

Lomofungin, an Inhibitor of Ribonucleic Acid Synthesis in Yeast Protoplasts: Its Effect on Enzyme Formation

S.-C. KUO, F. R. CANO, AND J. O. LAMPEN

Institute of Microbiology, Rutgers University, The State University of New Jersey, New Brunswick, New Jersey 08903

Received for publications 5 January 1973

Lomofungin, an antibiotic active against fungi, yeasts, and bacteria, rapidly inhibits synthesis of ribonucleic acid (RNA) but not protein by protoplasts of *Saccharomyces* strain 1016. With 40 μg of lomofungin/ml, RNA synthesis was almost completely halted after 10 min of incubation; protein synthesis continued for at least 40 min. Since lomofungin inhibits isolated RNA polymerases from yeast, but not in vitro protein synthesis, it is concluded that the primary action of lomofungin in yeast protoplasts is on RNA synthesis. Examination of the pulse-labeled RNA indicated that biosynthesis of both ribosomal precursor RNAs and messenger RNAs was severely inhibited after the protoplasts were incubated with lomofungin for 5 min, whereas formation of small-molecular-weight RNA (4 to 5s) was only slightly affected. Under these conditions, lomofungin almost completely prevented induction of α -glucosidase. Once the protoplasts had been induced, further production of the enzyme was not impaired by lomofungin until after 30 min of incubation, but was rapidly halted by cycloheximide (4 $\mu\text{g}/\text{ml}$). Lomofungin inhibition of invertase formation by protoplasts actively synthesizing the enzyme also became evident only after a lag of about 30 to 40 min, although synthesis was promptly halted by cycloheximide. These observations suggest the existence of relatively long-lived specific messenger RNAs for these enzymes.

Lomofungin, 1-carbomethoxy-5-formyl-4,6,8-trihydroxyphenazine (25), is an antibiotic produced by *Streptomyces lomendensis* var. *lomendensis*. It has been reported to inhibit the growth of gram-positive and gram-negative bacteria, yeast, and other fungi (13). From studies with a strain of *Saccharomyces cerevisiae*, Gottlieb and Nicolas (5) proposed that synthesis of ribonucleic acid (RNA) was preferentially sensitive to the antibiotic, whereas deoxyribonucleic acid (DNA) synthesis was less affected. Furthermore, in vitro studies showed that protein synthesis was not inhibited by lomofungin.

We have investigated the action of lomofungin on yeast protoplasts as well as on isolated yeast RNA polymerases (3) and conclude that the primary inhibitory effect is on the synthesis of RNA. The effect of lomofungin on the formation of several enzymes by protoplasts was examined. The kinetics of inhibition are consistent with the existence of relatively long-lived messenger RNAs (mRNAs) for invertase, α -glucosidase, and acid phosphatase.

MATERIALS AND METHODS

Growth of organisms and preparation of protoplasts. *Saccharomyces* strain 1016 was used; the properties of this mutant have been described (15, 16). The organism was grown as described previously (16) in modified Vogel's medium N (28) with 0.2 M glucose or 0.05 M maltose. To examine the inhibitory effect of lomofungin on growth, different concentrations of the antibiotic were added to exponential-phase cells; growth was followed by measuring the turbidity with a Klett-Summerson colorimeter (filter no. 66). A reading of 120 corresponds to about 4×10^7 cells of strain 1016 per ml.

Protoplasts were prepared from the exponential-phase cells in 0.6 M KCl medium and then washed with 0.8 M sorbitol medium as described by Kuo and Lampen (15, 16).

Conditions for enzyme formation by protoplasts and assays of enzyme activities. Washed protoplasts were suspended at 5×10^7 per ml in 0.8 M sorbitol medium with the indicated concentration of sugar as energy source and were incubated at 30 C in a reciprocating shaker (16). Samples were periodically removed from the incubation mixture and transferred to chilled tubes containing 4 volumes of ice-cold water

to lyse the protoplasts. The resulting suspensions were assayed for invertase, acid phosphatase, and α -glucosidase (15, 16).

Synthesis of nucleic acid and protein by protoplasts. Incorporation of ^{14}C -amino acids, ^3H -uridine, or ^{14}C -adenine into trichloroacetic acid-insoluble material was used as an index of protein and RNA synthesis. These precursors were added to protoplast suspensions which had been preincubated for 80 min at 30 C with 50 or 100 mM fructose; after specified times, 0.5-ml samples were removed and the reaction was stopped by adding 2 ml of 10% trichloroacetic acid. Incorporation of ^{14}C -amino acids into the protein fraction was measured according to Kuo and Lampen (15). For measurement of uridine incorporation, the acid-insoluble materials were kept at 0 C for 60 min, then collected on glass-filter paper (Whatman GF/A), and washed twice with 5 ml of ice-cold trichloroacetic acid (5%) containing 10 μg of cold uridine/ml. The dried filters were placed in 10 ml of toluene-Omnifluor (New England Nuclear Corp.) scintillation mixture, and the radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer.

Analysis of total RNA by polyacrylamide gel electrophoresis. Washed protoplasts were suspended at 5×10^7 per ml in 0.8 M sorbitol medium with 100 mM fructose as energy source and were incubated at 30 C for 80 min. To 2.5-ml volumes of the suspension, 25 μCi of ^3H -uridine/ml (specific activity, 40 Ci/mmol) was then added, and the mixtures were incubated for 5 min in the presence and absence of lomofungin (40 $\mu\text{g}/\text{ml}$). The samples were chilled rapidly by adding 1 ml of frozen, crushed 0.8 M sorbitol medium and were centrifuged for 2 min at $3,000 \times g$. RNA was extracted and purified as described by Udem and Warner (26) with the modification that 1:1 (vol/vol) phenol-chloroform was substituted for the phenol-cresol mixture.

RNA analysis by acrylamide gel electrophoresis was as described by Udem and Warner (26) and Bishop, Claybrook and Spiegelman (1). A 60- μg amount of purified RNA, 5 μg of *Escherichia coli* soluble RNA (Schwarz BioResearch, Inc.), and 4 μliters of 0.1% pyronine Y (as a visible marker), in a total volume of 30 μliters or less, was loaded onto the gels and electrophoresis was carried out at 50 ~ 60 V (or 5 mA per tube) for 80 min. After electrophoresis, the gels were frozen and sliced into 1.3-mm fractions with a razor blade device. The gel sections in the vial were treated with 0.2 ml of 0.3 M Nuclear-Chicago Solubilizer (in toluene) and 0.04 ml of 1 N NaOH. After overnight at room temperature, 5 ml of toluene-Omnifluor (New England Nuclear Corp.) scintillation mixture was added and the radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer.

For labeling the stable RNAs, strain 1016 (1.5×10^7 cells per ml) was grown in modified Vogel's medium with ^{14}C -uridine (0.2 $\mu\text{Ci}/\text{ml}$; specific activity, 50 mCi/mmol) for two generations (4 h). Protoplasts were prepared, and RNA was extracted and purified as for the pulse experiment with ^3H -uridine.

Preparation and use of poly U fiberglass filters for binding of polyadenylic acid (poly A)-con-

taining mRNAs. The procedure for preparation of polyuridylic acid (poly U) fiberglass filters with Whatman GF/C paper by ultraviolet irradiation was essentially according to Sheldon, Jurale, and Kates (21). Amounts of 3 to 6 μg of ^3H -RNA (pulse-labeled for 5 min) were dissolved in 2.0 ml of binding buffer [0.01 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.5)-0.12 M NaCl] and filtered slowly at 2 ~ 3 ml per min through the fiberglass filters. The filters were washed with 20 ml of binding buffer at 25 C, then by 20 ml of ice-cold 5% trichloroacetic acid, and finally by 10 ml of ice-cold 95% ethanol. The filters were dried; they were then suspended in 5 ml of toluene-Omnifluor (New England Nuclear Corp.) scintillation mixture, and radioactivity was counted.

Chemicals. All chemicals were of reagent quality. Adenine-8- ^{14}C (60 mCi/mmol), ^{14}C -uridine (56 mCi/mmol), and ^3H -uridine (20 Ci/mmol) were purchased from New England Nuclear Corp.; ^{14}C -protein hydrolysate, from Amersham/Searle Co.; and cycloheximide, from Calbiochem. Lomofungin was a generous gift of G. B. Whitfield of The Upjohn Co.

RESULTS

Effect of lomofungin on yeast growth, and on synthesis of RNA and protein by protoplasts. Lomofungin at a concentration of 5 to 40 $\mu\text{g}/\text{ml}$ halted growth (increased in turbidity) of exponentially growing cultures of *Saccharomyces* strain 1016 almost completely after 1 h (Fig. 1). Even at 1 μg per ml, there was a reduction in growth rate of about 50%.

Gottlieb and Nicolas (5) reported that the synthesis of RNA (and DNA by whole cells of *S. cerevisiae*) was strongly inhibited by lomofungin. We investigated this phenomenon in protoplasts of *Saccharomyces* 1016 and found that incorporation of ^3H -uridine into RNA was very sensitive to the drug, whereas incorporation of ^{14}C -amino acids into protein was relatively insensitive (Table 1). At 40 μg per ml, lomofungin produced an 85% decrease in RNA synthesis within the first 5 min of incubation; during the same period, synthesis of protein decreased only 12%.

The time course of inhibition of RNA and protein synthesis in protoplasts by lomofungin is presented in Fig. 2. Inhibition of RNA synthesis (uridine incorporation, Fig. 2A; adenine, Fig. 2C) was rapid and with 40 μg of lomofungin per ml was almost complete after 10 min of incubation. In the same protoplast suspension, total protein synthesis was not substantially affected after 20 min of incubation and continued for at least 40 min (Fig. 2B). Acrylamide gel analysis of the total RNA synthesized during 5-min pulses of ^3H -uridine demonstrated that most newly labeled RNA is of high molecular weight and heterogeneous in size; the amount of low-molecular-weight (4 to 5S) RNA probably repre-

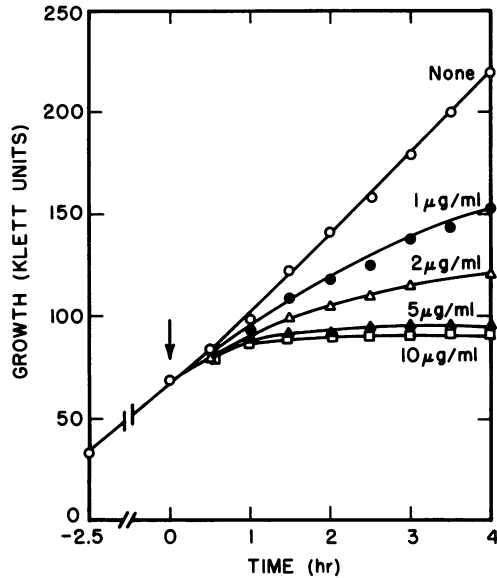


FIG. 1. Inhibition of growth of *Saccharomyces* strain 1016 by lomofungin. Various concentrations of lomofungin as indicated were added to exponentially growing cells at the arrow.

sents only about 2% of the labeled total RNA (Fig. 3A). The heterogeneous ^3H -RNA represents mainly ribosomal precursor RNAs and mRNA (13, 26). When RNA of cells which had been labeled with ^{14}C -uridine for several generations before protoplasting was examined, the stable RNA consisted almost entirely of the 25S and 18S ribosomal RNAs (rRNAs), although 4 to 5S RNA was also demonstrable (inset of Fig. 3A).

After treatment of the protoplasts with lomofungin (40 $\mu\text{g}/\text{ml}$) for 5 to 10 min, the formation of all large species of RNA during short exposures of ^3H -uridine was almost completely inhibited, whereas the peak of small RNA species was not much affected as compared with the control (Fig. 3B). R. S. S. Fraser, J. Creaner, and J. M. Mitchison (Nature [London], in press) in studies with *Shizosaccharomyces pombe* have also observed that lomofungin severely inhibits the synthesis of high-molecular-weight RNA, including the polydisperse RNA fraction, but not 4 to 5S RNA.

Since mRNA from yeast is polydisperse and not readily identified in an acrylamide gel electrophoresis pattern (Fig. 3), an independent procedure was used to detect the mRNA and determine the effect of lomofungin. Recently, McLaughlin et al. (19) demonstrated the occurrence of relatively homogeneous sequences of poly A of about 50 residues in the rapidly labeled mRNA of yeast. Although some mRNA

molecules may not contain poly A sequences, no poly A sequences are present in rRNAs (22). In the present studies, the poly A content of RNA pulse-labeled with ^3H -uridine for 5 min in the absence or presence of lomofungin was measured by hybridization of the poly A sequences to poly U that has been immobilized on a fiberglass filter by ultraviolet irradiation as described by Sheldon, Jurale, and Kates (21). During a 5-min pulse, the amount of poly A-containing ^3H -uridine incorporated into mRNA was approximately 10 to 15% of the total RNA labeling (Table 2). In the presence of lomofungin, the reduction in amount of ^3H -RNA retained on poly U filters (the mRNA) was closely correlated to the inhibition of total RNA labeling, showing that synthesis of mRNA and synthesis of rRNA are equally sensitive to the drug.

Effect of lomofungin on enzyme induction and synthesis by protoplasts. Yeast protoplasts are capable of synthesizing both internal and secreted enzymes under the conditions used in these studies, and the mannan-protein enzymes invertase and acid phosphatase are liberated into the incubation medium during the course of their synthesis (16). The present report deals with the effect of lomofungin on the induction and formation of several enzymes under conditions in which synthesis of rRNA and mRNA (but not protein) is promptly inhibited (Fig. 3; Table 2). Freshly prepared protoplasts produced very little invertase until 40 to 60 min after addition of a hexose (Fig. 4). Addition of lomofungin either at 40 min, when invertase production is beginning, or at 80 min,

TABLE 1. Effect of lomofungin on nucleic acid and protein synthesis by protoplasts^a

Lomofungin ($\mu\text{g}/\text{ml}$)	Radioactivity (counts per min per ml)	
	RNA	Protein
0	44,070 (100) ^b	54,402 (100) ^b
5	36,200 (82)	53,122 (99)
10	15,459 (35)	51,362 (95)
20	9,933 (22)	50,291 (91)
40	7,076 (16)	48,292 (88)

^a Protoplasts from cells grown in 0.05 M maltose were suspended at 5×10^7 cells per ml in 0.8 M sorbitol medium containing 100 mM fructose and incubated at 30 C (16). After 80 min of incubation, 2.5-ml volumes of the suspension received lomofungin as indicated and 10 μCi of uridine-5- ^3H (specific activity, 20 Ci/mmol) or 0.62 μCi of ^{14}C -protein hydrolysate. Samples were removed after 5 min of incubation and analyzed for radioactivity incorporated into RNA and protein.

^b Percentage of the control.

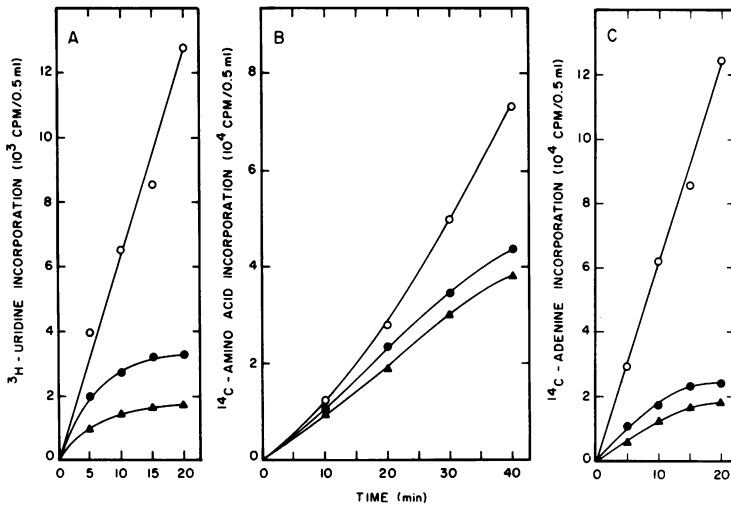


FIG. 2. Time course of lomofungin inhibition of RNA and protein synthesis. The conditions were the same as in Table 1. The protoplasts were preincubated for 80 min at 30 C; then lomofungin (20 or 40 $\mu\text{g/ml}$) and ^3H -uridine, ^{14}C -adenine, or ^{14}C -amino acids were added (0 min). (O) Control; (●) lomofungin, 20 $\mu\text{g/ml}$; (▲) lomofungin, 40 $\mu\text{g/ml}$.

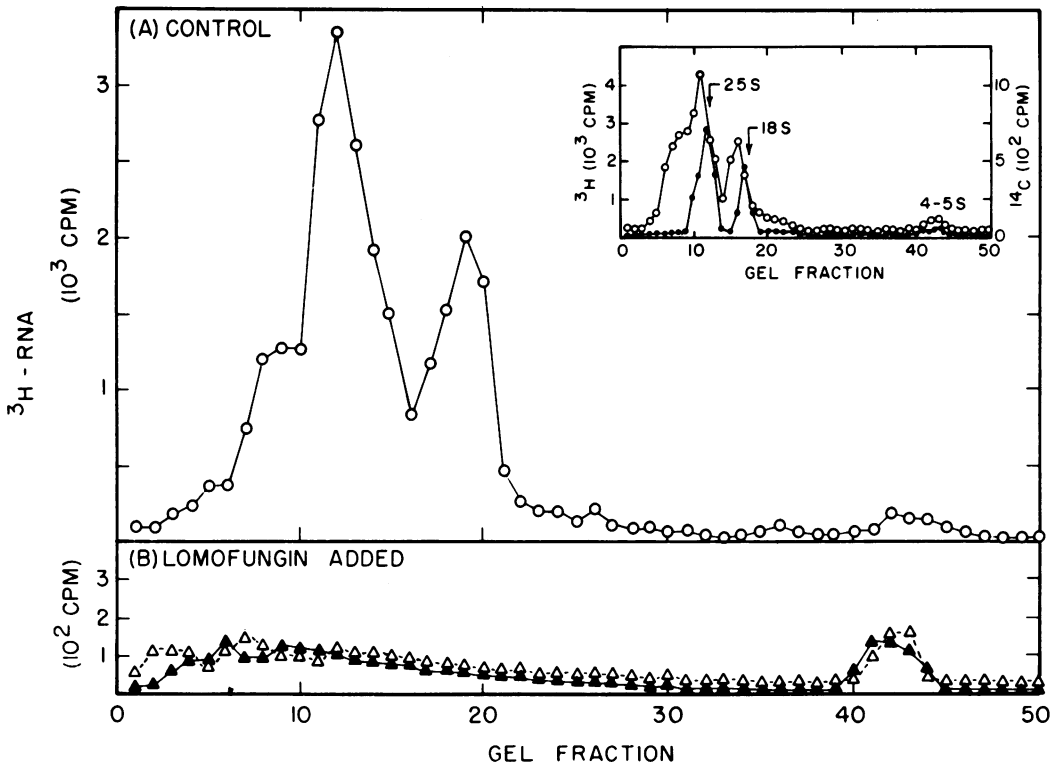


FIG. 3. Polyacrylamide gel electrophoresis of total RNA labeled by ^3H - or ^{14}C -uridine in the absence or presence of lomofungin. Protoplasts preincubated for 80 min were treated with lomofungin (40 $\mu\text{g/ml}$) for the indicated times. ^3H -uridine (25 $\mu\text{Ci/ml}$; specific activity, 40 Ci/mmol) was then added to 2.5-ml samples of the suspension and incubation was continued for 5 min. RNA was extracted and electrophoresis was performed as in Materials and Methods. Samples, approximately 60 μg of RNA, were analyzed on 6.5-cm, 2.5% acrylamide gels for 80 min at 50 V. Gels were frozen and cut into 1.3 mm slices; each slice was assayed for radioactivity. (A) Control (no lomofungin); (B) lomofungin added 5 min (Δ) or 10 min (\blacktriangle) before ^3H -uridine. In the inset of Fig. 3A, 5 μg of total ^{14}C -RNA labeled with ^{14}C -uridine for two generations and 60 μg of ^3H -RNA (^3H -uridine incorporated for 5 min) were loaded onto a gel for co-electrophoresis (see Materials and Methods). ^3H counts were corrected for cross-over from the ^{14}C counts. (O) ^3H -RNA; (●) ^{14}C -RNA.

TABLE 2. Effect of lomofungin on poly A-containing yeast RNA^a

Sample	Total ³ H-RNA ^b (counts/min)	Poly A-containing RNA (counts/min)
Control	3,788 (100) ^c	585 ^d (100) ^c
Protoplasts preincubated with lomofungin for 5 min	465 (12.3)	85 (14.5)
Protoplasts preincubated with lomofungin for 10 min	400 (10.5)	43 (7.5)

^a Protoplasts were prepared and labeled for 5 min with 25 μ Ci of ³H-uridine/ml, and the RNA was extracted and purified as described in Materials and Methods. Detection of poly A sequences by their retention on a poly U fiberglass filter was essentially as described by Sheldon, Jurale, and Kates (21).

^b Approximately 6.0 μ g of RNA of each sample was employed and analyzed for poly A sequences.

^c Percentage of the control.

^d Average of duplicate experiments.

when the maximal rate has been essentially reached, halted invertase production only after a lag of about 30 min, although the total amount of the enzyme formed in the presence of lomofungin was about twice as great with the more active protoplasts. Addition of cycloheximide (4 μ g/ml), which is known to inhibit cytoplasmic protein synthesis at the ribosomal level (18, 22), almost immediately halted invertase formation (Fig. 4).

The derepressed formation of another external enzyme, acid phosphatase, was also affected by lomofungin only after a lag of 20 or 30 min (J. O. Lampen, S.-C. Kuo, and F. Cano, *Int. Symp. on Yeast Protoplasts*, 3rd, October 1972, Salamanca, Spain, in press); however, when this antibiotic was added under repressed conditions (high phosphate medium), subsequent derepression of acid phosphatase synthesis was prevented.

It was important to determine whether lomofungin could inhibit the induction and synthesis of α -glucosidase since this is an intracellular, carbohydrate-free enzyme (6, 14). α -Glucosidase synthesis by protoplasts is readily induced by maltose (16, 27). Protoplasts were first incubated for 70 min in a medium containing 20 mM fructose plus a mixture of amino acids (0.5%). During this period, some invertase was synthesized and liberated into the suspending medium (Fig. 5). The protoplasts were then spun down and suspended in fresh medium to which was added 20 mM maltose as inducer for α -glucosidase. Lomofungin (40 μ g/ml) was

added at the same time as the maltose (70 min) or 40 min later. The induction of α -glucosidase was almost completely prevented by lomofungin (Fig. 5A). Once the protoplasts had been induced, further synthesis of the enzyme was not severely impaired until after 30 to 40 min of incubation with lomofungin, but was rapidly inhibited by cycloheximide, 4 μ g/ml (Fig. 5A). As would be anticipated from the results shown in Fig. 4, invertase synthesis by the same protoplasts (Fig. 5B) became inhibited only after 40 min of incubation with the lomofungin, regardless of whether it was added with the maltose (70 min) or 40 min later, and cycloheximide produced rapid inhibition.

DISCUSSION

Our results confirm and extend the findings of Gottlieb and Nicolas (5) and show that RNA synthesis of yeast protoplasts is rapidly and preferentially inhibited by lomofungin. With high concentrations of the drug (40 μ g/ml), RNA synthesis was almost completely halted in 10 min, whereas protein synthesis continued at a substantial rate for at least 40 min. Analysis of the total RNA synthesized during short exposures to ³H-uridine indicated that formation of both ribosomal precursor RNAs and mRNA was severely inhibited, but the low-molecular-weight RNA (4 to 5S) was not. The concept that the primary site of lomofungin action is the

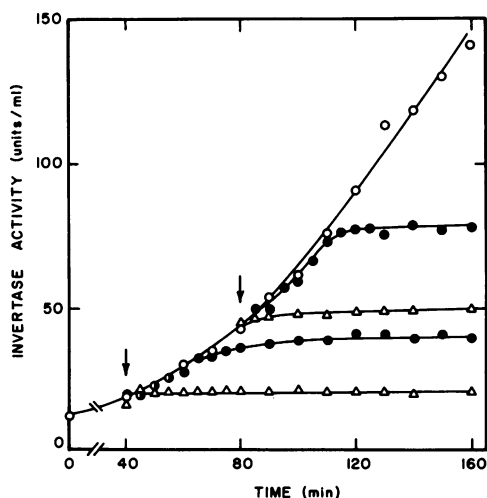


FIG. 4. Effect of lomofungin and cycloheximide on the synthesis of invertase by protoplasts. Protoplasts from cells grown in 0.05 M maltose medium were suspended at 5×10^7 per ml in 0.8 M sorbitol medium (16) containing 50 mM fructose and were incubated at 30 C. (●) Lomofungin (40 μ g/ml) or (Δ) cycloheximide (4 μ g/ml) was added at 40 or 80 min (arrows).

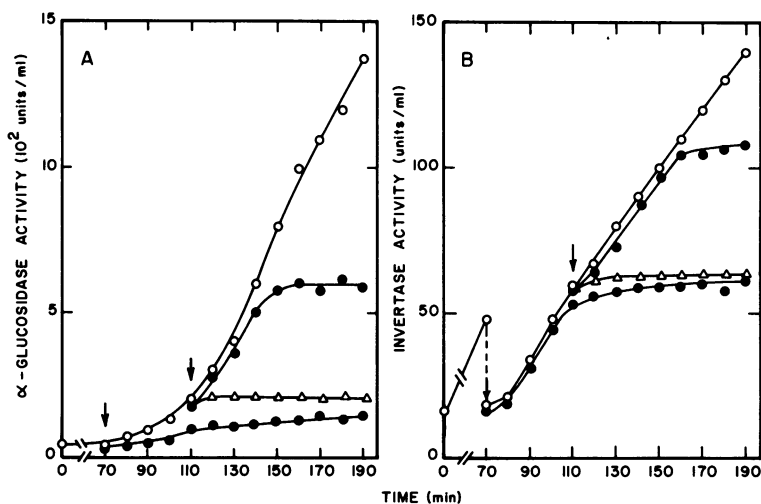


FIG. 5. Inhibition by lomofungin of the induction and synthesis of α -glucosidase and invertase. Protoplasts from cells grown in 0.2 M glucose medium were suspended at 5×10^7 per ml in 20 mM citrate-phosphate buffer, pH 6.0, containing 0.8 M sorbitol, 20 mM fructose, and 0.5% Casamino Acids. After incubation at 30 C for 70 min, the protoplasts were spun down and suspended in fresh medium to which 20 mM maltose (inducer) was added. Lomofungin (40 μ g/ml) was added (arrows) either at the same time or after 40 min in the presence of maltose (110 min). At various time intervals, samples were removed, lysed, and dialyzed overnight against phosphate buffer (1 mM, pH 6.8) at 4 C; they were then assayed for total α -glucosidase and invertase activities. (O) Control; (●) lomofungin added; (Δ) cycloheximide (4 μ g/ml) added at 110 min.

synthesis of RNA was further supported by in vitro studies, in that protein synthesis by a cell-free system was insensitive to levels of lomofungin as high as 100 μ g/ml (5) and the yeast RNA polymerases were inhibited by lomofungin (3).

Antibiotics such as actinomycin D and rifampin inhibit the process of transcription and consequently of mRNA formation. The appearance of enzyme in the presence of these antibiotics is assumed to be coded for by pre-existing messengers, and the stability of mRNA for a particular enzyme is then deduced from the rate of decline of enzyme formation after the onset of inhibition of RNA synthesis (2, 4, 9, 10, 17). Unfortunately, these antibiotics are relatively ineffective in yeast because of either poor permeability or failure to inhibit yeast RNA polymerases (8, 20, 24). For example, incorporation of 14 C-uridine into RNA by *Saccharomyces* 1016 was reduced only 50% in the presence of 50 μ g of actinomycin D per ml after 30 min of incubation (data not presented in detail). It is possible to increase the permeability of the yeast cells or protoplasts to these inhibitors by a detergent such as Brig 58 (11) or with a polyene antibiotic, e.g., amphotericin B (20); however, when one wishes to study the formation of extracellular enzymes, alteration of the plasma membrane, with possible direct effects on the

release process, is an undesirable complication. Thus, lomofungin should prove useful for examining the extent of transcriptional control of enzyme synthesis and secretion in yeast protoplasts.

The observation that lomofungin at 40 μ g/ml prevented almost completely the induction of α -glucosidase (Fig. 5) or derepression of acid phosphatase (Lampen et al., in press) supports the concept that synthesis of new mRNA (i.e., transcription) is involved in the induction or derepression of these two enzymes. However, if the protoplasts were actively synthesizing α -glucosidase, acid phosphatase, or invertase when lomofungin was added, further production of the enzymes did not decrease until after 30 to 40 min, that is, 20 to 30 min after mRNA synthesis had almost ceased. During this period, continued synthesis of mRNA was apparently not essential for the de novo formation of these enzymes. Addition of cycloheximide produced prompt inhibition. Although our findings are consistent with the existence of relatively long-lived mRNA for these enzymes examined, further studies will be required to explain the phenomenon.

One cannot decide from the available data whether synthesis of the enzymes eventually halted because the supply of mRNA molecules became rate-limiting or whether there was a

generalized decay of protein synthesis, since synthesis of rRNAs was also inhibited and the rate of ^{14}C -amino acid incorporation was beginning to decrease at about the same time. Singer and Penman (23) recently reported that the decay of protein synthesis by HeLa cells in the presence of actinomycin D was not due to the degradation of messenger molecules, but to a failure in the initiation of translation.

ACKNOWLEDGMENTS

We thank Jacqueline Richman for skilled technical assistance and R. S. S. Frazer, J. Creanor, and J. M. Mitchison for the opportunity to see their results in advance of publication.

This research was supported by Public Health Service grant AI-04572 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

- Bishop, D. H. L., J. R. Claybrook, and S. Spiegelman. 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. *J. Mol. Biol.* **26**:373-378.
- Both, G. W., J. L. McInnes, B. K. May, and W. H. Elliott. 1971. Insensitivity of *Bacillus amyloliquefaciens* extracellular protease formation to rifampicin and actinomycin D. *Biochem. Biophys. Res. Commun.* **42**:750-757.
- Cano, F. R., S.-C. Kuo, and J. O. Lampen. 1973. Lomofungin, an inhibitor of deoxyribonucleic acid-dependent ribonucleic acid polymerases. *Antimicrob. Ag. Chemother.* **3**:723-728.
- Davies, J. W. 1969. The stability and cell content of penicillinase messenger RNA in *Bacillus licheniformis*. *J. Gen. Microbiol.* **59**:171-184.
- Gottlieb, D., and G. Nicolas. 1969. Mode of action of lomofungin. *Appl. Microbiol.* **18**:35-40.
- Halvorson, H., and L. Ellias. 1958. The purification and properties of an α -glucosidase of *Saccharomyces italicus* Y 1225. *Biochim. Biophys. Acta* **30**:28-40.
- Halvorson, H. O., and C. Heredia. 1967. Protein biosynthesis in yeast, p. 107-132. In A. K. Mills and Sir H. Krebs (ed.), *Aspects of yeast metabolism*. Blackwell Scientific Publishers, Oxford.
- Hartwell, L. H., and C. S. McLaughlin. 1968. Temperature-sensitive mutant of yeast exhibiting a rapid inhibition of protein synthesis. *J. Bacteriol.* **96**:1664-1671.
- Hartwell, L. H., and B. Magasanik. 1963. The molecular basis of histidase induction in *Bacillus subtilis*. *J. Mol. Biol.* **7**:401-420.
- Hartwell, L. H., and B. Magasanik. 1964. The mechanism of histidase induction and formation in *Bacillus subtilis*. *J. Mol. Biol.* **10**:105-119.
- Hereford, L. M., and L. H. Hartwell. 1971. Defective DNA synthesis in permeabilized yeast mutants. *Nature N. Biol.* **234**:171-172.
- Hutchison, H. T., L. H. Hartwell, and C. S. McLaughlin. 1969. Temperature-sensitive yeast mutant defective in ribonucleic acid production. *J. Bacteriol.* **99**:807-814.
- Johnson, L. E., and A. Dietz. 1969. Lomofungin, a new antibiotic produced by *Streptomyces lomondensis* sp. n. *Appl. Microbiol.* **17**:755-759.
- Khan, N. A., and N. R. Eaton. 1967. Purification and characterization of maltase and α -methylglucosidase from yeast. *Biochim. Biophys. Acta* **146**:173-180.
- Kuo, S.-C., and J. O. Lampen. 1971. Osmotic regulation of invertase formation and secretion by protoplasts of *Saccharomyces*. *J. Bacteriol.* **106**:183-191.
- Kuo, S.-C., and J. O. Lampen. 1972. Inhibition by 2-deoxy-D-glucose of synthesis of glycoprotein enzymes by protoplasts of *Saccharomyces*: relation to inhibition of sugar uptake and metabolism. *J. Bacteriol.* **111**:419-429.
- Leive, L., and V. Kollin. 1967. Synthesis, utilization and degradation of lactose operon mRNA in *Escherichia coli*. *J. Mol. Biol.* **24**:247-259.
- McKeehan, W., and B. Hartesty. 1969. The mechanism of cycloheximide inhibition of protein synthesis. *Biochem. Biophys. Res. Commun.* **36**:625-630.
- McLaughlin, C. S., J. R. Warner, E. Edmonds, E. Nakazato, and M. H. Vaughan. 1973. Polyadenylic acid sequences in yeast messenger ribonucleic acid. *J. Biol. Chem.* **248**:1466-1471.
- Medoff, G., G. S. Kobayashi, C. N. Kwan, D. Schlessinger, and P. Venkov. 1971. Potentiation of rifampicin and 5-fluorocytosine as antifungal antibiotics by amphotericin B. *Proc. Nat. Acad. Sci. U.S.A.* **69**:196-199.
- Sheldon, R., C. Jurale, and J. Kates. 1971. Detection of polyadenylic acid sequences in viral and eukaryotic RNA. *Proc. Nat. Acad. Sci. U.S.A.* **69**:417-421.
- Siegel, M. R., and H. G. Sisler. 1964. Site of action of cycloheximide in cells of *Saccharomyces pastorianus*. II. The nature of inhibition of protein synthesis in a cell-free system. *Biochim. Biophys. Acta* **87**:83-89.
- Singer, R. H., and P. Penman. 1972. Stability of HeLa cell mRNA in actinomycin. *Nature (London)* **240**:100-102.
- Sošková, L., J. Soška, and A. Svoboda. 1970. The effect of 5-fluorouracil on cells and regenerating protoplasts of *Saccharomyces cerevisiae*. *Folia Microbiol. (Prague)* **15**:442-447.
- Tipton, C. D., and L. R. Kennehart, Jr. 1970. Lomofungin. I. Degradation studies of a new phenazine antibiotic. *J. Amer. Chem. Soc.* **92**:1425-1426.
- Udem, S. A., and J. R. Warner. 1971. Ribosomal RNA synthesis in *Saccharomyces cerevisiae*. *J. Mol. Biol.* **65**:227-242.
- Van Wijk, R. 1968. Alpha-glucosidase synthesis, respiratory enzymes and catabolite repression in yeast. IV. A Studies on the level of protein synthesis at which repression and induction of alpha-glucosidase synthesis occur. *Proc. Kon. Ned. Akad. Wetensch. Ser. C* **71**:293-313.
- Vogel, H. J. 1956. A convenient growth medium for *Neurospora* (medium N). *Microbiol. Genet. Bull.* **13**:42-43.