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Conversion of pregnenolone to DHEA by human 17α-hydroxylase/17,20-lyase (P450c17)

Evidence that DHEA is produced from the released intermediate, 17α-hydroxypregnenolone

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Most previous studies using reconstituted systems and fast kinetics suggest that the conversion of pregnenolone to dehydroepiandrosterone (DHEA; the precursor of androgen and estrogen biosynthesis) by P450c17 does not require the release of the intermediate 17α-OHPreg (a precursor of cortisol biosynthesis). With such a mechanism, it is difficult to conceive how high amounts of DHEA may be produced in some cells or tissues, such as the testis and cells from the adrenal reticularis, while in other tissues such as the fasciculata zone, high levels of 17α-OHPreg are synthesized. In this report, we address this matter using intact transfected cells, which better reflect the actual cellular conditions. Furthermore, by using transfected cells, we can conveniently analyze human enzymes, as we are not restricted by the availability of human tissues as in the case of methods using purified or partially purified enzymes. Using intact HEK-293 cells transfected with human P450c17 in culture, we showed, in a time course study of the transformation of pregnenolone, that there is an accumulation of 17α-OHPreg, and that, subsequently, the accumulated 17α-OHPreg decreases with a concomitant increase in DHEA production. The DHEA/17α-OHPreg ratio changes from 0.1 :1 after 1 h incubation to 50 : 1 after 20 h. This result strongly suggests that the transformation of Preg to DHEA proceeds through two steps in which DHEA is produced from the released intermediate 17α-OHPreg. We also show that high levels of substrate vs. enzyme concentration will lead to high hydroxylase activity whereas the reverse will increase the lyase activity. The result is in good agreement with recent observations suggesting that surrounding enzymes and steroids could modulate the lyase activity. Cotransfection of vectors expressing cytochrome b5 and NADPH cytochrome P450 reductase indicates that both are required for an optimum production of DHEA.

Keywords: cortisol; DHEA; 17α-hydroxylase/17, 20-lyase; intracrinology; steroidogenesis.

Cytochrome P450c17 (17α-hydroxylase/17,20-lyase) exhibits a dual enzymatic activity: 17α-hydroxylase and 17,20-lyase activities. It catalyzes the transformation of pregnenolone (preg) and progesterone (prog) into 17α-hydroxypregnenolone (17α-OHPreg) and 17α-hydroxyprogesterone (17α-OHProg), and then into dehydroepiandrosterone (DHEA) and 4-androstenedione (4-dione), respectively. As 17α-OHPreg and 17α-OHProg are precursors of cortisol biosynthesis, while DHEA and 4-dione are precursors of sex steroid formation, cytochrome P450c17 is thus a key enzyme controlling the biosynthesis of glucocorticoids and sex steroids. Although P450c17 was purified [1–3] and cloned [4,5] two decades ago, it is still unclear which mechanism drives this unique enzyme toward the synthesis of glucocorticoids or sex steroids, depending on the cell type in which it is expressed. One of the major difficulties is probably the discrepancy between the proposed mechanism and the actual physiological observations. Indeed, it is well recognized that the 17α-hydroxylase and 17,20-lyase activities of the enzyme could be affected differentially by metabolites produced in the steroidogenic pathway [6,7], thus suggesting that these two reactions could be separate. On the other hand, based on results obtained with reconstituted systems of enzymes purified from tissues [4] or from overproduction in yeast [8] or bacteria [9], it has been suggested that the transformation of preg into DHEA proceeds through two successive reactions without dissociation of the intermediate [10–14]. However, it is difficult to conceive a differential control of the hydroxylase and lyase activities of P450c17 in a model where the intermediates are not dissociated from the active site.

Another difficulty in the study of P450c17 is the difference in the substrate specificity of the enzyme between small laboratory animals and humans. Indeed, in small animals P450c17 is more active on 4-ene steroids [15–18], while in the human and in primates, it is more active on 5-ene steroids [5,15–19]. This probably explains why the human and some primates produce high levels of circulating DHEA [20,21] necessary for the

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Abbreviations: P450c17, 17α-hydroxylase/17,20-lyase; DHEA, dehydroepiandrosterone or 5-androsten-3β-ol-17-one; preg, pregnenolone or 5-pregnen-3β-ol-20-one; prog, progesterone or 4-pregnen-3,20-dione; 17α-OHPreg, 17α-hydroxypregnenolone or 3β,17α-dihydroxy-5-pregnen-20-one; 17α-OHProg, 17α-hydroxyprogesterone or 17α-hydroxy-4-pregnene-3,20-dione; HEK-293, transformed human embryonic kidney 293 cells; P450-Red, NADPH cytochrome P450 reductase; Cyt-b5, cytochrome b5; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ5→Δ4isomerase; 4-dione, 4-androstene-3,17-dione.

Enzymes: NADPH-cytochrome P450reductase (P450-Red; EC 1.6.2.4); 17α-hydroxylase/17,20-lyase (P450c17; EC 1.14.99.9).

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formation of sex steroids in peripheral tissue [22]. As small animals do not produce high amounts of DHEA, and in young people most of the sex steroids are produced by the gonads, the short life span and small size of these animals suggest that the gonads are the main source of sex steroids. Furthermore, human P450c17 catalyzes selectively the 17α-hydroxylation of preg and weakly prog. strongly suggesting that substrates such as dihydroprogesterone, pregnan-3α-ol-20-one and pregnan-3β-ol-20-one are poor substrates for human P450c17.

So far, because of the availability of enzymatic sources, most of the mechanistic studies on P450c17 were performed using enzyme purified or partially purified from animal sources. In this study we have used human enzymes in order to have more appropriate conditions. Also, we have shown previously that although purified enzymes are the preferred material for studying kinetic and binding interactions as well as catalytic properties, the use of intact transfected cells in culture is more suitable for characterizing physiological activity [23,24], especially when there is a fine differential control of the activities and a subtle combination of substrate, interacting metabolites, and coenzyme. In this report, we describe the mechanistic study of P450c17 using human expressed enzyme and intact transfected cells in culture. We were able to show that 17α-hydroxylase and 17,20-lyase reactions are distinct steps of a single enzyme, depending upon the available substrate, preg or prog and 17α-OHPreg or 17α-OHProg, respectively.

MATERIALS AND METHODS

Construction of P450 reductase, cytochrome b5 (Cyt-b5) and P450c17 expression vectors

The cDNA fragment containing the entire coding region of human NADPH-cytochrome P450reductase (P450-Red, EC 1.6.2.4) [25,26] was isolated and amplified by PCR from a λgt11 human placental cDNA library (Clontech Laboratories) using the following primers: 5′-GGAATTCAACATGGGA-GACTCCCACGTGGACACCAG-3′ and 5′-GGAAATCTGAT-TACAGGCCGGAGTCTGTGG-3′. The cDNA sequence has been obtained from Genbank database under the accession number s90469 in which we have added the ATG initiation codon to produce human functional P450-Red. The amplified fragment was then subcloned into a pCMV expression vector. Human liver cDNA was prepared by reverse transcription of 500 ng of poly(A)$^+$ RNA using 200 U Superscript II RT (Gibco BRL). Poly(T) primers and 100 ng human cytochrome b5 cDNA was amplified by PCR using *Taq* DNA polymerase (Perkin-Elmer Cetus) and the oligo-primer pair (5′-GGGGTC-GACATGGCAGAGCAGTCGGACGAGGCCGTG-3′, 5′-GGG-GAATTCTCAGTCCTCTGCCATGTATAGGCG-3′). The cDNA was then subcloned into a pCMV expression vector. The cDNA coding for human P450c17 (E.C. 1.14.99.9) was kindly provided by Y. Tremblay (CHUL Research Center, Quebec).

Transient expression in transformed human embryonic kidney (HEK-293) cells

Vectors expressing P450c17 (pCMV-P450c17) and P450-Red (pCMV-P450-Red) were transfected into HEK-293 cells by the calcium phosphate procedure [27] and cytochrome b5 (pCMV-cyt-b5) was transfected using the Ex-gene kit according to the manufacturer's instructions (MBI Fermentas, Amherst, New York, USA). Cells were initially plated at 5×10^5 cells per well in 6-well Falcon flasks and grown in Dulbecco's modified

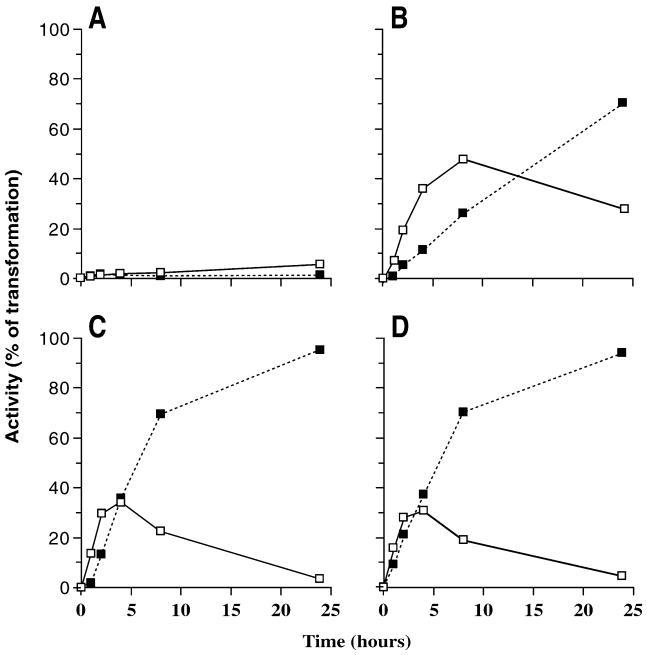

Fig. 1. Time course study of differential 17α-hydroxylase and 17,20-lyase activities catalyzed by various amounts of P450c17 in intact transfected HEK-293 cells in culture. Cells transfected with 0 (A), 0.1 (B), 0.5 (C), and 2 μg (D) vector expressing human P450c17 were seeded into six-well plates at a density of 5×10^5 cells per well. Five nanomolar [^{3}H]preg was added to freshly changed culture medium to assess 17α-hydroxylase and 17,20-lyase activities by quantification of 17α-OHPreg (□) and DHEA (■), respectively. After incubation for the indicated time periods, the media were collected and extracted. The amount of product and substrate was determined as described; 100% transformation corresponds to 10 nmol per 10^6 cells.

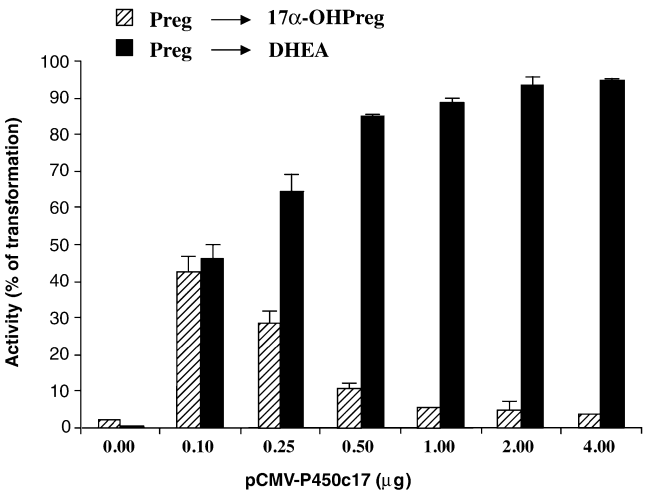

Fig. 2. Relative variation of 17α-hydroxylase and 17,20-lyase activities with increasing amounts of P450c17. HEK-293 cells were transfected with the indicated amounts of pCMV-P450c17 and were analyzed for their ability to catalyze the transformation of [^{3}H]preg to 17α-OHPreg (hatched bars) and DHEA (filled bars) after overnight incubation (16 h). Experimental conditions were as described in Fig. 1.

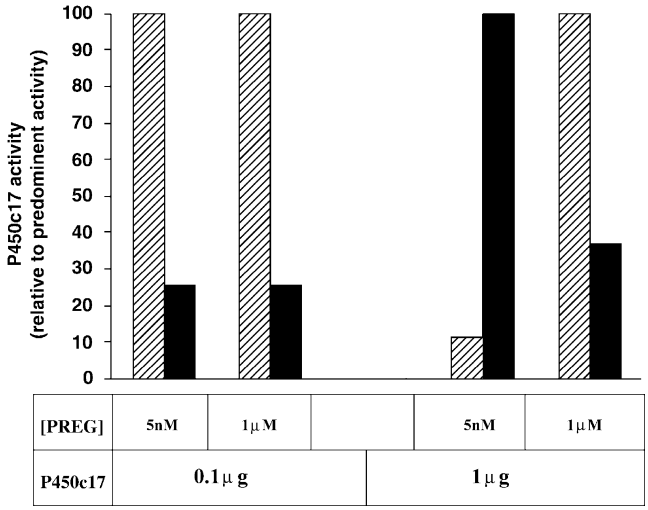

Fig. 3. Modulation of 17α-hydroxylase and 17,20-lyase activities by the substrate. HEK-293 cells were transfected with 0.1 and 1 μg of pCMV-P450c17 and analyzed for their ability to catalyze the transformation of 5 nM and 1 μM of [^{3}H]preg to 17α-OHPreg (hatched bars) and DHEA (filled bars) after overnight incubation (16 h). Experimental conditions were as described in Fig. 1.

Eagle's medium (GIBCO) supplemented with 10% (v/v) calf fetal serum (Hyclone, Logan, Utah, USA) at 37 °C under a 95% air, 5% CO_2 humidified atmosphere.

Assay of enzymatic activity

The determination of the activities was performed in intact cells transiently transfected with P450c17 and/or P450-Red and/or cyt-b5. [^{3}H]Preg was added to freshly changed culture medium in a 6-well culture plate. After incubation, the culture medium was collected and the steroids were extracted twice with 2 mL ether. The organic phases were pooled and evaporated to dryness. The steroids were solubilized in 50 μL dichloromethane, applied to a Silica Gel 60 TLC plate (Merck) before separation by migration in the toluene/acetone (4 : 1) solvent system. Substrates and metabolites were identified by comparison with reference steroids, revealed by autoradiography and quantified using the PhophorImager System (Molecular Dynamics, Inc.).

RESULTS

Analysis of the transformation of preg by transfected P450c17 in cultured cells

As illustrated in Fig. 1, HEK-293 cells transfected with P450c17 initially catalyze the transformation of preg into 17α-OHPreg, which accumulates. At early incubation times preg is abundant and because the transformation rate to 17α-OHPreg is rapid [8] the enzyme will preferentially use this substrate to produce 17α-OHPreg which will rapidly be formed and released from P450c17 resulting in accumulation. Subsequently, the accumulated 17α-OHPreg decreases with a concomitant increase of DHEA production. Figure 1 also shows that increasing amounts of P450c17 speeds up the formation of DHEA, leading to the formation of an earlier and lower peak of accumulated from the released 17α-OHPreg.

Effect of an increasing level of P450c17

As suggested by the data described above, the ratio between the substrate concentration and the level of P450c17 could play an important role in the control of the production of 17α-OHPreg or DHEA. To characterize this effect further we have transfected increasing amounts of vector expressing P450c17 into HEK-293 cells and assessed their ability to metabolize [^{3}H]preg. As illustrated in Fig. 2, increasing the level of P450c17 increased the production of DHEA while decreasing the formation of 17α-OHPreg. The result thus suggests that the ratio between the concentration of substrate and enzyme could play an important role in the modulation of the relative importance of 17α-hydroxylase and 17,20-lyase activities.

Effect of the substrate preg

To analyze further the differential effect of the substrate concentration and of the concentration of P450c17 on the 17α-hydrolyase and 17,20-lyase activities, we determined the relative production of 17α-OHPreg and DHEA at various concentrations of preg and various amounts of transfected P450c17. As illustrated in Fig. 3, when 100 ng of pCMV-P450c17 is transfected, the ratio between 17α-OHPreg and DHEA is the same whether in the presence of 5 nM or 1 μM of preg, probably because the level of enzyme is low and the enzyme is in a substrate-saturated condition at 5 nM preg.

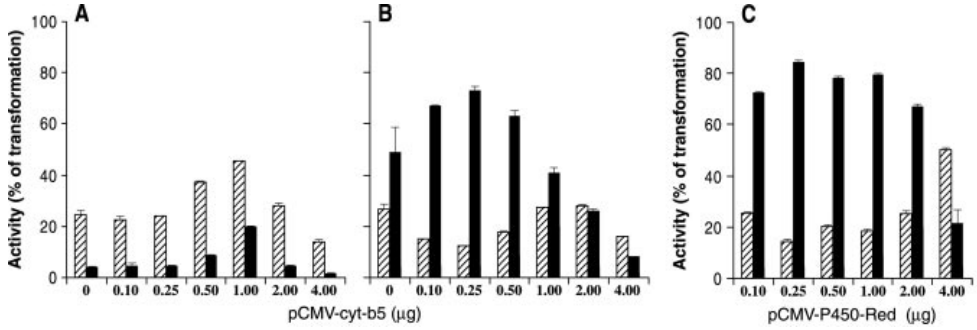

Fig. 4. Modulation of 17α-hydroxylase and 17,20-lyase activities by P450-Red and cyt-b5. HEK-293 cells were transfected with: 0.1 μg pCMV-P450c17 and the indicated amount of pCMV-cyt-b5 (A); 0.1 μg pCMV-P450c17 and 1 μg PCMV-P450-Red and the indicated amount of pCMV-cyt-b5 (B); and 0.25 μg pCMV-P450c17 and the indicated amount of pCMV-P450-Red (C). The transfected cells were analyzed for their ability to catalyze the transformation of 5 nM of [^{3}H]preg to 17α-OHPreg (hatched bars) and DHEA (filled bars) after overnight incubation. In (A) and (B), the transfection was performed using EX-gene transfection kit and in (C) the transfection was performed using the calcium phosphate procedure. Transfections and enzymatic assays were determined as described in Materials and methods.

Therefore, the production of 17α-OHPreg is much higher than DHEA because the transformation rate is higher. In contrast, when 1 μg of pCMV-P450c17 is used, the enzyme is no longer in a substrate-saturated condition (as with 5 nM preg), and the production of DHEA is higher than that of 17α-OHPreg. However, in the presence of 1 μM preg, the enzyme is still in a saturated condition, and the production of 17α-OHPreg is higher than that of DHEA.

Effect of P450-Red and of cyt-b5

Previous studies on P450c17 suggest that increasing the electron flow to the enzyme will increase the 17,20-lyase activity compared to the 17α-hydroxylase activity. Some studies attribute this effect to P450-Red, others suggest the predominant role of cyt-b5. To assess these effects in an intact cell model, we cotransfected P450c17 with various amounts of P450-Red and/or cyt-b5, and analyzed their ability to transform Preg into 17α-OHPreg and DHEA. As shown in Fig. 4, the cotransfection of P450-Red or cyt-b5 increases the production of DHEA. P450-Red shows a higher effect than cyt-b5, and adding cyt-b5 together with P450-Red produced a combined positive effect. However, an excess of cyt-b5 and P450-Red will diminish P450c17 activity. As mentioned previously, this effect could result from competition between cyt-b5 and P450c17 for electrons from limiting amounts of P450-Red [8]. These results strongly suggest that P450-Red and cyt-b5 could modulate the lyase activity through an optimum rearrangement of all the components of the P450c17 enzymatic complex namely P450c17, P450-Red, cyt-b5 as well as the concentration of cofactor and substrate.

DISCUSSION

The present report describes the characteristics of the human P450c17 transfected in intact cells. We have shown that the production of DHEA from preg through P450c17 comes mainly from the 17,20-lyase activity on the 17α-OHPreg released from the enzyme. As the transformation of preg to 17α-OHPreg is rapid, when preg is in excess, it will be rapidly transformed to 17α-OHPreg which will be released from the active site of the enzyme. Because the 17,20-lyase activity is slower, the formation of DHEA will appear at a later time and will result from the released intermediate. We therefore observe a sharp decrease of 17α-OHPreg with a reciprocal increase of DHEA. In other terms, 17α-OHPreg should be considered the physiological substrate for the 17,20-lyase activity resulting in the formation of DHEA thus suggesting that the reaction catalyzed by the human P450c17 follows two distinct steps with a dissociation of the intermediate from the enzyme. If DHEA were produced without release of the intermediate from the active site, the increase of DHEA production would not correspond to the decrease of 17α-OHPreg. This decrease of 17α-OHPreg is the result of transformation to DHEA by P450c17 and is not due to time dependant degradation of 17α-OHPreg (confirmed by Figs 2–4, in which experiments have the same incubation time). This result is somehow in contrast with previous studies that suggested that the 17α-hydroxylase and 17,20-lyase activities occur in succession, without dissociation of the intermediate from the enzyme [10–14]. The discrepancy is probably due to the fact that previous studies were performed using reconstituted P450 systems with purified components from various species. In such systems, the component to be studied (in this case the P450c17) is generally added in limited amounts, while other components are given in excess. Such a method, which is used widely for studying kinetics, is useful for characterizing a target parameter but does not allow us to see the subtle interactions of enzymes with dual activities. The use of intact transfected cells in culture, which better reflect cellular conditions, is more suitable for characterizing the dual activity of P450c17, especially when these activities are differentially controlled. Indeed, in our conditions, using a concentration of preg closer to its physiological concentration (5 nM), a low level of reductase component and an intracellular concentration of cofactor, we could clearly see that DHEA is produced from accumulated 17α-OHPreg. The result thus suggests that the ratio between the concentration of substrate and enzyme could play an important role in the modulation of the relative importance of 17α-hydroxylase and 17,20-lyase activities. Again, the rate of 17α-hydroxylase activity being higher, in conditions of excess preg, this substrate will be used preferentially by P450c17 and 17α-OHPreg will accumulate. In contrast, when the enzyme concentration is high and the preg concentration is low, P450c17 molecules are available to use the excess 17α-OHPreg that is released from the active site to produce DHEA. Control conditions in which nontransfected cells were incubated with preg provide evidence that 17α-OHPreg and DHEA are not metabolized by other enzymes in the cells. Previous studies by Couch *et al*. [7] using human microsomes also suggest that DHEA is produced from the released 17α-OHPreg. As our system used transfected human enzymes, it is possible that this mechanism is specific to humans, which produce large amounts of DHEA [21].

In agreement with the observations of Yanagibashi and Hall [28], and Miller *et al*. [29] who report the importance of the role of P450-Red and cyt-b5 in the increase of the 17,20-lyase activities, our results also show the positive effects of P450-Red and cyt-b5. In addition, our intact transfected cell models show that there is an optimum concentration of these components that is required. At this stage, it is not possible to determine whether the positive effect of cyt-b5 and P450-Red is due to a specific interaction with P450c17 in the position that allows the increase of the 17,20-lyase activity, or if it is due to the optimum combination that increases the total P450c17 activity, and thus increases the conversion of 17α-OHPreg to DHEA.

In conclusion, we could clearly show that these two activities proceed in distinct steps. In contrast with other enzymes possessing dual activities, in which only the end product is important, in the reaction catalyzed by P450c17 both the intermediate and the end product are crucial. They constitute precursors for different classes of hormones, namely, glucocorticoids and sex steroids. P450c17 is unique in having its dual activity differentially modulated by various components of the enzymatic complex. Our findings will allow us to study more efficiently the dual activity of P450c17 and its relationship to various components of the enzymatic system. Recent findings by Rainey *et al*. [30] show that the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) could play an important role in adrenarche. This finding is in agreement with our results showing that DHEA comes from released 17α-OHPreg. Together these results suggest that 17,20-lyase and 3β-HSD activities compete for the common subtrate 17α-OHPreg and thus modulate the production of DHEA. In the reticularis zone the 3β-HSD level decreases allowing further transformation of 17α-OHPreg to DHEA. In contrast, the high levels of 3β-HSD in the fasciculata and glomerulosa increases the transformation of 17α-OHPreg to 17α-OHProg thus increasing the formation of cortisol. The fact that DHEA is produced from the released intermediate is in agreement with numerous observations

reported recently about the effect of enzymes involved in the metabolization of preg, such as 21α-hydroxylase [31] and 3β-HSD [30,31] in the modulation of the production of DHEA.

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REFERENCES

1. Nakajin, S., Shively, J.E., Yuan, P.M. & Hall, P.F. (1981) Microsomal cytochrome P-450 from neonatal pig testis: two enzymatic activities (17 alpha-hydroxylase and c17,20-lyase) associated with one protein. *Biochemistry* **20**, 4037–4042.
2. Nakajin, S. & Hall, P.F. (1981) Microsomal cytochrome P-450 from neonatal pig testis. Purification and properties of A C21 steroid side-chain cleavage system (17 alpha-hydroxylase-C17,20 lyase). *J. Biol. Chem.* **256**, 3871–3876.
3. Kominami, S., Shinzawa, K. & Takemori, S. (1982) Purification and some properties of cytochrome P-450 specific for steroid 17 alpha-hydroxylation and C17–C20 bond cleavage from guinea pig adrenal microsomes. *Biochem. Biophys. Res. Commun.* **109**, 916–921.
4. Chung, B.C., Picado-Leonard, J., Haniu, M., Bienkowski, M., Hall, P.F., Shively, J.E. & Miller, W.L. (1987) Cytochrome P450c17 (steroid 17 alpha-hydroxylase/17,20 lyase): cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. *Proc. Natl Acad. Sci. USA* **84**, 407–411.
5. Zuber, M.X., Simpson, E.R. & Waterman, M.R. (1986) Expression of bovine 17 alpha-hydroxylase cytochrome P-450 cDNA in nonsteroidogenic (COS 1) cells. *Science* **234**, 1258–1261.
6. Nishikawa, T. & Strott, C.A. (1984) Steroid concentrations in the outer and inner zones of the adrenal cortex of the guinea pig. *J. Steroid Biochem.* **20**, 1123–1127.
7. Couch, R.M., Muller, J. & Winter, J.S. (1986) Regulation of the activities of 17-hydroxylase and 17,20-desmolase in the human adrenal cortex: kinetic analysis and inhibition by endogenous steroids. *J. Clin. Endocrinol. Metab.* **63**, 613–618.
8. Auchus, R.J., Lee, T.C. & Miller, W.L. (1998) Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J. Biol. Chem.* **273**, 3158–3165.
9. Imai, T., Globerman, H., Gertner, J.M., Kagawa, N. & Waterman, M.R. (1993) Expression and purification of functional human 17 alpha-hydroxylase/17,20-lyase (P450c17) in *Escherichia coli*. Use of this system for study of a novel form of combined 17 alpha-hydroxylase/17,20-lyase deficiency. *J. Biol. Chem* **268**, 19681–19689.
10. Yamazaki, T., Ohno, T., Sakaki, T., Akiyoshi-Shibata, M., Yabusaki, Y., Imai, T. & Kominami, S. (1998) Kinetic analysis of successive reactions catalyzed by bovine cytochrome p450 (17alpha,lyase). *Biochemistry* **37**, 2800–2806.
11. Swinney, D.C. & Mak, A.Y. (1994) Androgen formation by cytochrome P450 CYP17. Solvent isotope effect and pL studies suggest a role for protons in the regulation of oxene versus peroxide chemistry. *Biochemistry* **33**, 2185–2190.
12. Kuhn-Velten, W.N., Bunse, T. & Forster, M.E. (1991) Enzyme kinetic and inhibition analyses of cytochrome P450XVII, a protein with a bifunctional catalytic site. Quantification of effective substrate concentrations at the active site and their significance for intrinsic control of the hydroxylase/lyase reaction sequence. *J. Biol. Chem.* **266**, 6291–6301.
13. Kominami, S., Inoue, S., Higuchi, A. & Takemori, S. (1989) Steroidogenesis in liposomal system containing adrenal microsomal cytochrome P-450 electron transfer components. *Biochim. Biophys. Acta* **985**, 293–299.
14. Higuchi, A., Kominami, S. & Takemori, S. (1991) Kinetic control of steroidogenesis by steroid concentration in guinea pig adrenal microsomes. *Biochim. Biophys. Acta* **1084**, 240–246.
15. Fevold, H.R., Lorence, M.C., McCarthy, J.L., Trant, J.M., Kagimoto, M., Waterman, M.R. & Mason, J.I. (1989) Rat P450 (17 alpha) from testis: characterization of a full-length cDNA encoding a unique steroid hydroxylase capable of catalyzing both delta 4- and delta 5-steroid-17,20-lyase reactions. *Mol. Endocrinol.* **3**, 968–975.
16. Namiki, M., Kitamura, M., Buczko, E. & Dufau, M.L. (1988) Rat testis P-450 (17) alpha cDNA: the deduced amino acid sequence, expression and secondary structural configuration. *Biochem. Biophys. Res. Commun.* **157**, 705–712.
17. Conley, A.J., Graham-Lorence, S.E., Kagimoto, M., Lorence, M.C., Murry, B.A., Oka, K., Sanders, D. & Mason, J.I. (1992) Nucleotide sequence of a cDNA encoding porcine testis 17 alpha-hydroxylase cytochrome P-450. *Biochim. Biophys. Acta* **1130**, 75–77.
18. Tremblay, Y., Fleury, A., Beaudoin, C., Vallee, M. & Belanger, A. (1994) Molecular cloning and expression of guinea pig cytochrome P450c17 cDNA (steroid 17 alpha-hydroxylase/17,20 lyase): tissue distribution, regulation, and substrate specificity of the expressed enzyme. *DNA Cell Biol.* **13**, 1199–1212.
19. Lin, D., Harikrishna, J.A., Moore, C.C., Jones, K.L. & Miller, W.L. (1991) Missense mutation serine106→proline causes 17 alpha-hydroxylase deficiency. *J. Biol. Chem.* **266**, 15992–15998.
20. Adams, J.B. (1985) Control of secretion and the function of C19-delta 5-steroids of the human adrenal gland. *Mol. Cell. Endocrinol.* **41**, 1–17.
21. Labrie, F., Dupont, A. & Belanger, A. (1985) Complete androgen blockade for the treatment of prostate cancer. *Important Adv. Oncol.* 193–217.
22. Labrie, F. (1991) Intracrinology. *Mol. Cell. Endocrinol.* **78**, C113–C118.
23. Dufort, I., Rheault, P., Huang, X.F., Soucy, P. & Luu-The, V. (1999) Characteristics of a highly labile human type 5,17beta-hydroxysteroid dehydrogenase. *Endocrinology* **140**, 568–574.
24. Luu-The, V., Zhang, Y., Poirier, D. & Labrie, F. (1995) Characteristics of human types 1, 2 and 3 17 beta-hydroxysteroid dehydrogenase activities: oxidation/reduction and inhibition. *J. Steroid Biochem. Mol. Biol.* **55**, 581–587.
25. Baron, J., Taylor, W.E. & Masters, B.S. (1972) Immunochemical studies on electron transport chains involving cytochrome P-450. The role of the iron-sulfur protein, adrenodoxin, in mixed-function oxidation reactions. *Arch. Biochem. Biophys.* **150**, 105–115.
26. Kominami, S., Ochi, H., Kobayashi, Y. & Takemori, S. (1980) Studies on the steroid hydroxylation system in adrenal cortex microsomes. Purification and characterization of cytochrome P-450 specific for steroid C-21 hydroxylation. *J. Biol. Chem.* **255**, 3386–3394.
27. Kingston, R.E., Chen, C.A. & Okayama, H. (1991) In *Current Protocols in Molecular Biology* (Ausubel, E.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. & Smith, K.S., eds), pp. 9.1.1–9.1.9. Wiley & Sons, New York.
28. Yanagibashi, K. & Hall, P.F. (1986) Role of electron transport in the regulation of the lyase activity of C21 side-chain cleavage P-450 from porcine adrenal and testicular microsomes. *J. Biol. Chem.* **261**, 8429–8433.
29. Miller, W.L., Auchus, R.J. & Geller, D.H. (1997) The regulation of 17,20 lyase activity. *Steroids* **62**, 133–142.
30. Gell, J.S., Carr, B.R., Sasano, H., Atkins, B., Margraf, L., Mason, J.I. & Rainey, W.E. (1998) Adrenarche results from development of a 3beta-hydroxysteroid dehydrogenase-deficient adrenal reticularis. *J. Clin. Endocrinol. Metab.* **83**, 3695–3701.
31. Byrne, G.C., Perry, Y.S. & Winter, J.S. (1985) Kinetic analysis of adrenal 3 beta-hydroxysteroid dehydrogenase activity during human development. *J. Clin. Endocrinol. Metab.* **60**, 934–939.