# INHIBITION BY CYCLOHEXIMIDE OF PROTEIN AND RNA SYNTHESIS IN MUCOR ROUXII

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Cycloheximide (actidione) has been shown to inhibit protein synthesis and to a variable extent, RNA synthesis in various fungi, as well as mammalian cells, but not in bacteria, (Fiala and Davis, 1965; Fukuhara, 1965; de Kloet, 1965; Siegel and Sissler, 1965; Sussman, 1965; Wettstein _et al._, 1964).

In an atmosphere of $CO_2$ and $N_2$, cells of _Mucor rouxii_ are spherical, yeast-like and reproduce by budding. However, after exposure to air for a few hours, they are converted to hyphae (Bartnicki-Garcia, 1963). It was shown (Haidle and Storck, in manuscript) that this conversion is accompanied by protein and RNA synthesis. In the present paper, it is demonstrated that cycloheximide inhibits protein and RNA synthesis, as well as the morphological change.

_Materials and Methods_: Yeast-like cells of _Mucor rouxii_ NRRL 1894 were obtained by incubating sporangiospores in submerged culture in a glucose-mineral salts medium (Bartnicki-Garcia, 1962) supplemented with nicotinic acid, thiamine and casein hydrolysate. During growth, the culture was continually flushed with a mixture of 0.1 l/min $CO_2$ and 0.3 l/min $N_2$. Cycloheximide was a gift from the Upjohn Company, Kalamazoo, Michigan.

For incorporation of labeled amino acids and uridine, the yeast-like cells were exposed to air and tracer at the same time. Samples, 1.0 ml, were removed at the indicated times and mixed with an equal volume of 20% (w/v) trichloracetic acid (TCA) and incubated in the cold for 30 min. The precipitates were collected on Schleicher and Schuell membrane filters (type B-6) and were washed 6 times with 5 ml volumes of cold 10% TCA. Radioactivity was measured in a liquid scintillation spectrometer.

RNA hydrolysates were prepared by treating perchloric acid precipitated cells with ribonuclease, followed by alkaline hydrolysis of the solubilized material (Haidle and Storck, In manuscript). The base composition of RNA was determined by chromatography on Dowex 1-X8 formate resin as described elsewhere (Henney and Storck, 1963; Storck, 1965; Haidle and Storck, In manuscript).

Results: The effect of increasing concentrations of cycloheximide upon $C^{14}$-L-phenylalanine incorporation is presented in Fig. 1. This curve represents percent inhibition after 4 hr exposure of yeast-like cells to air, tracer and antibiotic. In the absence of cycloheximide, 4 hr are required for the onset of filamentous growth. Fig. 2 shows the time course of incorporation of $C^{14}$-L-isoleucine during the conversion in the presence and absence of the antibiotic. Isoleucine is incorporated exponentially in the absence of cycloheximide and 5% of the yeast-like cells produce germ tubes after 4 hr; this percentage increases to 80% after 10 hr. In the presence of 20 μg/ml cycloheximide, 5% of the cells produce germ tubes after 6 hr. When 200 μg/ml cycloheximide is present, germ tubes are produced only after 10 hr, but 90% of the population never produce germ tubes. Uridine incorporation, repre-

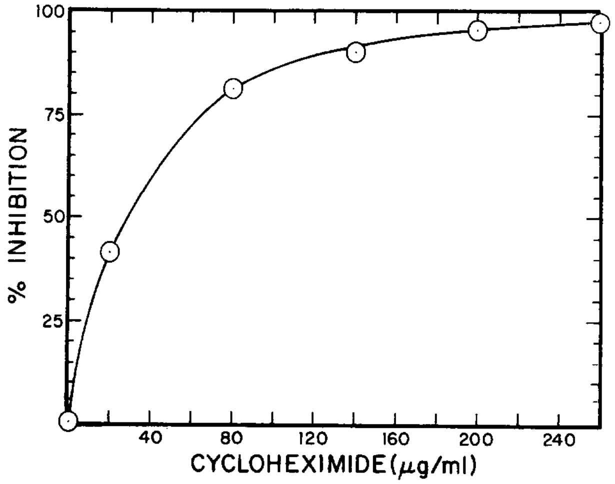

Fig. 1 Effect of various concentrations of cycloheximide on $C^{14}$-L-phenylalanine incorporation. Yeast-like cells were exposed to air, tracer (0.066 μc/ml of culture; 66 mc/mmole) and cycloheximide and incubated for 4 hr at 25 C with stirring.

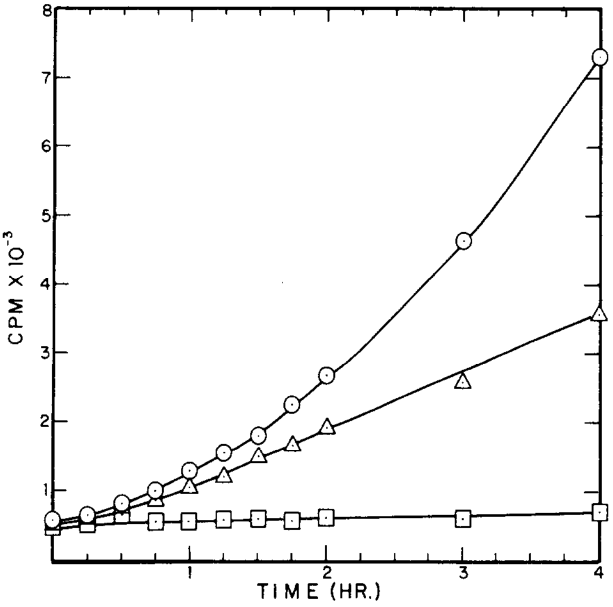

Fig. 2 Incorporation of $C^{14}$-L-isoleucine in the presence and absence of cycloheximide. Yeast-like cells were exposed to air and tracer (0.2 μc/ml of culture; 6.16 mc/mmole) at 0 time. Symbols: O-control, no cycloheximide; △ - 20 μg/ml cycloheximide; □ - 200 μg/ml cycloheximide.

senting RNA synthesis, is presented in Fig. 3. The discontinuities observed in these curves are typical for RNA synthesis during the conversion (Haidle and Storck, In manuscript). The amount of inhibition after 4 hr is 40 to 50% compared with the 95% observed for amino acid incorporation. There is very little inhibition of uridine incorporation during the first 45 min of the conversion.

Table 1 shows the difference in the base composition of RNA in the presence and absence of cycloheximide. When the antibiotic is present, the base composition of RNA resembles that of DNA. The base ratio in the absence of the antibiotic is typical for bulk RNA of both the yeast and hyphal forms.

Using standard manometric techniques measuring $O_2$ uptake, there was 66% inhibition of respiration of yeast-like cells in the presence of cycloheximide, whereas there was only 21% inhibition of respiration of hyphae.

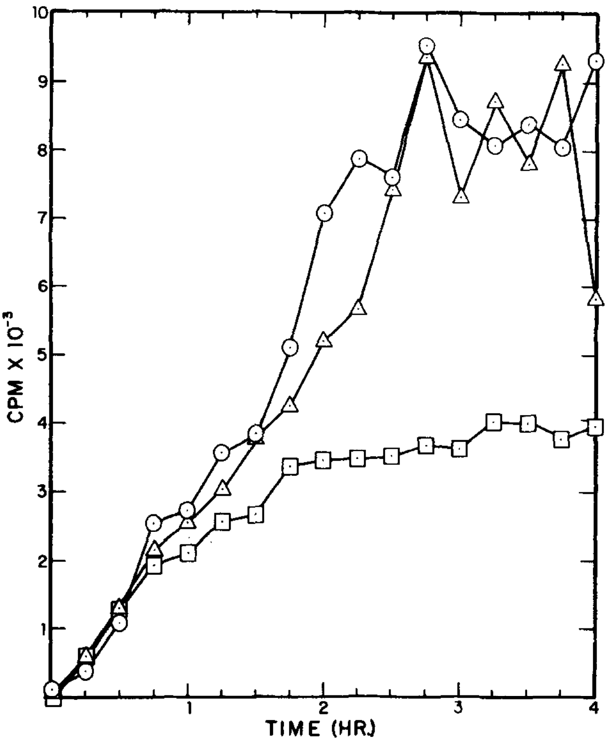

Fig. 3 Incorporation of $H^3$-uridine in the presence and absence of cycloheximide. Yeast-like cells were exposed to air and tracer (0.4 μc/ml of culture; 6.58 c/mmole) at 0 time. Symbols: O - control, no cycloheximide; △ - 20 μg/ml cycloheximide; □ - 200 μg/ml cycloheximide.

Table 1. Base composition of RNA in the presence and absence of cycloheximide.

| | Nucleotides (Mole %) | | | | |
|---|---|---|---|---|---|
| | C | A | U(T) | G | G+C |
| - Cycloheximide* | 19.5 | 29.2 | 24.8 | 26.5 | 46.0 |
| + cycloheximide** | 21.7 | 29.7 | 27.4 | 21.2 | 42.9 |
| DNA *** | 19.5 | 30.5 | 30.5 | 19.5 | 39.0 |

* Determined on $P^{32}$ content of nucleotides. Yeast-like cells were exposed to air at 0 time and tracer (35 μc of $P^{32}$/ml of culture) at 20 min. The incorporation was terminated at 40 min.

** Yeast-like cells were exposed to air and tracer (same as above) at 0 time in the presence of 250 μg/ml cycloheximide. The incorporation was terminated at 40 min.

*** Determined by CsCl buoyant density centrifugation and melting point analyses (Storck, 1966)

Discussion: Our results represent yet another example of the inhibition of the synthesis of both protein and RNA in a filamentous fungus which is capable of growing in the yeast form. Although there is general agreement in the literature concerning the inhibitory action of cycloheximide on protein biosynthesis, the situation with respect to RNA synthesis is not clear. Sussman (1965) found no inhibition of uridine incorporation in the slime mold *Dictyostelium discoideum* and Siegel, and Sissler (1964) observed a stimulation of RNA synthesis in *Saccharomyces pastorianus*. Studies on the mode of action of the antibiotic, using *in vitro* protein synthesizing systems, indicate that there is an interference in the transfer of amino acids from transfer RNA to protein (Siegel and Sissler, 1965; Wettstein et al., 1964)and also an inhibition of the breakdown of the polysome structure, which would effectively prevent the "read out" of the messenger. Fiala and Davis (1965), working with *Neurospora crassa*, have presented evidence to show that the inhibitory action on RNA synthesis is exerted mainly on ribosomal RNA and perhaps results from the inability to methylate this RNA in the presence of the antibiotic.

It is perhaps appropriate to point out the similarities and differences between our results and those of other workers. Fukuhara (1965) has studied the induction of respiratory enzymes when *S. cerevisiae* is transferred from an anaerobic to an aerobic environment. He observed a 95% inhibition of protein and a 30% inhibition of RNA synthesis and close to 100% inhibition of the synthesis of cytochrome c. We have noted a similar degree of inhibition of protein and RNA synthesis when yeast-like cells are exposed to air in the presence of cycloheximide. In addition, we observed a higher degree of inhibition of respiration in antibiotic-treated yeast-like cells than in hyphae, which can be interpreted as the suppression of the *de novo* synthesis of respiratory enzymes in yeast-like cells. Fukuhara (1965) has also presented evidence for the accumulation of a high turnover RNA with a base ratio differing from that of bulk RNA, which is in agreement with the work of de Kloet (1965) who found that there was an accumulation of DNA-like RNA in *S. carlsbergensis*. These data agree with our observation of the preferential synthesis of RNA which mimics DNA and which we believe is of the messenger type. Siegel and Sissler (1964), however, found that RNA synthesized in the presence of cycloheximide has a base composition similar to that of bulk RNA.

Finally, in the presence of cycloheximide, the morphological conversion of yeast-like cells to filamentous cells is inhibited which is similar to the inhibition of morphogenesis in *D. discoideum* (Sussman, 1965). Since we have shown that protein and RNA synthesis accompany the conversion and that cycloheximide inhibits both of these syntheses, it is assumed that protein and/or RNA synthesis are necessary for the yeast to filament change and that this change involves more than merely the change in shape of the cell wall.

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