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THE REVERSAL OF INHIBITION OF PROTEIN SYNTHESIS BY DOUBLE-STRANDED RNA IN LYSED RABBIT RETICULOCYTES WITH FRUCTOSE 6-PHOSPHATE

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SUMMARY

Fructose 6-phosphate (1.4 mM - 3.0 mM) effectively prevents the inhibition of protein synthesis in unfractionated rabbit reticulocyte lysates by the presence of double-stranded RNA (poly rI:poly rC, 1 μg/ml). Glucose 6-phosphate, but not fructose 1,6-diphosphate, is equally as effective as fructose 6-phosphate. The data suggest that fructose 6-phosphate prevents the formation of a protein synthesis inhibitor induced by double-stranded RNA.

INTRODUCTION

Cell-free protein synthesis in extracts of interferon treated cells (1-3) or rabbit reticulocytes (4-7) is inhibited by low concentrations of double-stranded RNA (dsRNA). The mechanism involved in the inhibition of protein synthesis is related to the inactivation of an initiation protein factor (8-19) and/or the activation of a nuclease (20-28), mediated by the oligonucleotide pppA2'p5'A2'p5'A (29-33). This trinucleotide is synthesized from ATP in the presence of dsRNA and Mg^{2+} (29,31).

Previous studies have demonstrated that sugar phosphates may play an important role in protein biosynthesis (34-36). The present communication shows that the addition of fructose 6-phosphate (1.4 mM - 3.0 mM) prevents the inhibitory action of dsRNA. This effect appears to be related to the reduced accumulation of a protein synthesis inhibitor induced by poly rI: poly rC.

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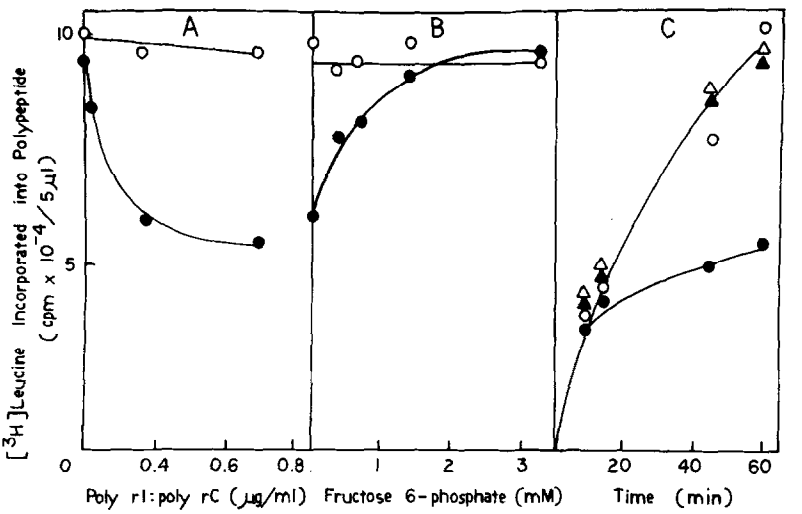

Figure 1. Effect of fructose 6-phosphate on the reversal of the inhibition of protein synthesis by poly rI:poly rC in lysed rabbit reticulocytes. (A): ●—●, minus fructose 6-phosphate; o—o, plus fructose 6-phosphate (3 mM). (B): o—o, minus poly rI:poly rC; ●—●, plus poly rI:poly rC (1 µg/ml). (C): kinetics of protein synthesis, Δ—Δ, control; ●—●, control plus poly rI: poly rC (1 µg/ml); ▲—▲, control plus fructose 6-phosphate (3 mM); o—o, control plus poly rI:poly rC (1 µg/ml) and fructose 6-phosphate (3 mM). In (A) and (B) leucine incorporation into polypeptides was determined by removing 5 µl aliquots after incubation for 60 min, 30°C.

MATERIALS AND METHODS

Poly rI:poly rC was obtained from Sigma Chemical Co. and dissolved in 200 mM NaCl and 7 mM phosphate buffer (pH 7). Cell-free protein synthesis assays were done with the addition of creatine phosphate (15 mM) and creatine phosphokinase (45 units/ml)(35). Other components of the assays were as described previously (34). The concentration of leucine was 10 µM (specific activity 7 Ci/mmol).

The synthesis of the low molecular weight inhibitor was accomplished by incubating the hemin (10 µM) supplemented lysate with 1 mM Mg:ATP and 1 µg/ml poly rI:polyrC at 30°C for 60 min. At the end of incubation, the reaction mixture was diluted 10-fold with water and heated for 5 min at 95°C. The denatured proteins were pelleted by centrifugation in a Brinkmann 3200 centrifuge for 5 min. The heat-treated supernatant was assayed at a 150-fold dilution for ability to inhibit amino acid incorporation. The reticulocyte lysate was preincubated with the heat-treated supernatant for 20 min at 30°C before the addition of the supplemented components (salts, ATP, GTP, energy regenerating system and amino acids) for protein synthesis.

RESULTS AND DISCUSSION

The maximum inhibition of protein synthesis by poly rI:poly rC is observed at 1 µg/ml (Fig. 1A,closed circles). The addition of 3 mM fructose

TABLE I. Effect of Fructose 6-Phosphate on the Accumulation and Expression of a Protein Synthesis Inhibitor Induced by dsRNA.

	Condition Used	Inhibition
		(%)
Experiment A[a]		
	minus dsRNA (control) minus fructose 6-phosphate	0
	minus dsRNA plus fructose 6-phosphate	0
	plus dsRNA minus fructose 6-phosphate	55
	plus dsRNA plus fructose 6-phosphate	0
Experiment B[b]		
	minus fructose 6-phosphate	55
	plus fructose 6-phosphate	50

[a]The protein synthesis inhibitor was synthesized as described in Materials and Methods. After the synthesis of the inhibitor, the heat-treated supernatant was prepared and the inhibitory activity was assayed in the lysed rabbit reticulocyte system. The amount of radioactivity in the control sample was 100,000 cpm after an incubation at 30°C for 60 min.

[b]The inhibitor was formed as described in experiment A and the inhibitory activity was assayed in the presence or absence of 3 mM fructose 6-phosphate.

6-phosphate, while exerting no inhibitory effect by itself, completely prevented the inhibition of protein synthesis by poly rI:poly rC (Fig. 1A, open circles). Maximal reversal of inhibition of protein synthesis by dsRNA is observed at 2 mM fructose 6-phosphate (Fig. 1B, closed circles). Figure 1C (closed circles) shows that the incorporation of leucine into polypeptide is linear for the first 15 min even in the presence of inhibitory concentration of poly rI:poly rC After 15 min, the rate of protein synthesis is slower and a 50% inhibition of protein synthesis is observed at the end of 60 min incubation. Fructose 6-phosphate (Fig. 1C, open circles) reverses the inhibitory effect of poly rI: poly rC and allows protein synthesis to proceed at a rate identical to the control (Fig. 1C, open triangles). In this experiment, fructose 6-phosphate is present simultaneously with dsRNA in the protein synthesizing system;

TABLE II. Effect of Glucose, Fructose, and Sugar Phosphates on the Synthesis of a Double-stranded RNA-Induced Inhibitor of Protein Synthesis

Addition	Polypeptide Synthesis[b]
	cpm
Control (minus poly rI:poly rC)	70,200
Plus poly rI:poly rC and	
No other addition	20,000
Glucose	29,700
Fructose	35,300
Glucose 6-phosphate	72,100
Fructose 6-phosphate	74,000
Fructose 1,6-diphosphate	22,700
Glucose 1-phosphate	33,000
Fructose 1-phosphate	24,000
2-Deoxyglucose 6-phosphate	21,000
Ribose 5-phosphate	35,100

[a]The concentrations of sugars and sugar phosphates were 3 mM. The sugars and sugar phosphates were incubated with poly rI:poly rC, Mg:ATP and lysate for 60 min, 30°C for the formation of the inhibitor as described in Materials and Methods.

[b]The inhibitor formed was assayed for its ability to inhibit endogenous amino amino acid incorporation in the reticulocyte lysate as described in Materials and Methods. Incubations were at 30°C, 60 min. The [4,5-^{3}H]leucine incorporated into polypeptide was measured by removing 5 µl aliquots.

therefore, it is not known whether the restoration of protein synthesis by fructose 6-phosphate is due to a block in the formation of the protein synthesis inhibitor, in response to the presence of dsRNA (e.g., the synthesis of pppA2'p5'A2'p5'A) or if fructose 6-phosphate allows the formation of the inhibitor but not its expression in the protein synthesizing machinery. To distinguish these possibilities, two experiments were performed. In the first experiment, the lysate containing 1 µg/ml of poly rI:poly rC was incubated in the presence or absence of fructose 6-phosphate. The formation of the inhibitor was monitored by performing the protein synthesis assay in reticulocyte lysates.

This experiment shows that the formation of the inhibitor is completely prevented by the presence of fructose 6-phosphate (Table 1A). In the second experiment, fructose 6-phosphate was used to challenge the activity of the preformed inhibitor. As shown in Table 1B, the addition of fructose 6-phosphate cannot reverse the inhibitory action of the preformed inhibitor in protein synthesis.

The effect of glucose, fructose, and phosphorylated sugars on the action of dsRNA in protein synthesis is shown in Table II. Glucose 6-phosphate and fructose 6-phosphate, but not the other sugars or sugar phosphates, prevent the inhibition of protein synthesis by dsRNA. Experiments are currently in progress to study the mechanism by which fructose 6-phosphate and glucose 6-phosphate can overcome the inhibitory action of dsRNA on protein biosynthesis.

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