J. Microbiol. Biotechnol. (2001), **11**(6), 1011–1017

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Purification and Characterization of a Cytochrome P-450 from Pravastatin-Producing *Streptomyces* sp. Y-110

PARK, JOO-WOONG[1], JOO-KYUNG LEE[1], TAE-JONG KWON, DONG-HEE YI, YONG-IL PARK[2], AND SANG-MO KANG*

[1]Biotechnology Laboratory, Youngjin Research Center, Youngjin Pharmaceutical Co. Ltd., Pyungtaek-Si 451-860, Korea
[2]Environmental Microbiology R.U., Korea Research Institute of Bioscience and Biotechnology, Taejon 305-600, Korea
Department of Microbial Engineering, Konkuk University, Seoul 133-701, Korea

Received: July 20, 2001
Accepted: October 18, 2001

Abstract *Streptomyces* sp. Y-110 cytochrome P-450, induced by the addition of compactin-Na into the culture medium, was purified from the cell extract to apparent homogeniety, mainly by DEAE-Sepharose, hydroxyapatite, and Mono Q column chromatography. The specific activity of purified enzyme on its substrate, compactin-Na, was determined to be 15 nmol of pravastatin per mg protein. The molecular mass of this enzyme on SDS-PAGE was 37±0.5 kDa, pI was 4.5, and its CO difference spectrum showed maximum absorption peaks at 452 and 550 nm, respectively. The N-terminal amino acid sequence was determined to be Met>Thr>Cys>Thr>Pro>Val>Asn>Val>Thr>Val>The>Gly>Ala>Ala>Gly>Gln>Ile>Gly>Tyr>Ala>Leu. Its apparent K_m on compactin-Na was 1.294 $\mu M \cdot min^{-1}$, and V_{max} was 1.028 $\mu M \cdot min^{-1}$. The maximum substrate concentration (K_s) for reaction was 270 μM and thus $1/[K_s]$ was 3.7 μM. These physicochemical characteristics and kinetic behavior of this enzyme were compared and shown to be different from those of *Streptomyces* cytochrome P-450 enzymes reported, suggesting that this enzyme may be an additional member of the *Streptomyces* cytochrome P-450 family.

Key words: Cytochrome P-450, pravastatin, *Streptomyces* sp. Y-110, hydroxylation, enzyme purification, hypercholesterolemia

Pravastatin, an HMG-CoA reductase inhibitor suppressing the biosynthesis of cholesterol, is a potent therapeutic agent for hypercholesterolemia. A soil microorganism capable of modifying compactin to pravastatin by hydroxylation was isolated and identified [unpublished]. This strain was subsequently named as *Streptomyces* sp. Y-110.

*Corresponding author
Phone: 82-2-450-3524; Fax: 82-2-450-3517;
E-mail: kangsm@kkucc.ac.kr

Cytochrome P-450, a photohemeprotein, is widely distributed in mammals, plants [10], and microorganisms, plays an important role in oxidative metabolism of xenobiotics including pharmaceuticals, and is of great importance in medicinal, agricultural, and environmental industries. Cytochrome P-450 is a monooxygenase and, in its reductive form, binds CO, showing its absorption maxima at 447 and 452 nm, respectively. The monooxygenase reaction requires two different substrates and two oxygen atoms: $AH+BH_2+O_2=A\text{-}OH+B+H_2O$, where AH is a major substrate receiving one oxygen atom and BH_2 is an auxiliary one donating hydrogen atoms.

Typically, cytochrome P-450 can be divided into two different types, depending on the requirement of ferredoxin components; microsomal (Type I) and mitochondrial (Type II) [11]. Microsomal cytochrome P-450 (Type I) is generally present in the cytoplasmic reticulum of eukaryotes, and shows relatively broader substrate specificity and transfers electron from its donor, NADPH, to its substrates by the action of cytochrome P-450 reductase containing FAD or FMN. In contrast, mitochondrial cytochrome P-450 (Type II) is found in mitochondria and in some microorganisms with a narrower substrate specificity, and transfers electrons donated from NAD(P)H to its substrates by the action of ferredoxin reductase and ferredoxin-containing FAD. The prosthetic groups of cytochrome P-450 reductase are FAD FMN, those of ferredoxin as [2Fe-2S] cluster, and that of cytochrome P-450 as photoporphyrin IX.

Cytochrome P-450 of *Streptomyces* species was initially used to study physiologically active substances or secondary metabolites such as antibiotics. *Streptomyces* cytochrome P-450 shows a relatively broader substrate specificity and is a microsome type cytochrome P-450, which is closer to eukaryotic systems than *Pseudomonas* cytochrome P-450cam. Consequently, this enzyme has been preferentially used to study metabolic fates of drugs in eukaryotic organisms.

Streptomyces strains have been used for the production of industrially useful substances by hydroxylation [11].

Pravastatin, known as a therapeutic agent for hypercholesterolemia, has been successfully produced in industry by virtue of the hydroxylation reaction of cytochrome P-450. Cytochrome P-450sca of *Streptomyces carbophilus* is induced by exogenous compactin (ML-236B) and hydroxylates the 6-position of compactin, resulting in the formation of pravastatin [6]. This enzyme is one of the first prokaryotic two-components cytochrome P-450 that is composed of NADH-cytochrome P-450 reductase and cytochrome P-450, showing high homology to a microsome type cytochrome P-450 [13].

The isolation and characterization of a soil strain, *Penicillium* sp. Y-8515, that produces compactin, was previously reported [9]. Since then, another soil strain was isolated for its prominent activity to convert compactin into pravastatin and was identified as *Streptomyces* sp. Y-110 [unpublished]. In this study, we report the purification and physicokinetic characterization of a compactin-inducible cytochrome P-450 of *Streptomyces* sp. Y-110.

MATERIALS AND METHODS

Chemicals

Resins and the molecular marker proteins for enzyme purification were purchased from Pharmacia (Sweden). Hydroxyapatite was obtained from Bio Rad (U.S.A.). Dithiothreitol (DTT), ferredoxin, FMN, FAD, $NADP^+$ reductase, NADPH, NADH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and nicotinamide were from Sigma Aldrich (U.S.A.). Other chemicals were of chemical grade or higher.

Media and Culture

Bennett's agar medium was used for subculturing *Streptomyces* sp. Y-110. For the production of cytochrome P-450, unless otherwise stated, the GYP medium containing 1% (w/v) glucose, 1% yeast extract, 0.05% K_2HPO_4, and 0.01% $MgSO_4 \cdot 7H_2O$ at pH 7.0, was used. After the initial growth for 24 h in a 5-l jar-fermenter at 28°C, 200 rpm, and with the aeration rate of 0.5 VVM, cells were allowed for further growth for 18 h with 0.05% (w/v) compactin-Na that was added to the culture to induce cytochrome P-450. Cells were harvested and used as a source of the enzyme.

Quantification of Cytochrome P-450

Cytochrome P-450 concentration was measured by the established method of Omura and Sato [8]. Two milligrams of sodium dithionite were mixed with 3 ml of sample solution and an equal volume of the mixture was separately placed into two cuvettes, and the base line was obtained by using a Shimatsu split-beam spectrophotometer at 400–500 nm. The sample cuvette was saturated with 30 to 40 bubble drops of carbon monooxide at a rate of 1 bubble/sec. It was put back into the spectrophotometer and then the absorbance level was measured at 400–500 nm until the peak reached its maximum at 450 nm. The concentration of cytochrome P-450 was determined as follows:

$$[(A_{450-490})_{observed}-(A_{450-490})_{baseline}]/0.091 = \text{cytochrome P-450 (nm)/ml}$$

where A_{490} is a reference point and the extinction coefficient at 450 to 490 nm is 91 $mM^{-1}cm^{-1}$.

Hydroxylase Activity

Crude enzyme preparation: The hydroxylase reaction mixture contained a cell extract, 0.233 mM compactin-Na, 0.26 mM NADH or NADPH in a final volume of 200 µl of 100 mM potassium-PBS (pH 7.4) and was incubated at 30°C for 30 min. Hydroxylase activity was determined by measuring the pravastatin produced after the reaction.

Purified enzyme preparation (NADPH generation system): The reaction mixture for the purified hydroxylase activity contained a portion of the purified cytochrome P-450, 0.26 mM NADPH or NADH, ferredoxin-$NADP^+$ reductase (0.04 unit), 320 µg of ferredoxin, 14 mM glucose-6-phosphate, glucose-6-phosphate dehydrogenase (0.2 unit), 10 mM nicotinamide, 2.5 mM $MgCl_2$, and 0.233 mM compactin-Na in a final volume of 200 µl of 100 mM potassium-PBS, pH 7.4.

The reaction mixture was incubated for 30 min at 30°C, and the reaction was stopped by adding 10 µl of 6 M NaOH, and then the pravastatin produced was measured as described above [6].

Purification of Cytochrome P450

Purification of cytochrome P-450 was performed following a modified method of Matsuoka *et al.* [6] and Michael *et al.* [7]. Briefly, 200 g (wet weight) of cells were suspended in 400 ml of buffer A (100 mM potassium-PBS, 20% glycerol, 2 mM DTT, 0.1 mM EDTA, 0.2 mM PMSF, pH 7.4), disrupted by treating the cell suspension for 1 cycle at 5°C in a microfluidizer (Microfluidic Co., U.S.A.), and centrifuged at 100,000 ×g for 1 h. The supernatant was taken, dialyzed against buffer A, and concentrated in a UF system (MWCO 10,000) to 20 mg protein/ml.

Ion-Exchange Chromatography

A portion of the concentrated enzyme preparation was loaded onto the top of the DEAE-Sepharose (Fast Flow) column (5×20 cm) preequilibrated with buffer A and the column was washed with 3 volume of the same buffer. The enzyme bound was then eluted with a linear gradient of NaCl (0 to 0.3 M) in buffer A at a flow rate of 5 ml/min. Each fraction was assayed for the cytochrome P-450 activity at 417 nm by using CO difference spectrometry,

and active fractions were pooled and concentrated on a UF system.

Hydroxyapatite Chromatography

The combined fractions from the DEAE-Sepharose column was dialyzed against an excess volume of buffer B (20 mM potassium-PBS, 20% glycerol, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, pH 7.4) and applied to the hydroxyapatite column (2.5×20 cm) preequilibrated with the same buffer. The column was washed with 180 ml of buffer B and then eluted with buffer B, but with increasing concentrations of potassium-PBS (20 to 300 mM), at a rate of 0.5 ml/min. The cytochrome P-450-positive fractions were pooled, dialyzed in buffer B, and concentrated on a UF system.

Anion Exchange Chromatography

The pooled fractions from the hydroxyapatite column was loaded onto a Mono Q® HR 5/5 type anion-exchange column (5×50 mm) preequilibrated with buffer B. The column was washed with the same buffer and eluted with a linear gradient of NaCl (0 to 0.3 M) in buffer A at a flow rate of 1 ml/min. The active fractions were combined and concentrated as above. The combined fractions from the first Mono Q® column were applied to the 2nd Mono Q® column preequilibrated with buffer C (10 mM potassium-PBS, 20% glycerol, 1 mM DTT, pH 7.4) and the column was washed with the same buffer containing 0.15 M NaCl at 0.5 ml/min and then eluted with increasing concentrations of NaCl (0.15–0.3 M). The cytochrome P-450-positive fractions were dialyzed, concentrated in 50 mM potassium-PBS (pH 7.4) containing 20% glycerol, and stored at −70°C.

Homogeneity of the Cytochrome P-450

Nondenaturing polyacrylamide gel electrophoresis (n-PAGE) was performed on Phast-gel gradient (8–25%) under nonreducing condition by using Phastsystem (Pharmacia, Sweden). SDS-PAGE with Phast-gel gradient, 8–25%, was performed by following the procedure that was described by Laemmli [3]. The protein was stained with Coomassie Brilliant Blue G-250.

Determination of Molecular Weight and Isoelectric Point

The molecular weight of the purified cytochrome P-450 was determined by using Superose® 6HR column chromatography (1×3 cm). The molecular weight markers used were bovine serum albumin (MW 67 kDa), ovalbumin (45 kDa), chymotrypsin A (25 kDa), and ribonuclease A (13.7 kDa), and Blue dextran 2000 was used for column calibration. The subunit molecular weight was estimated by SDS-PAGE by using a Phast-gel gradient, 8–25%. The marker proteins used were phosphorylase b (MW 94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). The isoelectric point of the purified enzyme was determined according to the method of Allen *et al.* [1]. A portion of purified enzyme and pI marker proteins were electrophoresed on a Phast-gel (IEF 3-9) and were silver-stained by following the method of PhastSystem (Phamacia, Sweden).

Analysis of N-Terminal Amino Acid Sequence

After SDS-PAGE, proteins resolved on the gel were blotted to the PVDF membrane at 20 V for 90 min, following the method of Matsudaira [5]. The blotted proteins on PVDF membrane were stained with Coomassie Brilliant Blue R-250 and then detached from the membrane. The N-terminal amino acid sequence was determined by using a 491A protein sequencer (Perkin Ellmer, U.S.A.).

Kinetics

The kinetic parameters of the purified enzyme, K_m and V_{max}, were determined with varying concentrations of the substrate (0.23 to 1.74 mmol), compactin-Na, in an NADPH generation system [14].

RESULTS AND DISCUSSION

Induction of Cytochrome P-450 and Solubilization

Cells were collected from the culture supplemented with compactin-Na, which was added to induce cytochrome P-450, and were extracted according to the methods of Michael *et al.* [7] and Matsuoka *et al.* [6]. The cell extracts were centrifuged at 100,000 ×g and the supernatant was assayed for hydroxylase activity in the NADPH generation system. As shown in Table 1, the hydroxylase activity of *Streptomyces* sp. Y-110 cell extracts was 3-folds higher than that of *Streptomyces carbophilus* [6]. On the other hand, the crude enzyme preparation obtained from the cells cultured with compactin-Na showed its highest peak at 450 nm on the CO difference spectrum (Fig. 1), similar to *Streptomyces carbophilus* cytochrome P-450sac [6] and *Streptomyces griseus* cytochrome P-450soy [7].

Purification of Cytochrome P-450

The cell-free extracts of *Streptomyces* sp. Y-110 containing cytochrome P-450 activity were subjected to DEAE-Sepharose column chromatography and the bound enzyme

Table 1. Induction of hydroxylation activity.

Induction	Hydroxylation of compactin-Na to pravastatin-Na (nmol product · mg protein^{-1} · min^{-1})
None	0
Compactin-Na	1.8

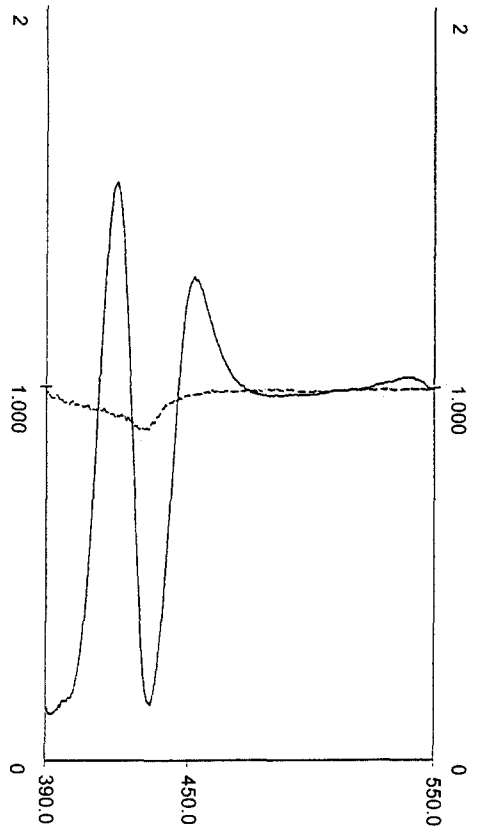

Fig. 1. CO difference spectrum of the crude enzyme preparation from cells cultured in the presence (—) or absence (---) of 0.05% compactin-Na.

was eluted with 0.3 M NaCl. Fractions were examined spectrophotometrically for the presence of heme proteins at 417 nm, showing two separate peaks, 1 and 2 (Fig. 2). CO difference spectra of peaks 1 and 2 showed their highest absorption level at 450 and 420 nm, respectively, suggesting that the peak 2 at 420 nm is attributable to the presence of a variant of cytochrome P-450, cytochrome P-420 (data not shown). The peak 1 fraction obtained from DEAE-Sepharose column was concentrated and applied

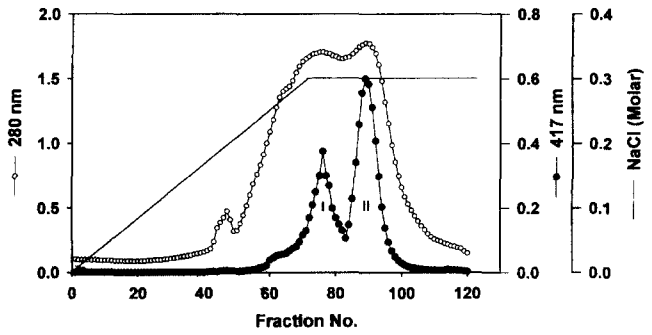

Fig. 2. DEAE-Sepharose column chromatography of the cytochrome P-450 from *Streptomyces* sp. Y-110.

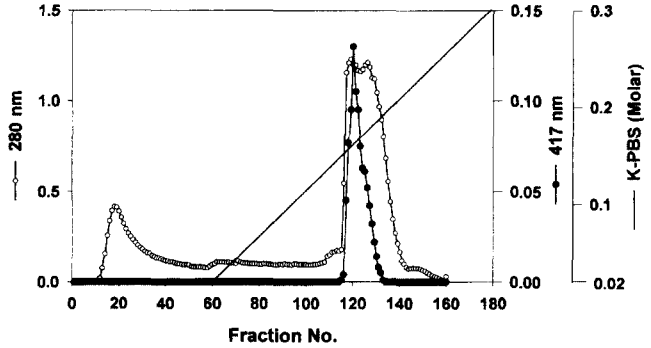

Fig. 3. Chromatography of the DEAE-Sepharose fraction (peak 1) on hydroxyapatite column.

to the hydroxyapatite column (Fig. 3) and the 417 nm sensitive fractions were then subjected twice to the Mono Q® column (Fig. 4). The cytochrome P-450 was finally purified with a specific activity of 15 nmol of pravastatin/mg protein, with 121-folds purification and 3.2% yield (Table 2). The purity of the purified cytochrome P-450 was evidenced by a single band on SDS-PAGE gel (Fig. 5).

Molecular Weight Determination

Molecular weight of the purified cytochrome P-450 was determined by Superose® 6HR column chromatography using marker proteins; the "y" value of marker proteins was $304.2910^{-0.0696x}$ (Fig. 6). The molecular weight of the purified *Streptomyces* sp. Y-110 cytochrome P-450 was 37.1 kDa. n-PAGE of this enzyme showed a single band (Fig. 5). Its molecular weight was shown to be 37.9 kDa when examined by SDS-PAGE; the "y" value of marker proteins on the standard curve was $22.06910^{-0.51016x}$ (Fig. 7), indicating that *Streptomyces* sp. Y-110 is a single polypeptide protein. The molecular weights of *S. carbophilus* cytochrome P-450sca, *S. griseus* cytochrome P-450soy, and *Nocardia* NH1 cytochrome P-450su1 and P-450su2 were 46±1, 48, and 44.3 and 44.4 kDa, respectively [12]. Therefore, it seems that, as far as its molecular weight

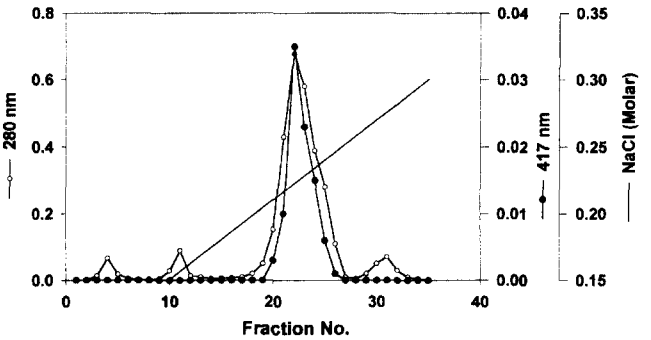

Fig. 4. The second Mono Q® ion-exchange column chromatography of the first Mono Q® column fractions.

Table 2. Summary of cytochrome P-450 purification from *Streptomyces* sp. Y-110.

Purification step	Protein (mg)	Total Cyt P-450 (nmol)	Specific activity (nmol · mg protein^{-1} · min^{-1})	Purification (fold)[a]	Yield (%)[b]
Supernatant	3735.0	463	0.124	1.0	100
DEAE-Sepharose	289.0	310	1.073	8.65	66.9
Hydroxyapatite	24.8	136	9.53	76.9	28.2
Mono Q(I)	14.3	44	12.5	100.8	9.6
Mono Q(II)	1.0	15	15.0	121.0	3.2

[a]Fold increase was determined based on specific content of cytochrome P-450.
[b]Yield was calculated based on total cytochrome P-450.
Symbol: Cyt P-450, cytochrome P-450.

is concerned, cytochrome P-450 of *Streptomyces* sp. Y-110 is likely to be a new species of cytochrome P-450.

Isoelectric Point

Using the Phast IEF 3–9 gel system, the isoelectric point of the purified *Streptomyces* sp. Y-110 cytochrome P-450 was determined to be 4.5 (Fig. 8). It has been reported that the pI of *B. megaterium* cytochrome P-450meg and *R. japonicum* cytochrome P-450c was 4.9 but that of *P. putida* cytochrome P-450cam was 4.5 [2]. In spite of the differences in microbial species, molecular weights, substrate specificities, and the identical pI value of 4.5 of *P. putida* cytochrome P-450cam and *Streptomyces* sp. Y-110 cytochrome P-450 suggested that both enzymes might contain similar levels of hydrophobic amino acids.

Spectral Characteristics

The spectral characteristics of the purified *Streptomyces* sp. Y-110 cytochrome P-450 were examined on a CO difference spectrum. It was reported that when hemeprotein was reduced with sodium dithionite, the maximum absorption peak of cytochrome P-450 appeared at near 417 nm and that of the absolute CO-ferrous form (CO-dithionite-reduced form) was at 449 nm [15]. The CO difference spectrum of *Streptomyces* sp. Y-110 cytochrome P-450 showed two peaks at 452 nm and 550 nm, respectively; however, no other significant peak was observed (Fig. 9).

N-Terminal Amino Acid Sequence

A sequence of the N-terminal end 20 amino acids of the purified *Streptomyces* sp. Y-110 cytochrome P-450 was determined, by using an amino acid sequence analyzer, to be as follows: Met>Thr>Cys>Thr>Pro>Val>Asn>Val>Thr>Val>The>Gly>Ala>Ala>Gly>Gln>Ile>Gly>Tyr>Ala>Leu. This sequence was compared with those of cytochrome P-450 enzymes previously reported [12] and showed no homology (Fig. 10). This result suggested *Streptomyces* sp. Y-110 cytochrome P-450 to be a new enzyme.

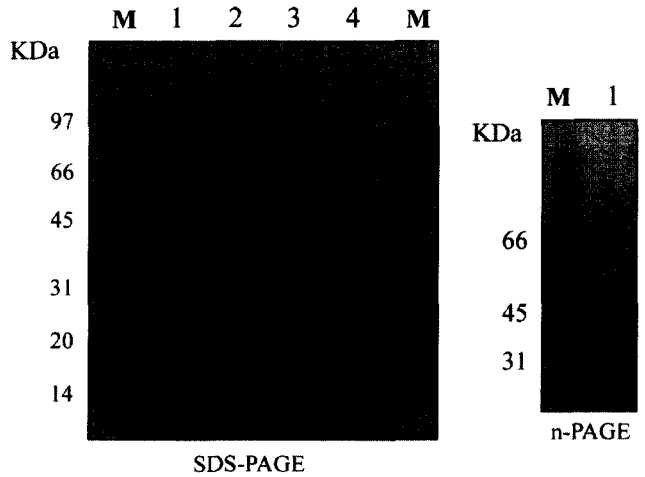

Fig. 5. SDS-PAGE and n-PAGE of cytochrome P-450 from *Streptomyces* sp. Y-110.
Lane M, standard low markers; Lane 1, eluate after Mono Q(II) chromatography; Lane 2, eluate after hydroxyapatite chromatography; Lane 3, eluate after ion-exchange chromatography; Lane 4, supernatant after centrifugation.

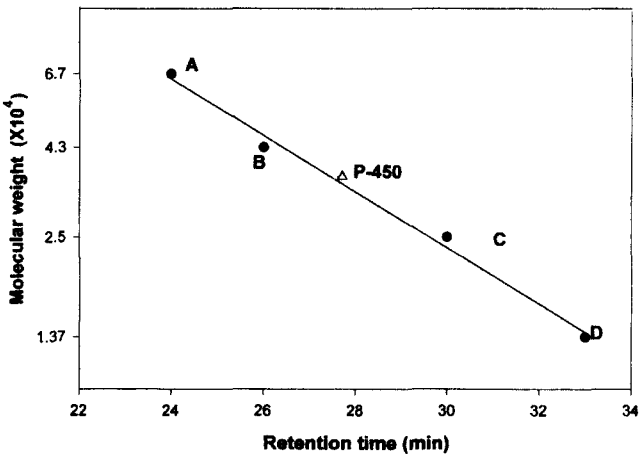

Fig. 6. Reationship between the retention times and molecular weights of cytochrome P-450.
The molecular weight markers were bovine serum albumin (67 kDa, A), ovalbumin (43 kDa, B), chymotrypsinogen A (25 kDa, C), and ribonuclease A (13.7 kDa, D)

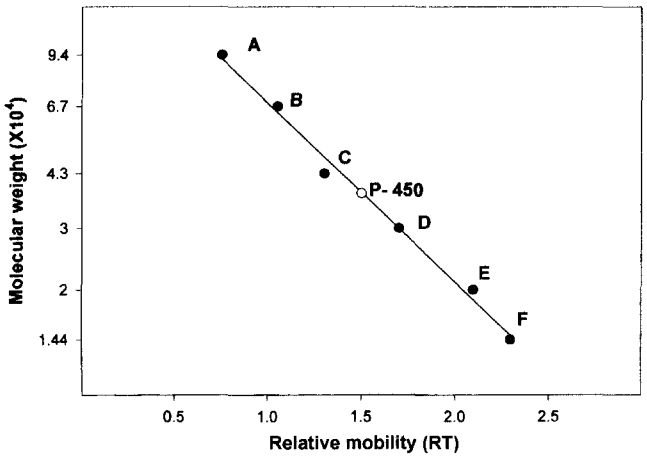

Fig. 7. SDS-PAGE of the purified cytochrome P-450.
The molecular weight markers were phosphorylase b (94 kDa, A), albumin (67 kDa, B), ovalbumin (43 kDa, C), carbonic anhydrase (30 kDa, D), trypsin inhibitor (20.1 kDa, E), and α-lactalbumin (14.4 kDa, F).

Hydroxylation Reaction by Purified Cytochrome P-450

The purified *Streptomyces* sp. Y-110 cytochrome P-450 *in vitro* catalyzed bioconversion of compactin-Na to pravastatin by hydroxylation reaction in the presence of ferredoxin, ferredoxin-NADP$^+$ reductase, NADPH-generating system, and O_2 (Table 3). Since cytochrome P-450 was purified to an apparent homogeneity, it required exogenous electron mediators for its *in vitro* hydroxylation activity, whereas the crude enzyme preparation did not (Table 1).

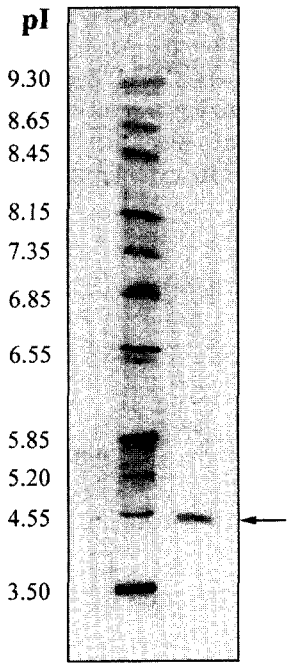

Fig. 8. Isoelectric focusing of the *Streptomyces* sp. Y-110 cytochrome P-450.
Standard proteins used were aminoglucosidase (pI, 3.50), soybean trypsin inhibitor (pI, 4.55), β-lactoglobulin A (pI, 5.20), bovine carbonic anhydrase B (pI, 5.85), human carbonic anhydrase B (pI, 6.55), myoglobin acidic band (pI, 6.85), myoglobin-basic band (pI, 7.35), lentil lectin-acidic band (pI, 8.15), lentil lectin-middle band (pI, 8.45), lentil lectin-basic band (pI, 8.65), and trypsinogen (pI, 9.30).

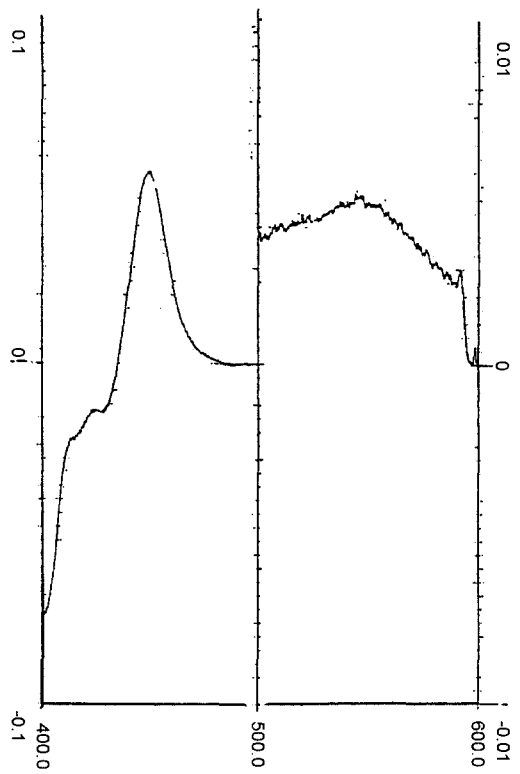

Fig. 9. CO difference spectrum of *Streptomyces* sp. Y-110 cytochrome P-450.

Kinetic Behavior of *Streptomyces* sp. Y-110 Cytochrome P-450

After the enzyme was incubated with varying concentrations of compactin-Na (0.01–0.1%), the Michaelis-Menten constant (K_m) and V_{max} were determined on a Lineweaver-Burk plot.

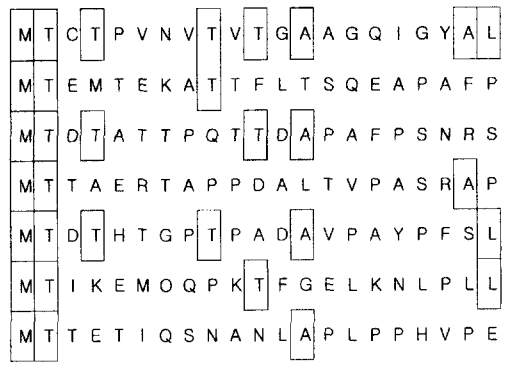

Fig. 10. Comparison of N-terminal sequences of various cytochrome P-450 enzymes.

Table 3. Hydroxylation of compactin-Na to pravastatin-Na by purified cytochrome P-450 in the presence of an electron transport system.

Reaction mixture	Hydroxylation of compactin-Na to pravastatin-Na
	nmol product nmol P-450^{-1} · min^{-1}
Complete system	4.75
Minus cytochrome P-450	0
Minus ferredoxin	0
Minus ferredoxin: NADP^{+} reductase	0

The K_m and V_{max} of this enzyme was 1.294 μM · min^{-1} and 1.028 μM · min^{-1}, respectively. The substrate concentration for its maximum reaction (K_s) was 270 M and thus 1/[K_s] was 3.7 μM. When compared to those of *S. carbophilus* cytochrome P-450sca-1 and P-450sca-2, 5.6 μM and 4.4 μM, respectively, and that of *P. putida* cytochrome P-450cam against its substrate, camphor, 0.47+0.07 μM [11], 1/[K_s] of *Streptomyces* sp. Y-110 cytochrome P-450 implied relatively lower substrate recognition to its substrate, compactin-Na. This was probably due to the fact that this inducible enzyme performed hydroxylation as well as detoxification reactions [4].

The physicochemical and kinetic characteristics of the purified *Streptomyces* sp. Y-110 cytochrome P-450 were different from other *Streptomyces* cytochrome P-450 enzymes. We earlier examined on fermentation parameters for the production of pravastatin from compactin by the in-house isolate, *Streptomyces* sp. Y-110 [unpublished]. Comparing to other microorganisms that are currently used for industrial production of pravastatin, the higher production of pravastatin from compactin by this strain [unpublished] and the significantly different physicochemical and kinetic characteristics of the cytochrome P-450 of this strain strongly suggest a possibility that strain Y-110 could fully be applicable for the industrial level production of pravastatin from compactin. The present results showed that the cytochrome P-450 of *Streptomyces* sp. Y-110 may be an additional member of the *Streptomyces* cytochrome P-450, although more detailed information on the structure of heme-binding protein, amino acid sequence, and electron mediators involved in the hydroxylation reaction are needed.

REFERENCES

1. Allen, R. C., C. A. Saravis, and H. R. Maurer. 1984. Isoelectric focusing multiparameter techniques, pp. 63–147. *In: Gel Electrophoresis and Isoelectric Focusing of Proteins.* Walter de Gruyter. Berlin, Germany.
2. Anders B., M. I. Sundberg, and J. A. Gustafasson. 1979. Purification and characterization of cytochrome P-450meg. *J. Biol. Chem.* **254:** 5264–5271.
3. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.
4. Masahiko, H., K. Ogawa, and H. Yoshikawa. 1993. Application of computer to monitoring and control of fermentation process: Microbial conversion of ML-236B Na to pravastatin. *Biotech. Bioengin.* **42:** 815–820.
5. Matsudaira, P. 1987. Sequence from picomole quantities of proteins electroblotted onto polyvinydene difluoride membranes. *J. Biol. Chem.* **262:** 10035–10038.
6. Matsuoka, T., S. Miyakoshi, K. Tanzawa, K. Nakahara, M. Hosobuchi, and N. Serizawa, 1989. Purification and chracterization of cytochrome P-450sca from *Streptomyces carbophilus. Eur. J. Biochem.* **184:** 707–713.
7. Michael, K. T., F. S. Sariaslani, and D. P. O'Keefe. 1989. Purification and characterization of a soybean flour-induced cytochrome P-450 from *Streptomyces griseus. J. Bacteriol.* **171:** 1781–1787.
8. Omura, T. and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* **239:** 2370–2378.
9. Park, J. W., J. K. Lee, T. J. Kwon, D. H. Park, and S. M. Kang. 2000. Production of compactin from *Penicillium* sp. Y-8515. *Kor. J. Appl. Microbiol. Biotechnol.* **28:** 291–297.
10. Park, S. H., Y. J. Chang, K. H. Kim, and S. U. Kim. 1999. Involvement of cytochrome P450 in (-)-(4R)-isopiperitenone oxidation by cell suspension cultures of *Mentha piperita. J. Microbiol. Biotechnol.* **9:** 147–149.
11. Sariaslani, F. S. 1991. Microbial cytochrome P-450 and xenobiotic metabolism. *Advance in Applied Microbiology* **36:** 132–178.
12. Sariaslani, F. S. and C. A. Omer. 1992. Actinomycete cytochrome P-450 involved in oxidative metabolism: Biochemistry and molecular biology. *Critical Reviews in Plant Sciences* **11:** 1–6.
13. Serizawa, N. and T. Matsuoka. 1991. A two component-type cytochrome P-450 monooxygenase system in a prokaryote that catalyzes hydroxylation of ML-236B to pravastatin, a tissue-selective inhibitor of 3-hydroxy-3-methyglutaryl coenzyme A reductase. *Biochimica et Biophysica Acta* **1084:** 35–40.
14. Wang, D. I. C., C. L. Cooney, A. L. Demain, P. Dunnill, A. E. Humphrey, and M. D. Lilly. 1979. *Fermentation and Enzyme Technology.* John Wiley & Sons Press. NY, U.S.A.
15. Waxman, D. J. and C. Walsh. 1982. Phenobarbital-induced rat liver cytochrome P-450 purification and characterization of two closely related isozymic forms. *J. Biol. Chem.* **257:** 10446–10457.