INHIBITION OF POLYPEPTIDE SYNTHESIS IN *E. COLI* CELL FREE SYSTEM BY INFLUENZA VIRAL PROTEIN

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1. Introduction

It is well known that *in vitro* protein synthesis directed by bacteriophage MS2 RNA is reduced by prior incubation of the RNA with MS2 coat protein [1–3]. This inhibition concerns exclusively the formation of non-coat proteins (viral RNA synthetase and maturation protein). In this paper, we studied the effect of viral protein, isolated from influenza virus, on polypeptide formation and related reactions using an *E. coli* cell free system. It was found that the viral protein markedly inhibits polypeptide formation, aminoacyl tRNA binding to ribosomes, and polysome formation.

2. Materials and methods

Ribosomes and supernatant enzymes (S-150) were prepared from *E. coli* B [4, 5]. Experiments for polypeptide formation, aminoacyl tRNA binding to ribosomes and formation of polysomes directed by synthetic mRNA were done with the systems as described [5–7]. ^{14}C-Phe-tRNA and ^{3}H-Lys-tRNA were prepared according to Kaji et al. [8]. Poly U and poly A were purchased from the Miles Laboratories, and *E. coli* B tRNA, ^{3}H-poly U (7.76 mCi/mmole) and ^{14}C-poly A (2.15 mCi/mmole) from the Schwarz Bio-Research Inc. ^{14}C-Phenylalanine (369 mCi/mmole) and ^{3}H-lysine (3960 mCi/mmole) were obtained from the New England Nuclear Corp. (counting efficiency, 80% for ^{14}C and 53% for ^{3}H). The Aichi strain of influenza virus A2 (300 ml, 2500 CCA) partially purified from 15 l of allantoic fluid was kindly provided by Drs. Diazo Nagaki, Yoshiteru Igarashi and Fumiaki Taguchi of Kitasato University, Tokyo, Japan. The virus was purified according to Barry and Davies [9] and Duesberg [10]. Isolation and purification of the viral protein from influenza virus were carried out as described by Duesberg and Robinson [11] and Duesberg et al. [12]. The purified viral protein (60 mg) was dissolved in 18 ml of 0.1% SDS (sodium dodecylsulfate) solution containing 0.1 M NaCl, 0.01 M tris-HCl (pH 7.4), 1 mM EDTA and 0.1% 2-mercaptoethanol and stored at −20°. The solution (10 ml) was precipitated with crystalline $(NH_4)_2SO_4$ at 80% saturation and adjusted to pH 7.4. The precipitate was dissolved in 1.5 ml of solution 1 containing 0.1% SDS and 1 mM acetic acid, dialyzed overnight against 300 ml of solution 1 at 4° and adjusted to a concentration of 8 mg protein/ml.

3. Results and discussion

In the first experiment performed to test the effect of influenza viral protein on polyphenylalanine synthesis, various amounts of the purified viral protein were preincubated with a constant amount of poly U as described in the legend of fig. 1, and then ^{14}C-Phe-tRNA was added to the mixture. As shown in fig. 1-A, with increasing amount of the viral protein, extent of the polyphenylalanine formation falls off rapidly. In a separate experiment, a similar result was observed in the case of polylysine formation. In contrast, as shown in fig. 1-B, albumin and histones did not alter the ac-

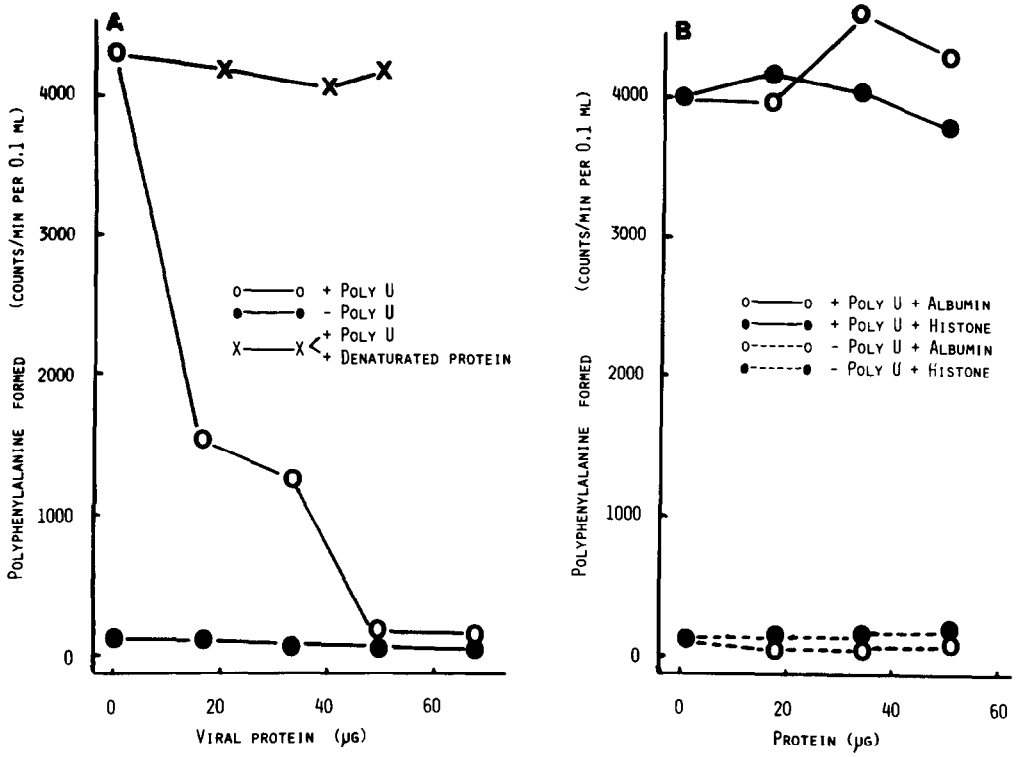

Fig. 1. Effect of the viral protein on the polyphenylalanine formation.
(A) The reaction mixture contained the following in μmoles/0.125 ml: Tris-HCl (pH 7.4), 10; magnesium acetate, 1.5; KCL, 8.5; 2-mercaptoethanol, 1.5; PEP, 0.6; GTP, 0.05. In addition, it contained 33 μg of ribosomes, 35 μg of S-150, 10 μg of poly U, 8 μg of SDS, 5 μg of pyruvate kinase and the viral protein as indicated in the figure. The mixture was incubated for 10 min at 37°, then 10,700 cpm of ^{14}C-Phe-tRNA was added and the incubation was continued for 30 min.
(B) The experiment was the same as in (A), except that albumin or histones as indicated in the figure were added instead of the viral protein. Bovine albumin (obtained from the Mann Research Laboratories) was dissolved in 1 mM Tris-acetate buffer (pH 7.1) containing 0.1% SDS. Purified calf thymus histones (kindly supplied by Dr. Yasukiyo Umemura) were dissolved in solution 1.

tivity of polyphenylalanine synthesis. This suggests that the viral protein has some functions which are different from that of albumin and histones. When the viral protein was subjected to the denaturation with ethanol at final 95% concentration, the inhibitory action by the protein did not take place (fig. 1-A, X—X). Thus, it appears that the inhibition is induced by a viral specific protein which is sensitive to the treatment with ethanol. As shown in table 1, the addition of influenza viral protein significantly inhibited the poly U-coded binding of Phe-tRNA to ribosomes. The binding activity for Phe-tRNA in the presence of viral protein was only about 20% of the control. The specific binding of Lys-tRNA was also inhibited by the viral protein to a similar extent as that of Phe-tRNA. Furthermore, the data shown in table 2 indicate that when ribosomes were incubated with the viral protein, the viral protein strongly reduced the polysome formation, that is, much less ^{3}H-poly U and ^{14}C-poly A were found around the region where polysomes would sediment. Under the described conditions, SDS partially inhibited the binding of aminoacyl tRNA (table 1), while SDS had no inhibitory effect on the polysome formation (table 2). In other experiments, since no degradation of ^{14}C-polyphenylalanine, ^{3}H-poly U and ^{14}C-Phe tRNA took place by the inclusion of viral protein, the possibility that the inhibition caused by the viral protein may be due to

Table 1
Inhibitory effect of the viral protein on the binding of aminoacyl tRNA to ribosomes.

Viral protein	Bound ^{14}C-Phe-tRNA (cpm)		(% of control)	Bound ^{3}H-Lys-tRNA (cpm)		(% of control)
	– Poly U	+ Poly U		– Poly A	+ Poly A	
–	60	2104	100	845	8132	100
+	74	557	23	548	1875	20
–*	75	2600		1050	10070	

The reaction mixture contained the following in μmoles/0.1 ml: Tris-HCl (pH 7.1), 10; magnesium acetate, 1.5; KCl, 7; 2-mercaptoethanol, 1. In addition, it contained 10 μg of poly U or poly A, 33 μg of ribosomes, 2 μg of SDS and 16 μg of the viral protein. The mixture was incubated for 10 min at 37°, then 6400 cpm of ^{14}C-Phe-tRNA or 15,000 cpm of ^{3}H-Lys-tRNA was added and the incubation was continued for 15 min at 24°.
* SDS was omitted from the reaction mixture.

Table 2
Inhibitory effect of the viral protein on the formation of ribosomes.

Viral protein	Bound ^{3}H-poly U (cpm)	(% of control)	Bound ^{14}C-poly A (cpm)	(% of control)
–	8257	100	7270	100
+	1075	13	1533	21
–*	8174		7197	

The reaction mixture contained the following in μmoles/0.4 ml: Tris-HCl (pH 7.4), 40; magnesium acetate, 6; KCl, 28; 2-mercaptoethanol, 4. In addition, it contained 132 μg of ribosomes, 8 μg of SDS, 64 μg of the viral protein and 49,000 cpm of ^{3}H-poly U or 61,000 cpm of ^{14}C-poly A. The mixture was incubated for 10 min at 37°, and then subjected to the sucrose density-gradient (10 to 30%) for 1 hr at 50,000 rpm in the Spinco SW-65 rotor. The bound poly U or poly A sedimenting faster than 70 S was measured.
* SDS was omitted from the reaction mixture.

possible contamination of this protein with protease, ribonuclease and esterase was made unlikely.

From these observations, we can conclude that the inhibitory effect of the viral protein isolated from influenza virus is exerted on the polysome formation and the tRNA binding induced by synthetic mRNA, thereby inhibiting the polypeptide formation. Phage coat protein has been reported to act as a repressor of phage protein synthesis *in vitro* [1–3]. On the other hand, the viral fiber protein from adenovirus is found to inhibit the synthesis of cellular macromolecules in adenovirus-infected cells [13, 14]. In these aspects, the data presented in this paper suggest the presence of an inhibitor in the viral structural proteins of influenza virus and may serve as useful model to study functional roles of animal viral protein on the reduction of host protein synthesis and the preferential synthesis of virus-specific macromolecules in virus-infected cells.

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