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Comparison of RNA Terminal Sequences of Phages f2 and Qβ: Chemical and Sedimentation Equilibrium Studies

Abstract. *The terminal fragment obtained by hydrolysis with ribonuclease T_1 of the ribonucleic acid from the bacteriophage Qβ has been isolated and purified. The results of chemical and enzymatic hydrolysis of this fragment and of the intact RNA itself indicate that the fragment has the composition (10 Cp, 4 Up), and that the RNA has the terminal sequence -GP(9 Cp, 4 Up) CpA. These conclusions are supported by the results of an application of the sedimentation-equilibrium method in which the molecular weight of the Qβ fragment was compared with that of the corresponding fragment from f2 phage RNA for which the terminal sequence, -GpUpUpApCpCpApCpCpCpA had previously been determined.*

In studies on the relation between function and primary structure of large RNA molecules we have concentrated initially on the development of new methods for the determination of nucleotide sequences near the terminals of these molecules (*1–3*). In the case of the RNA from bacteriophages, examination of the sequences at the 3′ terminals has been of particular interest since, with the assumption that the replicative mechanism in such organisms involves the synthesis of complementary strands of RNA in the usual 5′ → 3′ direction, these sequences would be expected to contain the initiation sites for this mechanism. Some of the enzymes involved in replication exhibit a unique specificity in that, for synthetic activity, they require intact homologous viral RNA as template (*4*); the basis for this specificity may reside in the nucleotide sequences at the 3′ terminals. We have reported (*3*) the terminal undecanucleotide sequence of f2 RNA as -GpUpUpApCpCpApCpCpCpA (5). The same terminal sequence has also been reported by De Wachter and Fiers (*6*) for the serologically related phage, MS2. We now report the characterization of the terminal sequence of the RNA obtained from the serologically unrelated phage Qβ and show that this virus has a terminal nucleotide sequence that differs from those of phages f2 and MS2.

For the determination of the terminal nucleoside of phage Qβ (*7*) the RNA [1110 O.D.U. at 260 nm, 50 nmole based on a chain length of 3050] was hydrolyzed with alkali as described (*1*) except that the separation of the terminal nucleoside from the salt and the nucleotides obtained from the reaction mixture was carried out on a DEAE-Sephadex A25 column (HCO_3^- form, 1.0 × 50 cm) in place of the DEAE-cellulose column. The nucleoside was identified by chromatography on a column of DEAE-Sephadex A25 (HCO_3^- form; *p*H 9.0; 0.4 by 100 cm). On such a column, control experiments have shown that the four nucleosides can be separated and quantitatively eluted with water. With this procedure, the Qβ RNA gave adenosine (38 nmole, 76 percent yield) as the only nucleoside.

Additional information on the terminal sequence was obtained by isolation of a larger fragment as described for f2 RNA (*2*). In this method the RNA is specifically cleaved with ribonuclease T_1, the product is oxidized with periodate, and the oxidized terminal oligonucleotide is selectively adsorbed on an aminoethylcellulose column. Subsequently, the absorbed material is released from the column by a β-elimination reaction, and thus the terminal fragment is recovered in a form which lacks the original terminal nucleoside. Application of this method to Qβ RNA gave an oligonucleotide which, on subsequent chromatography on DEAE-Sephadex at *p*H 8, was eluted at a position corresponding to a chain length considerably greater than that of the f2 fragment. In a typical experiment the ribonuclease digestion of 5350 O.D.U. (260 nm) of the RNA gave 12 O.D.U. at 260 nm (a yield of 50 percent) of the oligonucleotide.

The distribution of pyrimidine nucleotides in this product was determined by pancreatic ribonuclease hydrolysis. The fragment (2.53 O.D.U. at 260 nm) was treated with the enzyme (0.05 mg) for 15 hours at 25°C, and the products were analyzed as described (*3*). The only products obtained were cytidine 3′-phosphate (244 nmole) and uridine 3′-phosphate (99 nmole), yielding the ratio Cp : UP = 5.0 : 2.03. The size of the oligonucleotide and the identity of its terminal nucleotide were determined by a procedure (*2*) which consists of enzymatic dephosphorylation of the polynucleotide and subsequent alkaline hydrolysis, yielding the terminal group as a nucleoside. The Qβ oligonucleotide (2.9 O.D.U. at 260 nm) was treated with alkaline phosphatase (0.1 mg) in 0.03*M* tris chloride buffer, *p*H 8.2, for 3 hours at 37°C, and the product was hydrolyzed with 0.25*N* sodium

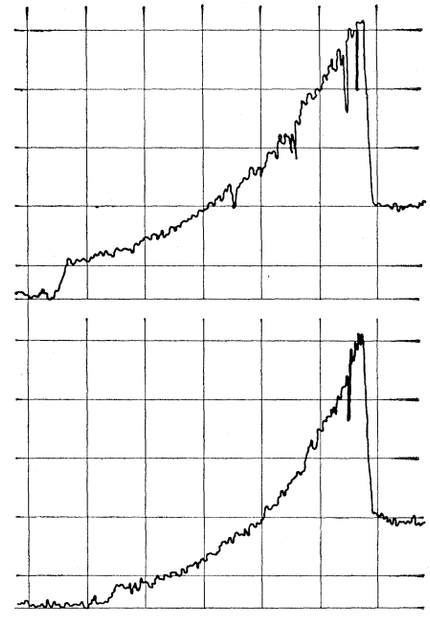

Fig. 1. Sedimentation equilibrium scanner traces for the two terminal oligonucleotides. Each was analyzed at 20°C in 0.2*M* potassium chloride–0.02*M* potassium phosphate buffer, *p*H 7, and at an initial concentration of 0.3 O.D.U. (260 nm) per milliliter. The scans were made at 260 nm after centrifugation for 20 hours. The Qβ fragment (upper trace) was run at 35,600 rev/min, and the f2 fragment at 50,740 rev/min.

hydroxide for 20 hours at 37°C. The mixture was neutralized with carbon dioxide and applied to a DEAE-Sephadex column (HCO_3^- form, *p*H 9.0, 0.4 by 100 cm). Elution with water gave cytidine (27 nmole) as the only nucleoside, and subsequent elution with 0.2*M* ammonium bicarbonate, *p*H 9.0, gave cytidine 2′(3′)-phosphate (252 nmole) and uridine 2′(3′)-phosphate (116 nmole) as the only nucleotides. The ratio of Cp : Up : C was 9.0 : 4.1 : 0.97, thus indicating a chain length of 14.

Application of the sedimentation equilibrium method to the analysis of this fragment and the corresponding oligonucleotide from f2 RNA (UpUpApCpCpApCpCpCp) confirmed the above result. The experiments were performed with a Spinco model E analytical ultracentrifuge and the split-beam photoelectric scanning techniques described by Schachman and Edelstein (*8*). Figure 1 shows typical scanner traces obtained at equilibrium and at the same temperature, with the use of short (3 mm) liquid columns as described by Van Holde and Baldwin (*9*). A comparison of the molecular weights of the two oligonucleotides should be governed by the relation

$$M_{Q\beta} = \frac{M_{f2}\left(\frac{d\ln c}{dr^2}\right)_{Q\beta}\left(\omega^2\right)_{f2}\left(1-\bar{v}\rho\right)_{f2}}{\left(\frac{d\ln c}{dr^2}\right)_{f2}\left(\omega^2\right)_{Q\beta}\left(1-\bar{v}\rho\right)_{Q\beta}}$$

If the partial specific volumes (v) of the two polymers are identical (*10*) and charge effects are similar, the ratio of the apparent molecular weights can be determined directly from the rotor speeds and the slopes of the plots shown in Fig. 2. Substitution in the above equation gave $M_{Q\beta} = 1.43\ M_{f2}$.

Accepting the molecular weight of the f2 fragment as 2805, the calculated value for the Qβ oligonucleotide is 4075. The theoretical molecular weight for the composition (10 Cp, 4 Up) is 4280 and for (9 Cp, 4 Up), 3975. Thus, the results of the chemical and physical experiments indicate that the Qβ terminal fragment is a tetradecanucleotide with the composition (9 Cp, 4 Up)Cp, and this evidence, combined with the known specificity of ribonuclease T_1 and the result of the alkaline degradation of the intact RNA, demonstrates that the terminal sequence of the Qβ RNA is -Gp(9 Cp, 4 Up)CpA.

That the Qβ RNA has a terminal sequence distinctly different from those of f2 and MS2 implies some interesting relations between the terminal sequences and the function of these RNA molecules. For example, these sequence differences may provide the molecular basis for the observed template specificities displayed by the Qβ and MS2 polymerases (*4*). Hori *et al.* (*11*) have found that, in addition to accepting Qβ RNA as a template for its synthetic activity, the Qβ polymerase also recognizes synthetic ribopolynucleotides, provided that the cytidine content is high. Eikhom and Spiegelman (*12*) have separated the Qβ polymerase system into two components and have shown that one of these can specifically synthesize polyguanylic acid when provided with polycytidylic acid as template. Thus, if the initial step in the replicative mechanism in vivo involves the synthesis of a complementary strand in an antiparallel conformation, the terminal sequence of the Qβ RNA, now found to possess a high cytidine content, may constitute the initiation site for the synthetic activity of the polymerase system.

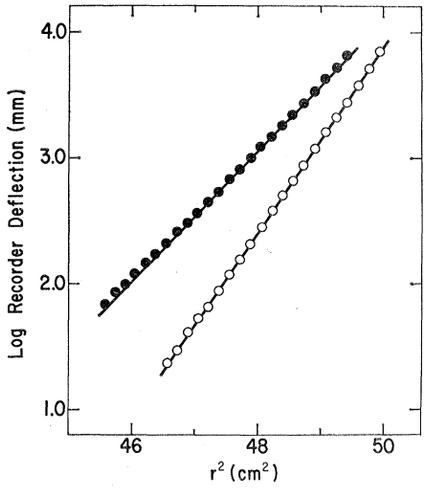

Fig. 2. Molecular weight determinations of the terminal oligonucleotides from f2 RNA (open circles) and Qβ RNA (solid circles). The data were obtained from the traces shown in Fig. 1, and the logarithm of the recorder deflection is plotted against the square of the distance from the axis of rotation. The slope $d(\ln c)/dr^2$ for the f2 fragment is 0.737 and that for the Qβ fragment, 0.527.

In studies on the replication in vitro of Qβ RNA, Bishop *et al.* (*13*) have observed that guanosine is the first nucleotide incorporated in the initial synthesis of the complementary strand, and, with the assumption of complementary copying, they concluded that the terminal nucleotide of Qβ RNA is cytidine. This conclusion is not borne out by our results, although it should be pointed out that our experiments were carried out on mature virus (containing RNA synthesized in vivo) whereas Bishop *et al.* base their conclusion on the synthetic capacity of the Qβ polymerase in vitro. The two conclusions can be accommodated. If we assume that the polymerase displays, in vitro, a specificity in its synthetic activity identical to that exerted in vivo, and that this activity involves, initially, complementary copying in an antiparallel fashion, we then must conclude that one or more of the terminal bases of the mature Qβ RNA are not copied by the replicative mechanism. Thus, if some of the terminal bases of the parental RNA are ignored by the polymerase in vivo, these bases must be added enzymatically to the progeny resulting from the replication of the rest of the chain. This proposed terminal addition could be the result of enzyme activity similar to or identical to that of the transfer RNA adenylate (cytidylate) pyrophosphorylase which has the capacity to add -CpCpA ends to unfinished transfer RNA molecules.

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5. Abbreviations: RNA, ribonucleic acid; DEAE, diethylaminoethyl; O.D.U., optical density unit; tris, tris(hydroxymethyl)aminomethane. Cp, cytidine 2′ or 3′ phosphate; Up, uridine 2′ or 3′ phosphate; C, cytidine; A, adenosine; G, guanosine. Sequences are indicated in the standard way by the linear array of nucleotides, with the 3′-hydroxyl terminus to the right. Nucleotides in parentheses are of still-undetermined sequence.
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