東北大学機関リポジトリ
TOUR
Tohoku University Repository

P-450; Structure, Function, and Regulation

著者	Fujii-Kuriyama Y., Sogawa K., Imataka H., Yokotani N.
journal or publication title	CYRIC annual report
volume	1989
page range	234-236
year	1989
URL	http://hdl.handle.net/10097/49540

III. 14. P-450; Structure, Function, and Regulation

Fujii-Kuriyama Y., Sogawa K., Imataka H. and Yokotani N.

Department of Chemistry, Faculty of Science, Tohoku University

P-450 is a collective term for a group of hemoproteins which catalyze the monooxigenase reactions of endogenous substrates such as many kinds of steroids, fatty acids and prostaglandings as well as exogenous substrates such as an almost infinite number of drugs and other lipophilic chemicals including environmental pollutants and chemical carcinogens. This prodigious metabolic versatility of the monooxigenase system is now known to result in part from the participation of multiple forms of P-450, the total number of which still remain unknown.[1] Generally speaking, P-450 species which are involved in the metabolism of endogenous substrate exhibit rigid substrate specificities, like most of other enzymes, whereas the so-called drug-metabolizing P-450s possess distinct but broad substrate specificity which in some cases overlap with one another, thus forming a sort of comprehensive protective system against toxic xenobiotics with hydrophobic nature.

Another very important feature of P-450 superfamily is that most of them, if not all, are inducible in tissue-specific and inducer-specific manners.[1] In response to inducing agents, either exogenous or endogenous, the synthesis of distinct forms of P-450 is known to be enhanced in specific tissues. This inductive response plays an important role in increasing the rate of metabolism of foreign compounds to detoxified forms or in some cases to harmful reactive intermediates to host animals, and in regulating the synthesis and degradation of steroid hormones and prostaglandins.

Therefore, our research efforts have been devoted this year to the three main projects related to the interest described above; 1) cDNA cloning of P-450 which catalyze the ω-hydroxylation of prostaglandings and fatty acids, 2) mechanisms of transcriptional regulation of P-450 gene, 3) analysis of defective genes of congenital P-450c21 deficiency.

1) cDNA cloning of P-450 which catalyze the ω-hydroxylation of prostaglandins and fatty acids

We had previously isolated cDNA of $P\text{-}450_{p\text{-}2}$ which shows ω-hydroxylating activity

toward PGA, by using an ologonucleotide probe synthesized on the basis of its partial amino acid sequence.[2] Since kidney microsomes are rich in the ω-hydroxylating activity of prostaglandins and fatty acids, we constructed cDNA library from mRNA of rabbit kidney and screened it with the P-450_{p-2} cDNA as a hybridization probe to obtain three kinds of cDNA clones. Sequence analysis of these cDNAs and their expression from the cDNA inserted into pAH83 showed that these cDNA clones are those of P-450_{ka-1}, P-450_{ka-2} and P-450_{kd}.[3] The sequences of these P-450s shows more than 80% similarity to one another, but less than 50 % similarity to those of other forms of P-450s, thus indicating that these ω-hydroxylating P-450s form a unique family of P-450.

2) Mechanism of transcriptional regulation of P-450 genes

Regulatory mechanisms of genes for drug-metabolizing P-450s and steroidogenic P-450s have been investigated by focusing attentions on cis-acting DNA elements in the upstream region of these genes and their cognate trans-acting factors. Drug-metabolizing P-450c gene was expressed remarkably by the administration of 3-methylcholanthrene or TCDD. Two kinds of the regulatory elements were necessary for a high level of inducible expression of the gene and were localized in the upstream region of the gene. One is designated XRE or xenobiotic responsive element and is distributed 5 times in the region ranging from -0.5 to -3kb to function as a inducible enhancer, while the other is BTE or basic transcription element and is localized at -44b to be involved in a basal level of the gene expression.

Trans-acting factors on the XRE and the BTE elements were investigated by gel mobility shift assay. The XRE-binding factor was found in the cytoplasm as a cryptic form. When an inducer such as 3-methylcholanthrene or TCDD was added, the binding protein was associated with the inducer to reveal the XRE-binding activity and translocated to the nuclei, leading to the induction of the mRNA synthesis.[4] The BTE-binding protein shows nuclear location and occurs ubiquitously in various cell lines. The BTE or BTE-analogous sequence is distributed in non-P-450 genes as well as other P-450 genes at the corresponding position. These findings, taken together, suggest that the BTE element and the cognate binding factor constitute a general machinary of transcriptional regulation.

Although the BTE sequence matches the consensus sequence of the GC box, the competition experiment using the gel shift assay clearly demonstrated that the BTE-binding factor is different from Sp 1. South-Western analysis using ^{32}P-labeled BTE sequence as a probe estimated the Mr of the BTE-binding protein to be about 56 kD cDNA clones for the binding protein have been isolated from rat cDNA library and sequence analysis of these cloned cDNAs demonstrated that the deduced primary structure contains three-time repeat of zinc finger motif with a stretch rich in basic amino acids on its N-terminal side, a

characteristic of DNA binding proteins. We are now continuing our effort to isolate a full length cDNA for the BTE-binding protein.

3) Analysis of defective genes of congenital P-450c21 deficiency

Congenital P-450c21 deficiency is one of the most common genetic diseases with an incidence of 1 in 15,000 births. In order to investigate a cause of high incidence of this genetic disease, we analyzed the defective genes of the patients by sequence analysis and COS cell expression system and identified two harmful mutations which reduced the P-450c21 activity remarkably. One is a base replacement of C with G in the 2nd intron and cause aberrant splicing in the maturation process of the mRNA, while the other is a cluster of three base replacements causing three amino acid changes in the primary structure of P-450c21. Interestingly, all these mutations were found to be present at the corresponding positions of the pseudogene whose location is near the functional P-450c21 gene. These findings may suggest that the mutations in the patient genes were brought in from the pseudogene by gene convention or homologous recombination event.[5)]

To find other critical mutations in the pseudogene, we examined the effects of each of the mutations in the pseudogene on the P-450c21 activity by constructing chimeric genes with a pseudogene mutation in the functional P-450c21 gene background. Additional 4 defective mutations were identified. Oligonucleotide probes containing each of these mutations were synthesized and their distribution in the patient genes are currently under investigation by DNA blot analysis using ^{32}P-labeled oligonucleotide probes.

References

1) Gotoh O. and Fujii-Kuriyama Y., "Frontier in Bistransformation" ed. by Ruckpaul K. and Rein H., Vol. 1, pp 195, Akademie-Verlag Berlin (1989).
2) Matsubara S., Yamamoto S., Sogawa K., Yokotani N., Fujii-Kuriyama Y., Haniu M., Shively J. E., Gotoh O., Kusunose Y. and Kusunose M., J. Biol. Chem. **262** (1987) 13366.
3) Yokotani N., Bernhardt R., Sogawa K., Kusonose E., Gotoh O., Kusunose M. and Fujii-Kuriyama Y., J. Biol. Chem. **264** (1989) 21665.
4) Fujisawa-Sehara A., Yamane M. and Fujii-Kuriyama Y., Proc. Natl. Acad. Sci. USA **85** (1988) 5859.
5) Higashi Y., Tanae A., Inoue H., Hiromasa T. and Fujii-Kuriyama Y., Proc. Natl. Acad. Sci. USA **85** (1988) 7486.