

## Effects of Lovastatin (Mevinolin) on Sterol Levels and on Activity of Azoles in *Saccharomyces cerevisiae*

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The hypocholesterolemic drug lovastatin (mevinolin) was found to be very effective in lowering the sterol levels of the wild-type yeast *Saccharomyces cerevisiae*. Lovastatin dramatically decreased the steryl ester content from 2.62 to 0.8  $\mu\text{g}/\text{mg}$  (dry weight), whereas the free sterol content decreased only from 2.79 to 2.24  $\mu\text{g}/\text{mg}$  (dry weight) when lovastatin was present in the medium at 10  $\mu\text{g}/\text{ml}$ . At higher concentrations (100  $\mu\text{g}/\text{ml}$ ), lovastatin nearly abolished the accumulation of steryl esters and decreased the free sterol concentration to less than 1.3  $\mu\text{g}/\text{mg}$  (dry weight). As a result of the lowered sterol levels, proportional amounts of exogenous sterol were taken up from the medium during aerobic, respiratory conditions. Nearly all of the exogenous sterol taken up was partitioned into the free sterol fraction. The inhibition of sterol esterification in the presence of lovastatin was dependent on heme synthesis. The result of these combined effects caused the MICs of three azole antifungal drugs (ketoconazole, clotrimazole, and miconazole) to be lowered from 6- to 32-fold when lovastatin was present in the medium at 10  $\mu\text{g}/\text{ml}$ .

The azole family of antifungal drugs is widely used for the treatment of many mycotic pathogens of plants, animals, and humans. The azoles act against fungi by interacting with cytochrome P-450, which is required for 14-demethylation of lanosterol (35, 36). The resulting accumulation of C-14-methylated sterols and the decreased availability of ergosterol affects membrane-bound enzymes and causes direct membrane and mitochondrial damage (32, 34). The inhibition of C-14 demethylase allows the accumulation of 14 $\alpha$ -methylergosta-8,24(28)-dien-3 $\beta$ ,6 $\alpha$ -diol, which is thought to interfere with fungal growth (33).

The number of systemic mycoses has risen dramatically in recent years because of the human immunodeficiency virus, which causes the acquired immune deficiency syndrome, and the increased use of immunocompromising drugs. However, the treatment of fungal infections with azoles has a number of limiting drawbacks. Most azoles are lipophilic drugs and are nearly insoluble in water. The extent of absorption, presystemic elimination, and plasma protein binding decreases the bioavailability to only a fraction of the administered dose. The high drug concentrations and long-term therapy required for effective control of systemic infections often results in serious side effects. Gastrointestinal, endocrine, and hepatic toxicities commonly occur during treatments. Efforts have only recently been made to develop antifungal compounds which are less hydrophobic, such as fluconazole, which has high bioavailability and cerebrospinal fluid penetration (8).

Under aerobic conditions, wild-type *Saccharomyces cerevisiae* synthesizes from 2 to 5% of its dry weight as ergosterol (13). Sterols are also extremely hydrophobic, and the low permeability of these lipophilic molecules for wild-type cells has been described previously (17, 22). This inability of *S. cerevisiae* to accumulate sterol from the medium has been termed "aerobic sterol exclusion." Conversely, under anaerobic conditions or in heme mutants, the synthesis of heme and sterol is precluded, and the cellular permeability to exogenous sterol uptake is substantial (12, 24, 31).

The cellular free sterol level of a heme mutant has been shown to contribute to aerobic sterol exclusion such that exogenous sterol uptake is inversely proportional to the endogenous sterol level (19, 20). Thus, by lowering the endogenous concentration of cellular sterol of a wild-type strain, it should be possible to increase the cells' permeability to exogenous sterol and, perhaps, increase the permeability, the susceptibility, or both to antifungal drugs.

In this study, we report the quantitative effects of the hypocholesterolemic drug lovastatin (mevinolin) on the free sterol and steryl ester fractions of *S. cerevisiae*. It is shown that the sterol levels can be lowered below the saturation state to allow exogenous sterol uptake in an aerobic environment. Additionally, we report the synergistic effect of lovastatin and different azoles in lowering the MICs of these antifungal agents.

### MATERIALS AND METHODS

**Yeast strains.** *S. cerevisiae* 2180-1A (MATa *SUC2 mal gal2, CUP1*) was obtained from the Yeast Genetic Stock Center, Berkeley, Calif. Strains JRY527, JRY1159, JRY1160, JRY1711, JRY1712, and JRY1714 were kindly supplied by Jasper Rine (University of California, Berkeley), and are described in Table 1. SGY688 was provided by P. Fernandez (E. R. Squibb & Sons, Princeton, N.J.). Strain FY14 was constructed during this study and is described below.

**Materials.** Glucose, yeast extract, and peptone were from Difco Laboratories (Detroit, Mich.). Cholesterol, oleic acid, palmitoleic acid, Tergitol Nonidet P-40, miconazole, ketoconazole, clotrimazole, 2,5-diphenyloxazole (PPO), and 1,4-bis(5-phenyloxazolyl)benzene (POPOP) were obtained from Sigma Chemical Co. (St. Louis, Mo.). [ $4\text{-}^{14}\text{C}$ ]cholesterol was purchased from Dupont, NEN Research Products (Boston, Mass). Solvents were from Fisher Scientific Co. (Raleigh, N.C.) and when necessary, were redistilled before use. Lovastatin was a generous gift of A. Alberts (Merck Sharp & Dohme Research Laboratories, Rahway, N.J.).

**Media and growth conditions.** The culture medium (YPD) consisted of 2% glucose, 1% peptone, and 1% yeast extract. Stock solutions of miconazole, ketoconazole, and clotrimazole were made in ethanol and transferred to 10 ml of

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TABLE 1. Sterol uptake assays of different *HMG* mutants<sup>a</sup>

Strain	Genotype	Sterol uptake ( $\mu\text{g}/\text{mg}$ [dry wt])
JRY527	<i>HMG1 HMG2</i>	0.074 $\pm$ 0.006
JRY1159	<i>hmg1::LYS2 HMG2</i>	0.122 $\pm$ 0.011
JRY1160	<i>HMG1 hmg2::HIS3</i>	0.096 $\pm$ 0.009
JRY1711	<i>hmg1-2 hmg2::HIS3</i> <i>hmg1-3 hmg2::HIS3</i>	0.165 $\pm$ 0.013
JRY1712	<i>hmg1-4 hmg2::HIS3</i> <i>hmg1-3 hmg2::HIS3</i>	0.159 $\pm$ 0.012
JRY1714	<i>hmg1-5 hmg2::HIS3</i> <i>hmg1-3 hmg2::HIS3</i>	0.136 $\pm$ 0.010

<sup>a</sup> Results are based on the averages of three sterol uptake assays. Strains JRY1711, JRY1712, and JRY1714 are diploids that exhibit intragenic complementation.

medium in test tubes at the appropriate concentrations. The ethanol concentrations never exceeded 0.75%, and controls were conducted to account for ethanol effects. Lovastatin was hydrolyzed in ethanolic NaOH (15% [vol/vol] ethanol and 0.25% [wt/vol] NaOH) at 60°C for 1 h and was then filter sterilized. Stock solutions of lovastatin were 20 mg/ml and were stored at -20°C. Test tubes and flasks were inoculated with 5  $\mu\text{l}$  of an overnight culture at the late logarithmic phase and were incubated at 28°C with constant shaking. Heme mutants were supplemented with cholesterol and unsaturated fatty acids from a mixture of oleic and palmitoleic acids (4:1 [vol/vol]) at a final concentration of 0.01%. Growth was monitored with a Klett-Summerson photoelectric colorimeter equipped with a green filter. MIC determinations were conducted in test tubes with YPD medium by using a 5- $\mu\text{l}$  inoculum from an overnight culture. Cultures were incubated at 28°C with constant shaking, and the MIC was recorded as the lowest concentration of antifungal agent at which no significant visible growth occurred after 3 days.

**Sterol uptake assay.** Whole-cell sterol uptake was measured by growing the cells to the stationary phase in YPD medium in the presence of 10  $\mu\text{g}$  of cholesterol per ml and [<sup>14</sup>C]cholesterol (specific activity, approximately 1,000 dpm/ $\mu\text{g}$ ). Cultures were pelleted and washed twice with Tergitol Nonidet P-40-ethanol (1:1 [vol/vol]) and once with distilled water. Washed cells were lyophilized overnight and weighed on an analytical balance (AE163; Mettler). Pellets were transferred to scintillation vials and minced, and the accumulated radioactivity was quantitated by suspending the cells with toluene-PPO-POPOP cocktail and counted in a scintillation counter (LS 5801; Beckman Instruments, Inc., Fullerton, Calif.).

**Sterol quantitation.** Cellular sterols were extracted from lyophilized cells following with dimethyl sulfoxide treatment (18). Separation of the free sterol and steryl ester fractions was achieved by thin-layer chromatography (27). The purified sterol fractions were analyzed by capillary gas chromatography in a gas chromatograph (5890A; Hewlett-Packard Co., Palo Alto, Calif.) equipped with a flame ionization detection system and a data acquisition and processing workstation (Maxima 820). The column was SPB-1 fused silica (film thickness, 0.25  $\mu\text{m}$ ; 30 m by 0.32 mm [inner diameter]; Supelco). Chromatography was performed with an oven temperature of 230°C and injector and detector temperatures of 280°C. Carrier gas was helium at 26 lb/in<sup>2</sup>, and the makeup gas was nitrogen.

**Construction of strain FY14.** A sterol auxotroph was

constructed to study the effects of lovastatin on sterol esterification such that heme competency could be controlled. Strain SGY688 (*MAT $\alpha$  hem3 ERG1::URA3 ura3 ade2 leu2 lys2*) was mated to FY1 (*MAT $\alpha$  hem1 ura3*) and allowed to sporulate. Segregants were isolated by micromanipulation and transferred onto YPD master plates supplemented with ergosterol and unsaturated fatty acids. Isolates were first screened for a heme mutation by determining their ability to grow on unsupplemented YPD medium. The *hem1* mutation was differentiated from the *hem3* mutation by determining the ability of isolates to grow in defined medium with ergosterol and 20  $\mu\text{g}$  of  $\delta$ -aminolevulinic acid (ALA) per ml in the absence of methionine and unsaturated fatty acids. Strains containing the *hem1* mutation were subsequently tested for the *ERG1::URA3* allele by growing them on 1  $\mu\text{g}$  of cholesterol per ml and shifting them to medium containing 75  $\mu\text{g}$  of ALA per ml to derepress 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase (EC 1.1.1.34). The lipids of the different isolates were extracted, purified by thin-layer chromatography, and analyzed by gas chromatography for elevated squalene concentrations (19). The same strains which were not exposed to ALA and strain SGY688 were also used as controls. Strain FY14 (*hem1 ERG1::URA3 ura3 ade2 leu2*) was used in this study.

## RESULTS AND DISCUSSION

The rate-determining enzyme of the ergosterol pathway in yeasts is HMG-CoA reductase (10, 16, 25). Studies have revealed that ergosterol acts as a negative-feedback regulator (4, 24), while heme and unsaturated fatty acids act to stimulate enzyme activity (9, 19). Lovastatin is an unmethylated derivative of compactin, both of which have been demonstrated to be competitive inhibitors of HMG-CoA reductase (1, 14).

Sterols exist in two different states in *S. cerevisiae*: as the free hydroxyl form and as esters of long-chain fatty acids. Free sterols are the membranous form, while steryl esters are extramembranous lipids, which are deposited within globules of the cytoplasm (11). The two sterol forms undergo a culture cycle-dependent conversion. The free sterol pool remains relatively constant, but the steryl ester pool is very low during exponential growth and increases sharply as the culture enters the stationary phase. Steryl esters are then hydrolyzed to free sterol and fatty acids for new membrane biogenesis during regrowth (3, 29).

It is known that in heme mutants the steady-state cellular concentration of free sterol can be adjusted within a 10-fold range by varying the concentration of exogenously supplied sterol (20). By using lovastatin, a competitive inhibitor of the rate-limiting enzyme of the ergosterol pathway, an attempt was made to lower the sterol concentration of *S. cerevisiae*. A wild-type strain, 2180-1A, was grown in YPD media with increasing concentrations of lovastatin and was harvested at the stationary phase. The lipids of the cell cultures were extracted, separated by thin-layer chromatography, and quantified by gas chromatography. Low levels of lovastatin (10  $\mu\text{g}/\text{ml}$ ) dramatically decreased the total steryl ester fraction from 2.62 to about 0.80  $\mu\text{g}/\text{mg}$  (dry weight) (Fig. 1). The free sterol fraction also decreased, but only from 2.79 to 2.24  $\mu\text{g}/\text{ml}$  (dry weight). At higher concentrations, the steryl ester fraction continued to decrease until a minimal level was reached with 100  $\mu\text{g}$  of lovastatin per ml. The free sterol fraction at higher concentrations of lovastatin decreased almost linearly about a concentration of 10  $\mu\text{g}$  of the hypocholesterolemic drug per ml. The growth rate and cell yield

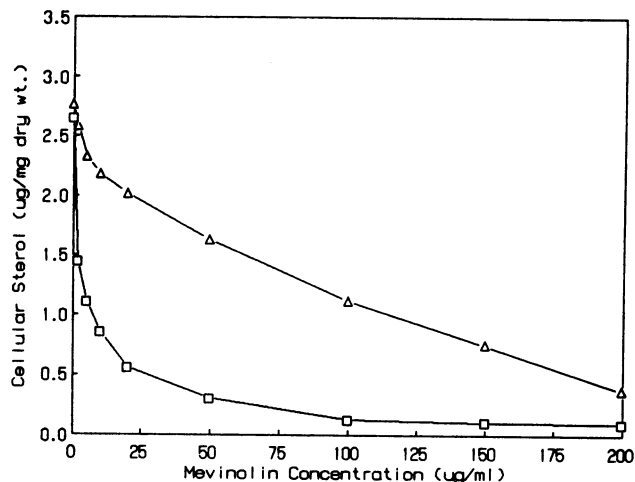


FIG. 1. Effect of lovastatin on cellular sterol fractions. The wild-type strain 2180-1A was grown in the presence of different concentrations of lovastatin and was harvested at the stationary phase. The lipids were extracted, separated by thin-layer chromatography, and quantified by gas chromatography. Results are reported as the average of three experiments.  $\Delta$ , Free sterol;  $\square$ , steryl ester.

were not significantly affected until a concentration of 75  $\mu\text{g}$  of lovastatin per ml or greater was present in the medium. At concentrations above 150  $\mu\text{g}$  of lovastatin per ml, the growth rate and cell yield were severely diminished (data not shown).

Since the capacity for sterol uptake in yeast sterol auxotrophs is known to be inversely proportional to the free sterol level when cells are below the free sterol saturation point (20), we measured the ability of the wild-type strain to accumulate sterol in the presence of lovastatin. An overnight culture of strain 2180-1A was used to inoculate flasks containing YPD,  $[4\text{-}^{14}\text{C}]$ cholesterol, and increasing concentrations of lovastatin. Cultures were then grown to the stationary phase and harvested. The cellular lipids were extracted and separated by thin-layer chromatography, and the radiolabeled cholesterol was quantified from the free sterol and steryl ester fractions.

When no lovastatin was present in the medium, cells were only able to accumulate 0.082  $\mu\text{g}$  of exogenous cholesterol per mg (dry weight). However, when the concentration of lovastatin was just 5  $\mu\text{g}/\text{ml}$ , cells were capable of taking up 0.812  $\mu\text{g}/\text{mg}$  (dry weight), nearly a 10-fold increase of sterol uptake over that of the control without the drug. As the concentration of lovastatin was increased to 100  $\mu\text{g}/\text{ml}$ , the uptake capacity for exogenous cholesterol was increased to about 2.85  $\mu\text{g}/\text{mg}$  (dry weight) (Fig. 2). That is, the permeability of the cell to exogenous sterol was inversely related to the lowered sterol levels conferred by lovastatin. Interestingly, nearly all of the exogenous cholesterol taken up was partitioned into the free sterol fraction. Thus, lovastatin severely decreased the accumulation of endogenous steryl esters (Fig. 1) and prevented the esterification of sterol taken up from the medium (Fig. 2).

Previously, uptake of exogenous sterol under aerobic conditions has been observed only in heme mutants and uptake control mutants (*upc*). We tested the effects of lovastatin on heme competency and the ability of yeast cells to respire and found no significant consequences. Strain 2180-1A was able to grow on 2% ethanol or glycerol in a

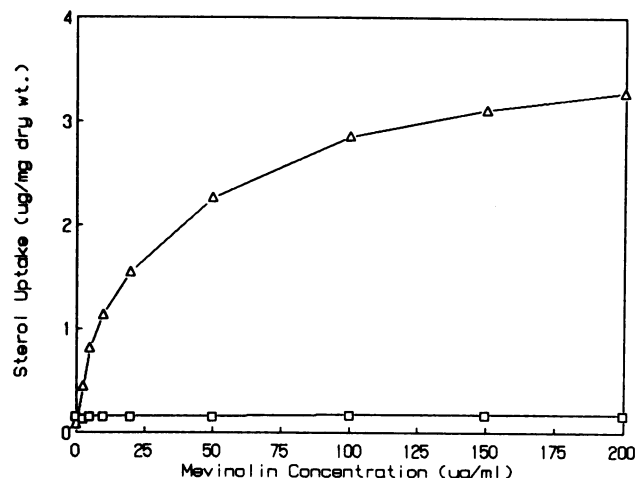


FIG. 2. Effect of lovastatin on sterol uptake capacity. Strain 2180-1A was grown in the presence of  $[4\text{-}^{14}\text{C}]$ cholesterol and different concentrations of lovastatin to examine the cellular permeability to exogenous sterol. Lipids were extracted and separated by thin-layer chromatography. The amounts of cholesterol partitioned into the free sterol ( $\Delta$ ) and steryl ester ( $\square$ ) fractions were measured by scintillation counting and are reported as the averages of three experiments. Greater than 95% of the cholesterol that was taken up was located in the free sterol fraction.

defined medium in the presence of 50  $\mu\text{g}$  of lovastatin per ml. This is the first report, to our knowledge, of a technique to manipulate wild-type *S. cerevisiae* to take up exogenous sterol during heme competency. Conversely, mutant strains of *S. cerevisiae* have been isolated that are resistant to the cytotoxic effects of 6-ketocholestanol and were found to have increased levels of HMG-CoA reductase activities. The elevated enzyme levels caused a parallel increase in sterol production and a decreased permeability to the oxygenated sterol analog, which was reported to account for the resistance phenotype (12).

To study the negative effect of lovastatin on sterol esterification, the sterol auxotroph FY14 (*hem1 ERG1::URA3 ade2 leu2*) was constructed. Sterol biosynthesis is precluded in this strain because of the insertionally inactivated *ERG1* gene. The *hem1* mutation renders the cell heme incompetent. Thus, cytochrome P-450 hemoproteins, which are required in the ergosterol pathway for the oxidative demethylation of lanosterol (2) and for the desaturation of the C-22 position, are not functional (15). Additionally, cytochrome *b5* hemoproteins, which are necessary for the desaturation of the C-5 position of the sterol molecule, are not active (23). Thus, the influence of lovastatin on esterification could be studied in a sterol auxotrophic background, and heme competency could be controlled with the addition of ALA to the medium to bypass the *hem1* mutation.

Strain FY14 was grown to the stationary phase under different growth conditions in the presence of  $[4\text{-}^{14}\text{C}]$ cholesterol and was then harvested. Cells were extracted, and the free sterol and steryl ester fractions were separated by thin-layer chromatography. Control cells incorporated about an equal amount of radiolabeled cholesterol in the free sterol and steryl ester fractions. When lovastatin was present in the medium at 50  $\mu\text{g}/\text{ml}$ , there was a small increase in the amount of free sterol that was incorporated and the steryl ester fraction was also increased slightly. This indicated that lovastatin does not directly inhibit steryl ester synthetase (acyl-coenzyme A: *o*-acyltransferase). When

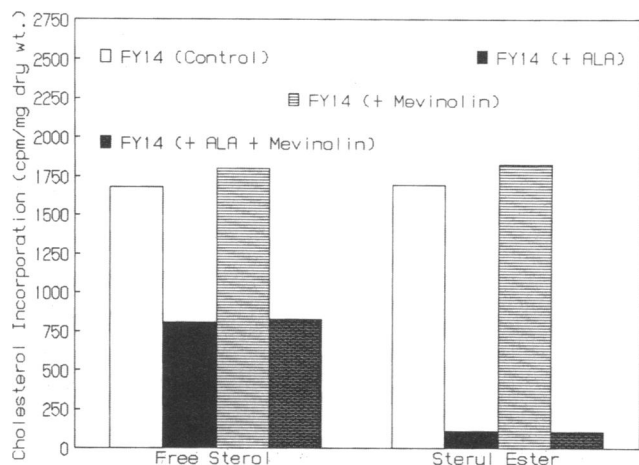


FIG. 3. Effect of lovastatin on sterol esterification by a sterol auxotroph. Strain FY14 was grown under different conditions of lovastatin (50  $\mu\text{g/ml}$ ), ALA (75  $\mu\text{g/ml}$ ), or both in the presence of [ $^{14}\text{C}$ ]cholesterol and was harvested at the stationary phase. The lipids were extracted and separated by thin-layer chromatography, and the amount of radiolabeled sterol in the free sterol and steryl ester fractions was quantified by scintillation counting. The results are averages of three experiments.

lovastatin was added to a culture that was additionally supplemented with 75  $\mu\text{g}$  of ALA per ml to allow heme competency, the amount of cholesterol incorporated into the free sterol fraction was 48% that of the control culture. The amount of cholesterol that was esterified was only 6% that of the control. However, when 75  $\mu\text{g}$  of ALA per ml was present in the medium in the absence of lovastatin, the incorporation of cholesterol into the free sterol and steryl ester fractions was 49 and 6% of the control, respectively (Fig. 3).

Thus, the inhibition of sterol esterification in FY14 was dependent on heme competency and appeared to be the result of incomplete sterol uptake. Therefore, the decrease of steryl esters in wild-type cells exposed to lovastatin may be the result of a decrease in the endogenous ergosterol concentration to a level below the free sterol saturation level such that esterification cannot occur (20). Apparently, the amount of sterol taken up from the medium is not sufficient to allow free sterol saturation. These phenomena are currently under investigation and will be reported elsewhere.

There are two genes in *S. cerevisiae* that encode for functional isozymes of HMG-CoA reductase. Cells carrying null mutations in both genes are inviable, while cells with a null mutation in just one gene can grow. It has been demonstrated that the gene encoding the *HMG1* enzyme normally contributes 83% of the total activity and the gene encoding the *HMG2* enzyme is responsible for 17% of the total activity (6, 7). Strains resistant to the growth inhibitory effects of lovastatin have been hypothesized to contain a mutation in the *HMG2* allele (5). We reasoned that cells with a mutation in *HMG1* or *HMG2* may have lower sterol levels and may not be efficient in saturating the free sterol fraction. Strains carrying either the *hmg1::LYS2* or *hmg2::HIS3* alleles were grown in YPD medium in the presence of [ $^{14}\text{C}$ ]cholesterol to assay for sterol uptake (Table 1). However, it was found that strains JRY1159 and JRY1160 did not take up a significant amount of sterol compared with that taken up by the parental control JRY527, even though JRY1159 is reported to have less than 23% of the HMG-CoA reductase activity of the control (6).

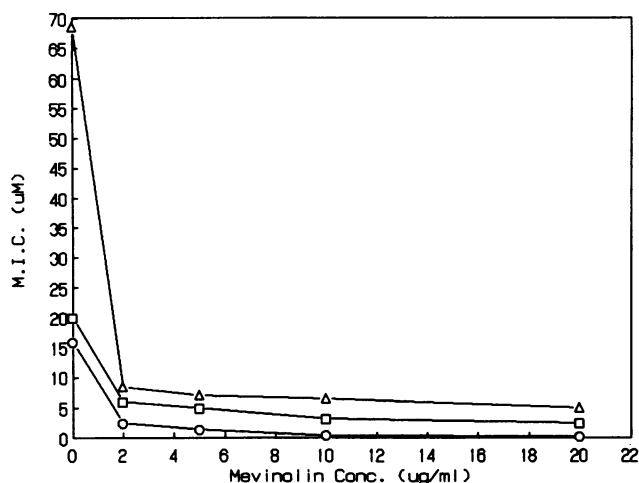


FIG. 4. Effect of lovastatin on the MICs of three azoles. Strain 2180-1A was grown in the presence of different concentrations of mevinolin; and the MICs of ketoconazole ( $\Delta$ ), clotrimazole ( $\square$ ), and miconazole ( $\circ$ ) were determined. The results are averages of three experiments. Lovastatin had a dramatic enhancement effect, such that the MIC was lowered from 6- to 32-fold when lovastatin was present in the medium at 10  $\mu\text{g/ml}$ .

Strains JRY1711, JRY1712, and JRY1714 are diploids with null mutations in *HMG2* and point mutations in *HMG1*. However, the point mutations display intragenic complementation and allow partial restoration of enzymatic activity (10 to 25% that of the wild type). When these strains were assayed for sterol uptake, they were found to take up a small amount of exogenous sterol. Each of the JRY strains esterified endogenous ergosterol during the stationary phase, revealing that sterol was available in a sufficient quantity to saturate the free sterol fraction and allow storage of excess sterol (data not shown).

Because very low concentrations of lovastatin were found to lower the sterol levels of wild-type *S. cerevisiae* and to increase the permeability to exogenous sterol, it occurred to us that the effectiveness of the antifungal azoles might also be increased. Others have reported a synergistic effect when ketoconazole is used in combination with different sterol synthesis inhibitors and found that *S. cerevisiae* is generally less susceptible to drug combinations than filamentous fungi are (28). Thus, strain 2180-1A was grown with different amounts of lovastatin to determine whether the MICs of some representative azoles would be decreased. When lovastatin was present in the medium at only 2  $\mu\text{g/ml}$ , the MICs of clotrimazole, ketoconazole, and miconazole were sharply decreased (Fig. 4). As the lovastatin concentration was increased to 10  $\mu\text{g/ml}$ , the MIC declined further until a plateau was reached for each azole. This enhancing effect was best demonstrated with miconazole, which had a 32-fold reduction in the MIC when 10  $\mu\text{g}$  of lovastatin per ml was present in the medium. The MICs of clotrimazole and ketoconazole were decreased 6- and 10-fold in the presence of 10  $\mu\text{g}$  of lovastatin per ml, respectively.

In conclusion, we proposed that the enhancement of lovastatin on the MICs of azoles may be the result of a combination of different effects. Lovastatin decreased the flow of intermediates through the sterol pathway and was shown to lower the intracellular steryl ester and free sterol levels dramatically. Moreover, the sterol that was taken up from the medium because of the lowered sterol levels was

not esterified. Thus, when azoles were added to the medium in the presence of lovastatin, the C-14 methyl sterols that accumulated could not be esterified and remained in the free sterol form. It is known that cellular membranes do not accommodate these methylated sterols favorably, which results in growth inhibition (30). Since the azoles are also very hydrophobic, the increased sterol permeability conferred by lovastatin may also cause increased permeability of the antifungal drugs, although this has not yet been tested directly.

The lowered cellular sterol content induced by lovastatin also leads to a decreased availability of ergosterol for critical cellular functions, such as sparking (26). A very recent study with *S. cerevisiae* has indicated that the fungistatic effect of fenpropimorph is not due to the accumulation of aberrant sterols, but is primarily caused by the depletion of sparking ergosterol (21). The synergism between the azoles and lovastatin may make well-tolerated dosages of the azoles more efficacious or may allow much lower azole concentrations to be required for the treatment of pathogenic fungal infections. The great advantage of this synergism is the low toxicity of lovastatin for humans, since cholesterol requirements can be satisfied by dietary sources.

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#### LITERATURE CITED

- Alberts, A. W., J. Chen, G. Kuron, V. Hunt, C. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, J. Hirschfield, K. Hoogsteen, J. Liesch, and J. Springer. 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl CoA reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci. USA* **77**:3957-3961.
- Aoyama, Y., and Y. Yoshida. 1978. The 14-demethylation of lanosterol by a reconstituted cytochrome P-450 system from yeast microsomes. *Biochem. Biophys. Res. Commun.* **88**:28-34.
- Bailey, R. B., and L. W. Parks. 1975. Yeast sterol esters and their relationship to the growth of yeast. *J. Bacteriol.* **124**:606-612.
- Bard, M., and J. F. Downing. 1981. Genetic and biochemical aspects of yeast sterol regulation involving 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Gen. Microbiol.* **125**:415-420.
- Bard, M., N. D. Lees, A. S. Burnett, and R. A. Parker. 1988. Isolation and characterization of mevinolin resistant mutants of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **134**:1071-1078.
- Basson, M. E., R. L. Moore, J. O'Rear, and J. Rine. 1987. Identifying mutations in duplicated functions in *Saccharomyces cerevisiae*: recessive mutations in HMG-CoA reductase genes. *Genetics* **117**:645-655.
- Basson, M. E., M. Thorsness, and J. Rine. 1986. *Saccharomyces cerevisiae* contains two functional genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Proc. Natl. Acad. Sci. USA* **83**:5563-5567.
- Berg, D., and M. Plempel. 1988. Sterol biosynthesis inhibitors. Pharmaceutical and agrochemical aspects. VCH Publishing, Chichester, England.
- Boll, M., M. Lowell, and J. Berndt. 1980. Effect of unsaturated fatty acids on sterol biosynthesis in yeast. *Biochim. Biophys. Acta* **620**:429-439.
- Brown, M. S., and J. L. Goldstein. 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21**:505-517.
- Clausen, M. K., K. Christiansen, P. K. Jensen, and O. Behnke. 1974. Isolation of lipid particles from baker's yeast. *FEBS Lett.* **43**:176-179.
- Downing, J. F., L. S. Burrows, and M. Bard. 1980. The isolation of two mutants of *Saccharomyces cerevisiae* which demonstrate increased activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Biochem. Biophys. Res. Commun.* **94**:974-979.
- Dulaney, E. L., E. O. Staply, and K. Simpf. 1954. Ergosterol production by yeasts. *Appl. Microbiol.* **2**:371-379.
- Endo, A., M. Kuroda, and K. Tanzawa. 1976. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236 and ML-236B, fungal metabolites having hypocholesterolemic activity. *FEBS Lett.* **72**:323-326.
- Hata, S., T. Nashino, M. Komori, and H. Katsuki. 1981. Involvement of cytochrome P450 in  $\delta^2$ -desaturation in ergosterol biosynthesis in yeast. *Biochem. Biophys. Res. Commun.* **103**:272-277.
- Kawaguchi, A. 1970. Control of ergosterol biosynthesis in yeast. *J. Biochem.* **67**:219-227.
- Lewis, T. L., G. A. Keesler, G. P. Fenner, and L. W. Parks. 1988. Pleiotropic mutations in *Saccharomyces cerevisiae* affecting sterol uptake and metabolism. *Yeast* **4**:93-106.
- Lorenz, R. T., K. Haecckler, G. Fenner, and L. W. Parks. 1989. Analysis of steryl esters. In W. D. Nes and E. J. Parish (ed.), *Analysis of sterols and biologically significant steroids*. Academic Press, Inc., New York.
- Lorenz, R. T., and L. W. Parks. 1987. Regulation of ergosterol biosynthesis and sterol uptake in a sterol-auxotrophic yeast. *J. Bacteriol.* **169**:3707-3711.
- Lorenz, R. T., R. J. Rodriguez, and L. W. Parks. 1986. Characteristics of sterol uptake in *Saccharomyces cerevisiae*. *J. Bacteriol.* **167**:981-985.
- Marcireau, C., M. Guilloton, and F. Karst. 1990. In vivo effects of fenpropimorph on the yeast *Saccharomyces cerevisiae* and determination of the molecular basis of the antifungal property. *Antimicrob. Agents Chemother.* **34**:989-993.
- Nicholas, R. O., and D. Kerridge. 1989. Correlation of inhibition of sterol synthesis with growth-inhibitory action. *J. Antimicrob. Chemother.* **23**:7-19.
- Osumi, T., T. Nishino, and H. Katsuki. 1979. Studies on the  $\delta^5$ -desaturation in ergosterol biosynthesis in yeast. *J. Biochem.* **85**:819-826.
- Pinto, W. J., R. Lozano, and W. R. Nes. 1985. Inhibition of sterol biosynthesis by ergosterol and cholesterol in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **836**:89-95.
- Quain, D. E., and J. M. Haslam. 1979. The effects of catabolite derepression on the accumulation of steryl esters and the activity of  $\beta$ -hydroxymethylglutaryl coenzyme A reductase in *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **111**:343-351.
- Rodriguez, R. J., and L. W. Parks. 1983. Structural and physical features of sterols necessary to satisfy bulk membrane and sparking requirements in yeast sterol auxotrophs. *Arch. Biochem. Biophys.* **225**:861-871.
- Skipski, G. P., A. F. Smolowe, R. C. Sullivan, and M. Barclay. 1965. Separation of lipid classes by thin-layer chromatography. *Biochim. Biophys. Acta* **106**:386-396.
- Sud, I. J., and D. S. Feingold. 1985. Effect of ketoconazole in combination with other inhibitors of sterol synthesis on fungal growth. *Antimicrob. Agents Chemother.* **28**:532-534.
- Taylor, F. R., and L. W. Parks. 1978. Metabolic interconversion of free sterols and steryl esters in *Saccharomyces cerevisiae*. *J. Bacteriol.* **136**:531-537.
- Taylor, F. R., and L. W. Parks. 1980. Adaptation of *Saccharomyces cerevisiae* to growth on cholesterol: selection of mutants defective in the formation of lanosterol. *Biochem. Biophys. Res. Commun.* **95**:1437-1445.
- Taylor, F. R., and L. W. Parks. 1981. An assessment of the specificity of sterol uptake and esterification in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **256**:13048-13054.
- Vanden Bossche, H., W. Lauwers, G. Williamsen, P. Marichal, F. Cornelissen, and W. Cools. 1984. Molecular basis for the

- antimycotic and antibacterial activity of N-substituted imidazoles and triazoles: the inhibition of isoprenoid biosynthesis. *Pest. Sci.* **15**:188–198.
33. **Watson, P. F., M. E. Rose, S. W. Ellis, H. England, and S. L. Kelly.** 1989. Defective sterol C-5 desaturation and azole resistance: a new hypothesis for the mode of action of azole antifungals. *Biochem. Biophys. Res. Commun.* **164**:1170–1175.
34. **Yamaguchi, H., K. Iwata, M. Nagano, and M. Osumi.** 1981. Ultrastructure of *Saccharomyces cerevisiae* treated with econazole. *J. Electron Microsc.* **30**:305–314.
35. **Yoshida, Y., and Y. Aoyama.** 1986. In K. Iwata and H. Vanden Bossche (ed.), *In vitro and in vivo evaluation of antifungal agents*, p. 123–134. Elsevier Science Publishers, Amsterdam.
36. **Yoshida, Y., and Y. Aoyama.** 1987. Interaction of azole antifungal agents with cytochrome P-450 purified from *Saccharomyces cerevisiae* microsomes. *Biochem. Pharmacol.* **36**:229–235.