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The human cytochrome P4507B1: catalytic activity studies

Sae-Bom Kim[a], Sonia Chalbot[a], Denis Pompon[b], Do-Hyun Jo[c], Robert Morfin[a,*]

[a] *Laboratoire de Biotechnologie, Conservatoire National des Arts et Métiers (CNAM), EA-3199, 2 rue Conté, Paris 75003, France*
[b] *Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91190 Gif sur Yvette, France*
[c] *Biotechnology, Ajou University, 5 Wonchon Dong, Paldal, Suwon 441-749, Korea*

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Abstract

The cytochrome P4507B1 (P4507B1) in the human hippocampus is responsible for the production of 7α-hydroxylated derivatives of dehydroepiandrosterone (DHEA) and other 3β-hydroxylated neurosteroids. Minor quantities of the 7β-hydroxylated derivatives are also produced. Neuroprotective action of these 7-hydroxysteroids was reported. Recombinant human P4507B1 was prepared from yeast coexpressing the human hippocampal P450 cDNA and the human P450 reductase genes. Microsomal P4507B1 activity was tested in the presence of NADPH and ^{14}C-labeled steroid substrates to deduce kinetic parameters and to study inhibitor responses. The K_M values obtained for DHEA, pregnenolone, epiandrosterone, 5α-androstane-3β,17β-diol and estrone were 1.90 ± 0.06, 1.45 ± 0.03, 1.05 ± 0.12, 0.8 ± 0.04 and 1.20 ± 0.26 μM, respectively. Production of limited amounts of 7β-hydroxylated derivatives was also observed, but only with DHEA, 5α-androstane-3β,17β-diol and epiandrosterone. K_M values determined for 7β-hydroxylation were identical to those for 7α-hydroxylation. The DHEA 7α-hydroxylation was inhibited by estrone and estradiol (mixed type inhibition) and by the [25–35] β-amyloid peptide (non-competitive inhibition). These results indicate that in human, the 7-hydroxylation catalysed by P4507B1 preferentially takes place on DHEA, 5α-androstane-3β,17β-diol and epiandrosterone with major and minor formation of 7α- and 7β-hydroxylated derivatives, respectively. Both estrogens and a β-amyloid component inhibit the P4507B1-mediated production of the 7-hydroxysteroid metabolites.

Keywords: 7α-Hydroxylation; DHEA; Pregnenolone; Estrone; Epiandrosterone; β-Amyloid

1. Introduction

The cytochrome P4507B1 (CYP7B1) present in various human tissues was found responsible for the 7α-hydroxylation of several 3β-hydroxysteroids, including oxysterols, dehydroepiandrosterone (DHEA), and epiandrosterone (EPIA) [1–4]. Importance of this steroid-hydroxylating process relies on animal studies showing alternate pathways for bile acid production and prostate growth regulation [5,6], promotion of immunity [7] and neuroprotection [8,9]. Works in humans include evidences for vital involvement of P4507B1 in oxysterol processing and gene localisation to chromosome 8q21.3 [10] and cDNA isolation from a λgt 10 cDNA library of human hippocampus that was cloned into pcDNA3.1/His A plasmid. This construction was used for expression and enzyme activity assays after transfection of human kidney 293/T cells [2]. We transferred the engineered CYP7B1 open reading frame to the pYeDP60 multicopy yeast expression vector and transformed the W(hR) yeast strain that over-expresses the human NADPH-cytochrome P450 reductase. Transformed yeast microsomes were then used as a source of CYP7B1 for steroid hydroxylation studies [11,12]. It was then shown that the yeast-expressed human CYP7B1 carried out both a major 7α-hydroxylation and a minor 7β-hydroxylation of DHEA [12], as already described in murine [13]. Other studies with DHEA and mouse brain microsomes showed that 7α- and 7β-hydroxylation levels were almost the same [14] and this led to suspect that either another P450 was responsible for 7β-hydroxylation, or that the 7α- to 7β-interconversion was mediated through an oxidoreductive

* Corresponding author. Tel.: +33 1402 72572; fax: +33 1402 72380.
E-mail address: morfin@cnam.fr (R. Morfin).

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mechanism, or that the native microsomal environment favoured the 7β-hydroxylation process. On the other hand, recent evidences obtained with various human brain region homogenates showed DHEA to be 7α-hydroxylated with no detectable trace of 7β-hydroxylation, and that such a 7α-hydroxylation was inhibited by estradiol (E_2) [4].

The aims of this work were to use yeast-produced human P4507B1 for investigation of both 7α- and 7β-hydroxylation processes with DHEA, EPIA, pregnenolone (PREG), 5α-androstane-3β, 17β-diol and estrone (E_1) substrates and to study the interference of E_1, E_2 and β-amyloid peptides that may modify these processes.

2. Materials and methods

2.1. Steroids, peptides and reagents

[4-^{14}C]-PREG (55 mCi/mmol) was produced (custom synthesis CFQ 6416) by Amersham (Amersham, Cardiff, Wales). [4-^{14}C]-DHEA (47.8 mCi/mmol), [4-^{14}C]-E_1 (52.5 mCi/mmol) and [4-^{14}C]-testosterone (48 mCi/mmol) were purchased from NEN (Paris, France), and [4-^{14}C]-EPIA (53.5 mCi/mmol) and [4-^{14}C]-5α-androstane-3β,17β-diol (53.5 mCi/mmol) were produced as previously described [12]. DHEA, EPIA, PREG, E_1, E_2, testosterone, 5α-androstane-3β,17β-diol β-NADP$^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were from Sigma–Aldrich (L'isle d'Abeau Chesnes, France). Custom chemical synthesis by Roowin S.A (Paris, France) provided mg quantities of chemically pure 7α-hydroxy-DHEA, 7α-hydroxy-EPIA, 7β-hydroxy-DHEA, 7β-hydroxy-EPIA, 7α-hydroxy-E_1. Both 5α-androstane-3β,7β,17β-triol and 5α-androstane-3β,7α,17β-triol were obtained after $NaBH_4$ reduction of 7β-hydroxy-EPIA and 7α-hydroxy-EPIA, respectively. All solvents (Merck, Darmstadt, Germany) were of the reagent grade. The β-amyloid peptides ([1–40], [1–28], [25–35]) were obtained from Biosource (Nivelles, Belgium) and were dissolved in 0.1% trifluoroacetic acid and diluted for the tests in 0.067 M phosphate buffer (pH 7.4), containing 1 mM EDTA.

2.2. P4507B1 production in microsomes of transformed yeast

The human cDNA of P4507B1 was provided by Chiang and co-workers [2]. The engineered open reading frame was transferred of the to the pYeDP60 multicopy yeast expression vector. Transformation was performed in the W(hR) yeast strain which over-expresses the human NADPH-cytochrome P450 reductase when grown in galactose-containing medium [11,15–17]. Reductase and P4507B1 were induced in the transformed yeast strain as previously described [17], except that the durations of derepression and of induction were 24 and 4 h, respectively. Yeast microsomes were prepared as previously reported [17] except that yeast disruptions were carried out in liquid nitrogen with a Freezer/Mill 6700 apparatus (Bioblock, France). The microsomal P4507B1 contents were measured according to Omura and Sato [18].

2.3. P4507B1-mediated 7α-hydroxylation of [4-^{14}C]-steroid substrates

Incubations were carried out as previously described [19]. Briefly, the [4-^{14}C]-steroid substrate (0.5 nmol) was added with the non-radioactive steroid in ethanol and dried under vacuum at the bottom of one 10 mL glass tube prior to addition of 0.7 mL 0.067 M phosphate buffer (pH 7.4), containing 1 mM EDTA, followed by a 0.1 mL suspension of yeast microsomes (32 pmol of P4507B1) and the NADPH regenerating system in 0.2 mL buffer. The tube was left open during the course of incubation with shaking at 37 °C for 10 min. Control incubations consisted in use of either no cofactor or no microsomes in the digests. Incubations were stopped after addition of 0.5 mL acetone followed by 2 mL ethyl acetate. The extraction process was continued with 2 mL ethyl acetate and was repeated three times. The extracts recovered were analyzed by thin layer chromatography (TLC) on ready to use silica 60 coated glass plates (Merck, Darmstadt, Germany) developed once in ethyl acetate. In this system, the R_f of compounds DHEA, 7β-hydroxy-DHEA and 7α-hydroxy-DHEA were 0.72, 0.30 and 0.20, respectively; the R_f of compounds PREG, 7β-hydroxy-PREG and 7α-hydroxy-PREG were 0.70, 0.35 and 0.25, respectively; the R_f of compounds EPIA, 7β-hydroxy-EPIA and 7α-hydroxy-EPIA were 0.70, 0.20 and 0.20, respectively; the R_f of compounds 5α-androstane-3β,17β-diol, 5α-androstane-3β,7β,17β-triol and 5α-androstane-3β,7α,17β-triol were 0.64, 0.18 and 0.18, respectively; the R_f of E_1, 7α-hydroxy-E_1 and 7β-hydroxy-E_1 were 0.84, 0.63 and 0.60, respectively. Quantitative scanning of the plates was carried out with use of a Berthold automatic TLC-linear analyzer (Perkin-Elmer, France). Counting of samples and dpm computation were carried out with a LKB-Wallac 1209 rack beta liquid scintillation counter fitted with external standard equipment and automatic background subtraction (Perkin-Elmer, France).

Due to the poor TLC separation of the 7α- and 7β-epimers in extracts of EPIA and 5α-androstane-3β,17β-diol digests, the mixtures of 7-hydroxylated compounds were eluted from the TLC plate and separated by high performance liquid chromatography (HPLC). A Shimadzu LC-6A HPLC apparatus (Shimadzu, Kyoto, Japan) fitted with a 26 cm C18-coated silica column (5 μm) (Ultrabase, SFCC, Neuilly Plaisance, France). The elution was carried out at 0.7 mL/min with methanol/water 7:3 (v/v) for the separation of 7β-hydroxy-EPIA from 7α-hydroxy-EPIA with collection of the fractions between 6.5 and 7 min, and between 7.5 and 8.5 min, respectively. The elution was carried out at 0.7 mL/min with methanol/water 6:4 (v/v) for the separation of 5α-androstane-3β,7β,17β-triol from 5α-androstane-3β,7α,17β-triol with collection of the fractions between 7

and 8 min, and between 9.5 and 11 min, respectively. The fractions collected were counted for quantification of the steroids.

2.4. Gas chromatography/mass spectrometry

Identification of the steroid metabolite produced was carried out by GC/MS analysis with an Agilent Technologies (Massy, France) system, including a 6980N network GC apparatus coupled with a 5973 network mass selective detector. The GC was fitted with a 30 m fused silica capillary column HP-5MS (i.d; 250 μm) coated with a 5% phenyl methyl siloxane phase (0.25 μm thickness). The system was used under the following conditions: injection of the sample (3 μL) was made at 80 °C and the temperature was increased one min later at 30 °C per min up to 240 °C; after 8 min at 240 °C another temperature increase was applied at 30 °C per min up to 300 °C, and the 300 °C was maintained for 30 min. Trimethylsilyl (TMS) ether derivatives of reference and of the steroid metabolites recovered from incubations were prepared by reacting the dried steroids in 30 μL pyridine with 20 μL bis-(trimethylsilyl)-trifluoroacetamide + 1% chlorotrimethylsilane (Sigma) at 60 °C for 60 min. The TMS derivatives were dried and taken up in toluene prior to injection on the GC column. The comparison of reference and metabolite mass spectra was carried out with the aid of the G1034C MS Chemstation software (Agilent Technologies, Massy, France) that provided the percent match between mass spectra.

2.5. Human P450-3A4 and testosterone 6β-hydroxylation measurements

The transformation of yeasts and their expression of the human P450-3A4 have been previously reported [20]. P450-3A4-containing microsomes of the transformed yeast were produced for incubation with the [4-^{14}C]-testosterone substrate in the presence of the NADPH regenerating system as described above. After extraction, the testosterone substrate was separated from the 6β-hydroxy-testosterone produced by TLC in $CHCl_3$/ethyl acetate/ethanol (56:14:10, v/v/v). The 6β-hydroxylation yields were computed after scanning of the plates in a Berthold automatic TLC-linear analyzer (Perkin-Elmer, France).

3. Results

3.1. Identification of the 7-hydroxylated metabolites produced

After incubation of the [4-^{14}C]-labeled steroid substrates with the human P4507B1, the 7α- and 7β-hydroxylated radiometabolites obtained were separated after TLC, localized on the plate by autoradiography and eluted with ethyl acetate/methanol. When necessary, such as for EPIA and 5α-androstane-3β,17β-diol, the eluted pool of 7α- and 7β-hydroxy steroids was separated after HPLC, and the TMS derivatives were prepared and analyzed by GC/MS. Comparison of retention times and of the mass spectra obtained, with those of authentic 7α- and 7β-hydroxysteroid-TMS, showed identical retention times and fragmentation patterns and matching mass spectra between references and metabolites (Table 1). The data ascertained that the radiometabolites obtained after DHEA, EPIA, and 5α-androstane-3β,17β-diol incubations were their respective 7α- and 7β-hydroxylated metabolites indeed, and that their production rates could be used for the measurement and kinetics of the P4507B1-mediated 7α- and 7β-hydroxylation of DHEA, EPIA, and 5α-androstane-3β,17β-diol.

In the case of [4-^{14}C]-PREG and [4-^{14}C]-E_1, no 7β-hydroxylated derivative could be detected. The TMS derivatives of the eluted 7α-hydroxy-PREG and 7α-hydroxy-E_1 were prepared and analyzed by GC/MS. Comparison of retention times and of the mass spectra obtained, with those of authentic 7α-hydroxy-PREG-TMS and 7α-hydroxy-E_1-TMS showed identical retention times and fragmentation patterns (Table 1). The data ascertained that only 7α-hydroxy-PREG and 7α-hydroxy-E_1 were produced, and that their production rates could be used for the measurement and kinetics of the P4507B1-mediated 7α-hydroxylation of PREG and E_1.

3.2. Kinetic parameters measurements

The K_M values obtained for the P4507B1-mediated 7α- and 7β-hydroxylations of the DHEA, EPIA and 5α-androstane-3β,17β-diol substrates were almost identical and are reported in Table 2. In contrast, velocities (shown as K_{cat}) were much lower for 7β-hydroxylation than for 7α-hydroxylation and reflect the low production of the 7β-hydroxylated metabolites. With EPIA, we observed a two-step substrate concentration-related kinetic. At low EPIA concentrations (1.0–4.0 μM), the K_M measured was 0.04 ± 0.01 μM, while higher EPIA concentrations (4.0–12.0 μM) resulted in a much higher K_M of 1.05 ± 0.12 μM.

The K_M and K_{cat} obtained for the P4507B1-mediated 7α-hydroxylation of PREG and E_1 are reported in Table 2. The low K_{cat} reflects the low production rates of the 7α-hydroxy-PREG and 7α-hydroxy-E_1 metabolites.

3.3. Inhibitions by estrogens and β-amyloid peptide components

The steroid estrogens (E_1 and E_2) and the β-amyloid peptides ([1–40], [1–28] and [25–35]) were selected for inhibition studies of the P4507B1-mediated 7α-hydroxylation of DHEA. Each inhibitor was used at two concentrations, and double reciprocal plots were constructed (Figs. 1 and 2). Both E_1 and E_2 at 5 and 10 μM exerted a mixed type inhibition

Table 1
GC/MS identification under TMS derivative form of the steroid metabolites produced after incubations of DHEA, PREG, EPIA, 5α-androstane-3β,17β-diol and E_1 with the human P4507B1

Steroid identified	Reference R_t (min)	Sample R_t (min)	M^+ ion (m/z)	100% ion (*m/z*)	Match of spectra (%)
7α-Hydroxy-DHEA-TMS	13.58	13.59	448	358	99
7β-Hydroxy-DHEA-TMS	14.95	15.05	448	358	96
7α-Hydroxy-PREG-TMS	15.76	15.72	476	386	96
7α-Hydroxy-EPIA-TMS	14.06	13.97	450	360	99
7β-Hydroxy-EPIA-TMS	15.65	15.57	450	435	99
5α-A-3β,7α,17β-triol-TMS	13.72	13.93	524	393	99
5α-A-3β,7β,17β-triol-TMS	15.90	15.87	524	434	99
7α-Hydroxy-E_1-TMS	14.84	14.78	430	340	99

R_t, Retention time.

of the 7α-hydroxylation process with concentration-related increase of K_M and decrease of V_{max} (Fig. 1A and B). The K_i calculated were of 2.34 ± 0.03 μM and 0.464 ± 0.006 μM for E_1 and E_2, respectively. The inhibitions exerted by the β-amyloid peptides differed according to the amino acid sequences used. For each β-amyloid peptide tested, control incubations were carried out with identical buffer and peptide medium constituents. Fragment [25–35] was the most efficient with a non-competitive inhibition exerted at 0.5 μM and 5 μM, resulting in unchanged K_M and lowered V_{max} (Fig. 2B). The K_i calculated was 10.96 ± 0.30 μM. In contrast [1–28] fragment yielded no inhibition when used at the 0.5 μM (not shown) and 5 μM concentrations (Fig. 2A). β-amyloid peptide [1–40] used at the same concentrations as [25–35] fragment, yielded a much less extensive non-competitive inhibition (Fig. 2A) that did not permit the calculation of the K_i.

In order to ascertain whether the non-competitive inhibition of [25–35] β-amyloid fragment was specific to P4507B1, the testosterone 6β-hydroxylation carried out by the yeast-expressed P450-3A4 was used for control experiments. Due to the high K_M of P450-3A4 for testosterone 6β-hydroxylation (140 μM) and absence of cytochrome b_5 in the medium, the yields in 6β-hydroxy-testosterone produced were low. Kinetic measurements in the presence of 5 and 10 μM [25–35] β-amyloid fragment yielded no difference with control experiments carried out in the absence of the β-amyloid fragment (data not shown).

4. Discussion

The W(hR) yeast strain used for expression of the human P4507B1 contained the human NADPH-P450-reductase expressed in its microsomes [15]. Native microsomes from the untransformed W(hR) strain contained no P450 detectable by the Omura and Sato procedure, and did not transform DHEA and the other steroids used in this study. After expression of the human P4507B1 in the transformed yeast, P450 contents could be measured in microsomes and the DHEA-7α-hydroxylating activity was obtained. This approach allowed the study of hydroxylations carried out by the human P4507B1 without interference of any other steroid-metabolizing human proteins.

Our data show that the yeast-expressed human P4507B1 carries out the NADPH-dependent 7α- and 7β-hydroxylation of DHEA and of other 3β-hydroxysteroid substrates. The production of both 7-hydroxylated metabolites has already been reported in mouse with use of the yeast-expressed mouse P4507b1 [21] and of the HeLa-expressed mouse P4507b1 [13]. Other species, such as *Fusarium moniliforme*, contain a DHEA-inducible P450 that carries out the 7β-hydroxylating activity as a side transformation to the DHEA-7α-hydroxylation process [22]. Furthermore, we report identical K_M values for the 7α- and 7β-hydroxylation of DHEA, EPIA and 5α-androstane-3β,17β-diol, respectively, and the 7β-hydroxylation did not occur for the PREG and E_1 substrates. These results taken together ascertain that the 7β-hydroxylation of DHEA by the P4507B1 was not an

Table 2
Kinetic parameters obtained with steroid substrates for the human P4507B1

Steroid Substrates	7α-Hydroxylation		7β-Hydroxylation	
	K_M[a]	K_{cat}[b]	K_M[a]	K_{cat}[b]
DHEA	1.90 ± 0.05	3.14 ± 0.10	2.38 ± 0.27	0.088 ± 0.009
EPIA	1.05 ± 0.12	0.167 ± 0.006	1.05 ± 0.12	0.073 ± 0.002
5α-Androstane-3β, 17β-diol	0.80 ± 0.04	0.54 ± 0.03	0.80 ± 0.04	0.073 ± 0.004
PREG	1.45 ± 0.03	0.22 ± 0.01	nd	nd
E_1	1.35 ± 0.30	0.007 ± 0.001	nd	nd

nd, Not detected.
[a] μM ± S.E.M.
[b] pmol. min^{-1}. pmol $P4507B1^{-1}$ ± S.E.M.

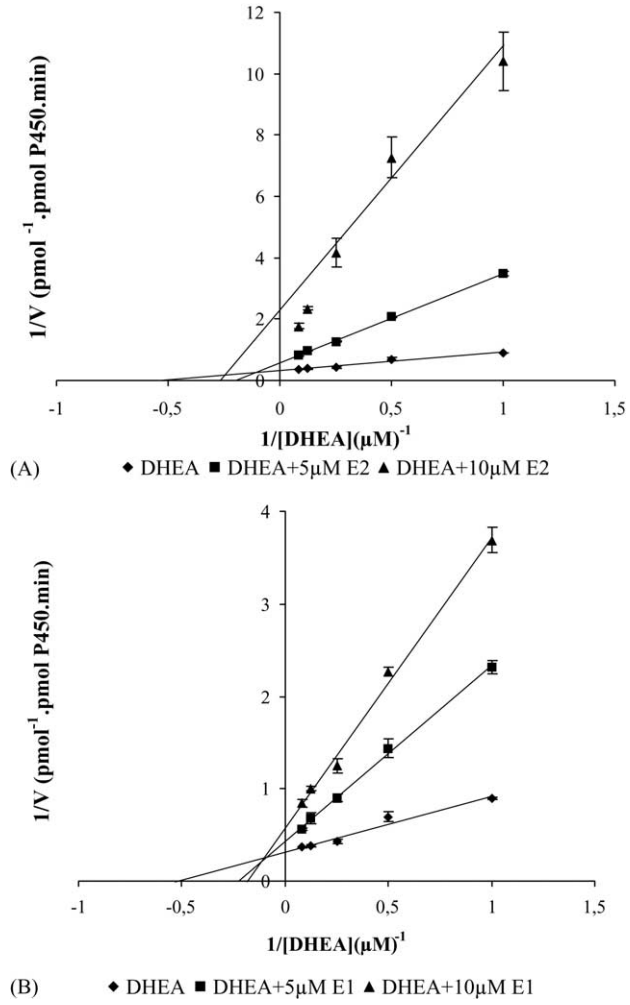

Fig. 1. Inhibitions by estradiol and estrone of the NADPH-dependent DHEA 7α-hydroxylation carried out by the yeast-expressed human P4507B1. Estradiol (E_2) at two concentrations exerted a mixed-type inhibition (A), estrone (E_1) at two concentrations exerted a mixed-type inhibition (B). Error bars indicate the SEM calculated from three different experiments.

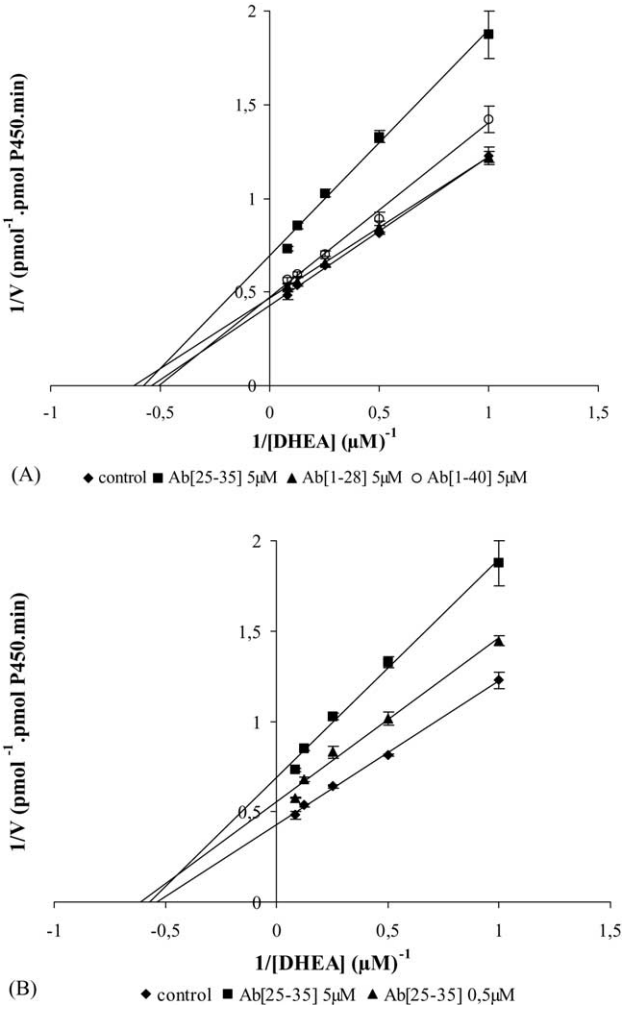

Fig. 2. Inhibitions by β-amyloid peptides of the NADPH-dependent DHEA 7α-hydroxylation carried out by the yeast-expressed human P4507B1. β-amyloid peptides ([1–40], [1–28] and [25–35]) were tested at 5 μM with a non-competitive inhibition exerted by [25–35] mainly and [1–40] barely (A); [25–35] β-amyloid peptide used at two concentrations clearly exerted a non-competitive inhibition (B). Error bars indicate the SEM calculated from three different experiments.

artifact, and suggest a wobble positioning of the substrate in the active site. It is well known that substitution borne in steroids at equatorial positions (such as 3β and 7β), are more thermodynamically stable than those borne at axial positions (such as 3α and 7α). In the P4507B1-catalysed 7α-hydroxylation process, the wobble positioning of the steroid substrate in the active site may result with a tendency to yield in part to the thermodynamic stability.

Because of the low efficiency of DHEA 7β-hydroxylation by P4507B1 in comparison with the measured almost equal concentrations of 7α- and 7β-hydroxy-DHEA in human plasma [23], the P4507B1-mediated production of 7β-hydroxy-DHEA cannot be the sole source for this metabolite. Nevertheless, the activity of the P4507B1 is necessary for 7β-hydroxy-DHEA production because knockout mice for this gene produce neither 7α-hydroxy-DHEA nor 7β-hydroxy-DHEA [24]. Thus, an oxidoreduction process starting with 7α-hydroxy-DHEA and the NADPH-dependent 11β-hydroxysteroid dehydrogenase type 1 was shown to be necessary for the production of 7β-hydroxy-DHEA [25].

The K_M measured for the 7α-hydroxylation of DHEA was the same as the one previously reported [11]. In addition, lower values were found for K_M all the other steroid substrates (with DHEA > PREG > E_1 > EPIA > 5α-androstane-3β,17β-diol), indicating that highest and lowest affinity for P4507B1 were for 5α-androstane-3β,17β-diol and DHEA, respectively. The biphasic saturation curve for EPIA cannot be explained, but indicate a putative very high affinity of P4507B1 for EPIA when present at the low concentrations usually present in human tissues. In contrast, the V_{max} (given as K_{cat} in pmol product formed .min^{-1}. pmol P4507B1^{-1}) were found highest for DHEA and lowest for E_1 (with DHEA > 5α-

androstane-3β,17β-diol > PREG > EPIA > E_1). These V_{max} values indicate that the high affinity of PREG, E_1, EPIA and 5α-androstane-3β,17β-diol for the P4507B1 did not result into a large production of their 7α-hydroxylated derivatives, and that they may be native competitors for the DHEA 7α-hydroxylating process.

The present findings on the 7α-hydroxylation process on DHEA and other steroids may be of importance for the regulation of endocrine actions. Thus, the 7α-hydroxylation of a 3β-hydroxylated metabolite of progesterone in the brain was outlined as a mechanism for the regulation of anesthetic steroids levels [26], and the 7α-hydroxylation of 5α-androstane-3β,17β-diol in the prostate was described as a mechanism responsible for the regulation of prostatic 5α-dihydrotestosterone levels and androgenic activity in mice [6]. Our evidences for a mixed type inhibition of E_1 and E_2 for the 7α-hydroxylation process imply that the native circulating estrogens may interfere with such regulations. It is known that in humans the circulating levels of 7α-hydroxy-DHEA are significantly lower in females than in males [23], and this may be due to the higher estrogen levels available in females. The possible occurrence of other polymorphisms within the P4507B1 open reading frame that could lead to modified affinities for the steroid substrates has not been investigated yet. The recent observation that prostate cancer was less frequently occurring in orientals than in caucasians led to the finding of a C–G polymorphism in the P4507B1 promoter region that was associated both with a higher transcription rate of P4507B1 and a lesser occurrence of prostate cancer [27]. Occurrence of such a polymorphism suggests that there may be other natural polymorphisms to be found in the open reading frame of P4507B1.

Aside of the regulatory actions for the P4507B1, the 7α- and 7β-hydroxysteroids produced in the brain were found responsible for neuroprotection [8,28]. Our finding that the β-amyloid peptide is a non-competitive inhibitor specific of the P4507B1-mediated 7α-hydroxylation leads to several conclusions. The availability of β-amyloid peptides in the human brain is known in Alzheimer's diseased patients where they are responsible for the onset of the plaque deposits associated to the disease and to the neuronal losses. A large inhibitory activity was exerted only by [25–35] fragment that contains a [31–35] helix [29] responsible for toxic aggregates formation [30]. A much lower inhibition potency and no inhibition were found with [1–40] peptide and [1–28] peptide, respectively. Peptide [1–40] contains both the aggregating [15–23] and [31–35] helixes [30], and [1–28] contains [15–23] helix only. Our inhibition data suggest that [31–35] helix only may be responsible for the non-competitive inhibition of the P4507B1. In the human hippocampal neurons where the P4507B1 is localized [11], inhibition of the neuronal P4507B1 by [25–35] peptide may result into lower levels of the 7α-hydroxy-DHEA produced and in a lower neuroprotection. This suggests that, as neuroprotection decreases, neurons are lost at a higher rate. Evidences that P4507B1 transcript levels are decreased in Alzheimer's diseased brain [31] indicate that the P4507B1 synthesis in neurons is decreased in turn.

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