Mechanism of Stimulation of in Vitro Protein Synthesis by Some Copolymers of Styrene, Vinyluracil, and Vinyladenine with Maleic Acid and Acrylic Acid†

Sophia Boguslawski, Paul E. Olson, and Mathias P. Mertes*

ABSTRACT: Copolymers of vinyl bases with acrylic acid and styrene or 1-vinyluracil with maleic acid were found to stimulate in vitro polyphenylalanine synthesis using a system extracted from *Escherichia coli* MRE600. Poly(styrene-maleic acid) was found to inhibit a ribosomal bound ribonuclease. Poly(1-vinyluracil,maleic acid), poly(1-vinyluracil,acrylic acid), and poly(9-vinyladenine,acrylic acid) were not inhibitors of the ribosome bound ribonuclease. The potent (up to fivefold) stimulation by these three polymers is due to the action of the polymers to interfere with ribosomal bound inhibitory protein. A protein, removed by washing ribosomes with 1 M ammonium chloride, characterized by M. J. Miller, A. Niveleau, and A. J. Wahba ((1974) *J. Biol. Chem. 249,* 3803) has been described as a potent inhibitor of in vitro poly(U)-coded protein synthesis using extracts of *Escherichia coli* MRE 600.

Synthetic analogues of nucleic acids, explored as potential chemotherapeutic agents, have shown some interesting biological properties. Polyvinyluracil and polyvinyladenine did not function as messages for in vitro protein synthesis; however, they did inhibit coded synthesis by their complimentary nucleic acids poly(A) and poly(U) (Reynolds et al., 1972). These polymers also inhibited murine leukemia virus replication in mouse-embryo cells (Pitha et al., 1973).

We have observed that some copolymers of styrene or vinyl bases with either acrylic acid or maleic acid did not inhibit in vitro protein synthesis, but, rather gave a substantial increase in poly(U)-coded polyphenylalanine synthesis (Olson et al., 1975). Poly(inosinic acid)-poly(cytidylic acid), a potent interferon inducer (Field et al., 1967), also showed stimulation of poly(U)-coded in vitro protein synthesis using a system isolated from *Escherichia coli* MRE 600.

While several mechanisms for this observed stimulation could explain the results, the most obvious would be that the copolymers inhibit an RNase in the system and prolong the effective life of the message. Another mechanism suggested by the preliminary studies is that the copolymers counteract the inhibitory effects of a ribosomal-bound protein, subunit I of Qβ replicase, thereby giving increased protein synthesis (Miller et al., 1974). This report summarizes the results of studies on these possibilities.

Materials and Methods

Poly(U), [^{3}H]poly(U), poly(A), and poly(I)-poly(C) were products of Miles Laboratories. [^{3}H]- and [^{14}C]phenylalanine and [^{3}H]lysine were products of Schwarz/Mann. *E. coli* B tRNA was purchased from Plenum Scientific; poly(acrylic acid) was from Aldrich Chemicals. DNase and other common chemicals were purchased from Sigma. The polymers used in this study have been described (Olson et al., 1975). *E. coli* MRE 600 cells were furnished by the Enzyme Laboratory, University of Kansas.

Preparation of E. coli Ribosomes and S-100. All isolations were carried out at 2 °C. Thawed *E. coli* MRE 600 cells (30 g) were disrupted by grinding with 45 g of washed sand and 20 ml of buffer A: 50 mM Tris-HCl (pH 7.8) with 10 mM magnesium chloride, 6 mM 2-mercaptoethanol, and 60 mM ammonium chloride. Alternatively, larger quantities were disrupted by sonication using the same buffer. The mixture was centrifuged (10 000*g*) for 15 min, DNase (5 μg/ml) and 2-mercaptoethanol (6 μmol/ml) were added to the supernatant, and the solution was stirred for 15 min. The solution was clarified by centrifugation for 20 min at 30 000*g*. Ribosomes were collected by centrifugation of the S-30 supernatant at 105 000*g* for 2 h. The S-100 fraction was frozen and stored in small aliquots at −70 °C. Ribosomes were resuspended in buffer A, centrifuged as described, and stored in small aliquots in a minimal amount of buffer A at −70 °C.

Ribosomes free of subunit I of Qβ replicase and crude subunit I fractionation were obtained by overnight extraction of ribosomes in a buffer containing 1 M ammonium chloride, 20 mM Tris-HCl (pH 7.8), 10 mM magnesium acetate, and 10 mM 2-mercaptoethanol. Ribosomes free of subunit I were collected by centrifugation as described, resuspended in buffer A, and stored in small aliquots at −70 °C.

Subunit I of Qβ replicase was purified from the wash by ammonium sulfate fractionation as described by Miller et al. (1974).

Assay of Poly(U)-Dependent Polyphenylalanine Synthesis. The reaction mixture contained 50 mM Tris-HCl (pH 7.8), 56 mM ammonium chloride, 6 mM 2-mercaptoethanol, 5 mM ATP, 0.5 mM GTP, 15 mM magnesium chloride, 120 μg of *E. coli* B tRNA, 5 μl of S-100, 1 A_{260} unit of ribosomes, poly(U) as indicated, 30 μM [^{3}H]phenylalanine (sp act. 0.5 mCi/μmol), and polymer added as indicated in a total volume of 0.1 ml. After incubation for 20 min at 37 °C, the reaction was terminated by adding approximately 2 ml of 10% trichloroacetic acid. The samples were heated to 95 °C for 10 min, cooled, filtered on glass filter pads, and the pads washed three times with 5% trichloroacetic acid and finally with ethanol. The dried filter pads were counted in a Beckman scintillation counter.

Assay of RNase Activity in E. coli Extracts. The reaction conditions were the same as those used for polypeptide syn-

† From the Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, Kansas 66045. *Received November 14, 1975.* Supported by Grants CA 16423 from the National Cancer Institute and GM 1341 from the Institute of General Medical Sciences, National Institutes of Health.

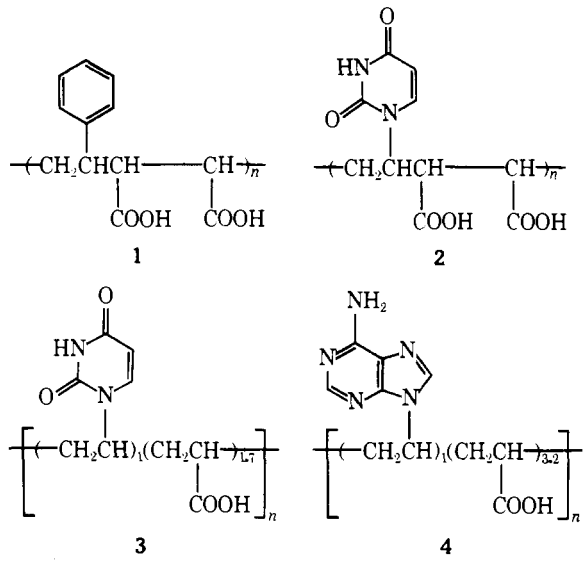

FIGURE 1: Copolymers of styrene-maleic acid, poly(S-MA) **1**; 1-vinyluracil-maleic acid, poly(vU,MA) **2**; 1-vinyluracil,acrylic acid, poly(vU,AA) **3**; and 9-vinyladenine,acrylic acid, poly(vA,AA) **4**.

thesis except for the absence of labeled amino acid and using 2.5 μg of [^{3}H]poly(U), specific activity 0.0148 μCi/μg. The time of incubation was as indicated. After incubation, the entire assay solution was applied to DEAE-cellulose disks (2.4 cm, Whatman DE81) and the disks were soaked for 5 min each in three different solutions containing 0.5 M disodium phosphate, rinsed in water, ethanol, dried, and the disks counted.

Phe-tRNA Synthesis in S-100 Extracts. The incubation mixture contained 0.1 M Tris-HCl (pH 7.5), 5 mM magnesium chloride, 2 mM ATP, 0.1 mM DTT, 80 mM ammonium chloride, 0.1 mM EDTA, 0.6 mg of *E. coli* tRNA, 50 μM [^{3}H]phenylalanine (0.5 mCi/μmol), and 5 μl of *E. coli* S-100 in a total volume of 0.1 ml. After incubation for 15 min at 37 °C, the reaction was terminated by adding 2 ml of 10% HCl. The samples were cooled, filtered on glass filter pads, washed with 5% HCl and ethanol, and the pads were dried and counted in a scintillation counter.

Results

Copolymers of the general structural types shown in Figure 1 were prepared by radical induced copolymerization (Olson et al., 1975). The copolymers poly(S-MA)[1] (**1**) and poly(vU,MA) (**2**) are in a 1:1 ratio of monomers; however, some polymerization involving the 5–6 double bond of the uracil ring is thought to be present in **2**. Compounds **3** and **4**, poly(vU,AA) and poly(vA,AA), are random copolymers with the calculated ratio of the monomers indicated in the formulas shown in Figure 1. Polymers were fractionated on Sephadex G-75 and those fractions in the void volume were used.

It was found that these polymers stimulated poly(U)-coded polyphenylalanine synthesis in a cell-free system from *E. coli* MRE 600. The extent of stimulation was dependent on the concentration of polymer and poly(U).

The polymers do not act as messages since, in the absence of poly(U), no incorporation was observed. The stimulation observed as acid insoluble radioactivity was true poly(phenylalanine) synthesis because the addition of the polypeptide synthesis inhibitor puromycin (5 μg) abolished incorporation of [^{3}H]phenylalanine in the acid precipitated material.

TABLE I: Stimulation of in Vitro Polyphenylalanine Synthesis by Polymers at Different Levels of Poly(U).[a]

μg of poly(U)	Additions	Concn (μg)	[^{3}H]Phe incorporated, pmol (% of control)
0.53	None		19
0.53	Poly(S-MA) (1)	6.1	46 (242)
0.53	Poly(vA,AA) (4)	19.6	96 (505)
1.59	None		27
1.59	Poly(S-MA) (1)	6.1	72 (266)
1.59	Poly(vA,AA) (4)	19.6	108 (400)
3.20	None		36
3.20	Poly(S-MA) (1)	6.1	67 (180)
3.20	Poly(vA,AA) (4)	19.6	91 (360)
7.20	None		51
7.20	Poly(vU,AA) (3)	16.8	98 (192)
14.4	None		68
14.4	Poly(vU,AA) (3)	16.8	92 (135)
28.8	None		87
28.8	Poly(vU,AA) (3)	16.8	100 (114)

[a] The blank (4 pmol), without poly(U), was subtracted from all samples. The preparation derived from *E. coli* MRE 600 was assayed according to procedures described under Methods.

Table I shows the stimulation of polyphenylalanine synthesis afforded by poly(S-MA) (**1**), poly(vU,AA) (**3**), and poly(vA,AA) (**4**) at different levels of poly(U). With very low levels of poly(U) (0.53 μg) up to fivefold stimulation was observed (compound **4**), while at higher poly(U) concentrations (28.8 μg) very little stimulation was found using poly(vU,AA) (**3**) during the 20-min incubation period.

A time-course study of the stimulatory effects of poly(S-MA) (**1**) and poly(vA,AA) (**4**) on polyphenylalanine synthesis revealed that during the first 10 min very little stimulation was observed. However, synthesis in the control reaction stopped at 20 min, while the synthesis in the presence of the polymers continued at the initial rate for 45 min. A tenfold increase in the amount of poly(U) did not alter the time course of the stimulated assays. The results in Figure 2 demonstrate that the synthesis in the sample with 1.1 μg of poly(U) stopped after 10 min, while using 11 μg of poly(U) synthesis continued for 25 min. Poly(vA,AA) (**4**) at both levels of message gave essentially the same pattern—synthesis continued at the initial rate for 45 min.

The fact that stimulation was greater at the lower level of poly(U) and that incorporation in the absence of polymer ceased after 10 min suggested that these polymers protected the message against degradation by nucleases. This possibility was examined by preincubation of limiting amounts of message (2.9 μg) with S-100 and ribosomes with or without polymers for 8 min after which [^{3}H]phenylalanine was added. It was found (Table II) that preincubation of message in the control samples gave only 10% of the incorporation found when poly(U) was not preincubated. In the presence of the polymers the samples retained almost full activity. Polyacrylic acid, ineffective in stimulating polyphenylalanine synthesis, did not preserve the message.

The presence of nucleases in the *E. coli* extracts was shown in an experiment in which [^{3}H]poly(U) was incubated under

[1] Abbreviations used are: poly(S-MA), poly(styrene-maleic acid); poly(vU,MA), poly(1-vinyluracil,maleic acid); poly(vU,AA), poly(1-vinyluracil,acrylic acid); poly(vA,AA), poly(9-vinyladenine,acrylic acid); ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; DEAE, diethylaminoethyl; DTT, dithiothreitol; EDTA, (ethylenedinitrilo)-tetraacetic acid.

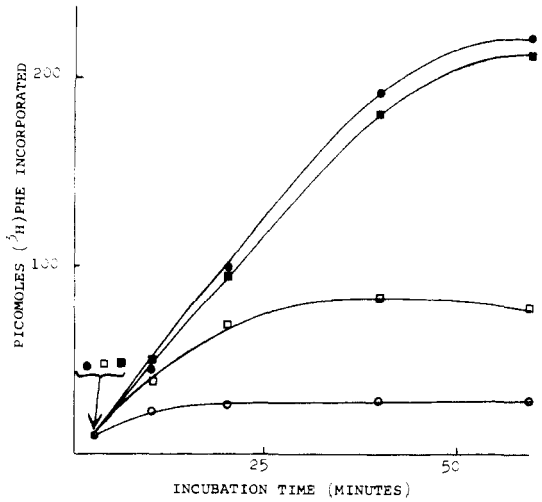

FIGURE 2: Time-course study for the in vitro incorporation of [^{3}H]Phe in the presence of polymers using extracts from *E. coli* MRE 600: 1.1 μg of poly(U) (○); 1.1 μg of poly(U) and 16.3 μg of poly(vA,AA) (**4**) (●); 11 μg of poly(U) (□); 11 μg of poly(U) and 16.3 μg of poly(vA,AA) (**4**) (■).

TABLE II: Effect of Polymers on in Vitro [^{3}H]Phenylalanine Incorporation after Preincubation.[a]

Additions	Concn (μg)	pmol of [^{3}H]Phe Incorporated after Preincubation −Poly(U)	+Poly(U)	% of Control
None		17	2	11
Poly(S-MA) (**1**)	6.1	68	76	114
Poly(vU,MA) (**2**)	14.2	100	40	40
Poly(vU,AA) (**3**)	10.0	92	82	89
	16.8	100	92	92
Poly(vA,AA) (**4**)	1.3	48	38	79
	19.6	104	112	107
Poly(I)-poly(C)	72	37	24	64
Poly(acrylic acid)	3.6	9	1	11

[a] The samples were incubated for 8 min at 37 °C with polymers as indicated. The control contained 2.9 μg of poly(U) added after preincubation (column 3). Poly(U) (2.9 μg) was added to those samples in column 4 before preincubation. [^{3}H]Phe was added to all samples after preincubation and the assay was run as described under Methods.

TABLE III: Degradation of [^{3}H]poly(U) by Ribosome and S-100 Preparations from *E. coli* MRE 600.[a]

Additions	Time of Reaction (min)	cpm	% of Control
None	8	7760	100
Ribosomes	8	2300	30
S-100	8	887	11
Ribosomes + S-100	0	7000	90
Ribosomes + S-100	2	4300	55
Ribosomes + S-100	5	2000	26
Ribosomes + S-100	8	1550	20
Ribosomes + S-100	10	1000	13

[a] Samples containing [^{3}H]poly(U) (2.5 μg) and all other additions used in poly(Phe) synthesis except phenylalanine were incubated for the respective times at 37 °C. The assay for [^{3}H]poly(U) concentration is described under Methods.

TABLE IV: Effect of Polymers on the Nucleolytic Activity of *E. coli* MRE 600 Extracts.[a]

Additions	Concn (μg)	% [^{3}H]Poly(U) Remaining after Incubation at 37 °C S-100	Ribosomes	S-100 + Ribosomes
None		11	30	20
Poly(S-MA) (**1**)	6.1	89	77	
Poly(vU,MA) (**2**)	5.3			15
Poly(vU,AA) (**3**)	16.8	13	31	
Poly(vA,AA) (**4**)	19.6	13	31	19
Poly(I)-poly(C)	72	13	31	
Poly(A)-poly(U)	92	100	100	
Poly(vinyl sulfate)	6.4	90	95	

[a] Samples containing [^{3}H]poly(U) and all other additions used in poly(Phe) synthesis except phenylalanine were incubated for 8 min at 37 °C. The assay for [^{3}H]poly(U) is described under Methods.

the same conditions used in protein synthesis except for the absence of phenylalanine. Table III shows extensive degradation of [^{3}H]poly(U) by nucleases in ribosomes and S-100 separately and their mixture. Poly(vinyl sulfate), recognized as an effective RNase inhibitor, preserved [^{3}H]poly(U) (Table IV). Poly(A)-poly(U) also was effective in preserving the message. Among the stimulatory polymers only poly(S-MA) (**1**) was an RNase inhibitor. No RNase inhibition was observed using the synthetic uracil or adenine containing polymers **2**, **3**, or **4** or with poly(I)-poly(C); [^{3}H]poly(U) remaining after incubation was the same as the control assays, in the absence of polymers. This was the first indication that two mechanisms for polymer stimulation were occurring; RNase inhibition was found only using poly(S-MA) (**1**). This was confirmed in a time course study of polyphenylalanine synthesis correlated with the level of poly(U). Assays using [^{3}H]poly(U) and unlabeled phenylalanine were run simultaneously with assays containing identical amounts of poly(U) and [^{3}H]phenylalanine. Radioactivity was determined for the times indicated in Figures 3 and 4. In Figure 3 the controls show that the level of poly(U) correlates well with the incorporation of [^{3}H]phenylalanine; synthesis essentially stops when the poly(U) level falls to about 10% of the initial concentration. In the presence of poly(S-MA) (**1**) the message is partially protected from degradation; about 20% remains after 50 min, while synthesis continued at the initial rate at 50 min. In contrast, poly(vA,AA) (**4**) does not preserve the message. Figure 4 shows no difference in the level of [^{3}H]poly(U) in the control or with added polymer. However, the strong stimulatory effect with **4** gave polyphenylalanine synthesis at the initial rate even at 50 min in the presence of very low levels of poly(U). It can be concluded from these results that poly(vA,AA) (**4**) stimulates protein synthesis by a different mechanism, not by RNase inhibition and further that poly(S-MA) (**1**) stimulation may occur by both mechanisms.

The possibility that these polymers act by affecting the charging of tRNA was ruled out in an experiment examining tRNA charging with [^{3}H]phenylalanine by *E. coli* S-100 extracts. [^{3}H]Phe-tRNA synthesis was 62 pmol in the control and 65 pmol in the presence of 12 μg of poly(vA,AA) (**4**).

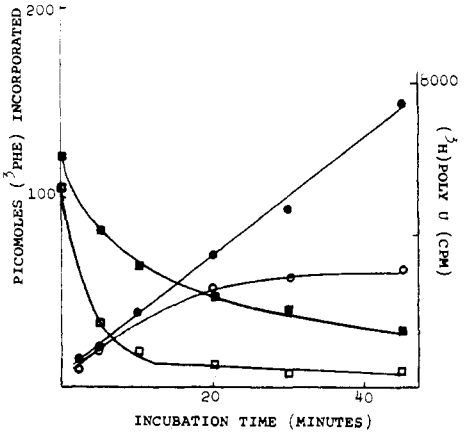

FIGURE 3: Correlation between polyphenylalanine synthesis and the level of poly(U) with and without poly(S-MA) (**1**) 6.1 μg: poly(Phe) synthesis, control (○); poly(Phe) synthesis with poly(S-MA) (●); [^{3}H]poly(U) levels in control (□); [^{3}H]poly(U) in samples containing poly(S-MA) (■). To measure [^{3}H]poly(U) levels identical assays using unlabeled Phe were used.

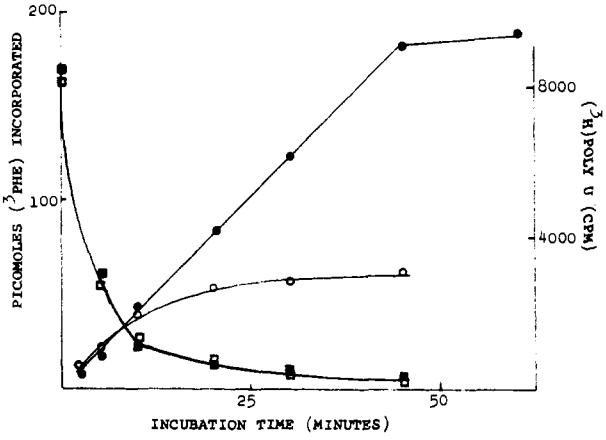

FIGURE 4: Correlation between polyphenylalanine synthesis and the level of poly(U) with and without poly(vA,AA) (**4**) (16.3 μg): poly(Phe) synthesis control (○); poly(Phe) synthesis with poly(vA,AA) (●); [^{3}H]-poly(U) level in control (□); [^{3}H]poly(U) level in samples containing poly(vA,AA) (■). To measure [^{3}H]poly(U) levels identical assays using unlabeled Phe were used.

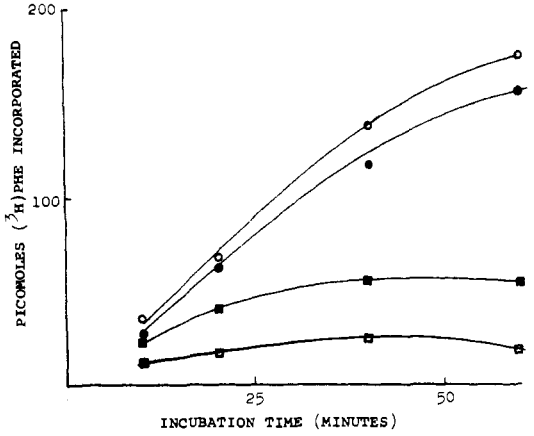

FIGURE 5: Time course of synthesis and inhibition of in vitro polyphenylalanine synthesis in *E. coli* by 45–55% ammonium sulfate fraction of ribosomal wash. Washed ribosomes (1.6 A_{260} units) and 0.8 μg of poly(U) were used as described under Methods; control (○), plus 16.3 μg of poly(vA,AA) (●), plus 90 μg of protein fraction (□), plus 16.3 μg of poly(vA,AA) and 90 μg of protein fraction (■). The blank, without message (3 pmol of [^{3}H]Phe incorporated) was subtracted from all values.

TABLE V: Dependence of Inhibition of in Vitro Polyphenylalanine Synthesis by Added Protein from the 45–55% Ammonium Sulfate Pellet of the Ribosomal Wash on the Concentration of the Message.[a]

	pmol of [^{3}H]Phe Incorporated	
μg of poly(U)	Control	90 μg of Protein Added
0.32	69	5.7
0.80	99	21.9
1.60	114	126
3.20	124	198
4.80	124	170

[a] Washed ribosomes, ribosomal protein fractionation, and in vitro polyphenylalanine synthesis are described under Methods.

The other possibility is that the stimulatory polymers counteract the effect of protein(s) or factor(s) present on *E. coli* ribosomes that inhibit translation by binding the message.

A translation inhibitor isolated from the 1 M ammonium chloride solution used in washing *E. coli* ribosomes has been reported (Wahba, et al., 1974). It has been characterized as subunit I of $Q\beta$ replicase and inhibits the translation of poly(U) and poly(U) sequences in natural mRNA.

To examine this possibility, ribosomes were extensively washed with 1 M ammonium chloride to release the inhibitory material(s). Figure 5 shows the results of a time-course study of [^{3}H]phenylalanine incorporation at low message levels (0.8 μg) using the washed ribosomes in the presence and absence of poly(vA,AA) (**4**). Synthesis is efficient and continues at the initial rate for 40 min. This contrasts with unwashed ribosomes that stops after 20 min. These results, coupled with the lack of stimulation afforded by poly(vA,AA) (**4**), support the proposal that the polymers may be interfering with an inhibitory material in addition to inhibiting (with poly(S-MA) (**1**) RNase released during incubation from ribosomes. Next we tried to isolate this inhibitory fraction from ribosomal wash. The first step in purification of subunit I is ammonium sulfate fractionation. The 45–55% pellet of the ribosomal wash was found to inhibit poly(U) translation and to be dependent on message levels. Phenylalanine incorporation in the washed ribosome system using varying levels of poly(U) reached saturation above 1.6 μg of poly(U) (Table V). The addition of 90 μg of the crude subunit I preparation strongly inhibited polyphenylalanine at low poly(U) levels. At saturating levels of poly(U) the subunit preparation was stimulatory probably due to the presence of initiation factors (IF_3) (Miller et al., 1974).

Polymers **2**, **3**, and **4** act as stimulants by inhibiting the action of the ribosomal protein (subunit I), since the addition of these polymers to the inhibited reaction gave stimulation (Table VI). Figure 5 shows these results using poly(vA,AA) (**4**). Further studies using varying amounts of the subunit I preparation confirm this effect (Figure 6).

The strong inhibitory effect of the subunit I preparation is only partially caused by subunit I itself. There also is strong nucleolytic activity in this preparation; 45 μg of protein from this preparation under conditions of protein synthesis completely degraded 2.4 μg of [^{3}H]poly(U) in 4 min. RNase II

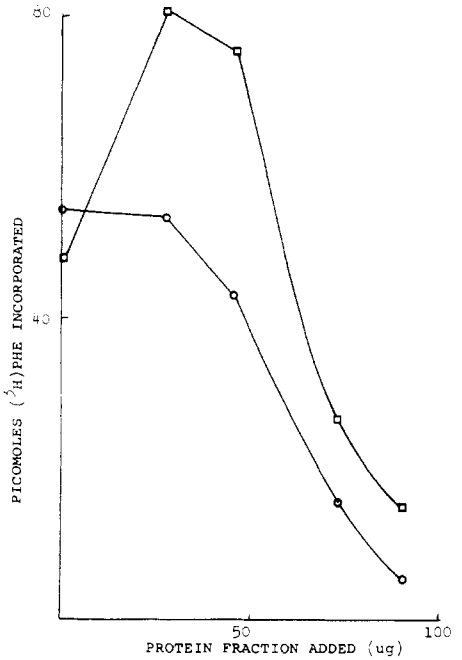

FIGURE 6: Concentration dependence of the inhibition of in vitro polyphenylalanine synthesis by 45–55% ammonium sulfate pellet from ribosomal wash. Washed ribosomes (1.6 A_{260} units) and 0.8 μg of poly(U) were used as described under Methods; Control (○), plus 9.8 μg of poly(vA,AA) (**4**) (□).

TABLE VI: Effect of Polymers on Polyphenylalanine Synthesis in the Presence of Added Ribosomal Protein Using Washed Ribosomes.[a]

Additions	Concn (μg)	pmol of [^{3}H]Phe incorporated
None		10.4
Poly(S-MA) (**1**)	2.6	11.2
Poly(vU,MA) (**2**)	3.5	17.0
Poly(vU,AA) (**3**)	14.7	17.8

[a] Washed ribosomes, 64 μg of the 45–55% ammonium sulfate fraction pellet, and 0.8 μg of poly(U) were used in the assay as described in the experimental section.

activity is reported to precipitate in the 40–60% ammonium sulfate fraction of *E. coli* ribosome washings (Spahr, 1964). Previous results (Figure 4) have shown that poly(vA,AA) (**4**) is not an inhibitor of RNase and that is why we cannot regain full translational activity by adding **4** to the subunit I preparation inhibited reaction.

Discussion

Poly(vinyluracil) is reported to be an effective inhibitor of in vitro poly(A)-coded translation and ribosomal binding of Lys-tRNA in the presence of ApApA. Similar effects were observed on poly(U)-coded translation and the UpUpU stimulated ribosomal binding of Phe-tRNA when polyvinyladenine was used. These actions are attributed to the complimentary binding of polyvinyluracil with poly(A) and polyvinyladenine with poly(U) (Reynolds et al., 1972).

We prepared some copolymers of styrene, 1-vinyluracil, and 9-vinyladenine with maleic acid and acrylic acid and examined them for inhibition of in vitro translation coded by messages containing bases complimentary to the synthetic polymers. The polymers shown in Figure 1 were, in fact, potent stimulants of poly(U)-coded polyphenylalanine synthesis using a system isolated from *E. coli* MRE 600.

Stimulation in this system by compounds **1**–**4** was concentration dependent for both the message and the polymers. These polymers did not change the rate of synthesis, since time studies showed that the polymer stimulation was not apparent until after 15 min when synthesis in the control ceased.

Since there is no change in the initial rate of synthesis, the observed stimulation could be the result of preservation of the message. The stimulant polymers would then exert their action by inhibiting the nucleolytic activity of a ribonuclease in the system. The system was shown to contain ribonuclease; however, direct measurements revealed that only poly(S-MA) (**1**) was an effective inhibitor of RNase action in degradation of poly(U). The uracil and adenine polymers, **2**, **3**, **4**, did not prevent the degradation of poly(U).

As seen in the results of Figure 5, compound **4** appears to act, not by preservation of the system or message, but by reversing the effects of an endogeneous inhibitor(s) released on incubation.

Wahba and co-workers and other laboratories have described such an inhibitor that can be released by washing *E. coli* MRE 600 ribosomes. This protein has been identified as subunit I of Qβ replicase and 30S ribosomal protein SI (Wahba et al., 1974; Miller et al., 1974; Groner et al., 1972; Lee-Huang and Ochoa, 1972). The results of this study show that compounds **2**, **3**, and **4** act as stimulants for in vitro poly(U)-coded translation by counteracting the inhibitory effects of a ribosomal-bound protein, presumably the same as that described by Wahba and co-workers.

It appears that polymers that inhibit this protein must contain a base (either adenine or uracil), since compound **1** containing a phenyl ring does not affect the inhibited reaction, but gives stimulation by inhibiting a ribosomal bound ribonuclease.

References

Field, A. K., Tytell, A. A., Lampson, G. D., and Hilleman, M. R., (1967), *Proc. Natl. Acad. Sci. U.S.A. 70,* 3581.

Groner, Y., Scheps, R., Kamen, R., Kolakofsky, D., and Revel, M. (1972), *Nature (London), New Biol. 239,* 19.

Lee-Huang, S., and Ochoa, S. (1972), *Biochem. Biophys. Res. Commun. 49,* 371.

Miller, M. J., Niveleau, A., and Wahba, A. J. (1974), *J. Biol. Chem. 249,* 3803–3807.

Olson, P. E., Fraher, P., Boguslawski, S., and Mertes, M. P. (1975), *Biochemistry 14,* 4892.

Pitha, P. M., Teich, N. M., Lowy, D. R., and Pitha, J. (1973), *Proc. Natl. Acad. Sci. U.S.A. 10,* 1204–1208.

Reynolds, F., Grunberger, D., Pitha, J., and Pitha, P. (1972), *Biochemistry 11,* 3261–3266.

Roberts, B. E., and Patterson, B. M. (1974), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2330–2334.

Schiff, N., Miller, M. J., and Wahba, A. J. (1974), *J. Biol. Chem. 249,* 3797–3802.

Spahr, P. F. (1964), *J. Biol. Chem. 239,* 3716.

Staehelin, T., and Falvey, A. K. (1971), *Methods Enzymol. 22C,* 433–436.

Wahba, A. J., Miller, M. J., Niveleau, A., Landers, T. A., Carmichael, G. C., Weber, K., Hawley, D. A., and Slobin, L. I. (1974), *J. Biol. Chem. 249,* 3314.