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INTERACTION OF LANOSTEROL TO CYTOCHROME P-450 PURIFIED FROM YEAST MICROSOMES: EVIDENCE FOR CONTRIBUTION OF CYTOCHROME P-450 TO LANOSTEROL METABOLISM

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SUMMARY: Interaction between lanosterol and cytochrome P-450 purified from microsomes of anaerobically-grown *Saccharomyces cerevisiae* was studied. Lanosterol (4,4,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol) stimulated the oxidation of NADPH by molecular oxygen in the presence of cytochrome P-450 and NADPH-cytochrome P-450 reductase both purified from *S. cerevisiae* microsomes. Lanosterol stimulated the reduction of cytochrome P-450 by NADPH with the cytochrome P-450 reductase, and induced Type I spectral change of cytochrome P-450. These observations suggest that lanosterol interacts to the substrate region of cytochrome P-450 of *S. cerevisiae*. Based on these facts, possible role of cytochrome P-450 in lanosterol metabolism in yeast cell is discussed.

Recent work in this laboratory has shown that the microsomal fraction from anaerobically-grown cells of *Saccharomyces cerevisiae* contains an electron-transfer system which is analogous to that of liver microsomes (1-8). The electron-transfer system consists of two electron-transport chains named "cytochrome b_5 pathway" and "cytochrome P-450 pathway", respectively. The cytochrome b_5 pathway mediates the oxidative desaturation of palmitoyl CoA, and plays an important role in unsaturated fatty acid biosynthesis in *S. cerevisiae* (4). On the other hand, at present the role of cytochrome P-450 pathway in a metabolic process resulting in the biosynthesis of a cellular component of *S. cerevisiae* is less understood. Alexander *et al.* (9) suggested that the conversion of lanosterol (4,4,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol) to zymosterol (5α-cholesta-8,24-dien-3β-ol) by cell-free system from *S. cerevisiae* is mediated, at least a part, by cytochrome

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P-450 based on the observed inhibitory effect of CO on the lanosterol metabolism. However, they have not presented any evidence for the interaction between lanosterol and cytochrome P-450.

Recently, we purified catalytically active preparations of cytochrome P-450 and NADPH-cytochrome P-450 reductase from microsomes of anaerobically-grown cells of *S. cerevisiae* (7,8). In this communication, we present evidence indicating that lanosterol binds to purified cytochrome P-450 of *S. cerevisiae* and stimulates the redox turnover of the cytochrome in the presence of the cytochrome P-450 reductase.

EXPERIMENTAL PROCEDURE

Cytochrome P-450 and NADPH-cytochrome P-450 reductase were purified from microsomes of anaerobically-grown cells of *S. cerevisiae* according to the methods of Yoshida *et al.* (7) and of Aoyama *et al.* (8), respectively. Cytochrome P-450-containing electron-transfer chain was reconstituted in a mixture consisted of 0.1 M potassium phosphate buffer, pH 7.2, 0.15 mM NADPH, 0.24 μM cytochrome P-450, 2.22 units[2] of NADPH-cytochrome P-450 reductase, 0.05% sodium cholate[3], and 0.001% Emulgen 913[3] (Kao-Atlas Co., Tokyo). Total volume of the mixture was made up to 2.0 ml. The rate of NADPH oxidation by O_2 in the presence of the reconstituted system was estimated in the above mixture at 30°C, and oxidation of NADPH was followed spectrophotometrically at 340 nm. The reduction of purified cytochrome P-450 by NADPH in the presence of the cytochrome P-450 reductase was observed with the above mixture except that the mixture was further added by glucose, glucose oxidase and catalase to achieve anaerobiosis (8). Reduction of cytochrome P-450 was followed spectrophotometrically as described previously (8). When the effect of lanosterol on the NADPH oxidation or the cytochrome P-450 reduction was determined, 5 μl of lanosterol emulsion (5 μmoles/ml of 1.0% Emulgen 913) was added to the reaction mixture. In the control experiment, 5 μl of 1.0% Emulgen 913 was added instead of the lanosterol emulsion.

Cytochrome P-450 and protein were determined by the methods of Omura and Sato (10) and of Lowry *et al.* (11), respectively.

RESULTS AND DISCUSSION

A reconstituted system consisted of cytochrome P-450 and NADPH-cytochrome P-450 reductase both purified from *S. cerevisiae* microsomes catalyzed NADPH oxidation by O_2. The rate of the NADPH oxidation could be increased by the addition of lanosterol, and both cytochrome P-450 and the cytochrome P-450 reductase as

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[2]The enzyme unit of NADPH-cytochrome P-450 reductase was defined as its cytochrome *c* reducing activity (8).

[3]These detergents were included in the cytochrome P-450 and the reductase preparations, and were necessary for supporting the activity of the reconstituted system

Table I. Oxidation of NADPH with the reconstituted system in the presence of lanosterol.

Reaction system	NADPH oxidized (nmoles/min)
Complete	3.22
" - P-450	1.11
" - P-450 reductase	0.00
" - lanosterol	1.08

The reaction mixture for the complete system consisted of 0.1 M potassium phosphate buffer, pH 7.2, 0.15 mM NADPH, 0.24 μM cytochrome P-450, 2.22 units of NADPH-cytochrome P-450 reductase, 0.05% sodium cholate, 0.001% Emulgen 913, and 5 μl of lanosterol emulsion (5 μmoles/ml of 1.0% Emulgen 913). Total volume of the mixture was made up to 2.0 ml, and oxidation of NADPH was measured as described under EXPERIMENTAL PROCEDURE.

well as lanosterol were required for the maximum NADPH oxidation (Table I). However, about one-third of the NADPH oxidation was observed even in the absence of cytochrome P-450 (Table I). The rate of NADPH oxidation without cytochrome P-450 (1.11 nmoles/min) was comparable to that observed in the absence of lanosterol (1.08 nmoles/min). The NADPH oxidation observed in the absence of cytochrome P-450 was not affected by lanosterol, and this activity must be due to slow autoxidation of NADPH-cytochrome P-450 reductase (6,8). Therefore, most of the NADPH oxidation with the reconstituted system observed in the absence of lanosterol may be due to the autoxidation of NADPH-cytochrome P-450 reductase. Accordingly, it can be said that the contribution of cytochrome P-450 to the NADPH oxidation with the reconstituted system was not evident unless lanosterol was added to the reaction mixture. In other words, lanosterol should stimulate the redox turnover of cytochrome P-450 in the reconstituted system, and this was supported by the following observations.

Purified *S. cerevisiae* cytochrome P-450 could be reduced by NADPH in the reconstituted system. The reduction rate was, however, very low (curve B of Fig. 1); the apparent first-order rate constant calculated from this trace was 0.07 min^{-1}. When lanosterol was added to the reaction mixture, the rate of cytochrome

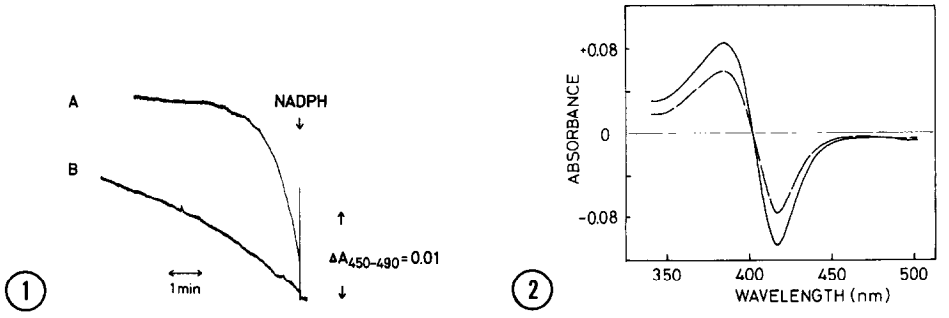

Fig. 1. Time course of the reduction of cytochrome P-450 by NADPH in the presence and absence of lanosterol. Curve A: Two ml of the reaction mixture consisted of 0.1 M potassium phosphate buffer, pH 7.2, 0.24 μM cytochrome P-450, 2.22 units of NADPH-cytochrome P-450 reductase, 0.05% sodium cholate, 0.001% Emulgen 913, 7.5 mM glucose, 2 units of glucose oxidase, 2600 units of catalase, and 5 μl of lanosterol emulsion (5 μmoles/ml of 1.0 % Emulgen 913) was placed in a spectrophotometer cuvette, and was saturated with CO. The mixture was incubated at 30°C for 2 min to achieve anaerobiosis. The reaction was then started by the addition of 3 μmoles of NADPH as indicated, and the increasing absorbance difference between 450 and 490 nm was followed at 30°C. Curve B: Experiment was carried out under same conditions as described for curve A except that lanosterol emulsion was omitted from the reaction system.

Fig. 2. Lanosterol-induced Type I spectral change of cytochrome P-450. Purified *S. cerevisiae* cytochrome P-450 dissolved in 0.1 M potassium phosphate buffer, pH 7.2, containing 20% glycerol and 0.05% Emulgen 913 to give a concentration of 10.9 μM was placed in both sample and reference cuvettes, and the base line was recorded. Then, difference spectra caused by the additions of 5 μl (— —) and 10 μl (———), respectively, of lanosterol emulsion (5 μmoles/ml of 1.0% Emulgen 913) were recorded.

P-450 reduction in the reconstituted system increased more than ten-fold (curve A of Fig. 1); the apparent first-order rate constant calculated from this trace was 1.0 min^{-1}. As shown in Fig. 2, lanosterol induced Type I spectral change of purified *S. cerevisiae* cytochrome P-450, and the magnitude of this spectral change depended on lanosterol concentration. These results clearly indicate that lanosterol interacted to *S. cerevisiae* cytochrome P-450 and increased the reactivity of the cytochrome with its reductase system.

It was reported for liver microsomal cytochrome P-450 that both stimulation of enzymatic reduction of the cytochrome and Type I spectral change of the cytochrome are observed when cytochrome P-450 binds with its substrate (12). Therefore, the line of evidence described above suggest that lanosterol is a substrate

for *S. cerevisiae* cytochrome P-450, and the stimulation of the NADPH oxidation with the reconstituted system by lanosterol (Table I) should be due to certain metabolism of lanosterol by the cytochrome accompanied with the utilization of electrons from NADPH. In addition, we found that squalene and some other sterols such as cholesterol, ergosterol and stigmasterol could stimulate neither the enzymatic reduction of cytochrome P-450 nor the NADPH oxidation with the reconstituted system. Therefore, the enzymatic reduction of cytochrome P-450 and the NADPH oxidation with the reconstituted system seemed to be stimulated specifically by lanosterol so far studied. This fact supports the above-mentioned possibility that lanosterol is a substrate for *S. cerevisiae* cytochrome P-450.

Lanosterol is metabolized to ergosterol via zymosterol by an enzyme system in yeast cell (13), and the conversion of lanosterol to zymosterol involves removal of the three methyl groups attached at C-4 and C-14 of the sterol nucleus (13). Recently, Alexander *et al.* (9) suggested a possibility that the demethylation of lanosterol to form zymosterol by a cell-free system from *S. cerevisiae* is mediated, at least a part, by cytochrome P-450. However, they inferred this possibility simply from the observed inhibitory effect of CO on the conversion of ^{14}C-lanosterol to 4,4-dimethylsterols, 4-methylsterols and zymosterol by a crude particulate fraction from *S. cerevisiae*, and they did not presented any evidence for the interaction between lanosterol and cytochrome P-450. By using purified preparation of cytochrome P-450 (7), we demonstrated here the interaction between *S. cerevisiae* cytochrome P-450 and lanosterol presumablly at the substrate region of the cytochrome. In addition we notice the fact that *S. cerevisiae* cytochrome P-450 interacted directly with lanosterol itself. This fact suggests a possibility that cytochrome P-450 contributes to the initial step of tha lanosterol metabolism. The initial step of the lanosterol metabolism in yeast is considered as the removal of the methyl group attached at C-14 of the sterol nucleus (13). So, it is highly likely that cytochrome P-450 contributes to the removal of the methyl group at C-14 of lanosterol. In fact, Mitropoulos *et al.* (14) reported that the removal of the methyl group at C-14 of lanosterol by cell-

free system from *S. cerevisiae* was inhibited by CO. They (14) also reported that the methyl group at C-14 of lanosterol was removed as formic acid. This fact suggests that the removal of the methyl group at C-14 of lanosterol consists of a few component reactions, *i.e.* two- or three-step oxidation of the methyl carbon and the elimination of the oxidized carbon, as the case of the oxidative 4α-demethylation of lanosterol by hepatic microsomes (15-19). It is, therefore, important to elucidate the relationship between cytochrome P-450 and each of the component reactions. This problem will be cleared when the lanosterol metabolite(s) formed by the reconstituted system is identified. Analysis of the lanosterol metabolite(s) is being in progress in our laboratory.

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