EFFECT OF CYCLOHEXIMIDE ON YEAST CELL WALL SYNTHESIS

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The yeast cell wall appears to consist of a complex insoluble glucan polymer embedded in an amorphous matrix. The glucan supports the shape of the cell and maintains its outline even when the cytoplasm and the other wall polymers have been removed with water or dilute alkali (Northcote and Horne, 1952). The matrix material appears to be mainly a mannan polymer which has been found covalently linked to peptides forming glycopeptides (Sentandreu and Northcote, 1968). Similar physical organization of cell wall has been found in a large number of fungal cells (Bartnicki-Garcia, 1968).

In bacteria the biosynthesis of the rigid peptidoglycan of the wall is not prevented by the presence of chloramphenicol (Hancock and Park, 1968; Mandelstam and Rogers, 1959) or other specific inhibitors of protein synthesis at the ribosomal level. Regeneration of yeast protoplasts is prevented by cycloheximide (Soskova, Svoboda and Soska, 1968; Necas, Svoboda and Kopecka, 1968) an inhibitor of ribosomal protein synthesis in some eucaryotic cells. This antibiotic stopped incorporation of radioactivity from L- (U-C^{14})-threonine in both cytoplasm and the cell wall

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of Saccharomyces cerevisiae while incorporation of radioactivity from D-(U-C^{14})-glucose was only partially halted (Sentandreu, 1968). This communication is concerned with some characteristics of the inhibition brought about by cycloheximide on yeast cell wall biosynthesis.

Materials and Methods

The organism used in this work was a Saccharomyces cerevisiae strain isolated from pressed baker's yeast. The growth medium contained: Yeast extract, 3 g; D-glucose, 20 g/l and growth conditions were similar to those already described (Sentandreu and Northcote, 1969).

Yeast cells (12.5 mg) in the early exponential phase were transferred to fresh medium diluted with water (1/4, v/v) and supplemented with cycloheximide (4 mg/l) and after 10 minutes at 28°, radioactive glucose (2.5 μCi; specific activity 320 mCi/mM) was added. After 30 minutes at 28° the cells were collected by centrifugation and washed with cold water (twice). Wall polysaccharides were extracted by a slight modification of the method of Northcote and Horne (1952). Yeast cells were treated with 2 N-sodium hydroxide at 100° for 2 hours in sealed ampoules. The supernatant obtained after centrifuging was retained for preparation of yeast mannan. The insoluble residue was extracted with 0.5 N-acetic acid at 100° for 2 hours and finally washed with water. To the alkaline supernatant solution, mannan (5 mg obtained from baker's yeast by the method described by Edwards, 1965) was added, the resulting solution was treated with ethanol (4 volumes) and the precipitated mannan purified by the precipitation with Fehling reagent (Edwards, 1965). Aliquots of the Cu^{++} mannan complex and of the insoluble glucan residue after acetic acid extraction were collected on glass fiber paper discs and counted in a

Table 1. Incorporation of radioactivity from D-(U-C^{14})-glucose into S. cerevisiae walls

	Control		Medium supplemented with cycloheximide	
	Counts/ min/mg cell	% of total radioactivity	Counts/ min/mg cell	% of total radioactivity
Glucan	1549	58.5	2138	93.1
Mannan	1093	41.5	159	6.9

The cells (12.5 mg) were incubated in medium diluted with water (1/4, v/v) and supplemented with cycloheximide (4 mg/l) and after 10 minutes at 28°, radioactive glucose (1%, w/v; 10 μCi) was added to the culture. After 30 minutes at 28 C the cells were harvested and fractionated as described in the text.

gas flow counter (Sentandreu, 1968).

The results presented in Table 1 show that the incorporation of radioactivity from D- (U-C^{14})-glucose into glucan was 58.5% and into mannan 41.5% of the total detected. When the cells were supplemented with cycloheximide the values obtained were 93.1% and 6.9% respectively. The presence of antibiotic dramatically reduced the incorporation of label into the mannose polymer. At the same time there was a significant increase in the glucan radioactivity.

To find experimental evidence that cycloheximide inhibition of mannan biosynthesis was parallel with a similar inhibition in peptide incorporation into the wall, the following experiment was carried out. Yeast cells (7 mg) were incubated in a medium supplemented with L-(U-C^{14})-threonine (2.5 μCi; specific activity 10 mCi/mM) in conditions similar to those described above. The cells after 30 minutes at 28° were

collected by centrifugation, washed with ice-cooled water and broken in a Ribi Cell Fractionator (Servall) at 25000 psi. The resulting suspension was centrifuged at 2000 g for 10 minutes and the walls washed with water and finally collected on glass fiber paper discs and counted in a gas flow counter.

Table 2. Incorporation of radioactivity from L-(U-C^{14})-threonine into S. cerevisiae walls

Walls from a medium supplemented with:	c/min/mg cells	%
Control	4285	100
Cycloheximide	188	4.4

Cells (7.0 mg) were incubated in a medium diluted with water (1/4, v/v) and supplemented with cycloheximide (4 mg/l) and after 10 minutes at 28° radioactive threonine (2.5 μC; specific activity 10 μCi/μm) was added to the culture. After 30 minutes at 28° the cells were harvested and broken in a Ribi Cell Fractionator as described in the text.

The results presented in Table 2 show that cycloheximide-treated cells incubated with radioactive threonine incorporated into their walls only 4.4% as much radioactivity incorporated as those incubated without antibiotic. These results showed that cycloheximide stopped the incorporation of peptide material into the cell wall and that this inhibition was parallel to that of the mannan synthesis.

The cycloheximide effect could be thought of as either a primary inhibition at peptide level of the glycoprotein formation or as a secondary one due to the failure of resynthesis of certain enzymes involved in

polysaccharide synthesis. To get some information about this point and in an attempt to determine the pool level (if any) of the wall peptides, cells (12 mg) in the early exponential phase were resuspended in the same medium used above (20 ml) supplemented with cycloheximide (4 mg/l) and D-(U-C^{14})-glucose (5 μCi) and incubated at 28°. Samples (4 ml) were withdrawn at intervals (2, 5, 10 and 25 minutes), the cells collected and washed by centrifugation and glucan and mannan polymers extracted as described above (Fig. 1).

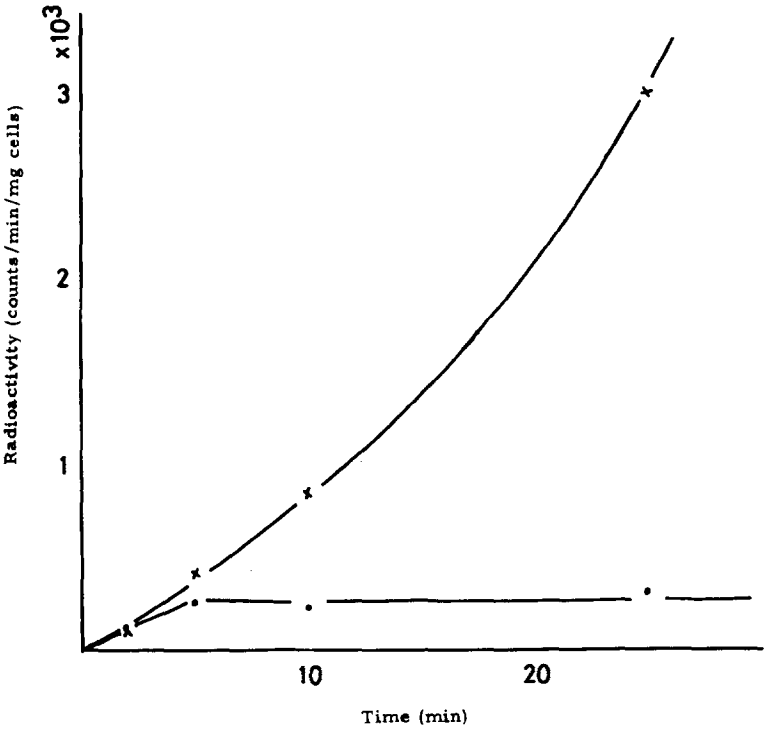


Fig. 1. Incorporation of radioactivity from D-(U-C^{14})-glucose into yeast mannan.

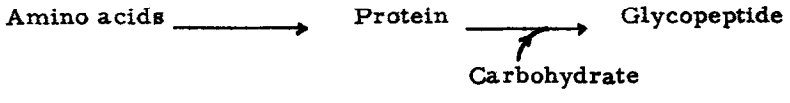
Control x Cycloheximide •

The amount of radioactivity from D-(U-C^{14})-glucose incorporated into mannan increased during a short period; after about 5 minutes the

curve flattened off and no further incorporation of radioactivity was detected. The incorporation of radioactive mannose may take place on those peptides which had already been partially glycosylated before the addition of radioactive glucose or in those peptides accumulated in a cellular pool.

Morris (1967) has reported that in Chlorella, cycloheximide inhibits protein synthesis but also interferes with polysaccharide synthesis after several hours. This interference is suggested to be a secondary effect due to the decay of those enzymes involved in the synthesis and which are not resynthesized in the presence of antibiotic. In our system, the speed of mannan-glycopeptides inhibition makes this possibility very unlikely.

Our findings suggest that the biosynthesis of the major constituents of yeast cell walls is the result of two processes. One of them is independent initially of protein synthesis and is involved in the production of the insoluble network of glucan (Necas, Svoboda and Kopecka, 1968; Garcia-Mendoza and Novais, 1968). This process might take place by means of the organized particles present on the outer plasmalemma surface as suggested by Moor and Muhlethaler (1963). A role of the plasmalemma in insoluble microfibrilar synthesis has also been found in Chlorella (Morris, 1967), and suggested in higher plants (Northcote and Lewis, 1968). The amorphous mannan-peptide matrix which embeds the insoluble glucan might be synthesized in the cytoplasm following the scheme:



Cycloheximide would interfere with the first step of this scheme. The mannan-peptides ordinarily synthesized may be carried to the site of

wall synthesis in vesicles which are seen to accumulate at the region of bud formation (Sentandreu and Northcote, 1969). The material of the vesicles could be passed across the plasmalemma by a process of reverse pinocytosis.

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