THE INHIBITION OF f2 RNA DIRECTED E. COLI PROTEIN SYNTHESIS BY POLY rI•rC IN VITRO[1]

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SUMMARY

Poly rI•rC inhibits both f2 RNA and T4 mRNA directed in vitro ^{3}H-lysine incorporation by an E. coli S-30 extract. The inhibition of f2 RNA directed cell-free protein synthesis is highly concentration dependent in that the inhibition is maximal at both high (10 μg/ml) and low (0.01 μg/ml) concentrations but minimal at intermediate concentrations (about 1 μg/ml). The inhibition is found to be due to an interaction between poly rI•rC and f2 RNA. Our results suggest that the conformation of f2 RNA might play a role in poly rI•rC inhibition.

There have recently been a number of reports that various heterologous RNA's have an inhibitory effect on in vitro protein synthesis (1,2,3,4). We have been studying products induced by T4 bacteriophage infection of E. coli as selective inhibitors of translation. In a control experiment, the effect of poly rI•rC was studied. The purpose of this paper is to report the results of this experiment. In brief, we find that poly rI•rC is a potent inhibitor of in vitro protein synthesis when f2 bacteriophage RNA is used. T4 mRNA directed in vitro protein synthesis is also inhibited, but to a lesser degree. There are indications that some cistrons of the f2 RNA are inhibited more than others by poly rI•rC.

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[1]Abbreviations: Poly rI•rC - homopolymeric nucleotide duplexes of polyribo-inosinic acid•polyribocytidilic acid; Poly rI - polyriboinosinic acid homopolymer; Poly rC - polyribocytidilic acid homopolymer.

MATERIALS AND METHODS

Bacteriophage f2 was grown and purified as described by Davern (5) with E. coli K 38 as the host cell. Phage RNA was extracted by the procedure of Webster et al. (6). Purified phage RNA was lyophilized and dissolved in glass-distilled water.

Bacteriophage T4 mRNA was prepared according to Young and Van Houwe (7). Infection of E. coli B with wild type T4 phage was carried out at 30°C for 20 minutes in M9S medium.

The S-30 extract was prepared from E. coli Q-13 cells grown in M9S medium to a cell density of 5×10^8 cells/ml. Harvested cells were washed in TM buffer and were broken by sonication. The crude extract was centrifuged at 30,000xg for 30 minutes to remove cell debris. The 30,000xg supernatant was used for ^{3}H-lysine incorporation.

The in vitro incorporating was described by Webster et al. (6). The incubation mixture was the following: 3 mM ATP, 0.2 mM GTP, 10 mM PEP, 10-20 µg/ml pyruvic kinase, 10 mM glutathione, 0.1 mM of each amino acid except lysine, 9 mM magnesium acetate, 30 mM ammonium chloride and 50 mM Tris-HCl, pH 7.8. The S-30 extract was added (3 mg/ml) to this incubation mix, and the mixture allowed to incubate at 37°C for 10 minutes. Following the preincubation period, f2 RNA (300 µg/ml) and ^{3}H-lysine (0.05 mM, 340 µCi/µ mole) were added, and the reaction allowed to continue at 37°C for 20 minutes. The time course of ^{3}H-lysine incorporation was linear up to 20 minutes. The reaction mixture was terminated by the addition of 5% TCA and then heated at 95°C for 15 minutes. The precipitate was collected on Whatman glass fiber filter paper and the papers were washed with 5% TCA plus 0.005 M lysine, dried, and counted in toluene-based scintillation fluid.

^{3}H-lysine was purchased from Schwarz/Mann. Poly rI, poly rC and poly rI·rC were obtained from Biopolymer, Inc.

RESULTS AND DISCUSSION

The inhibitory effect of poly rI·rC on in vitro protein synthesis is

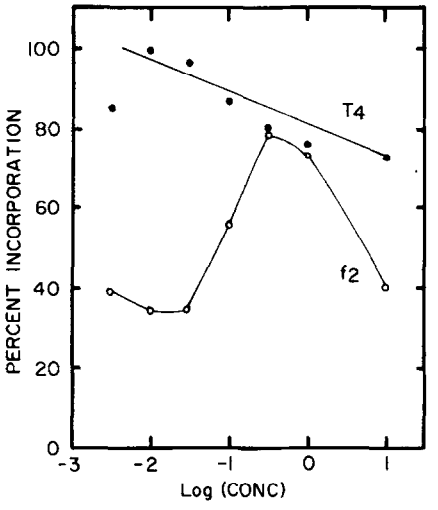

Figure 1. Effect of poly rI•rC on ^{3}H-lysine incorporation by E. coli S-30 system. The reaction conditions are as described in Materials and Methods except that T4 mRNA concentration is 800 ug/ml. Concentrations of poly rI•rC in ug/ml is expressed in log scale. ^{3}H-lysine incorporation in the absence of poly rI•rC is taken as 100% incorporation. —•—•—, T4 mRNA directed protein synthesis; —o—o—, f2 RNA directed protein synthesis.

shown in Figure 1. Poly rI•rC at 10 μg/ml inhibits T4 mRNA directed cell-free protein synthesis by 25%. Lower concentrations of poly rI•rC are less inhibitory. At 0.01 μg/ml, this inhibition is completely abolished. When f2 RNA is used as message, however, the inhibitory pattern is quite different. More than 50% inhibition is observed in the presence of poly rI•rC at both low (0.005-0.01 μg/ml) and high (10 μg/ml) concentrations. However, the inhibition is minimal at intermediate concentrations (about 0.5-1 μg/ml). It is interesting to note that this peculiar concentration dependent inhibition of protein synthesis by poly rI•rC has also been observed in reticulocyte lysate system by Ehrenfeld and Hunt (3). They found that poly rI•rC inhibits protein synthesis at 0.01 μg/ml and this inhibition is not observed at 10 μg/ml. The mechanism of poly rI•rC inhibition on protein synthesis in an E. coli system at such low concentrations is not clear at present. Further experiments were carried out to test the possibility that the inhibition might be due to the double strandedness of the polymer used. Single stranded homopolymers, poly rI and poly rC,

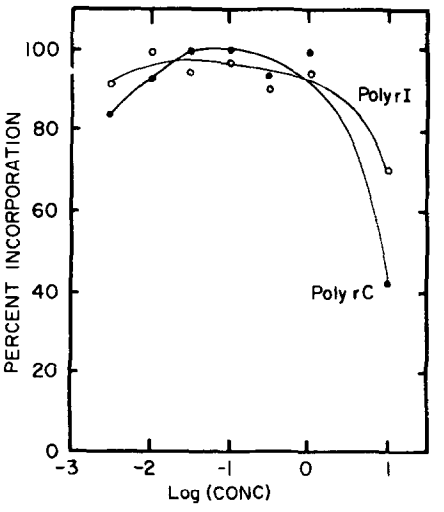

Figure 2. Effect of poly rI and poly rC on f2 RNA directed ^{3}H-lysine incorporation. The reaction conditions are as described in Materials and Methods. Concentrations of poly rI and rC in μg/ml are expressed in log scale. ^{3}H-lysine incorporation in the absence of any synthetic polyribonucleotides is taken as 100% incorporation. —•—•—, incorporation in the presence of poly rI; —o—o—, incorporation in the presence of poly rC.

are added separately at different concentrations to the reaction mixtures with f2 RNA as message. Poly rI or poly rC has no effect at low concentrations (Fig. 2). At concentrations above 1 μg/ml both poly rI and poly rC are strongly inhibitory. These results indicate that the inhibition of E. coli protein synthesis at low concentrations is peculiar to poly rI•rC, since both poly rI and poly rC are inhibitory at high concentrations but are ineffective at low concentrations. Furthermore, the inhibition by poly rI•rC appears specific for f2 RNA at low concentrations but non-specific at high concentrations where both f2 RNA and T4 mRNA directed protein synthesis are inhibited.

The inhibition observed at low concentrations of poly rI•rC may represent an interaction between poly rI•rC and ribosomes, initiation factors, tRNA or mRNA. First three possibilities were eliminated since such interactions should produce non-specific inhibition regardless of mRNA source. Our results (Fig. 1) show that low concentrations of poly rI•rC specifically inhibits f2 RNA directed protein synthesis but not T4 mRNA directed protein synthesis. Reac-

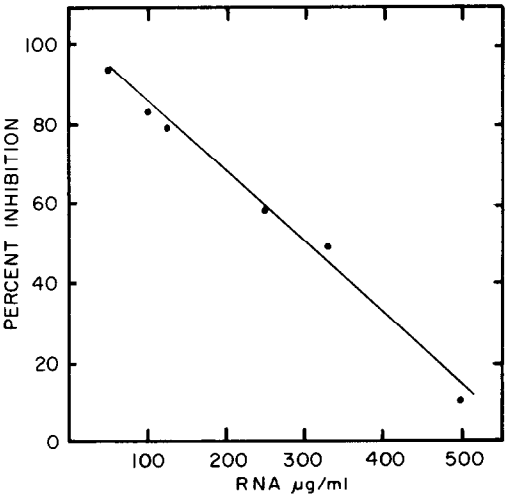

Figure 3. Effect of changing f2 RNA concentrations on poly rI•rC inhibition. The reaction conditions are as described in Materials and Methods except that varying amounts of f2 RNA were used. Poly rI•rC concentration was fixed at 0.01 µg/ml in all reaction mixtures. ^{3}H-lysine incorporation directed by f2 RNA (500 µg/ml) in the absence of poly rI•rC is taken as zero percent inhibition.

tions were therefore carried out at varying amounts of f2 RNA but fixed poly rI•rC concentration (0.01 µg/ml). The results (Fig. 3) clearly indicate that poly rI•rC interacts with f2 RNA since high concentrations of f2 RNA overcome this inhibition.

It has been shown that the conformation of f2 RNA plays a role in translational regulation (8,9,10,11). The interaction between poly rI•rC and f2 RNA, therefore, may also depend on the conformation of f2 RNA. To test this hypothesis, f2 RNA was heated for 3 minutes at 60°C or 70°C and ^{3}H-lysine incorporation was carried out in the presence or absence of low concentration of poly rI•rC. It has been shown that native f2 RNA directs primarily coat protein synthesis, 70°C treated-f2 RNA directs primarily replicase synthesis, while 60°C treated-f2 RNA directs the synthesis of all three proteins (12). The data from Table 1 indicate that poly rI•rC only inhibits native f2 RNA directed protein synthesis but has no effect when heat-treated f2 RNA is used as message. It is evident, therefore, that the conformation of f2 RNA plays a role in

poly rI•rC inhibition. Furthermore, this inhibition might be specific for the coat protein cistron which is presumably synthesized predominantly by the native f2 RNA. Further experiments to clarify this observation are now in progress.

Double stranded RNA is known to occur in bacteriophage T4 infected E. coli cells (13). The role of the T4 induced double stranded RNA, if any, is not clear. Our results indicate that double stranded poly rI•rC specifically interact with f2 RNA in E. coli protein synthesis at very low concentrations. The significance of this inhibition remains to be seen.

Table 1. Effect of poly rI•rC on native and heat-treated f2 RNA directed protein synthesis. The reaction conditions are as described in Materials and Methods. f2 RNA was heated to the indicated temperatures for 3 min. and quickly cooled to 4°C before using for ^{3}H-lysine incorporation. Poly rI•rC concentration was fixed at 0.01 ug/ml. ^{3}H-lysine incorporation directed by native f2 RNA in the absence of poly rI•rC is taken as 100% incorporation.

Message	Poly rI•rC (0.01 ug/ml)	Incorporation (c.p.m.)	% Incorporation
Native f2 RNA	-	2346	100
	+	1652	69
Heated f2 RNA	-	1171	49
(60°C - 3 min.)	+	1148	48
Heated f2 RNA	-	1232	51
(70°C - 3 min.)	+	1243	52
No RNA		60	—

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