HFI with ^{3}H-Qβ RNA annealed with polymers*

Table 1 indicates that ^{3}H-Qβ RNA preincubated with poly (A) at 80°C completely lost the binding ability while only partial decrease in the ability was shown when the RNA was incubated under the same conditions without poly (A) or when RNA and poly (A) were heated separately and mixed together at 30°C just prior to the binding assay.

Table 2 indicates that RNA preincubated with synthetic polymers other than poly (A) still sustained 50–100% of the HFI binding ability. These results indicate that only RNA annealed with poly (A) completely lose to interact with HFI and support that the factor binds to Qβ RNA at the site(s) abundant in uridylate residue. These results are consistent with the fact that poly (U) specifically interferes with the binding of HFI to Qβ RNA.

The binding of ^{3}H-Qβ RNA to HFI was, however, considerably low as compared with that of control when the RNA was annealed with poly (U) at 0.1 M NaCl and also when annealed with poly (I) at both 0 and 0.1 M NaCl and was separated from free polymer to minimize inhibition by it. It might be, therefore, possible that residual polymer still contained, interferes with the binding or that RNA annealed with these polymers partially lost the binding ability.

Qβ replicase has been shown to form a tight complex with Qβ RNA in the presence of HFI, but not to form such the complex without the factor or with heterologous RNA [3,9,10]. In addition, it has been shown that

Table 2
Binding of HFI to ^{3}H-Qβ RNA annealed with polymers other than poly (A)

RNA samples	NaCl (M) added at annealing	^{3}H-Qβ RNA retained (cpm)
^{3}H-Qβ RNA	0	9813
	0.10	7444
^{3}H-Qβ RNA annealed with poly (C)	0	8057
	0.10	8254
^{3}H-Qβ RNA annealed with poly (I)	0	5567
	0.10	5006
^{3}H-Qβ RNA annealed with poly (U)	0	10100
	0.10	4600

When ^{3}H-Qβ RNA was annealed with poly (C), incubation was carried out as described in (b) of table 1 except that poly (A) was substituted for by 2 nmoles of poly (C). When annealed with poly (I) and poly (U), incubation was carried out as described in (b) of table 1 except that 5 μg of ^{3}H-Qβ RNA was annealed with 10 nmoles of poly (I) or poly (U). The mixture was then applied on 1 × 70 cm Sephadex G-200 column and eluted with 0.01 M Tris–HCl pH 7.6–0.05 M NaCl in order to remove unbound polymers. Binding was carried out as described in table 1.

one mole of the factor per mole of RNA molecule is required for the synthesis of Qβ RNA [5]. These results strongly suggest that the binding of HFI to Qβ RNA could be attributed to facilitate the enzyme to make a specific, tight complex with Qβ RNA and could occur preferentially at the uridylate-rich site(s) on the RNA molecule.

Recently, Weber et al. [11] have determined the nucleotide sequence of the enzyme binding site of Qβ RNA. The fragment, derived from the region near the 5′-terminus of coat protein ribosome binding site, is found to comprise of 100 nucleotides and is abundant in uridine nucleotide (about 40% of total nucleotides). At present, it is not known whether HFI binds preferentially to the U-rich replicase binding site.

Acknowledgements

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