Seminalplasmin inhibits transcription and translation of ϕ80 DNA in vitro

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Seminalplasmin, an antimicrobial protein from bovine seminal plasma that has been earlier shown to inhibit transcription in whole cells and by purified RNA polymerase in vitro, but not translation in whole cells, is now shown to inhibit both transcription and translation independently of each other, in a coupled transcription-translation system from *E. coli* using ϕ80d*pho*A*lac*Z DNA as the template.

Seminalplasmin *Translation inhibition* *Transcription inhibition*
Coupled transcription–translation

1. INTRODUCTION

Seminalplasmin, an antimicrobial protein of bovine seminal plasma, active on several gram-positive and gram-negative bacteria and yeasts, has been shown to inhibit transcription in whole cells of *Escherichia coli, Candida albicans* and the Zajdela ascitic hepatoma, in protoplasts of *C. albicans* and in nuclei of *Saccharomyces cerevisiae* ([1–3]; K.H. Scheit and P.M. Bhargava, unpublished). It has also been shown to inhibit transcription of a variety of templates in vitro by purified *E. coli* RNA polymerase [4], purified yeast RNA polymerases A and B ([2]; K.H. Scheit and P.M. Bhargava, unpublished) and several reverse transcriptases [2,5].

Here, we show that seminalplasmin inhibits transcription as well as translation in vitro in a system derived from *E. coli* using ϕ80d*pho*A*lac*Z DNA as the template.

2. MATERIALS AND METHODS

Seminalplasmin was prepared free of any ribonuclease activity [4] and estimated spectrophotometrically as in [1].

The in vitro coupled transcription–translation system used was the same as in [6]. The system essentially consists of salts, vitamins, an energy generating system and cAMP in a Tris–acetate buffer, the ϕ80d*pho*A*lac*Z (in some experiments, T_7) DNA and the S-30 fraction from *E. coli* deleted for *lac*Z [6,7]. The reaction was carried out in a total volume of 0.1 ml, at 37°C for the desired period. Incorporation of [^{14}C]UTP (527 mCi/mmol) or [^{32}P]UTP (486 mCi/mmol) was used to measure transcription, and of [^{14}C]leucine (240 mCi/mmol) or [^{3}H]phenylalanine (46.7 Ci/mmol) to measure translation. The formation of β-galactosidase and alkaline phosphatase was assayed by their enzyme activities [6,7]. The concentrations stated are final concentrations in the incubation mixture.

Transcription was measured as in [6,7]. For measurement of acid-insoluble radioactive protein synthesis two methods were used. In the first method, a 20-μl aliquot was spotted on a Millipore disc, washed 4-times for 10 min, each time with 5 ml of 10% cold trichloroacetic acid, and then for 10 min with 10% trichloroacetic acid at 90°C followed by ethanol; the disc was then dried and counted. In the second method, 3 ml of cold 5% trichloroacetic acid was added to the total reaction

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mixture followed by 0.1 ml of 0.5% BSA after 15 min at 4°C, the mixture was heated at 100°C for 25 min and then filtered through a Millipore membrane filter. The precipitate was washed 3-times with cold 5% trichloroacetic acid (5 ml each time), followed by ethanol (10 ml). For measurement of radioactivity in ^{3}H-containing samples, 0.1 ml of water was added, followed by 1 ml of NCS tissue solubiliser to the Millipore filter in the vial, and the sample counted after 30 min following the addition of 10 ml of Aquasol scintillant.

For measurement of poly(U)-directed polyphenylalanine synthesis, poly(U) (0.25 μg) was used instead of the DNA; all amino acids were eliminated except [^{14}C]phenylalanine (0.002 μmol and 2.2×10^{8} cpm/ml).

To uncouple transcription from translation, the in vitro reaction was carried out in the absence of amino acids. After 10 or 20 min of incubation at 37°C under vigorous shaking, transcription was stopped by adding rifampicin and translation was started by the addition of the 20 amino acids (one of which, leucine, was ^{14}C-labelled) with or without seminalplasmin. At various time intervals a 10-μl aliquot was spotted on Whatman no.3 disc, washed and counted as above.

To check if seminalplasmin used was free of nuclease activity, the ϕ80 DNA (9.75 mg/ml) was transcribed in vitro for 25 min using the S-30 fraction (10.4 mg protein/ml) and [^{14}C]UTP (0.002 μmol and 2.1×10^{6} cpm/ml). The reaction was stopped after 70 min by heating at 90°C for 5 min followed by rapid chilling in a dry-ice–acetone bath. The mixture was brought back to 37°C and seminalplasmin (70 μg/ml) was added. At various time intervals, a 20 μl aliquot was spotted on a Millipore disc and processed as above. A control without seminalplasmin was used.

3. RESULTS AND DISCUSSION

The extent of transcription of ϕ80d*pho*A*lac*Z DNA in the coupled in vitro transcription–translation system was proportional to the amount of DNA used (11–44 μg/ml), showing that DNA was limiting (table 1). The addition of seminalplasmin at time zero elicited at 35–50% inhibition during the first 10 min of incubation at all DNA concentration used. This implies that the percentage inhibition was the same irrespective of the amount of RNA synthesized (table 1).

No degradation of the transcript occurred at 37°C in the presence of seminalplasmin, above the 18% observed in the control (table 2). Thus, the effect of seminalplasmin cannot be explained by the presence of a nuclease as a contaminant. Since the inhibition by seminalplasmin was not relieved by increasing the concentration of the DNA 4-fold (table 1), the inhibition cannot be due to binding with the template.

Table 1

Effect of seminalplasmin on transcription in vitro using increasing amounts of ϕ80d*pho*A*lac*Z DNA

DNA (μg/ml)	Transcription of [^{14}C]-UTP into RNA (cpm)		Inhibition of synthesis of RNA by seminal-plasmin (%)
	− Seminal-plasmin	+ Seminal-plasmin	
0	200	100	–
11	1398	871	37.7
33	3906	2090	46.5
44	5279	2858	46.0

The in vitro synthesis of RNA was carried out for 10 min and measured as described in section 2. The concentration of the S-30 fraction was 9 mg protein/ml, and of seminalplasmin 25 μg/ml. The cpm given are for 20 μl of the reaction mixture

Table 2

Degradation of [^{14}C]UTP-labelled transcript of ϕ80d*pho*A*lac*Z DNA during incubation with seminalplasmin

Time of incubation	Acid-insoluble radioactivity (cpm)		% degradation	
	− seminal-plasmin	+ seminal-plasmin	− seminal-plasmin	+ seminal-plasmin
0 min	2400	2300	0	4.2
30 min	2050	2150	14.6	10.4
60 min	1980	2050	17.5	14.6

Preformed [^{14}C]UTP-labelled transcript of the DNA was incubated at 37°C for the specified period, alone or with seminalplasmin (70 μg/ml), and the acid-insoluble radioactivity measured, as described in section 2. The cpm given are for 20 μl of the reaction mixture

The inhibition, by seminalplasmin, of the transcription of the ϕ80 DNA (fig.1A) and of T_7 DNA (fig.1B) was partial (~40%) during the first 10 (fig.1A) to 20 (fig.1B) min. However, when the incorporation of the labelled precursor into DNA, in the presence of seminalplasmin, reached about 50% of the control (cf. table 1 and fig.1A,B), further synthesis of RNA stopped completely. If seminalplasmin was added to the transcribing ϕ80d*pho*A*lac*Z DNA after 10 min (fig.1C), its inhibitory effect was as immediate and complete as of rifampicin. These results suggest that the inhibition of transcription by seminalplasmin may be due to its binding to a complex of RNA polymerase either with the template or with some other factor present in the reaction mixture, the complex formation taking 10–20 min.

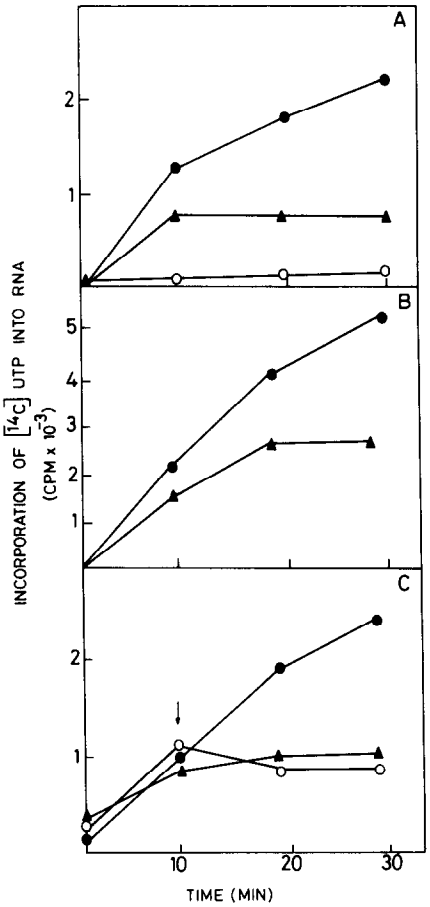

Fig.1. Effect of seminalplasmin added at time zero (fig.1A,1B) or at 10 min (fig.1C) on the incorporation of [^{14}C]UTP into RNA in an in vitro system using ϕ80d*pho*A*lac*Z DNA (fig.1A,1C) or T_7 DNA (fig.1B) as the template. The experimental conditions were the same as in table 1 excepting that the concentrations of the various components used, were as follows: ϕ80d*pho*A*lac*Z DNA, 11 μg/ml; T_7 DNA, 20 μg/ml; S-30 fraction, 11.4 mg/ml; rifampicin, 40 μg/ml; seminalplasmin, 25 μg/ml. (●—●) control; (○—○) + rifampicin; (▲—▲) + seminalplasmin. The arrows indicate the time at which seminalplasmin or rifampicin was added. The cpm given are for 20 μl of the reaction mixture.

At low concentrations (10 μg/ml), seminalplasmin did not inhibit poly(U)-directed synthesis of polyphenylalanine, and at higher concentrations (40 μg/ml), only 27% inhibition was observed (table 3). However, if the template was ϕ80d*pho*A*lac*Z DNA, seminalplasmin had a strong effect on protein synthesis measured either by [^{3}H]leucine incorporation (table 4) or by enzymatic activity; in the latter case, synthesis of β-galactosidase (using 12 μg of the DNA and 10 mg (protein) of the S-30 fraction/ml, and an incubation time of 70 min) was inhibited by 70 and 94%, and of alkaline phosphatase by 62 and 100%, by 10 and 25 μg of seminalplasmin (added at time zero), respectively. The above inhibitions could be due to a primary effect

Table 3

Effect of seminalplasmin on poly(U)-directed polyphenylalanine synthesis in vitro

Conc. of the inhibitor (μg/ml)		Incorporation of phenylalanine into acid-insoluble material (cpm)	Inhibition of polyphenylalanine synthesis (%)
Seminalplasmin	Rifampicin		
0	–	19450	0
6	–	19860	0
10	–	19430	0
30	–	17180	12
40	–	14250	27
–	40	17710	9

The reaction was carried out for 63 min as described in section 2. The final concentration of [^{3}H]phenylalanine in the reaction mixture was 2.2 × 10^8 cpm and 0.002 μmol/ml, and of the S-30 fraction 8.8 mg protein/ml. The incorporation into polyphenylalanine was estimated following precipitation with 3 ml of cold 5% trichloroacetic acid as in section 2

Table 4

Inhibition by seminalplasmin added at time zero, of translation in an in vitro coupled transcription–translation system from *E. coli*

Seminalplasmin (μg/ml)	Incorporation of [^{3}H]leucine into protein (cpm)	% Inhibition of incorporation
0	400	0
7	120	70
12	60	85
22	40	90
37	0	100
74	0	100
0	440	0

The in vitro protein synthesis was carried out in a total volume of 0.2 ml, essentially as described in section 2. To the ϕ80d*pho*A*lac*Z DNA (9 μg/ml) was added the in vitro mix containing [^{3}H]leucine (1.79 μg/ml) with or without seminalplasmin. After 3 min at 37°C, the synthesis was started by adding the S-30 fraction. After 1 h at 37°C under vigorous shaking, the reaction was stopped by adding chloramphenicol and samples were withdrawn to measure the incorporation as in section 2, following precipitation with 3 ml of cold 5% trichloroacetic acid

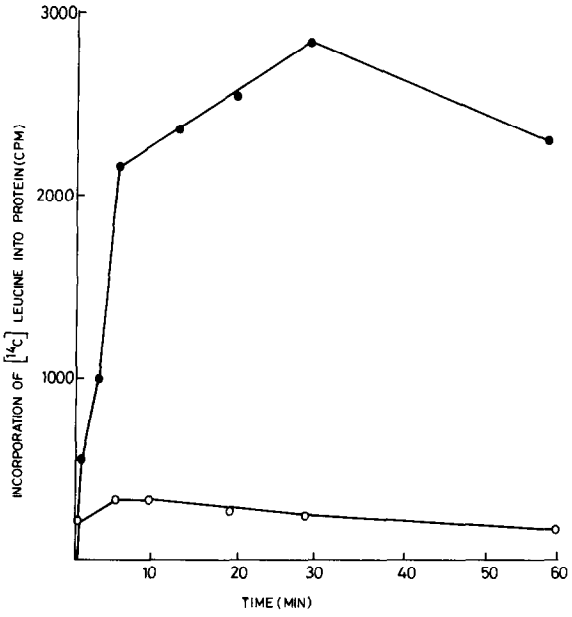

Fig.2. Effect of seminalplasmin on the translation of a 20-min transcript of ϕ80d*pho*A*lac*Z DNA in an in vitro coupled transcription–translation system from *E. coli*. The experimental conditions were the same as described in section 2 except that the total volume of the incubation mixture for transcription was 84 μl instead of 100 μl, and that all the amino acids were eliminated from the premix; the concentration of DNA was 12 μg/ml, and of the S-30 fraction, 4.8 mg protein/ml. The transcription was continued for 20 min and then stopped with rifampicin (80 μg/ml). Translation was started by addition of the 20 amino acids (in 12 μl) along with the labelled amino acid ([^{14}C]leucine, 13 × 10^5 cpm and 0.0027 μmol/ml) with or without seminalplasmin (25 μg/ml). During translation the total volume of the incubation mixture was 100 μl. The radioactivity incorporated into protein in the stated time was determined in a 10-μl aliquot following treatment with trichloroacetic acid on a Millipore disc as in section 2. (●—●) – seminalplasmin; (○—○) + seminalplasmin.

of seminalplasmin on transcription or translation or both, as it takes about 12 min to synthesize the mRNA for β-galactosidase and 20 min for alkaline phosphatase [7].

To distinguish between the above possibilities, the effect of seminalplasmin on an uncoupled translation of transcripts from ϕ80d*pho*A*lac*Z DNA was analyzed. Fig.2 shows that seminalplasmin almost completely inhibited the incorporation of [^{14}C]leucine into protein in the absence of any synthesis of RNA. Similar inhibition of translation by seminalplasmin was observed on a 10-min as on a 20-min transcript. A direct action of seminalplasmin on translation is further supported by experiments in which seminalplasmin was added at the start of the coupled synthesis (table 4).

The possibility of non-specific inhibition of translation is ruled out by the observation that even high concentrations of seminalplasmin inhibited poly(U)-directed polyphenylalanine synthesis by less than 30% (table 2).

Our results show that in the in vitro coupled transcription–translation system seminalplasmin inhibits transcription of ϕ80d*pho*A*lac*Z and T_7 DNA templates in the presence of the S-30 fraction and the triphosphates, and that this inhibition is immediate and complete if seminalplasmin is added 10–20 min after the commencement of transcription, and only partial during this period. It was found in [4] that when purified RNA polymerase instead of the S-30 fraction was used, the inhibitory effect of seminalplasmin was observ-

ed only if it was incubated with the polymerase prior to the addition of the template (T_7 or calf thymus DNA or synthetic polydeoxyribonucleotides) and triphosphates. These observations put together suggest that in the *E. coli* S-30 fraction a time-dependent association of RNA polymerase with certain other component(s) of the system may be required for seminalplasmin to exhibit complete inhibition of transcription.

The results reported here also show that seminalplasmin immediately and efficiently inhibits translation of ϕ80d*pho*A*lac*Z DNA into proteins and specifically into β-galactosidase and alkaline phosphatase. However, seminalplasmin has only a partial effect on translation of poly(U) to polyphenylalanine. It is possible that seminalplasmin may affect translation by binding specifically to either natural initiation codons in mRNAs or to an initiation factor.

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REFERENCES

[1] Reddy, E.S.P. and Bhargava, P.M. (1979) Nature 279, 725–728.
[2] Bhargava, P.M. (1981) Biochem. Soc. Transac. 9, 540–543.
[3] Reddy, E.S.P. (1981) Ph.D. thesis, Andhra University, Waltair.
[4] Scheit, K.H., Reddy, E.S.P. and Bhargava, P.M. (1979) Nature 279, 728–731.
[5] Reddy, E.S.P., Reddy, E.P.K., Das, M.R. and Bhargava, P.M. (1983) Biochem. J., in press.
[6] Inouye, H., Pratt, C., Beckwith, J. and Torriani, A. (1977) J. Mol. Biol. 110, 75–87.
[7] Pratt, C. (1980) J. Bacteriol. 143, 1265–1274.