ACTION OF RIBONUCLEASE BS-1 ON A DNA-RNA HYBRID

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SUMMARY

Evidence has been obtained that ribonuclease BS-1, structurally and catalytically related to ribonuclease A, is capable of degrading a DNA-RNA hybrid. This finding has been discussed with regard to the recently reported biological effects of the enzyme.

INTRODUCTION

Enzymes capable of degrading the polyribonucleotidic strand of DNA-RNA hybrid molecules have been described. They can act non-specifically, as does ribonuclease III from E.coli, which is able to degrade both double-stranded polyribonucleotides (1, 2) and the RNA strand of DNA-RNA hybrids (3), or specifically on the polyribonucleotidic strand of the hybrid structure, as in the case of ribonuclease H (4). Both RNAase III and RNAase H, moreover, are inactive against single-stranded RNA (1-4).

Ribonuclease BS-1, purified from bull seminal plasma and characterized by a phosphotransferase mode of action (5, 6), has been found to have a dimeric structure made up of two identical subunits (7), the primary structure of which is strictly homologous to that of pancreatic ribonuclease A (8). This enzyme catalyzes the degradation of both single-stranded RNA and polyribonucleotides bearing a secondary structure, like viral double-stranded RNA and the poly(A)·poly(U) complex (9). Needless to say, it is inactive towards double-stranded DNA (9). Recently it has been shown that ribonuclease BS-1 has a significant antispermatogenic activity (10, 11) and a degenerative effect on animal tumour cells (12, and Leone, E., personal communication).

In the present work we have obtained evidence that ribonuclease BS-1 is also active on a DNA-RNA hybrid.

MATERIALS AND METHODS

Calf thymus DNA was purchased from Worthington, unlabelled ribonucleoside triphosphates from Sigma, tritium labelled uridine triphosphate (NET 380, sp. act., 36 Ci/mmole) was from NEN Chemicals GmbH, ribonuclease A (E.C. 2.7.7.16) from Sigma, Type XII A, electrophoretically purified deoxyribonuclease (E.C. 3.1.4.5) from Worthington, ribonuclease BS-1 was purified as described elsewhere (5).

Preparation of DNA-dependant RNA polymerase. DNA-dependant RNA polymerase (E.C. 2.7.7.6) was prepared from purified nuclei of the hepatopancreas of Octopus vulgaris Lam (13, and manuscript in preparation). Essential steps include: sonication of nuclei in the presence of 0.4 M ammonium sulphate, centrifugation at 126,000 x g, precipitation of the supernatant with ammonium sulphate at 55% saturation, chromatography on DEAE-cellulose. The second peak eluted by ammonium sulphate gradient is inhibited by α-amanitin; it has been designed as polymerase II. One unit of the enzyme catalyzes the incorporation of 1 pmole of UMP into an acid-insoluble product under the conditions described below.

Preparation of the DNA-RNA hybrid. DNA-RNA hybrid was prepared by the following procedure. 1 ml of incubation mixture contained: 50 μmoles Tris-HCl, pH 8.0; 12 μmoles $MgCl_2$; 2 μmoles $MnCl_2$; 5 μmoles NaF; about 40 μmoles ammonium sulphate; 1 μmole DTT[+]; 50 μg denatured DNA; 0.25 μmoles each of ATP, CTP, GTP; 0.005 μmoles 5,6[^{3}H]UTP; enzyme, 100 units. DNA was denatured in 0.1 M NaOH (10 min at 25°C). Incubation was carried out for 30 min at 30°C. The extent of the reaction was determined by adding to small aliquots of the incubation mixture, at 0°C, two volumes of chilled 15% CCl_3COOH. After 10-15 minutes at 0°C, the precipitates were filtered through 'Millipore' membranes (HAWP00010, Millipore Filter Co.), and washed extensively with 5% CCl_3COOH. Radioactivity was counted in a Packard Tri-Carb liquid scintillation counter as already described (9). The product of the polymerase reaction, if diluted 1:10 with distilled water, was 97.5% digested by ribonuclease A (50 μg/ml, 30 min at 25°C). The product was either used directly (preparation A), under proper ionic strength conditions

[+]Abbreviations used: DTT, dithiothreitol; SSC, 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0.

(SSC), or extracted with phenol as follows. After addition of 1/10 volume of 10 times concentrated SSC, the incubation mixture was extracted three times with 1 volume of phenol, which had been equilibrated with SSC. The phenol was then removed from the aqueous phase by shaking five times with 1.5 volumes of peroxide-free ether. Ether was finally expelled by a stream of nitrogen. The phenol-extracted material was either used directly (preparation B) or precipitated with 2 volumes of absolute ethanol. After standing overnight at -30°C, the sample was centrifuged at 30,000 x g for 30 min at 0°C, and the sediment dissolved in SSC (preparation C). No acid-insoluble radioactivity was detected in the supernatant.

Ribonuclease assay. Incubations with ribonuclease A or ribonuclease BS-1 were carried out in 1 ml of SSC at 30°C (standard conditions). Two volumes of chilled 15% CCl_3COOH were added, at 0°C, to stop the reaction. The precipitates were filtered and acid-insoluble radioactivity which remained on the filters was counted as described (9). Other experimental details are given in the legends to figures and tables.

RESULTS

The action of ribonuclease BS-1 on the preparation A as substrate is shown in Fig. 1. The product of the RNA polymerase reaction with denatured DNA as the template was significantly degraded by the enzyme, whereas, under identical conditions, it appeared to be almost completely resistant to ribonuclease A. Further evidence that the product is a DNA-RNA hybrid has been obtained by the following experiments.

1. The phenol-extracted sample (preparation B) was subjected to digestion by ribonuclease A and ribonuclease BS-1 with results not significantly different from those shown in Fig. 1. This is shown by the data in Table I. However, after heat treatment under conditions of low ionic strength (see legend to Table I), it became completely sensitive to ribonuclease A in SSC. 2. The phenol-extracted material was first incubated with pancreatic deoxyribonuclease at low ionic strength and then subjected to ribonuclease A digestion under standard salt conditions. As one can see in Table II, the DNAase-treated substrate became totally digestable

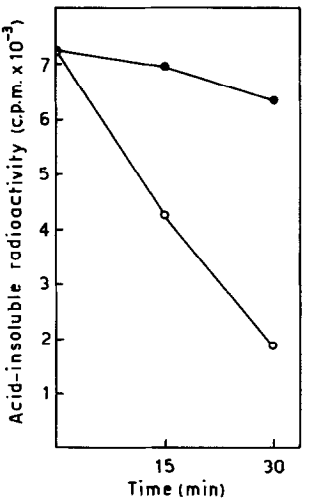

Fig. 1. Activity of ribonuclease BS-1 or ribonuclease A towards the RNA synthesized on denatured DNA. 0.2 ml of preparation A (see Methods) were brought to 1 ml with 1.25 x SSC, and incubated with RNAase BS-1 (o—o) or RNAase A (●—●), 20 μg/ml each, at 30°C.

Table 1. Degradation of the DNA-RNA hybrid by RNAase BS-1 and RNAase A

	Conditions	Acid-insoluble radioactivity (c.p.m.) determined after incubation of: 15 min	30 min
(a)	Control (no enzyme)	4737	4790
	+RNAase A (20 μg/ml)	3786	3289
	+RNAase BS-1 (20 μg/ml)	1339	585
(b)	Control (no enzyme)	4653	4634
	+RNAase A (20 μg/ml)	133	102

(a) Aliquots of preparation B were incubated under standard conditions (see Methods section for details).

(b) Aliquots of preparation B, diluted 1:20 with water, were denatured by heating at 100°C for 10 min. After quick cooling, concentrated SSC was added up to standard concentration and incubation with ribonuclease A, at 30°C, was carried out.

Table II. Effect of DNAase treatment on the DNA-RNA hybrid

Conditions	Acid-insoluble radioactivity c.p.m.
-DNAase, -RNAase	2314
+DNAase, -RNAase	2076
-DNAase, +RNAase A	1167
+DNAase, +RNAase A	21
+DNAase, +RNAase BS-1	17

Incubation with DNAase (40 μg/ml) was carried out in 0.5 ml of 10 times diluted SSC, containing 10 mM $MgCl_2$. After 30 min at 37°C, volumes of incubation mixtures were brought to 1 ml with 2 times concentrated SSC, and 20 μg of RNAase A or RNAase BS-1 were added. Incubations were carried out for 30 min at 30°C.

by ribonuclease A; needless to say, it was also completely degraded by ribonuclease BS-1.

The effect of increasing concentrations of ribonuclease BS-1 on the DNA-RNA hybrid is shown in Fig. 2. In this case, the ethanol precipitated hybrid (preparation C) was incubated with the enzyme also after heat denaturation. A control with ribonuclease A was also included. Loss of radioactivity from the hybrid structure was not complete even at relatively high ribonuclease BS-1 concentrations. This can be tentatively explained by the possibility that short purine-rich RNA fragments, not digested by the enzyme, stick to the DNA strand co-precipitating with it.

DISCUSSION

Ribonuclease BS-1 is able to degrade both single- and double-stranded RNA (9). This property has been related to its dimeric structure (14). In this paper a novel property of the enzyme has been reported: its activity towards a DNA-RNA hybrid. The mechanism of this action is still unknown. Since the enzyme is inactive on DNA, presumably degradation of the hybrid consists in the breakdown of its RNA moiety. Studies on the fate of the DNA strand are currently in progress.

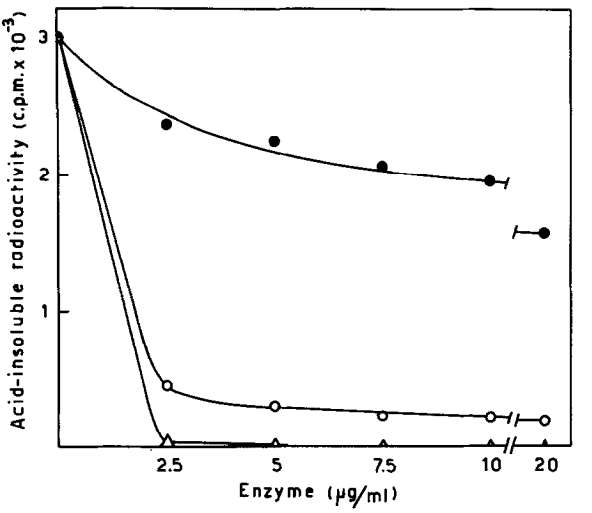

Fig. 2. Degradation of the DNA-RNA hybrid as a function of ribonuclease concentration. Incubations were carried out for 15 min at 30°C under standard conditions (see Methods). ●—●, RNAase A; o—o, RNAase BS-1; Δ—Δ, RNAase BS-1 incubated with denatured DNA-RNA hybrid (preparation C). Denaturation was performed as described under Table I (b).

The finding that ribonuclease BS-1 is capable of degrading a DNA-RNA hybrid may have some interest with regard to the rather peculiar biological effects of the enzyme. It has been recently demonstrated, in fact, that ribonuclease BS-1, in contrast to ribonuclease A, which it resembles, on the other hand, in several structural and catalytic properties, has a definite antispermatogenic activity (10, 11). Moreover, degenerative changes have been described to occur in Crocker tumour cells, in mice, in the presence of the seminal enzyme (12).

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REFERENCES

1. Robertson, H.D., Webster, R.E., and Zinder, N.D. (1967) J. Virol. 12, 718-719.
2. Libonati, M., Viñuela, E., and Weissmann, C. (1967) FEBS Meet. Abstr. 4th, Oslo, Comm. n. 576.
3. Robertson, H.D., Webster, R.E., and Zinder, N.D. (1968) J. Biol. Chem. 243, 82-91.
4. Hausen, P., and Stein, H. (1970) Eur. J. Biochem. 14, 278-283.
5. D'Alessio, G., Floridi, A., De Prisco, R., Pignero, A., and Leone, E. (1972) Eur. J. Biochem. 26, 153-161.
6. Floridi, A., D'Alessio, G., and Leone, E. (1972) Eur. J. Biochem. 26, 162-167.
7. D'Alessio, P., Parente, A., Guida, C., and Leone, E. (1972) FEBS Lett. 27, 285-288.
8. Suzuki, H., Greco, L., Parente, A., Farina, B., La Montagna, R., and Leone, E. (1972) Acta Vitaminol. Enzimol. 26, 213-214.
9. Libonati, M., and Floridi, A. (1969) Eur. J. Biochem. 8, 81-87.
10. Dostál, J., and Matoušek, J. (1972) J. Reprod. Fert. 31, 273-274.
11. Leone, E., Greco, L., Rastogi, R.K., and Iela, L. (1973) J. Reprod. Fert. 34, 197-200.
12. Matoušek, J. (1973) Experientia, 29, 858-859.
13. Taniguchi, T., and Libonati, M. (1973) Int. Congr. Biochem. 9th, Stockholm, Comm. n. 3i 42.
14. D'Alessio, G., Zofra, S., and Libonati, M. (1972) FEBS Lett. 24, 355-358.