STIMULATION OF AMINO ACID INCORPORATION INTO PROTEIN BY PHAGE DNA AND AN RNA POLYMERASE FRACTION*

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DNA has been implicated in protein synthesis in cell-free systems by the observation that DNase inhibits amino acid incorporation into protein with extracts of Escherichia coli (Tissières, Schlessinger, and Gros, 1960; Matthaei and Nirenberg, 1961) and by the demonstration that DNA stimulates β-galactosidase formation with UV-irradiated extracts of Escherichia coli (Eisenstadt and Novelli, 1961; Novelli, Kameyama, and Eisenstadt, in press.)

This communication describes a phage DNA stimulation of the incorporation of valine-C^{14} into protein with fractions of Salmonella typhimurium extracts. The stimulation by the phage DNA is dependent on the addition of purified RNA polymerase with polymerase-poor fractions.

Experimental:

A histidine auxotroph of Salmonella typhimurium strain LT-7 was grown to early log phase in nutrient broth. The cells were ground with alumina and the paste was extracted with 0.01 M Tris buffer, pH 7.8, containing 0.01 M magnesium acetate. The

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extract was centrifuged at 30,000 x g for twenty minutes and the supernatant solution was centrifuged again at 30,000 x g for thirty minutes. The 30,000 x g supernatant fraction was further centrifuged to remove DNA and the RNA polymerase as follows: first, the ribosomes were sedimented by centrifugation for ninety minutes at 105,000 x g. They were washed once and resuspended in the buffer above. The supernatant solution from the initial ninety-minute centrifugation was centrifuged for six hours at 105,000 x g. Two-thirds of the supernatant solution was removed by aspiration and is referred to as 6-hour supernatant fraction. The ribosome and 6-hour supernatant fractions together have 25% of the RNA polymerase of the 30,000 x g supernatant fraction as measured by the amount of ATP-C^{14} incorporation into RNA which is inhibited by DNase. Also, not more than 25% of the DNA of the 30,000 x g supernatant solution is found in the two fractions. Previous studies had shown this separation of components in extracts of E. coli (Stevens, 1960).

The first experiment in Table I shows that the 30,000 x g supernatant fraction stimulates an incorporation of valine-C^{14} into protein in the presence of ATP, an ATP-generating system, UTP, CTP, GTP, and a mixture of amino acids. Omission of any one of the four ribonucleoside triphosphates reduces the incorporation. The reaction is stimulated 3-fold by the inclusion of the amino acid mixture. The incorporation of valine-C^{14} is inhibited almost completely by chloramphenicol and by RNase and 50% by DNase.

In experiment 2 of Table I, the effect of the addition of a purified fraction of the RNA polymerase of E. coli and of sever-

Table I

Characteristics of Valine-C^{14} Incorporation into Protein with the 30,000 x g Supernatant Fraction of Salmonella typhimurium

	System	Valine-C^{14} Incorp., cpm
1.	Complete	620
	-ATP	272
	-ATP, generating system	150
	-UTP	270
	-CTP	400
	-GTP	247
	-amino acid mixture	169
	+chloramphenicol	3
	+RNase	4
	+DNase	280
2.	Complete	652
	+RNA polymerase	1250
	+T2 DNA	2440
	+T6 DNA	2270
	+S. typhimurium DNA	783
	+Calf Thymus DNA	780
	+T2 DNA + RNA polymerase	2440
	+T6 DNA + RNA polymerase	2210
	+S. typhimurium DNA + RNA polymerase	950
	+Calf Thymus DNA + RNA polymerase	1170

The complete system contained in a final volume of 0.50 ml: ATP, 0.001 M; UTP, CTP, and GTP, 5×10^{-4} M; phosphocreatine, 0.01 M; creatine kinase, 20µg; 19 amino acids minus valine, 5x10-5 M; valine-C^{14}, 5×10^{-5} M, 3×10^{6} cpm per µmole; magnesium acetate, 0.01 M; Tris buffer, pH 7.8, 0.05 M; potassium chloride, 0.10 M. Additions were: Chloramphenicol, 50 µg; RNase (Armour) 1 µg; DNase (Worthington) 10 µg; T2 DNA, 0.20 O.D. units at 260 mµ (All DNA concentrations are expressed as O.D. units at 260 mµ in the remainder of the paper); T6 DNA, 0.20; S. typhimurium DNA, 0.27; calf thymus DNA, 0.20; RNA polymerase, a 100-fold purified fraction from E. coli (Stevens, 1961), 140 µg. In experiment 1, 1.42 mg of 30,000 x g supernatant fraction was added, in experiment 2, 1.16 mg. The reaction mixtures were incubated for 45 minutes at 37°C and the reactions were terminated by the addition of 2 ml of 5% TCA. The precipitates were washed successively with 5% TCA, 5% TCA at 90°C for 15 minutes, and ethanol: ether, 3:1. They were then washed 5 times with 5% TCA on millipore filters and the filters were placed on planchets and counted. Zero-time values were subtracted.

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al DNA preparations is shown. Addition of the polymerase fraction leads to a 2-fold increase in the incorporation of valine-C^{14}. Addition of T2 DNA or T6 DNA leads to a 3-4-fold

increase in the incorporation. Addition of S. typhimurium DNA or calf thymus DNA increases the incorporation only slightly. Addition of the polymerase fraction together with DNA does not further increase the extent of the incorporation of valine-C^{14} with the 30,000 x g fraction.

Table II shows the results of studies of valine-C^{14} incorporation into protein and ATP-C^{14} incorporation into total nucleic acid with the ribosome and 6-hour supernatant fractions. As shown, the incorporation of valine-C^{14} is not stimulated by DNA alone. Addition of an RNA polymerase fraction alone leads to only a small increase in the incorporation. The addition of the RNA polymerase fraction together with phage DNA leads to a significant stimulation of the incorporation of valine-C^{14}. Addition of bacterial DNA together with the polymerase fraction does not increase the valine-C^{14} incorporation. Incorporation of ATP-C^{14} is increased by the addition of the polymerase fraction alone, but to a much larger extent by the addition of the polymerase together with the phage DNA. Addition of the polymerase together with bacterial DNA increases the incorporation of ATP-C^{14} only slightly more than the polymerase alone.

It has been found that the incorporation of valine-C^{14} under any of the conditions shown in Table II is dependent on the presence of both the ribosome and supernatant fractions and is inhibited almost completely by chloramphenicol and by RNase. Addition of DNase does not effect the incorporation in the absence of DNA and the polymerase, but completely inhibits the stimulation by the two components.

Table II

Effect of DNA and RNA Polymerase Fraction on the Incorporation of Valine-C^{14} and ATP-C^{14} by Ribosomes and 6-Hour Supernatant Fraction

Source of DNA	Valine-C^{14} Incorp., cpm		ATP-C^{14} Incorp., cpm	
	- Polymerase	+	- Polymerase	+
None	80	96	172	484
T2	74	235	228	2728
T4	80	179	236	1544
T6	77	200	246	1616
E. coli	72	95	272	604
Erwinia carotovora	59	92	168	496
S. typhosa	80	100	216	680

Each incubation vessel contained the complete system described in Table I in a final volume of 0.5 ml. with ATP-C^{14}, 150,000 cpm per μmole. The DNA samples added were as follows: T2, 0.20; T4, 0.10; T6, 0.13; *E. coli*, 0.24; *Erwinia*, 0.22; *S. typhosa*, 0.29. The RNA polymerase was a 350-fold purified fraction from *E. coli*, 17 μg. 0.23 mg of 6-hour supernatant, and 0.30 mg of ribosomes were added to each tube. The reaction mixtures were incubated for 45 minutes at 37°C, and the reactions were terminated by the addition of 2 ml of 5% TCA. The precipitates were washed 5 times with 5% TCA in the cold, and then with 2 ml. of 5% TCA for 20 minutes at 90°C. The supernatant from the 90°C TCA wash was extracted twice with ether, and an aliquot was plated for determination of ATP-C^{14} incorporated into hot TCA extractable material. The precipitate was further washed with ethanol-ether and plated as described in Table I for valine-C^{14} activity.

Preparations of the RNA formed in the RNA polymerase reaction with T2 DNA have been examined for their stimulation of valine-C^{14} incorporation. A stimulation has been found with several preparations, but the stimulation is only 10-20% of that obtained when DNA and the RNA polymerase fraction are added to the incubation mixture.

The results suggest that phage DNA promotes valine-C^{14} incorporation into protein by stimulating the RNA polymerase reaction. The greater stimulatory capacity of the phage DNA than bacterial DNA may perhaps be attributed to size or homogeneity.

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