Preferential Inhibition by Poly(Adenylic Acid) of Minus-Strand Synthesis by Bacteriophage Q β Ribonucleic Acid Replicase

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(Received 19 February 1976)

Among the homopolymers examined, poly(A) was found to inhibit preferentially the synthesis of the minus strand by bacteriophage Q β RNA replicase in the presence of host factor. A specific interaction of poly(A) with the host factor is suggested to be a principal cause for the observed preferential inhibition by poly(A) of the host-factor-requiring Q β RNA replicase reaction.

Initiation of RNA synthesis by bacteriophage Q β RNA replicase (hereafter referred to as 'Q β replicase') can be inhibited, by possibly different mechanisms, with a variety of components, such as the polyanion polyethylene sulphonate (Kondo & Weissmann, 1972*a*), the triphenylmethane dye aurintricarboxylic acid (Blumenthal & Landers, 1973), guanosine 3',5'-bisdiphosphate (Landers *et al.*, 1974), excess of Q β plus strand (Kondo & Weissmann, 1972*b*), synthetic homopolymers (Haruna & Spiegelman, 1966; Kondo & Weissmann, 1972*b*) and tobacco-mosaic-virus RNA (Okada *et al.*, 1969; Kondo & Weissmann, 1972*b*).

The present experiments were undertaken to gain more information on a possible mechanism by which synthetic homopolymers inhibit Q β replicase reactions. It was found that poly(A) inhibited preferentially the synthesis of the minus strand directed by Q β plus strand. However, poly(C) and poly(U) seemed to inhibit efficiently the syntheses of both plus and minus strands by Q β replicase.

Experimental

Q β replicase host factor and Q β RNA

Q β replicase (1400 units/mg; 1 unit = 1 nmol of GMP incorporated/20 min) and heat-stable host factor were prepared as previously described (Kondo *et al.*, 1970; Kondo & Weissmann, 1972*a*). Q β plus strand was purified from the bacteriophage particles (Weissmann *et al.*, 1968) and Q β double-stranded RNA was prepared by the method of Billetter *et al.* (1966).

Q β replicase reaction

A reaction mixture (10–40 μ l) containing 80 mM-Tris/HCl (pH 7.4), 12 mM-MgCl₂, 1 mM-EDTA, nucleoside triphosphates (0.8 mM each, except one) for radioactive labelling, Q β replicase, host factor and template, as specified in the Figure legends and Table

1, was incubated at 37°C. Acid-insoluble radioactivity was determined by using glass-fibre discs (Whatman GF/C, 25 mm) (Kondo & Weissmann, 1972*a*).

RNA hybridization

Strand species of the enzyme product were determined as follows. The replicase product was made 0.1% with respect to sodium dodecyl sulphate, and about 0.1 vol. of Chelex (Na⁺ form; Bio-Rad Laboratories, Richmond, CA, U.S.A.). RNA was extracted with 2 vol. of phenol at room temperature (12°C) and dialysed first against 1 $\times$ SSC (150 mM-sodium citrate/15 mM-NaCl, pH 7.0) and then against 0.5 mM-EDTA (pH 7.0). After dialysis, RNA was directly used for the annealing assay described previously (Weissmann *et al.*, 1968; Kondo & Weissmann, 1972*b*).

Homopolymer concentration

The concentration of homopolymers (Miles Laboratories, Kankakee, IL, U.S.A.) was determined spectrophotometrically assuming the following molar extinction coefficients (at pH 12): for adenosine, 14900 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ at 259.5 nm; for cytidine, 9100 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ at 271 nm; and for uridine, 8500 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ at 262 nm.

Results and Discussion

Fig. 1 demonstrates a differential effect of poly(A) on the Q β replicase reaction with either plus (Fig. 1*a*), minus (Fig. 1*b*) or both types of strands (Fig. 1*c*) as template. Fig. 1(*b*) shows that the synthesis of the plus strand was significantly resistant to the poly(A) inhibition as determined by a reaction using denatured double-stranded RNA as template in the absence of host factor, in which only the minus strand could elicit RNA synthesis (Feix *et al.*, 1968). By contrast, the plus-strand-directed RNA synthesis, which required host factor, was strongly inhibited by

Fig. 1. Differential effect on $Q\beta$ replicase reaction of homopolymers

The reaction mixture (10 μ l) contained 0.7 μ g of $Q\beta$ replicase, 2 μ g of host factor (for *a* and *c*), 0.11 μ g of plus strand (for *a*) or 0.28 μ g of denatured double-stranded RNA (for *b* and *c*), 7.7 nmol of [³²P] ATP (1.1×10^4 c.p.m./nmol), homopolymers as indicated in the Figure, and other components as specified in the Experimental section. The 100% values without homopolymer were 6235, 2626 and 6375 c.p.m. for (*a*), (*b*) and (*c*) respectively. ○, With poly(U); Δ, with poly(A); □, with poly(C).

poly(A) (Fig. 1*a*). On the other hand, poly(C) and poly(U) inhibited to a roughly similar extent the $Q\beta$ replicase reaction directed by either plus or minus

strand (Figs. 1*a* and 1*b*). The same characteristics in the inhibition by these homopolymers were also observed when the ratio homopolymer/template was 1:2 or less (M. Kondo, unpublished work).

The $Q\beta$ replicase reaction with denatured double-stranded RNA as template seemed to be more resistant to the homopolymer inhibition (Figs. 1*b* and 1*c*) than was the one using the pure single-stranded RNA as template (Fig. 1*a*). This could possibly be due to a simultaneous presence of both plus and minus strands in a reaction, for a similar resistance of the $Q\beta$ replicase reaction to excess of template was also

Fig. 2. Effect of poly(A) and poly(C) on the kinetics of RNA synthesis by $Q\beta$ replicase

The reaction mixture (20 μ g) contained 1.4 μ g of $Q\beta$ replicase, 8 μ g of host factor, 0.27 μ g of plus strand (for *a*) or 1.12 μ g of denatured double-stranded RNA (for *b*), 14.2 nmol of [³²P]ATP (1.5×10^5 c.p.m./nmol), and other components as specified in the Experimental section. When present, 0.2 μ g of poly(A) and 0.33 μ g of poly(C) were included. ○, Control without homopolymers; Δ, with poly(A); □, with poly(C).

Table 1. *Product analysis of the Q β replicase reaction in the presence or absence of poly(A)*

The reaction mixture (40 μ l) contained 2.9 μ g of Q β replicase, 1.12 μ g of denatured double-stranded RNA, 16 μ g of host factor, and other components as specified in the Experimental section. After 1 min preincubation, the reaction mixture received 12.3 nmol of [^{32} P]ATP (3.8×10^4 c.p.m./nmol), and was incubated for further 5 min. A 30 μ l portion was then removed and immediately diluted to 100 μ l with 10 mM-Tris/HCl, pH 7.5. Product RNA was purified and analysed as described in the Experimental section. (1) Annealed radioactivity was corrected for the values obtained after RNAase (ribonuclease) digestion (5 μ g/ml, 30 min at 25°C) of the previously heat-denatured samples, namely 74 and 47 c.p.m. for the reaction without and with poly(A) respectively. (2) The radioactivity was adjusted according to the AMP incorporation obtained after 5 min incubation, namely 8423 and 5800 c.p.m./2 μ l for the reaction without and with poly(A) respectively.

Poly(A) addition (μ g)	Input radioactivity [c.p.m. (%)]	RNAase-resistant radioactivity [c.p.m. (%)]			Strand species in product [c.p.m. (%)] (2)	
		(1)				
		Strand input (μ g)			Plus	Minus
		3.3	6.6	13.2		
None	7321 (100)	1937 (26.5)	2048 (28)	1723 (23.7)	4625 (73.9)	1634 (26.1)
0.4	4311 (100)	182 (4.2)	195 (4.5)	193 (4.5)	4121 (95.6)	193 (4.4)

observed when denatured double-stranded RNA was used for template (Kondo & Weissmann, 1972*b*, and unpublished work). Nevertheless, it could be concluded that poly(A) was significantly different from other homopolymers in its inhibitory effect on the Q β replicase reaction, depending on whether or not it required host factor.

Fig. 2 shows the kinetics of AMP incorporation by Q β replicase in the presence or absence of homopolymers, which was directed by either plus strand (Fig. 2*a*) or denatured double-stranded RNA in the absence of host factor (Fig. 2*b*). In these experiments the ratio homopolymer/template was about 0.74:1 (plus strand) and 0.6:1 (minus strand, assuming an average minus-strand content in the double-stranded RNA of about 30%) for poly(A), and 1.2:1 (plus strand) and 1:1 (minus strand) for poly(C). Under these conditions, poly(A) inhibited preferentially synthesis of the minus strand, but not that of plus strand, in contrast with the poly(C) effect. The differential inhibition by poly(A) was further confirmed by the hybridization experiment (Table 1), in which the RNA product [synthesized by denatured double-stranded RNA (as template)] was analysed for content of the strand species by the annealing technique. Table 1 shows that the presence of poly(A) in a Q β replicase reaction caused a specific decrease in the minus-strand content in the RNA product of 90% as compared with the value obtained in the absence of poly(A), whereas it could decrease the plus-strand content only by 10%.

It was shown that host factor inhibited preferentially poly(A)-directed protein synthesis *in vitro* (Carmichael *et al.*, 1975), and also was able to bind tightly to poly(A)-cellulose, from which host factor was released only after elution with 8 M-urea in 1 M-NaCl (Carmichael, 1975). On the other hand, Q β

replicase, as well as its largest subunit, could bind tightly to poly(C)- and poly(U)-cellulose, but not so tightly to poly(A)-cellulose (Carmichael, 1975). Moreover, the largest subunit was found to inhibit specifically poly(C)- and poly(U)-dependent protein syntheses *in vitro*, but not poly(A) translation (Miller & Wahba, 1974). Thus it became clear that host factor could specifically interact with poly(A), to which host factor seemed to remain bound very tightly. Therefore a specific complex-formation between host factor and poly(A) was suggested to be a principal cause for the preferential inhibition by poly(A) of the host-factor-requiring Q β replicase reaction. In support of this prediction, the extent of inhibition by poly(A) of the plus-strand-directed RNA synthesis was indifferent to the ratio of homopolymer to template, whereas that by poly(C) or poly(U) was rather dependent on this ratio (Haruna & Spiegelman, 1966; M. Kondo, unpublished work).

Since the minus-strand-directed Q β replicase reaction is independent of host factor (Feix *et al.*, 1968), poly(A) would not be expected to exert any significant effect on this enzyme reaction. The present results were compatible with this interpretation. However, since both poly(C) and poly(U) could interact strongly with Q β replicase (Carmichael, 1975), these homopolymers were expected to interfere with the Q β replicase reaction regardless of whether it required host factor or not. Although the mechanism of inhibition by the latter homopolymers is not yet understood, this prediction is consistent with the present results. At least for poly(C), it can be assumed that it inhibits the Q β replicase reaction by competition with natural template RNA for an initiation site of the enzyme molecule, since poly(C) itself, but not poly(U), is an excellent template for Q β replicase (Hori *et al.*, 1967).

I thank the Belgian National Funds for Scientific Research for grants, and J. Boons for his technical assistance. Part of this work was carried out at the Institute of Molecular Biology, University of Zurich.

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