PREFERENTIAL INHIBITION OF SYNTHESIS AND METHYLATION OF RIBOSOMAL RNA IN NEUROSPORA CRASSA BY ACTIDIONE.[1]

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Reports from several laboratories indicate that the general effect of the antibiotic actidione (cycloheximide) in Saccharomyces cerevisiae (Kerridge, 1958) in S. pastorianus (Siegel, 1963; Siegel and Sisler, 1964a, 1964b) and in mammalian cell cultures (Bennett et al., 1964) is the inhibition of protein and DNA synthesis. Studies with Staphylococcus aureus, an organism whose growth is not sensitive to actidione, have shown that this antibiotic inhibits the formation of inducible β-galactosidase (Creaser, 1955). In this paper we report that actidione preferentially inhibits the synthesis and methylation of ribosomal RNA in Neurospora crassa. A substantial effect on the methylation of soluble RNA is noted.

N. crassa ATCC 38706, a methionine-requiring mutant, was maintained on slants composed of the agar-organic medium described by Horowitz (1947). Liquid cultures were prepared by inoculating 22.5 x 10^7 conidia into 400 ml of mineral salts-sucrose medium (Horowitz, 1947) contained in 2.8 liter Fernbach flasks, supplemented with DL-methionine at a level of 50 μg/ml. Cultures were grown on a rotary shaker at 28°.

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Under these conditions the middle of the logarithmic growth phase was reached in 16.5 hours. Uridine-2-C^{14} (specific activity 30 mc/mM) or S-adenosyl-L-methionine-methyl-C^{14} (SAM, specific activity 39.4 mc/mM) were added after 13.5 hours growth and the tissue was incubated 3 hours longer to midlog phase. Actidione was added to the growth flasks at a level of 100 μg/ml five minutes prior to the addition of the radioactive compounds. The mycelium was harvested on a sintered glass funnel and washed with a total of one liter of cold medium. RNA was extracted from tissue disrupted by glass beads in 0.01 M Tris-HCl buffer, pH 7.1, 0.01 M in $MgCl_2$ by the phenol method as described by Somberg (1964). After overnight precipitation in 66% alcohol, 2% potassium acetate, pH 5.0, the RNA was washed several times by centrifugation and resuspension at 0° with 66% ethanol, 2% potassium acetate, pH 5.0. The RNA was then dissolved in 3% sucrose, 10^{-4} M $MgCl_2$ and applied to a linear

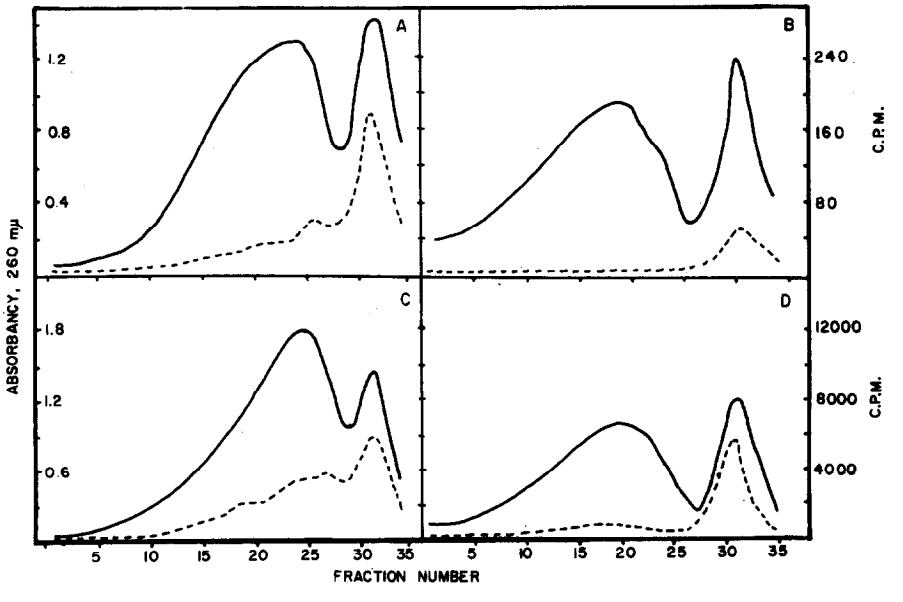

Figure 1. Sucrose density gradient profiles which show the effect of actidione on the uptake of uridine-2-C^{14}, or methyl label from methyl-C^{14}-S-adenosylmethionine, into N. crassa RNA. Conditions are given in the text. Solid line, absorbancy; dashed line, radioactivity. A, uptake of methyl label in control cells; B, uptake of methyl label in actidione-treated cells; C, uptake of uridine in control cells; D, uptake of uridine in actidione-treated cells.

5-20% sucrose gradient with Mg^{++} at a constant concentration of 10^{-4} M. The samples were centrifuged in a swinging bucket head (Spinco SW 39) at 38,000 r.p.m. for 180 minutes at 4°, the tubes punctured, and absorbancies and count of 2-drop fractions determined.

Profiles are shown in Fig. 1. Specific activities of ribosomal and soluble RNAs, calculated from values obtained by integration of the curves in Fig. 1 are given in Table I. Synthesis, as reflected in uridine-2-C^{14} incorporation into ribosomal and soluble RNAs in the actidione system is 58% and 16% inhibited, respectively. It is of interest to note the almost complete inhibition of ribosomal RNA methylation and the substantial inhibition of S-RNA methylation in the actidione system. The decreased methylation of RNA may indicate that RNA which is synthesized in the presence of actidione is not "normal", although actual inhibition by actidione of RNA methylases cannot be ruled out. Comparable results were obtained when L-methionine-methyl-C^{14} was substituted for SAM.

TABLE I

Specific Activities of Ribosomal and Soluble RNAs from Figure 1.

	Control, CPM/OD Unit	Actidione, CPM/OD Unit	Per Cent INHIBITION
Ribosomal RNA, uridine label	1911	803	58
Soluble RNA, uridine label	4604	3900	15
Ribosomal RNA, SAM label	26.8	1.5	94
Soluble RNA, SAM label	96.5	30.7	68

Gordon, Boman and Isaksson (1964) have recently reported that chloramphenical produces an *in vivo* inhibition of RNA methylation in an auxotroph of *E. coli*, and that the effect is predominantly localized in ribosomal RNA. The similarity of results indicates that actidione may act in a manner analogous to chloramphenical and cause the accumulation

of small ribonucleoprotein particles in which the RNA is submethylated. This possibility is currently being investigated.

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