Mutation of Histidine 373 to Leucine in Cytochrome P450c17 Causes 17α-Hydroxylase Deficiency*

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We identified a new homozygous missense mutation His373 → Leu in the CYP17 gene of two sisters with 17α-hydroxylase deficiency with an elevated plasma aldosterone concentration by sequencing their genomic DNAs amplified by polymerase chain reaction. Using polymerase chain reaction-based site-directed mutagenesis, we prepared a DNA that encoded the Leu373 mutant protein. COS-1 cells transfected with the mutant DNA, despite having an RNA hybridizable to the P450c17 cDNA, did not show 17α-hydroxylase and 17,20-lyase activities. Also, the cells were devoid of 11β-hydroxylase and aldosterone synthase activities. To examine the mechanism by which the single amino acid change His373 → Leu eliminates activity, we expressed N-terminally modified P450c17 proteins with and without the Leu373 mutation in Escherichia coli and performed spectral studies. Membrane preparations from E. coli cells expressing the wild-type form of the modified enzyme showed an absorption peak at 449 nm upon addition of carbon monoxide in the reduced state and produced characteristic substrate-induced difference spectra, whereas those from the cells expressing the mutant form did not show these spectral changes. The 17α-hydroxylase and 17,20-lyase activities were observed only in E. coli cells expressing the wild-type enzyme. These results show that the His373 → Leu mutant does not incorporate the heme prosthetic group properly and suggest a critical role of His373 in heme binding.

Steroid 17α-hydroxylase (P450c17) has a dual function of catalyzing the 17α-hydroxylation of pregnenolone and progesterone and the 17,20-cleavage of the corresponding hydroxylated steroids (1, 2). The enzyme is encoded by a single gene CYPl7 that is located on chromosome 10q24-q25 (3, 4). The gene consists of eight exons that have been completely sequenced (5) and is expressed in the adrenal and gonadal glands (6). Deficiency of 17α-hydroxylase leads to decreased production of glucocorticoids and sex steroids, and this in turn increases ACTH secretion. This hormonal imbalance causes hypertension, pseudohermaphroditism in the male, and primary amenorrhea and hypogonadism in the female (7).

Since the first description by Biglieri et al. (8) in 1966, well over 120 cases with 17α-hydroxylase deficiency (17-OHD) have been reported (7, 9). Patients with this disease are usually characterized by a very low or undetectable plasma level of aldosterone. The depletion of aldosterone is thought to result from sodium retention; expansion of the extracellular fluid volume suppresses the renin-angiotensin system, thereby decreasing aldosterone secretion from the adrenal. However, several cases with this disease in Japan and the United States have been shown to have elevated aldosterone levels with suppressed renin activities (9). Whereas the molecular defects have been elucidated recently in several 17-OHD cases, no structural analysis has been done for the CYPl7 gene of 17-OHD patients with elevated serum aldosterone concentrations. We report here two Japanese sisters with 17-OHD associated with hyperaldosteronism caused by a homozygous missense mutation of His373 to leucine in the CYPl7 gene.

MATERIALS AND METHODS

Patients—The patients described in this report are two Japanese sisters who live in Nagano Prefecture in central Japan. Their clinical features were described in detail previously (10). These two sisters, currently 24 and 20 years old, sought medical attention for hypertension and sexual infantilism at ages 17 and 13, respectively. Both had a 46 XY karyotype, and neither showed pubertal development. Increased responses of luteinizing and follicle-stimulating hormones to luteinizing hormone-releasing hormone were observed in both cases. They had high plasma levels of 17-deoxysteroids including aldosterone, low to normal levels of 17α-hydroxysteroids, and low levels of sex steroids. The concentrations of 17-deoxysteroids returned to normal levels by administration of dexamethasone. Their plasma concentrations of aldosterone were high, but the plasma renin activity was not increased. Their father and grandmother had hypertension but not hypogonadism, and their mother and other sister were normotensive and showed normal sexual development.

Preparation of Genomic DNA—Peripheral blood was collected from each member of the family, from a normal person, and from a 17-OHD patient unrelated to the family. Leukocytes were isolated by Ficoll-Hypaque density gradient centrifugation and lysed in 10 mM Tris-HCl (pH 8.0), containing 100 mM NaCl, and 0.1 mg/ml proteinase K.

1 The abbreviations used are: ACTH, adrenocorticotropic hormone; 17-OHD, 17α-hydroxylase deficiency; bp, base pair(s); kb, kilobase(s); IPTG, isopropyl-1-thio-β-D-galactopyranoside; Mops, 3-(N-morpholino)propanesulfonic acid; PCR, polymerase chain reaction; 17-OH pregnenolone, 17α-hydroxy pregnenolone; 17α-hydroxyprogrenolone; 16-OH progesterone, 16α-hydroxy progesterone; 17α-hydroxyprogesterone; 18-α corticosterone, 18-hydroxycorticosterone; DMEM, Dulbecco's modified Eagle's medium.
ase K. After standing overnight at 37 °C, DNA was extracted with phenol/chloroform and precipitated with ethanol. The DNA was dissolved in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

**Southern Blotting**—Samples of genomic DNA were digested with EcoRI, BamHI, and HindIII, and fractionated by electrophoresis on a 0.8% agarose gel. Following electrophoresis, the DNA fragments were transferred to a nitrocellulose membrane (Hybond N, Amersham Corp.) and probed with a 32P-labeled full-length human P450c17 cDNA (6). Radiolabeling of the cDNA probe was carried out by the random primer labeling method (11) using [α-32P]dCTP (111 TBq/mmol, ICN Biomedicals, Inc.). The filter that had been prehybridized in 6 × SSC, 5 × Denhardt’s reagent, 1% SDS, 0.1 mg/ml heat-denatured salmon testis DNA at 67 °C for 6 h was incubated with hybridization solution containing 6 × SSC, 1% SDS, and the 32P-labeled probe (10 6 cpm/ml) at 67 °C for 16 h. The filter was washed twice with 2 × SSC, 1% SDS, and twice with 0.1 × SSC, 0.1% SDS at 65 °C for 30 min.

**DNA Amplification—Exons 1–8 of the CYP17 gene were individually amplified by PCR (12). Oligonucleotides used as primers were synthesized on an Applied Biosystems 381A DNA synthesizer and are shown in Table I. Each PCR reaction mixture (100 μl) contained 1 μg of genomic DNA, 50 μl of each primer, 200 μM each of dATP, dGTP, dTTP, and dCTP, 6 mM MgCl2, and 5 units of Taq DNA polymerase in 10 mM Tris-HCl (pH 8.0). All amplifications were done in a thermal cycler (Perkin-Elmer Cetus Instruments) with the following program: 1) denaturation at 96 °C for 2 min; 2) followed by 40 cycles of denaturation (96 °C, 15 s), annealing (55 °C, 30 s), and extension (72 °C, 1 min); and 3) final extension (72 °C, 2 min). The PCR products were electrophoresed on a 1% agarose gel, and the DNAs with expected sizes were recovered from the gel using Suprec-01 (Takara Shuzo) and used as templates for asymmetric PCR amplification. Asymmetric PCR was done with a molar ratio of primers of 201 (13).

To achieve allele-specific amplification of exon 6, the following primers were synthesized: primer W, 5′-GAGGATCACCAGTGGCCTTGTG-3′, and primer M, 5′-GGGATGCAACGTTGGCCTTAGA-3′, where the underlined A was the substituted nucleotide found in the genes of the patients. Using these primers and primer 5′-6′ (Table I), asymmetric PCR was carried out under the same conditions described above except that the number of cycles of denaturation, annealing, and extension was reduced to 20.

Each single-stranded DNA amplified by PCR was sequenced by the dideoxy method (14), using the Sequenase version 2.0 kit (U. S. Biochemical Corp.). A sequencing primer of 17-20 bp was synthesized (TGT-3′, and primer M, 5′-GGGATGCAACGTTGGCCTTAGA-3′), and primer M, 5′-GGGATGCAACGTTGGCCTTAGA-3′, where the underlined A was the substituted nucleotide found in the genes of the patients. Using these primers and primer 5′-6′ (Table I), asymmetric PCR was carried out under the same conditions described above except that the number of cycles of denaturation, annealing, and extension was reduced to 20.

**Constitution of Eukaryotic Expression Vectors**—The vector expressing the wild-type P450c17 protein in COS-1 cell was constructed by ligating the P450c17 cDNA to the EcoRI site of the eukaryotic expression vector pcDL-SRa 296 (15). The P450c17 cDNA encoding the His93 to Leu (the changed nucleotide is underlined) and contains a HincII site (indicated by boldface letters). The conditions for PCR amplification (underlined letters are changed nucleotides). The conditions for PCR were the same as those described above under "Construction of Eukaryotic Expression Vectors." The PCR products were digested with XbaI, and the resulting 306-bp blunt end XbaI fragment was inserted into the blunt end NdeI-XbaI site of pCWori+ (19, 20). Barnes et al. (21) succeeded in producing large amounts of bovine P450c17 in E. coli by modifying the 5′-sequence of the bovine P450c17 cDNA; the second codon was changed from TGG (Trp) to GCT (Ala), and silent mutations were introduced to codons 4–7 so as to make the 5′-sequence AT-rich. Thus, we prepared a human P450c17 cDNA in which the nucleotide sequence of the first seven codons was identical to that of the modified bovine cDNA. A 38-base 5′-primer, 5′-TGGGTCTGTATGATTAGCATTTGGCCTTAGA-3′, corresponding to amino acids 139–146 were synthesized and used for PCR amplification. The PCR products were digested with XbaI, and the resulting 306-bp blunt end XbaI fragment was inserted into the blunt end NdeI-XbaI site of pCWori+ (19, 20). Finally, the expression plasmids were constructed by ligating the 1.4-kb XbaI-BglII fragment of P450c17 cDNA (encoding His93 to Leu without the Leu373 mutation) into the XbaI site of pCWori+ carrying the PCR product described above. The cDNAs encoding the modified wild-type and the modified Leu373 mutant P450c17s are designated modP17-W and modP17-M, respectively.

**Bacterial Expression of Recombinant Plasmids, Preparation of Membranes, and Spectral Studies**—An overnight culture of E. coli DH5α transformed with modP17-W or M was grown at 37 °C in LB medium containing 50 μg/ml ampicillin. A 4-ml aliquot thereof was used to inoculate 200 ml of Terrific broth (22) containing ampicillin. The cells were harvested by centrifugation, washed with 50 mM Mops buffer (pH 7.5) containing 100 mM KCl, 1 mM EDTA, and 1 mM dithiothreitol, and resuspended in the same buffer. For preparation of membranes, the cells were treated with 0.2 mg/ml lysozyme, and the resulting spheroplasts were lysed by sonication. After brief centrifugation at 7,000 × g to remove unbroken cells and debris, the supernatant was made to 6 mM MgCl2 and again centrifuged at 60,000 × g for 50 min at 4 °C. The pellet was suspended in the Mops buffer and homogenized. An aliquot of 20 μg of protein was made up to 6 ml with the same buffer containing 10 mM glucose, and the mixture was divided equally between two cuvettes. Several grains of sodium dithionite were added to each cuvette, and the base-line spectrum was recorded. CO was then bubbled through the sample cuvette to obtain the difference spectrum. Anomalously high CO binding (24) were measured similarly by adding a steroid substrate dissolved in ethanol to the sample cuvette and an equal volume of ethanol to the reference cuvette. The difference spectrum was re-
corded between 350 and 550 nm in a Gilford RESPONSE spectrophotometer.

Western Blot Analysis—Proteins were treated with 1% SDS, 5% 2-mercaptoethanol at 100 °C for 2 min before electrophoresis. SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli (25) using an 8% polyacrylamide gel. The separated proteins were electrottransferred to nitrocellulose membrane (Schleicher and Schuell). The membrane was incubated for 1 h at room temperature in 20 mM Tris-HCl (pH 7.5), 137 mM NaCl, 0.1% Tween 20 (TBS-T) containing 5% dry milk to block excess binding sites, followed by washing with TBS-T three times. The membrane was then incubated for 1 h with anti-porcine P450c17 antibody (Oxygene, Dallas) that had been diluted with TBS-T and treated with membrane preparations from untransformed E. coli DH5α. After washing three times with TBS-T, the antigen-antibody complex was reacted with goat anti-rabbit IgG antibody conjugated with horseradish peroxidase (Amersham Corp.) and was washed three times with TBS-T. The antigen-antibody complex was detected by chemiluminescence after addition of luminol and hydrogen peroxide (ECL, Amersham Corp.).

Steroid Metabolism by E. coli Expressing P450c17—E. coli DH5α cells were cultured and induced as described above. At 24 h after addition of IPTG, a radiolabeled steroid ([^3]C)pregnenolone, [^3]H)pregnenolone, or [^3]H)progesterone) was added to 2 ml of the culture, and the mixture was incubated for 24 h at 30 °C with gentle shaking. The products were extracted and analyzed by TLC as described above.

RESULTS

Southern Blot Analysis—The patients described here are two sisters who were diagnosed as having 17-OHD from steroid profiles and other symptoms (10). Genomic DNAs obtained from leukocytes of the two patients and an unaffected person were each digested with restriction endonucleases EcoRI, BamHI, and HindIII. Fig. 1 shows Southern blot analysis of the DNA digests. In each case, EcoRI yielded fragments of 5.7 and 6.8 kb; BamHI yielded fragments of 1.7, 5.8, and 16 kb; and HindIII yielded fragments of 1.25, 2.35, and 11 kb. These results indicate that there is no large deletion or insertion in either allele of the P450c17 gene of these patients.

In order to determine if there exist base changes in the P450c17 gene of the patients, each exon of the gene was amplified by PCR and sequenced. Only exon 6 of the patients was found to contain a homozygous missense mutation changing codon 373 from histidine (CAC) to leucine (CTC) (Fig. 2A). The sequence ladders for other members of the family examined all showed a heterozygous pattern CAC/CTC for codon 373, indicating that they have the same mutation in one allele and the normal sequence in the other (Fig. 2B). We also found, in all subjects examined, silent mutations in exons 1 and 5: His46, CAT → CAC; Ser65, TCT → TCG; Asp280, GAT → GAC. The same silent mutations were reported by Kagimoto et al. (26) in a patient with 17-OHD and a normal control.

Allele-specific Amplification—To confirm the results of the sequence analysis, we performed allele-specific PCR amplification of genomic DNA (Fig. 3). The 5'-primer used was 5′-CAAGCCTGGCTGTTTGAACTG-3′, and the 3′-primer was either W, containing the normal sequence, or M, containing the mutated nucleotide (Table I). Using either 3′-primer, a DNA of expected size (682 bp consisting of part of intron 4, exon 5, intron 5, and part of exon 6) was produced for the heterozygotes. In contrast, only when the 3′-primer was W, but not M, could we amplify the DNA from a normal control and a patient unrelated to the family. Similarly, we could amplify the DNA from the patients only when the 3′-primer was M.

Enzyme Activity of the His373→Leu Mutant Expressed in COS-1 Cells—To estimate the catalytic activity of the His373→Leu mutant, we prepared a cDNA encoding the mutant protein by PCR-based site-directed mutagenesis and expressed it in COS-1 cells. Northern blot analysis revealed that the cells transfected with the vector containing either the wild-type or mutant cDNA produced comparable amounts of RNA hybridizable to the P450c17 cDNA probe, whereas the cells transfected with the control vector produced no hybridizable RNA (Fig. 4). When incubated with Δ substrates, the cells transfected with the wild-type P450c17 cDNA demon-strated catalytic activity toward Δ substrates. The DNA amplified by PCR was subjected to dideoxy sequencing. In the sequencing of exon 6 of a normal person, Patient 1, and Patient 2. B, nucleotide sequences of exon 6 of patients' grandmother, father, mother, and elder sister.
Fig. 3. Allele-specific amplification. Genomic DNAs from a normal control, sister, Patient 1, Patient 2, other members of the family, and a 17-OHD patient unrelated to the family were each hybridized to primers 5-5' and W (lanes W), or to primers 5-5' and M (lanes M). After PCR amplification, samples were subjected to electrophoresis on a 1% agarose and stained with ethidium bromide. Molecular weight markers were λ phage DNA cut with HindIII and φX174 DNA cut with HaeIII.

Table I

Oligonucleotide sequences used to amplify exons 1–8 of the human P450c17 gene

| Exon | Name | Primer sequence | Size of PCR product |
|------|------|-----------------|---------------------|
| 1    | 1-5' (37) | TTGGCAAGCCTCTTTTACTC | 419 bp |
| 1    | 1-3' (45) | TCTGAAACGCTGAAAGCTTC | 269 bp |
| 2    | 2-5' (19) | TGCTGTAAGTTTCTCAGC | 354 bp |
| 3    | 3-5' (31) | TCTTACCCCTACCCC | 236 bp |
| 4    | 4-5' (68) | AGCTAAGATCCGCCTCAG | 311 bp |
| 5    | 5-3' (42) | GTCAAGATCCGGCTCAG | 250 bp |
| 5    | 5-3' (44) | GGGTGAATGTTCCAGT | 215 bp |
| 6    | 6-5' (16) | ACACACTGTCACCCAC | 404 bp |
| 7    | 7-3' (34) | TGAATGCATCATGGGCT | 419 bp |
| 8    | 8-5' (52) | TGGGTGTGAGATTCCTACAG | 18S |

Fig. 4. Northern blot analysis. Total RNAs were prepared individually from COS-1 cells transfected with pCWL-SRα 296 carrying the normal (Normal) and the His<sup>73</sup> → Leu mutant (Mutant) P450c17 cDNA and from cells transfected with unmodified pCDL-SRα 296 (Vector). Each lane contained 15 μg of RNA. Hybridization was done with a 32P-labeled human P450c17 cDNA probe. The locations of 18 and 26 S ribosomal RNA are indicated.

The numbers in parentheses indicate the number of bases of intronic DNA lying between the primer and the intron/exon junction.

Expression of Modified P450c17 cDNA in E. coli and Spectral Study—To obtain sufficient amounts of the wild-type and mutant P450c17 for spectral studies, we attempted to produce these proteins in E. coli. To this end, we constructed a pcWori+ vector containing the coding sequence of the wild-type or mutant human P450c17 cDNA. The vector, however, failed to produce detectable amounts of P450c17 protein in E. coli DH5α. Barnes et al. (21) also observed that the pcWori+ vector constructed by introducing the coding sequence of bovine P450c17 cDNA did not produce the P450 protein. They found that modification of the nucleotide sequence corresponding to N-terminal seven amino acids led to production of large amounts of the recombinant protein. Thus, we modified the 5' sequences of the wild-type and mutant human P450c17 cDNAs as described by Barnes et al. (21); the second codon was changed from TGG (Trp) to GCT (Ala), the third codon from GAG (Glu) to CTG (Leu), the fifth codon from GTG (Val) to TTG (Leu), and the seventh codon from CTC (Leu) to GTT (Val). As shown in Fig. 6, Western blot analysis revealed that E. coli transformed with the vectors containing the modified cDNAs produced proteins immunoreactive to anti-porcine P450c17 antibody in the presence of IPTG. Membrane fractions prepared from E. coli transformed with the pcWori+ carrying the modified wild-type cDNA showed, in the reduced state, a peak at 449 nm upon addition of CO and a characteristic substrate-induced difference spectrum when 17-OH pregnenolone, progesterone, or 17-OH progesterone was added. (Only the data for pregnenolone are shown in the figure.) On the other hand, membrane preparations from E. coli transformed with the mutant vector failed to produce these spectral changes (Fig. 7). These results suggest that the single amino acid substitution His<sup>73</sup> → Leu causes a defect in heme binding.

Barnes et al. (21) showed that E. coli contained an electron transport system that could support the activity of recombi-
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**Fig. 5.** Enzyme activity in COS-1 cells. COS-1 cells were transfected with pcDL-SRa 296 (Vector), with pcDL-SRa 296 expressing the normal P450c17 (Normal), or with pcDL-SRa 296 expressing the His373 → Leu mutant (Mutant). A, cells were incubated with 1 μM [14C]pregnenolone (Preg) (10^5 cpm/nmol) or with 1 μM 17-OH [3H]pregnenolone (17OH Preg) (5 × 10^4 cpm/nmol). Incubation time was 14 h unless otherwise indicated. B, cells were incubated for 14 h with 1 μM [14C]progesterone (Prog) (10^5 cpm/nmol) or with 1 μM 17-OH [3H]progesterone (17OH Preg) (5 × 10^4 cpm/nmol). The products were analyzed by TLC as described under “Materials and Methods.” DHEA, dehydroepiandrosterone; 16OH Prog, 16-OH progesterone; Andro, androstenedione.

**Fig. 6.** Western blot analysis of bacterially expressed P450c17 proteins. Lane 1, 1 μg of sheep adrenal microsomal protein; lanes 2 and 3, membrane preparations (50 μg of protein) from E. coli DH5α transformed with pCWmod17-W and cultured in the absence and presence of IPTG, respectively; lanes 4 and 5, membrane preparations (50 μg of protein) from E. coli DH5α transformed with pCWmod17-M and cultured in the absence and presence of IPTG. Molecular weight standards were electrophoresed on the same gel and stained with Coomassie Brilliant Blue.

**Fig. 7.** Spectral study. E. coli membranes were prepared from cells transformed with pCWmod17-W and -M and treated with sodium dithionite as described under “Materials and Methods.” After the baseline was recorded, the content of the sample cuvette was gassed with CO for 2 min (A). Also shown is the difference spectrum after addition of 40 μM pregnenolone (B).

nanty expressed bovine P450c17. We also observed the conversion of pregnenolone to 17-OH pregnenolone, of progesterone to 16-OH and 17-OH progesterone, and of 17-OH pregnenolone to dehydroepiandrosterone in E. coli cells expressing the N-terminally modified wild-type human P450c17. (As reported by Barnes et al. (21), 17-OH progester-
one was not converted to androstenedione.) \textit{E. coli} expressing the mutant form of the enzyme showed none of these activities (Fig. 8).

**DISCUSSION**

This is the first report of molecular analysis of the \textit{CYP17} gene in a family with 17α-hydroxylase deficiency accompanied by elevated plasma aldosterone concentrations. We have found a new homozygous missense mutation His^{373} → Leu in two Japanese siblings with this subtype of the disease. We also documented heterozygosity of this mutation in the patients' family members. The plasma levels of basal and ACTH-stimulated deoxycorticosterone, corticosterone, 18-OH corticosterone, and 18-OH deoxycorticosterone are reported to be high in 17-OHD heterozygotes (27). Although these clinical features are useful for diagnosis, they are obviously not definitive. We established the diagnosis by allele-specific PCR amplification of genomic DNAs.

To date, 11 different genetic lesions have been reported in patients with 17-OHD. Three of these were nonsense mutations causing immature chain termination (29–31); two were small duplications of 4 and 7 bp, respectively, resulting in the change in the reading frame (26, 32); two were small deletions (28, 33); and one was a 518-bp deletion with a 469-bp insertion (34). Amino acid replacement mutations were described in only three patients; two were compound heterozygotes (Arg^{406} → Cys/Glu^{461} → Stop (30) and Pro^{542} → Thr/Arg^{590} → Stop (31), respectively), and only one was homozygous for a single amino acid change (Ser^{406} → Pro) (17). Thus, our patients are

**Fig. 8. Enzyme activity in \textit{E. coli}.** \textit{E. coli} DH5α cells transformed either with pCWmod17-W or -M were induced with IPTG. At 24 h after addition of IPTG, \textsuperscript{[14C]}pregnenolone (10⁶ cpm/ nmol) (A), \textsuperscript{[14C]}progesterone (10⁶ cpm/ nmol) (B), 17-OH \textsuperscript{[3H]}pregnenolone (5 × 10⁶ cpm/nmol) (C), or 17-OH \textsuperscript{[3H]}progesterone (5 × 10⁶ cpm/nmol) (D) was added to 2 ml of culture medium to a concentration of 1 μM. The mixture was incubated for 24 h, and the products were analyzed by TLC as described under "Materials and Methods."
only the second examples of a homozygous single amino acid replacement.

The Leu737 mutant expressed in COS-1 cells lacked the 17α-
hydroxylase and 17,20-lyase activities. To gain insight into
the mechanism by which this single amino acid mutation
eliminated activity, we attempted to produce large amounts
of P450c17 proteins in E. coli and perform spectral studies.
Although pCWori+ vectors constructed by insertion of the
coding sequences of the wild-type and mutant forms of cDNA
failed to produce detectable amounts of P450c17 proteins in
E. coli, modification of the 5′-nucleotide sequences as de-
scribed by Barnes et al. (21) affected production of the recom-
munant proteins (Fig. 6). Spectral changes characteristic of
P450 (CO and substrate-induced difference spectra in the
reduced state), however, were observed only with the mem-
brane preparations from E. coli expressing the modified wild
type (Fig. 7). It is generally accepted that the fifth ligand of
heme iron is a conserved cysteine residue interacting with the
heme propionate may be crucial for heme incorporation.

While the Leu737 mutant expressed in COS-1 or E. coli cells
is devoid of the activities of P450c17, the patients plasma
contained considerable amounts of 17α-hydroxysteroids (10).
Thus, it is possible that the Leu737 mutant might have retained
a small amount of activity, which we could not detect by our
in vitro methods. A similar discrepancy between the enzyme
activities in vivo and in vitro has been reported by Yanase et
al. (31).

The reason our patients have an elevated plasma aldoste-
rone level is not clear. The mutant enzyme is not able to
synthesize aldosterone from deoxycorticosterone. Recently,
Lifton et al. (37) studied the molecular basis of glucocorticoid-
suppressible hyperaldosteronism and found a chimeric 11β-
hydroxylase/aldosterone synthase gene. This gene is thought
to arise from gene duplication by unequal crossing over, fusing
the 5′ regulatory region of 11β-hydroxylase gene to the coding
sequence of the aldosterone synthase gene. Like glucocorti-
coid-suppressible hyperaldosteronism patients, the plasma
aldosterone level in our patients appears to be controlled by
ACTH. Analysis of the 11β-hydroxylase and the aldosterone
synthase genes should be done in our patients.

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