Molecular Engineering Study on Electron Transfer from NADPH-P450 Reductase to Rat Mitochondrial P450c27 in Yeast Microsomes*

(Received for publication, June 3, 1996, and in revised form, July 12, 1996)

Toshiyuki Sakaki‡, Shiro Kominami‡, Koji Hayashi‡, Megumi Akiyoshi-Shibata‡, and Yoshiyasu Yabusaki‡

From the ‡Biotechnology Laboratory, Sumitomo Chemical Co., Ltd. Takatsukasa, Takarazuka, Hyogo 665 and the †Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima, Hiroshima 724, Japan

We have reported the localization on yeast microsomes for a modified P450c27 (mic-P450c27) that contains the microsomal targeting signal of bovine P450c17 in front of the mature form of rat mitochondrial P450c27 (Sakaki, T., Akiyoshi-Shibata, M., Yabusaki, Y., and Ohkawa, H. (1992) J. Biol. Chem. 267, 16497–16502). In this study, we found that mic-P450c27 could be reduced by NADPH in the yeast microsomes without supplement of its physiological redox partners, adrenodoxin and NADPH-adrenodoxin reductase. In order to elucidate the direct electron transfer from NADPH-P450 reductase to mic-P450c27, we carried out simultaneous expression of mic-P450c27 and yeast P450 reductase. The reduction rate of mic-P450c27 was increased by overproduction of yeast P450 reductase, roughly in proportion to the reductase content in the microsomes. In addition, we constructed a fused enzyme between mic-P450c27 and yeast P450 reductase. The reduction rate of heme iron in the fused enzyme was too rapid to be measured. These recombinant yeast microsomes showed a notable 27-hydroxylation activity toward 5α-cholestan-3α,7α,12α-triol in the absence of adrenodoxin and adrenodoxin reductase. Finally, we purified mic-P450c27 from the recombinant yeast microsomes and reconstituted the hydroxylation system in liposomal membranes using the purified mic-P450c27 and yeast NADPH-P450 reductase. Mic-P450c27 was reduced by NADPH and showed its monoxygenase activity on the reconstituted system. Therefore, yeast NADPH-P450 reductase alone was found to transfer two electrons from NADPH to mic-P450c27. These results clearly show that mic-P450c27 not only localizes on the microsomes but also functions as a microsomal cytochrome P450 that accepts electrons from NADPH-P450 reductase.

Cytochrome P450 represents a large group of structurally related hemoproteins that catalyze the monoxygenase reactions of a wide variety of both endogenous and exogenous compounds. P450 monoxygenases can be classified into two types based on the electron supplying proteins to P450. In class I P450s (1), which include most of bacterial soluble P450s and mitochondrial P450s, the electron transport chain involves an FAD-containing enzyme, ferredoxin reductase, and an iron-sulfur enzyme, ferredoxin, to transfer electrons from NADH or NADPH to the terminal P450. In class II P450s, which include microsomal P450s and bacterial P450s (2), electrons are transferred from NADPH to P450 by the catalysis of NADPH-P450 reductase that contains one molecule each of FAD and FMN. Although detailed studies have been carried out for the interaction between the P450s and their partner proteins in the both electron transfer systems (1, 3, 4), the interaction between mitochondrial P450s and the NADPH-P450 reductase or between microsomal P450s and the ferredoxin has not been well demonstrated yet. Recently, Jenkins and Waterman (5) reported a very interesting finding for the electron transfer system to heterologously expressed P450c17 in Escherichia coli. They purified both an FMN-containing flavodoxin and an FAD-containing NADPH-flavodoxin reductase from E. coli and found that these enzymes could support 17α-hydroxylation activity of bovine adrenal microsomal P450c17, which explains well why P450c17 expressed in E. coli is functionally active without P450 reductase (6).

From an evolutionary standpoint, microsomal P450 is thought to have diverged from a bacterial soluble P450, followed by the appearance of mitochondrial P450 from microsomal P450 (7). Alteration of soluble P450 to microsomal one could be explained by the addition of an amino-terminal hydrophobic sequence to the soluble P450 and that of microsomal P450 to mitochondrial one could be attributed to the substitution of the signal sequence for the localization into the mitochondrial inner membranes. Indeed, we have succeeded in conversion of subcellular localization of P450c27 from yeast mitochondria to microsomes only by altering its amino-terminal targeting signal (8). This microsomal modified P450c27 (referred to as mic-P450c27) was active in both 25-hydroxylation of 1α-hydroxyvitamin D₃ and 27-hydroxylation of cholestan-3α,7α,12α-triol (THC) when reconstituted in vivo and in vitro with adrenodoxin (ADX) and NADPH-adrenodoxin reductase (ADR) (8). However, no possible schemes have been suggested for the evolution or alteration of the electron transfer partners to P450.

In the present study, we examined by the use of molecular engineering methods whether mic-P450c27 could accept electrons efficiently from yeast NADPH-P450 reductase. In addition, we have constructed a highly active fused enzyme between mic-P450c27 and yeast P450 reductase. These findings clearly indicated that yeast P450 reductase can transfer electrons from NADPH directly to mic-P450c27.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Sumitomo Pharmaceuticals Research Center, Kasugana, Osaka 554, Japan.

¶ To whom correspondence should be addressed: Biotechnology Laboratory, Sumitomo Chemical Co., Ltd., 4-2-1 Takatsukasa, Takarazuka, Hyogo 665, Japan. Tel.: 81-797-74-2058; Fax: 81-797-74-2133.

1 The abbreviations used are: THC, 5α-cholestan-3α,7α,12α-triol; ADR, NADPH-adrenodoxin reductase; ADX, adrenodoxin.
EXPERIMENTAL PROCEDURES

Materials—DNA modifying enzymes, restriction enzymes, and DNA sequencing kit were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). 5-fluorodeoxyuridine-5′-triphosphate (FUDR-TP) and dATP-TP were from Amerham Japan (Tokyo, Japan), 1,2-epoxypropane diol (glycidyl ether, Sigma, St. Louis, MO), and phospholipids (phosphatidylcholine, bovine, grade II) were from Lipid Products (Nutfield, Surrey, UK). Linker DNAs were synthesized by using an Applied Biosystems 380A DNA synthesizer. E. coli HB101 (Takara Shuzo Co., Ltd.) and Saccharomyces cerevisiae AH22 (9) were used as the recombinant host strains. The yeast expression vector pAAH5N (10) was used to construct expression plasmids. The expression plasmid pAMS25 for microsomal P450c27 was constructed as described previously (8).

Recombinant DNA Procedures—Recombinant DNA procedures were performed as described previously (9). Sequencing of the synthesized linker DNA and the reaction region of the fusion enzyme DNA was performed as described previously (11). Recombinant E. coli and S. cerevisiae strains were cultivated in L broth and concentrated SD medium, respectively, as described (12). Polymerase chain reaction was carried out with Pfu DNA polymerase (Strategene Cloning Systems, CA) using Perkin Elmer PC2000 apparatus.

Measurement of P450 Hemoprotein—P450 homoprotein content in whole cells or subcellular fractions was determined by a reduced CO difference spectrum (13) using an extinction coefficient of 91 mM−1cm−1 (13).

Western Blot Analysis—Total cellular proteins from the recombinant yeast strain were subjected to an electrophoresis on SDS-polyacrylamide gel and then transferred electrophoretically from the gel to a nitrocellulose filter (9). The reaction mixture was probed with anti-mic-P450c27 antiserum (8) or antipeptide NADPH-P450 reductase IgG (14), followed by 125I-labeled Protein A as described previously (9).

Purification of Mic-P450c27 and Reconstitution of the Hydroxylation System in Liposomal Membranes—Microsomal fraction was prepared from the recombinant yeast strains as described previously (9). Mic-P450c27 was purified from AH22/pAMS25 microsomes to an electrophoretically homogeneous form by the same procedures as reported (8). The preparation (12 nmol of P450/mg of protein) was incorporated into liposomal membranes, and the hydroxylation system was reconstituted in vitro with yeast NADPH-P450 reductase (specific activity of 150 units/mg protein) provided by Dr. Y. Aoyama, Soka University (15). The molar ratio of microsomal P450, P450 reductase, and phospholipid was 1:1:3.000.

THC 27-Hydroxylation Activity—The monooxygenase activity of the microsomal fraction was measured as follows. The reaction mixture contained each of the microsomal fractions prepared from the recombinant yeast strains (0.025–0.1 μM P450), 100 mM Tris-HCl (pH 7.8), 0.5 mM EDTA, and [15]H[3H]THC (20, 50, 100, or 200 μM). The reaction was initiated by addition of NADPH to a final concentration of 0.5 mM. After incubation at 37°C for 3 or 5 min, the reaction was stopped by vigorous mixing with CH2Cl2. The organic phase was recovered and dried up. The resulting residue was solubilized with acetonitrile for high performance liquid chromatography with monitoring the radioactivity on a Flow-one α system (Packard). The THC 27-hydroxylation activity on the reconstituted liposomal membrane was similarly measured. The cellular THC 27-hydroxylation activity of the recombinant yeast was measured by incubation of the cell culture with 10 μM [15]H[3H]THC at 30°C as described previously (8).

Reduction of Heme Iron—Stopped flow analysis was performed at 10°C using a dual-wavelength stopped-flow device (Unisoku Co., Ltd., Hirakata, Japan) as described previously (16, 17). The reaction mixture contained the microsomal fraction (0.2 μM P450 or its fused enzyme), 20 mM potassium phosphate (pH 7.4), 20% glycerol, 50 mM glucose, 20 units/ml glucose oxidase, 200 units/ml catalase, and 100 μM THC. The reaction was initiated by rapid mixing of the microsomes with an equal volume of 80 μM NADPH in the presence of CO, and the change in the absorbance difference between 450 and 490 nm was recorded. A computer program was employed to fit the kinetic data as described (17).

Other Methods—Cytochrome c reductase activity derived from NADPH-P450 reductase or the fused enzyme was measured as described previously (14, 18). Protein concentration was determined by the method of Lowry et al. (19), using bovine serum albumin as a standard.

RESULTS

Construction of Yeast Expression Plasmids—The expression plasmid pAMS25R for simultaneous expression of mic-P450c27 and yeast NADPH-P450 reductase was constructed by inserting the NotI fragment of pAMS25 containing the expression unit for mic-P450c27 into the unique NotI site of the plasmid pARR3N (10). The plasmid pF250 for the fused enzyme between mic-P450c27 and yeast P450 reductase was constructed by adapting the procedures for the construction of pAFCR1 (17). A BamHI-Xhol fragment (250 base pairs) encoding the carboxy-terminal portion of mic-P450c27 was amplified by polymerase chain reaction. The resulting BamHI-Xhol fragment was doubly inserted with the HindIII-BamHI fragment prepared from pAMS25 between the HindIII and Xhol sites of pBlueScript II. The plasmid pGyR encoding yeast NADPH-P450 reductase (20) was digested with PvuII to insert a linker containing an Xhol site. Both HindIII-Xhol fragments from the resulting plasmids that encode mic-P450c27 and P450 reductase, respectively, were doubly inserted into the unique HindIII site of the expression vector pAAH5N in the correct orientation.

Fig. 1 schematically represents the three expression plasmids used in the present study, pAMS25 for mic-P450c27, pAMS25R for both mic-P450c27 and yeast P450 reductase, and pP250 for the fused enzyme between mic-P450c27 and yeast P450 reductase. The individual expression plasmids were introduced into S. cerevisiae AH22 cells to obtain the corresponding recombinant yeast strains.

Expression of Mic-P450c27 and Its Fused Enzyme in Yeast—Fig. 2 shows Western blot analysis of whole cellular proteins prepared from the recombinant yeast strains. A clear band reactive with anti-mic-P450c27 antiserum was detected in both AH22/pAMS25 and AH22/pAMS25R at the position of apparent molecular mass of about 55 kDa (Fig. 2, left, lanes 2 and 3). AH22/pF250 shows a protein band reactive with both anti-mic-P450c27 antiserum and anti-P450 reductase IgG (Fig. 2, left, lane 4 and right, lane 2). The apparent molecular mass of the protein was about 130 kDa, which agrees with the structure of the fused enzyme as deduced from its cDNA sequence. The expression levels of mic-P450c27 in AH22/pAMS25 and AH22/pAMS25R and the fused enzyme in AH22/pF250 were estimated to be about 2.0, 1.6, and 1.1 × 105 molecules/cell, respectively, on the basis of reduced CO difference spectra of the recombinant yeast cells.

P450 and NADPH-P450 Reductase Contents in the Microsomal Fraction of AH22/pAMS25R. The molar ratio of mic-P450, P450 reductase, and phospholipid was 1:1:3.000.
Heterologous Electron Transfer in the P450 System

Fig. 2. Western blot analysis of whole cellular proteins prepared from recombinant yeast. Whole cellular proteins (2.0 × 10^6 cells) prepared from recombinant yeast cells were analyzed by gel transfer immunoblot with anti-mic-P450c27 antiserum (left) and anti-yeast P450 reductase Ig (right). Left, lanes 1, AH22/pAH5N (control); 2, AH22/pAMS25; 3, AH22/pAMS25R; 4, AH22/pF250. Right, lanes 1, AH22/pAH5N (control); 2, AH22/pF250. Mic-c27, YR, and FE indicate the migrating points of mic-P450c27, yeast P450 reductase, and the fused enzyme, respectively.

Mal Fraction Prepared from the Recombinant Yeast Cells—Mic-P450c27 expressed in yeast was localized in the microsomal fraction as reported previously (8). The fused enzyme exhibited a subcellular distribution quite similar to mic-P450c27. Thus, we determined the P450 and P450 reductase contents in the yeast microsomal fractions based on reduced CO difference spectra and NADPH-cytochrome c reductase activity, respectively (Table I). While the P450 content in AH22/pAMS25 and AH22/pAMS25R microsomes was about 24-fold higher than in AH22/pAMS25 microsomes, the ratio of mic-P450c27 to P450 reductase contents was roughly estimated to be 1.06 and 1.17 in AH22/pAMS25 and AH22/pAMS25R microsomes, respectively. The AH22/pF250 microsomes exhibited the highest cytochrome c reductase activity, suggesting that the reductase portion of the fused enzyme can reduce cytochrome c more efficiently than P450 reductase itself. These results are consistent with those obtained for the fused enzyme between rat P4501A1 and yeast P450 reductase (17).

NADPH-dependent Heme Reduction in Mic-P450c27 and the Fused Enzyme—Fig. 3A shows the reduced CO difference spectra of AH22/pAMS25 microsomes in the presence of 100 μM THC when reduced by addition of NADPH or sodium hydrosulfite. Most of mic-P450c27 in AH22/pAMS25 microsomes was reduced by NADPH within 2 min. Similarly, almost all of mic-P450c27 on the reconstituted membrane was reduced within 1 min (Fig. 3B). Thus, it seems most likely that mic-P450c27 was reduced by endogenous yeast NADPH-P450 reductase present in the microsomes.

The reduction rate of the heme iron by NADPH in the presence of substrate was also examined by stopped flow analysis. The rate constant k (s^{−1}) in AH22/pAMS25 and AH22/pAMS25R microsomes was estimated to be 0.046 and 0.67, respectively (Table II). Therefore, the reduction rate of the heme iron in mic-P450c27 was found to be roughly proportional to the yeast P450 reductase content in the microsomes. The reduction rate constant of mic-P450c27 was slightly larger than the reduction rate constant for rat microsomal P4501A1 similarly expressed in the yeast microsomes (17). In contrast, the reduction rate of the heme iron of the fused enzyme was too rapid to be measured, as was the case for the fused enzyme between rat P4501A1 and yeast P450 reductase (17). These results also strongly support the finding that yeast P450 reductase is an electron donor enzyme that can transfer electrons directly to mic-P450c27.

THC 27-Hydroxylation Activity—We reported previously that AH22/pRXMS25 strain containing mic-P450c27 and mature forms of bovine ADX and ADR converts THC into its 27-hydroxylated product 5β-cholestan-3α,7α,12α,27-tetrol (8). The substrate THC was also added to the cell culture of AH22/pAMS25R and AH22/pF250 strains. After 14 h incubation, 5β-cholestan-3α,7α,12α,27-tetrol was detected in the culture supernatants of both strains (data not shown). Both mic-P450c27 and its fused enzyme were localized on the yeast endoplasmic reticulum membranes and/or cytoplasm. The mitochondrial electron donor protein(s) could not be involved in the mic-P450c27-dependent activity in the cell culture of AH22/pAMS25R and AH22/pF250 strains.

Fig. 3. Reduced CO difference spectra of the microsomal fraction prepared from AH22/pAMS25 cells (A) and mic-P450c27 purified from the reconstituted membrane with NADPH-P450 reductase and phospholipid (B). Reduced CO difference spectra were measured by addition of NADPH (---) and dihydrosulfite (------) in 0.1 M potassium phosphate (pH 7.0) as described under "Experimental Procedures."

| Strain       | P450 pmol/mg protein | Cytochrome c reductase activity nmol/min/mg protein |
|--------------|----------------------|-----------------------------------------------|
| AH22/pAMS25  | 110                  | 61                                            |
| AH22/pAMS25R | 90                   | 1460                                          |
| AH22/pF250   | 58                   | 2640                                          |

Values are the mean ± S.D. of at least three separate experiments. ND, not determined.
Heterologous Electron Transfer in the P450 System

TABLE III

Kinetic parameters of mic-P450c27 and its fused enzyme for THC 27-hydroxylation in recombinant yeast microsomes and in vitro reconstituted system

| Preparation     | \( K_{\text{m}} \) for THC (µM) | \( V_{\text{max}} \) (mol product/min/mol P450) |
|------------------|-------------------------------|-----------------------------------------------|
| AH22/pAMS25R microsome     | 36 ± 3                       | 21 ± 1                                        |
| AH22/pF250 microsome      | 42 ± 8                       | 110 ± 6                                       |
| Reconstituted membrane    | 59 ± 13                      | 6.0 ± 0.8                                     |

THC 27-hydroxylation activity in AH22/pAMS25, AH22/pAMS25R, and AH22/pF250 microsomes was further examined using stopped flow analysis. Reconstituted systems from these microsomes showed a notable THC 27-hydroxylation activity, whereas AH22/pAMS25 microsomes showed little activity. The kinetic parameters, apparent \( K_{\text{m}} \) (µM) and \( V_{\text{max}} \) (mol product/min/mol P450) values, were calculated by Lineweaver-Burk plots (Table III). The kinetic parameters for AH22/pAMS25R microsomes could not be estimated because of its low activity. Mic-P450c27 and its fused enzyme had nearly the same \( K_{\text{m}} \) values for THC, whereas the fused enzyme showed five times larger \( V_{\text{max}} \) values than mic-P450c27. These results strongly suggest the two electrons essential for the P450c27-dependent THC hydroxylation are transferred from NADPH through NADPH-P450 reductase to mic-P450c27. The \( V_{\text{max}} \) value of the fused enzyme (110 min\(^{-1}\)) was about 2-fold higher than for mic-P450c27 in the AH22/pAMS25 microsomes in the presence of ADX and ADR (8). This high monooxygenase activity of the fused enzyme is likely due to a more efficient electron transfer from the reductase part to the P450 part of the fused enzyme. Efficient electron transfer has been observed in several fusion constructs between microsomal P450 and NADPH-P450 reductase (11, 12, 17, 21–23).

The direct electron transfer from NADPH-P450 reductase to mic-P450c27 was definitively demonstrated in the membrane reconstituted system using the purified mic-P450c27 and yeast P450 reductase. The apparent \( K_{\text{m}} \) value for THC of mic-P450c27 in the liposomes containing mic-P450c27 and the reductase (1:1 in molar ratio) was similar to that of AH22/pAMS25R microsomes, although the \( V_{\text{max}} \) value was about a third of that obtained in the microsomes that contain the reductase about 1.7 times more than mic-P450c27.

DISCUSSION

P450c27 was first purified from rat liver mitochondria by Okuda et al. (24), who demonstrated the requirement of ADX and ADR for its activity in a reconstituted system. For the interaction of P450s with their electron donor proteins, Bernhardt and Gunsalus (25) examined heterologous electron transfer from the bacterial electron donor putidaredoxin to rabbit microsomal P4502B4 and found very low affinity of the bacterial redoxin for the microsomal P450. Jenkins and Waterman (5) reported the electron transfer from NADPH to heterologously expressed microsomal P450c17 through E. coli flavodoxin and flavodoxin reductase system, although the 17α-hydroxylation activity supported by the E. coli reductase was 10-fold less efficient as compared with purified rat P450 reductase. This flavodoxin and flavodoxin reductase system can be replaced by another ferredoxin and ferredoxin reductase system from spinach for human P4501A2 and 3A4 expressed in E. coli (26, 27). Recently, Black et al. (28) reported the monooxygenase activity of the fusion construct of P450c27 with NADPH-P450 reductase expressed in COS-1 cells, although the mitochondrial localization of the fused enzyme was absolutely required for its activity. However, because the mitochondrial fraction of COS-1 cells contains an electron transfer system consisting of ferredoxin and ferredoxin reductase, any other protein(s) than P450 reductase may participate in the electron transfer from NADPH to P450c27 part. Thus, no direct evidence has been reported for heterologous electron transfer from the microsomal electron donor P450 reductase to mitochondrial P450.

On the expression of mic-P450c27 in yeast, we have previously concluded that endogenous yeast NADPH-P450 reductase was insufficient for mic-P450c27 to show monooxygenase activity, because 1α-hydroxyvittamin D₃ 25-hydroxylation activity could not be detected without the addition of both ADX and ADR (8). However, to our surprise, stopped flow analysis revealed that the reduction rate of mic-P450c27 heme iron in AH22/pAMS25R microsomes is somewhat more rapid than that observed for rat P4501A1 in AH22/pAMC1 microsomes (17). These findings suggest the existence of the electron donor protein(s) to mic-P450c27 on the yeast microsomal membrane. In order to confirm the electron transfer from NADPH-P450 reductase to mic-P450c27, we first constructed the simultaneous expression plasmid pAMS25R for mic-P450c27 and yeast P450 reductase. The resulting AH22/pAMS25R strain contained a 24-fold increased amount of the reductase as compared with AH22/pAMS25 cells. The reduction rate of mic-P450c27 heme iron in AH22/pAMS25R microsomes was 17-fold more rapid than mic-P450c27 in AH22/pAMS25 microsomes, roughly in proportion to the reductase content. In addition, AH22/pAMS25R microsomes showed THC 27-hydroxylation activity without addition of ADX and ADR. These results strongly support our hypothesis that mic-P450c27 can accept two electrons from P450 reductase in the P450 reaction cycle.

Next, we constructed the fused enzyme between mic-P450c27 and yeast NADPH-P450 reductase to further investigate the electron transfer from NADPH to P450 reductase to mic-P450c27. The rate of the first electron transfer from NADPH to the heme iron was too rapid to be measured, as was the case for the fused enzyme between rat P4501A1 and yeast P450 reductase (17). In addition, the fused enzyme showed a 5-fold higher THC 27-hydroxylation activity than mic-P450c27 in AH22/pAMS25R microsomes. These observations also strongly suggested the direct electron transfer from the reductase part to the heme iron in the mic-P450c27 part of the fused enzyme. To exclude the possibility of contamination of other electron transfer proteins in the yeast microsomes, mic-P450c27 was purified from the recombinant yeast microsomes. The purified preparation of mic-P450c27 in the liposomal membranes reconstituted with yeast P450 reductase showed a significant THC 27-hydroxylation activity. This clear evidence that electrons are transferred directly from NADPH to mic-P450c27 through P450 reductase. The discrepancy between the observation of Okuda et al. (24) and that of the present study may be attributable to the amount of P450 reductase used for reconstitution. As compared with the reconstituted system used by Okuda et al. (24), we employed equal or more amounts of yeast P450 reductase for the reconstitution of the liposomal membranes and for coexpression in the present study.

Mic-P450c27 has the microsomal targeting signal derived from the microsomal P450c17 that is absent in the mature form of native mitochondrial P450c27. Therefore, it seems likely that the amino-terminal hydrophobic sequence of mic-P450c27 plays an important role not only in its localization to the microsomal membrane but also in its membrane topology resulting in efficient interaction with NADPH-P450 reductase. The redox potential of mitochondrial P450s is quite similar to that of microsomal P450s. Therefore, in the view of electrochemistry there is no difficulty for the electron transfer from...
The direct electron transfer from microsomal P450 reductase to mic-P450c27 is also of interest from the evolutionary aspect of P450 superfamily. Comparison of primary structures of more than 200 P450 species revealed that microsomal P450s have diverged from an ancestral form of a soluble bacterial P450 and mitochondrial P450s then evolved from a microsomal P450 (7). We substantiated the conversion of P450c27 localization from mitochondria to microsomes by altering its amino-terminal targeting signal (8), showing that only the amino-terminal sequences of 20–40 residues decide its subcellular localization. Thus, this novel technique for construction of a highly active single chain enzyme consisting of mitochondrial P450 and microsomal NADPH-P450 reductase is also of interest from the evolutionary aspect.

Acknowledgments—We express our gratitude to Dr. Y. Aoyama of Sokaha University and Dr. Y. Yoshida of Mukogawa Women’s University for providing yeast NADPH-P450 reductase and anti-yeast NADPH-P450 reductase Ig. We also thank Y. Matsuda for expert technical assistance.

REFERENCES

1. Ravichandran, K. G., Boddupalli, S. S., Hasemann, C. A., Peterson, J. A., and Deisenhofer, J. (1993) *Science* **261**, 731–736
2. Falco, A. J., and Ruttiger, R. T. (1987) *Life Sci.* **40**, 1769–1754
3. Coplin, V. M., and Vickery, L. E. (1997) *J. Biol. Chem.* **266**, 18606–18612
4. Furuya, H., Shimizu, T., Hirano, K., Hatano, M., and Fujii-Kuriyama, Y. (1989) *Biochemistry* **28**, 6848–6857
5. Jenkins, C. M., and Waterman, M. R. (1994) *J. Biol. Chem.* **269**, 27401–27408
6. Barnes, H. J., Arlotto, M. P., and Waterman, M. R. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 5597–5601
7. Nebert, D. W., Nelson, D. R., Estabrook, R. W., Feyereisen, R., Fujii-Kuriyama, Y., Gonzalez, F., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Loper, J. C., Sato, R., Waterman, M. R., and Waxman, D. J. (1991) *DNA Cell Biol.* **10**, 1–14
8. Sakaki, T., Akiyoshi-Shibata, M., Yabusaki, Y., and Ohkawa, H. (1992) *J. Biol. Chem.* **267**, 16497–16502
9. Oeda, K., Sakaki, T., and Ohkawa, H. (1985) *DNA (N. Y.)* **4**, 203–210
10. Murakami, H., Yabusaki, Y., Sakaki, T., Shibata, M., and Ohkawa, H. (1990) *J. Biochem.* (Tokyo) **108**, 859–865
11. Sakaki, T., Shibata, M., Yabusaki, Y., and Ohkawa, H. (1987) *DNA Cell Biol.* **6**, 21–39
12. Sakaki, T., Shibata, M., Yabusaki, Y., Murakami, H., and Ohkawa, H. (1990) *DNA Cell Biol.* **9**, 603–611
13. Omura, T., and Sato, R. (1964) *J. Biol. Chem.* **239**, 2570–2578
14. Anyama, Y., Yoshida, Y., Kubota, S., Kumaoka, H., and Furumichi, A. (1978) *Arch. Biochem. Biophys.* **185**, 362–369
15. Kominami, S., Inoue, S., Higuchi, A., and Takemori, S. (1989) *Biochim. Biophys. Acta* **985**, 293–299
16. Kominami, S., Ogawa, N., Morimune, R., De-ying, H., and Takemori, S. (1992) *J. Steroid Biochem. Mol. Biol.* **42**, 57–64
17. Sakaki, T., Kominami, S., Takemori, S., Ohkawa, H., Akiyoshi-Shibata, M., and Yabusaki, Y. (1994) *Biochemistry* **33**, 4033–4038
18. Murakami, H., Yabusaki, Y., and Ohkawa, H. (1987) *DNA (N. Y.)* **5**, 1–10
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
20. Yabusaki, Y., Murakami, H., and Ohkawa, H. (1988) *Biochem. (Tokyo) 103, 1004–1010
21. Murakami, H., Yabusaki, Y., Sakaki, T., Shibata, M., and Ohkawa, H. (1987) *DNA (N. Y.)* **6**, 189–197
22. Yabusaki, Y., Murakami, H., Sakaki, T., Shibata, M., and Ohkawa, H. (1988) *DNA (N. Y.)* **7**, 701–711
23. Shibata, M., Yabusaki, Y., Murakami, H., and Ohkawa, H. (1990) *DNA Cell Biol.* **9**, 27–36
24. Okuda, K., Masumoto, O., and Ohyama, Y. (1988) *J. Biol. Chem.* **263**, 18113–18142
25. Bernhardt, R., and Gunsalus, I. C. (1992) *Biochem. Biophys. Res. Commun.* **187**, 310–317
26. Dong, M.-S., Yamazaki, H., Guo, Z., and Guengerich, F. P. (1996) *Arch. Biochem. Biophys.* **327**, 11–19
27. Yamazaki, H., Ueng, Y.-F., Shimada, T., and Guengerich, F. P. (1995) *Biochemistry* **34**, 6380–6389
28. Black, S. M., Harikrishna, J. A., Szklarz, G. D., and Miller, W. L. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 7247–7251
29. Lambeth, J. D., and Pember, S. O. (1983) *J. Biol. Chem.* **258**, 5596–5602
30. Guengerich, F. P. (1983) *Biochemistry* **22**, 2811–2820
31. Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C., Gotoh, O., Okuda, K., and Nebert, D. W. (1993) *DNA Cell Biol.* **12**, 1–51
32. Porter, T. D., and Kasper, C. B. (1988) *Biochemistry* **27**, 1682–1687
Molecular Engineering Study on Electron Transfer from NADPH-P450 Reductase to Rat Mitochondrial P450c27 in Yeast Microsomes
Toshiyuki Sakaki, Shiro Kominami, Koji Hayashi, Megumi Akiyoshi-Shibata and Yoshiyasu Yabusaki

J. Biol. Chem. 1996, 271:26209-26213.
doi: 10.1074/jbc.271.42.26209

Access the most updated version of this article at http://www.jbc.org/content/271/42/26209

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 10 of which can be accessed free at http://www.jbc.org/content/271/42/26209.full.html#ref-list-1