HISTIN, AN RNA POLYMERASE INHIBITOR ISOLATED FROM HISTOPLASMA CAPSULATUM

George Boguslawski*, Gerald Medoff,
David Schlessinger, and George S. Kobayashi

Departments of Medicine and Microbiology
Washington University School of Medicine
660 South Euclid, St. Louis, Missouri 63110

Received March 21,1975

Abstract

The dimorphic fungus Histoplasma capsulatum contains an extractable inhibitor of RNA polymerase in its mycelial phase. The inhibitor, called histin, binds to the inhibited enzymes: column chromatography cannot restore polymerase activity to extracts prepared from a mixture of mycelial cells and yeast phase cells.

Histin is specific in that it preferentially affects those RNA polymerases which are resistant to the toxin from Amanita phalloides, α-amanitin. This relative specificity for some RNA polymerases is also found when histin is tested against the enzymes from Xenopus laevis and Escherichia coli as well as from H. capsulatum.

Introduction

The pathogenic fungus Histoplasma capsulatum reversibly converts from a mycelial phase at 23° to a yeast phase at 37°. We have previously shown that the conversion may involve a modification of the RNA-synthesizing machinery of the cell (1); therefore, an analysis of its RNA polymerase was begun. We found that the yeast phase of H. capsulatum had at least three RNA polymerases which could be separated by chromatography on phosphocellulose columns (2). The major RNA polymerase species (PC I) was inhibited strongly by α-amanitin, whereas the other two were resistant.

* Present address: Department of Microbiology, University of Kansas, Lawrence, Kansas 66045

The findings in the yeast phase prompted the study of the RNA polymerases of the mycelial phase of H. capsulatum. Here we report that when extracts were prepared from mycelial, only trace amounts of RNA polymerase could be detected. The mycelial cells have proved to contain a novel potent inhibitor of RNA polymerase activity. The inhibitor exhibits a marked specificity in its action against various RNA polymerases which extends beyond the fungal system. This suggests a possible role for the inhibitor.

Materials and Methods

Organism.

The yeast phase cells of H. capsulatum [Down's strain, mating type (-) from our laboratory] were grown as before (2) using an initial inoculum density of 10^7 cells/ml. The maintenance and growth of the mycelial phase of the same strain is described elsewhere (Boguslawski et al., submitted for publication). Cells were harvested by filtration, washed with distilled water and extracts prepared as described (2).

RNA polymerases.

Ribonucleic acid polymerases from H. capsulatum were prepared by phosphocellulose chromatography and assayed as described (2). The nomenclature of RNA polymerases from H. capsulatum was discussed in the same paper and is followed throughout this report. Xenopus laevis RNA polymerases I_A, II_B, and III were isolated and partially purified by chromatography on DEAE-Sephadex and CM-Sephadex as previously described (3). Each polymerase was subsequently concentrated and further purified by chromatography on phosphocellulose (R.G. Roeder, personal communication). Escherichia coli RNA polymerase was purified through the phosphocellulose step as described (4). The RNA polymerases from X. laevis and from E. coli were kindly donated by Dr. R. G. Roeder of the Washington University School of Medicine, St. Louis, Missouri.

Inhibitor assay.

Inhibition was tested by pipeting aliquots of mycelial extracts into the RNA polymerase reaction mixture prior to enzyme addition and comparing the incorporation of [^{3}H] UMP into RNA with the incorporation obtained in the absence of the inhibitor. Total reaction volume was 0.125 ml. When crude preparations of the inhibitor were used, corrections were made for the residual RNA polymerase activity. Purified inhibitor preparations contained no polymerase activity. Both crude and purified preparations of the inhibitor were stable for months when stored at -20°.

Results

RNA polymerase activities from yeast and mycelium.

When the extract from the yeast phase cells was chromatographed on a phosphocellulose column, it yielded three well separated peaks of activity (Fig. 1). In contrast, mycelial extracts contained only very little RNA polymerase activity, and in a single peak. It is important to note that the

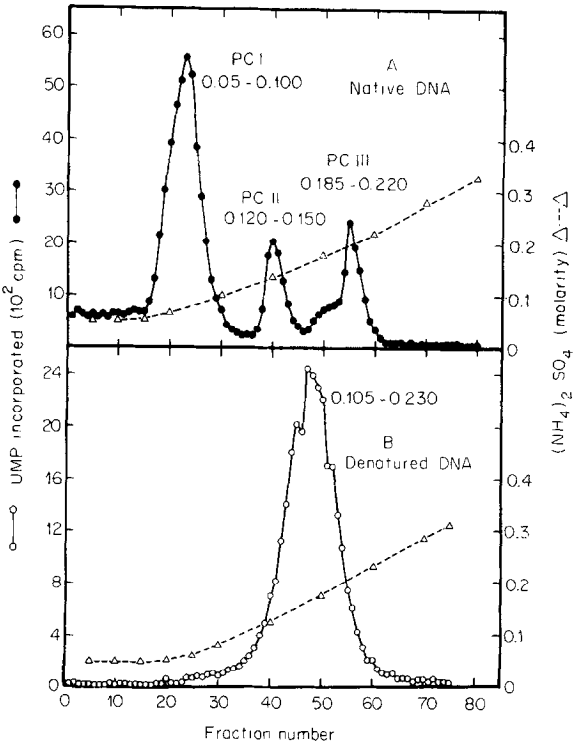

Figure 1. Phosphocellulose chromatography of RNA polymerase from the yeast and mycelial phase of H. capsulatum. Slightly purified extracts of yeast (63 mg protein, panel A) and mycelium (83 mg protein, panel B) were prepared, chromatographed and assayed as described in reference 2. Column bed volumes were 20 ml and 16 ml, respectively. The RNA polymerase activity of yeast fractions was assayed using native calf-thymus DNA (●——●). The activity of mycelial fractions was assayed with denatured DNA (○——○). $(NH_4)_2SO_4$ concentration (------).

yeast enzymes were assayed with native DNA, whereas the mycelial RNA polymerase was assayed with denatured DNA, a more active template. When native DNA was used as a template for the mycelial enzyme, activity was barely detectable.

The two chromatographic patterns shown in Figure 1 represent two separate column runs. On the basis of the position of elution in the salt gradient, the mycelial polymerase does not correspond to any of the yeast enzymes; it elutes between the yeast phase enzymes PC II and PC III.

The chromatographic profile obtained with mycelial extract suggested to us that mycelium could contain an inhibitor of RNA polymerase. This suggestion was supported by the result of an experiment in which mycelial and yeast phase cells were extracted jointly or separately and RNA polymerase activities compared. The yeast phase extract contained high levels of RNA polymerase, whereas the mycelial extract had very little activity. The extract prepared from a 1:1 mixture of cells also showed almost no activity and the residual activity was totally resistant to α-amanitin. In contrast, the α-amanitin sensitive activity in yeast constitutes about 80% of the total (data not shown). The residual activity in the extract from mixed cells, therefore, resembles the mycelial activity. In addition, when mixed cell extract was chromatographed on phosphocellulose column (not shown), only a single peak of activity was seen, corresponding exactly to the peak obtained with the mycelial extract alone (Fig. 1). This observation suggested that the inhibitory substance was strongly bound to RNA polymerase and could not be removed by column chromatography.

The single peak of activity in mycelial and mixed cell extracts probably represented the minute proportion of RNA polymerase which did not undergo inactivation in the crude extract. From these results we inferred the presence of an inhibitor of RNA polymerase in mycelial extracts and have called it "histin".

Specificity of inhibition

The three yeast phase enzymes PC I, PC II, and PC III were tested for sensitivity to the crude inhibitor preparation. As can be seen in Fig. 2, there were differences among the enzymes in response to increasing histin concentrations. Although all three polymerases were totally inhibited at very high levels, enzymes PC II and PC III were much more sensitive than was PC I.

The findings shown in Figure 2 were extended to other RNA polymerase systems, using more purified preparations of the inhibitor (Table I). RNA

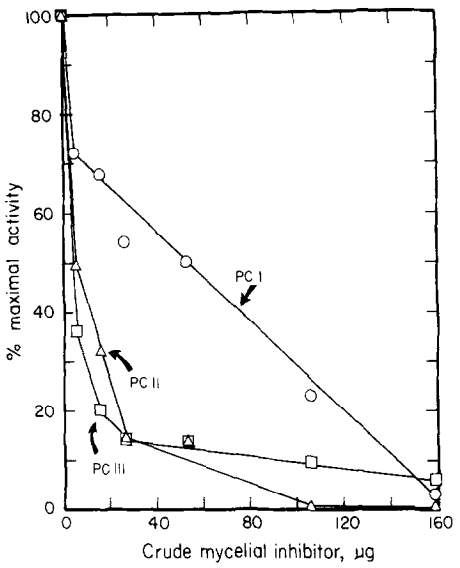

Figure 2. Differential inhibition of yeast RNA polymerases by a crude histin preparation. Aliquots of yeast enzyme fractions were tested against increasing amounts of histin using native DNA as template. (○—○) PC I; (△—△) PC II; (□—□) PC III. Maximal activity (100%) represents 561 cpm, 460 cpm, and 1,582 cpm, respectively.

polymerase from E. coli was strongly inhibited by histin, and RNA polymerases from X. laevis embryos showed differential sensitivity to histin. Table I also includes data for enzymes from the yeast phase of H. capsulatum.

When α-amanitin was employed instead of histin, H. capsulatum enzyme PC I was inhibited 50% by 3 µg/ml of α-amanitin, whereas PC II and PC III were not affected even at 64 µg/ml. These results contrast with the action of histin (Table I). Moreover, E. coli polymerase was strongly inhibited by histin whereas, in agreement with published data (6), it was totally resistant to α-amanitin.

Discussion

Although many naturally occurring and synthetic inhibitors of RNA polymerase have been described (5-10), α-amanitin is the most widely studied, and has been extensively used for the characterization of transcription in cells

Table I

Effect of purified* mycelial inhibitor on the activity of RNA polymerase from various organisms.

Organism	RNA Polymerase	Amount of Inhibitor (ug)	Counts/min.	Percent Activity Remaining
H. capsulatum yeast phase	PC I	-	898	100.0
		0.45	886	98.7
		1.80	828	92.5
		18.00	705	78.5
	PC II	-	5197	100.0
		0.45	1409	27.1
		1.80	661	12.7
		18.00	376	7.2
	PC III	-	1227	100.0
		3.20	117	9.5
		16.00	67	5.5
X. laevis embryo	I_A	-	33184	100.0
		0.45	16659	50.2
		4.50	3553	10.7
		18.00	3389	10.2
	II_B	-	1012	100.0
		1.80	1335	131.9
		4.50	1439	142.2
	III	-	7164	100.0
		1.80	2498	34.9
		4.50	1889	26.4
		18.00	1748	24.4
E. coli	Core enzyme	-	21300	100.0
		0.90	6045	28.4
		1.80	4638	21.8
		4.50	3025	14.2

*The inhibitor was purified about 12-fold by heat treatment (Boguslawski et al, submitted for publication).

and extracts (6, 11-15). Histin, with a spectrum of activity complementary to α-amanitin, may also be useful for studies of transcription.

Several results suggest that α-amanitin and histin probably act at different sites, or different subunits, of RNA polymerase. For example, E. coli polymerase is inhibited only by histin, whereas H. capsulatum PC I polymerase is inhibited by α-amanitin and only weakly by histin. The Class I, II, and III RNA polymerases from X. laevis showed α-amanitin sensitivities identical to those reported (16) for the analogous enzymes from mouse plasmacytoma cells (R.G. Roeder, personal communication). Thus, polymerase I_A (the predominant Class I enzyme) was totally insensitive to one mg of α-amanitin per ml, polymerase II_B (the major Class II enzyme) was 50% inhi-

bited by 0.004 μg/ml concentration of the toxin, and polymerase III was inhibited by 50% at 20 μg/ml. Instead, histin was most effective at low levels against polymerase I_A and III (Table I). The inverse correlation between the sensitivity to α-amanitin and histin is thus strong, though not absolute.

Whether histin has any inhibitory effect against RNA polymerases of the yeast or mycelial phases in whole cells is at present unknown. It is possible that histin may have no effect on the mycelial phase, or that its level in the cell might somehow determine whether a transition to the yeast phase can occur. Although there is no clear case in which inhibitors function in organisms undergoing transitions from one phase to another, there are modifications reported in RNA polymerases during sporulation of Bacilli (17) and during the life cycle of some fungi (18, 19).

We have continued to purify and characterize histin, and will report elsewhere (Boguslawski et al., submitted for publication) evidence that it is a small, heat stable, acidic protein. The availability of purified material in the future should permit a clearer determination of its cellular location and function.

Acknowledgments

We are grateful to Dr. R. G. Roeder for generous gifts of RNA polymerases from X. laevis and E. coli and for helpful advice.

This work was supported by USPHS grants AI 10622 and AI 06213; training grants AI 00459 and AM 05611 and grants from the John A. Hartford Foundation and the Research Corporation (Brown-Hazen Fund). George Boguslawski was supported by Special N.I.H. Fellowship 1F22-AI 00284.

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