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Purification, Molecular Cloning, and Genomic Organization of Human Brain Long-Chain Acyl-CoA Hydrolase[1]

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An acyl-CoA hydrolase, referred to as hBACH, was purified from human brain cytosol. The enzyme had a molecular mass of 100 kDa and 43-kDa subunits, and was highly active with long-chain acyl-CoAs, *e.g.* a maximal velocity of 295 μmol/min/mg and K_m of 6.4 μM for palmitoyl-CoA. Acyl-CoAs with carbon chain lengths of C_{8-18} were also good substrates. In human brain cytosol, 85% of palmitoyl-CoA hydrolase activity was titrated by an anti-BACH antibody, which accounted for over 75% of the enzyme activity found in the brain tissue. The cDNA isolated for hBACH, when expressed in *Escherichia coli*, directed the expression of palmitoyl-CoA hydrolase activity and a 44-kDa protein immunoreactive to the anti-BACH antibody, which in turn neutralized the hydrolase activity. The hBACH cDNA encoded a 338-amino acid sequence which was 95% identical to that of a rat homolog. The hBACH gene spanned about 130 kb and comprised 9 exons, and was mapped to 1p36.2 on the cytogenetic ideogram. These findings indicate that the long-chain acyl-CoA hydrolase present in the brain is well conserved between man and the rat, suggesting a conserved role for this enzyme in the mammalian brain, and enabling genetic studies on the functional analysis of acyl-CoA hydrolase.

Key words: acyl-CoA thioesterase, amino acid sequence, gene structure, human brain, purification.

Long-chain acyl-CoA hydrolases/thioesterases [EC 3.1.2.2] include a number of enzymes which catalyze the hydrolysis of fatty acyl-CoAs to the corresponding free fatty acids and CoASH. This enzyme activity is widely distributed among organisms and cell types, and is found in several subcellular compartments. Of these enzymes, the thioesterase domain of the multifunctional fatty acid synthase (FAS) has been well characterized. It terminates chain elongation by cleaving the acyl thioester of the 4′-phosphopantetheine prosthetic group linked to the ACP domain (reviewed in Ref. *1*). The production of medium chain fatty acids in lactating mammary glands of certain species is also known to be directed by a specific acyl-CoA hydrolase, designated as thioesterase II (*2, 3*). This enzyme, although not an integral component of FAS, can gain access to its catalytic core and release the growing acyl chain from the 4′-phosphopantetheine moiety to modulate the product chain-length specificity of FAS. A similar enzyme has been found in the water fowl uropygial gland (*4*). However, no physiological functions have been attributed to many other acyl-CoA hydrolases, including bacterial thioesterases I and II (*5-7*), and a certain member of the carboxylesterase multigene family (*8*).

Since the first description appeared in the literature in 1958, it has been well documented that there is a markedly high level of hydrolytic activity toward long-chain acyl-CoAs in the brains of mammals (*9-16*), suggesting some specific role of the enzyme in this tissue. However, its physiological significance has remained unclear. In previous studies, we purified long-chain acyl-CoA hydrolase, referred to as rBACH, from rat brain cytosol and demonstrated, by immunoblot analysis, the ubiquitous distribution of its homologs in mammalian brains, including that of man (*17*). Immunohistochemistry revealed the neuronal localization of this enzyme in rat brain (*17*). Moreover, molecular cloning and sequence analysis confirmed that it was a novel acyl-CoA hydrolase (*18*). Broustas *et al.* (*19, 20*) also purified and cloned this enzyme from the rat brain, designating it as ACT. Their careful investigation of its catalytic

[1] Sequence data obtained from this study have been deposited in the DDBJ, EMBL, and GenBank databases under Accession No. D88894.

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[3] RT-PCR confirmed the expression of both rBACH and rLACH1 in the brain, and in the liver after the treatment of rats with peroxisome proliferators (Yamada *et al.*, unpublished data).

[4] Immunohistochemistry of adult rat testis localized rBACH in the spermatocyte, but not in Sertoli cells (Yamada *et al.*, unpublished data).

Abbreviations: ACP, acyl carrier protein; hBACH and rBACH, human and rat brain acyl-CoA hydrolase; FAS, fatty acid synthase.

properties further revealed the specific nature for the hydrolysis of acyl-CoAs (*19*). Thus, these studies (*17-20*) were the first to clearly define the catalytic and molecular properties of the long-chain acyl-CoA hydrolase present in the mammalian brain. However, until the present one, there has been no report dealing with the purification or molecular cloning of brain acyl-CoA hydrolase from any animals other than rats. Therefore in this study, for a better understanding of the mammalian brain long-chain acyl-CoA hydrolase, we purified the human homolog, temporarily termed hBACH, as the second example after that of the rat, and isolated its cDNA clone. This allowed us to analyze the structural organization of the hBACH gene using the human genome database.

MATERIALS AND METHODS

Materials—Human brain autopsy samples were obtained 12-25 h after clinical death from males aged 32-67 years at the Tokyo Medical Examiner's Office (Tokyo) and stored at −80°C. The antibody against rBACH was previously described (*17*). Calcium phosphate gel-cellulose was prepared according to the method of Koike and Hamada (*21*). Sephadex G-25 and G-150, Blue-Sepharose CL-6B, and Sephacryl S-200 HR were purchased from Pharmacia. Fatty acyl-CoAs were obtained from Sigma. Other chemicals were of the highest grade commercially available.

Enzyme Assay—Acyl-CoA hydrolase activity was measured by spectrophotometry as described previously (*17, 22*). The standard reaction mixture contained 20 μM palmitoyl-CoA, 0.1 mM 5,5′-dithio-bis(2-nitrobenzoic acid), 100 mM sodium phosphate (pH 7.4), and the enzyme in a final volume of 1 ml. After incubation at 30°C for 1.5 min, the reaction was started by adding the substrate, and then the absorbance at 412 nm was monitored continuously for 1.5-3 min. The molar absorption coefficient ε_{412} = 13,600 $M^{-1} \cdot cm^{-1}$ (*22*) was used to calculate the sulfhydryl group released from the acyl-CoA. Enzyme activity (U) is expressed as micromoles of acyl-CoA hydrolyzed/min at 30°C.

Purification Procedure—Brain tissue homogenates were prepared with 3 volumes of 0.25 M sucrose containing 1 mM EDTA and 10 mM Tris-HCl (pH 7.5), and centrifuged at 25,000 × *g* for 10 min. The supernatants were further centrifuged at 105,000 × *g* for 60 min, and the resulting supernatants were used as the brain cytosol. These procedures were carried out at 0-4°C, whereas the following ones were performed at room temperature (about 25°C). Unless otherwise noted, the buffers used were adjusted to pH 7.5 and contained 1 mM EDTA.

Cytosol prepared from 150 g of human brain tissue was heated at 61-62°C for 3 min, and after cooling to 10°C on ice, the pH of the cytosol was adjusted to 5.4 by the addition of 0.5 M acetic acid (*22*). After centrifugation at 25,000 × *g* for 10 min, the supernatant was neutralized to pH 7.5 with 0.5 M KOH and then precipitated with ammonium sulfate. The precipitate obtained (40-60% saturation) was dissolved and desalted in 10 mM potassium phosphate on a Sephadex G-25 column (2.6 × 6.5 cm), and then applied to a column containing calcium phosphate gel-cellulose (3.6 × 10 cm) equilibrated with the same buffer. This column was washed with 10 bed volumes of 30 mM potassium phosphate and then eluted with 120 mM potassium phosphate. The active fractions were pooled and 70% saturated with ammonium sulfate. The precipitate was dissolved in 10 mM potassium phosphate and then developed on a Sephadex G-150 column (2.6 × 95 cm) with 50 mM potassium phosphate containing 1 M ammonium sulfate. The inclusion of ammonium sulfate prevented a considerable loss of enzyme activity on high dilution. The active fractions were pooled, and the enzyme was precipitated with 70% ammonium sulfate, dissolved in 10 mM potassium phosphate and then desalted on a Sephadex G-25 column (Pharmacia PD-10) equilibrated with the same buffer. This preparation was applied to a Blue-Sepharose CL-6B column (2.3 × 7 cm) equilibrated with the same buffer. After the column had been washed with 10 bed volumes of 30 mM potassium phosphate, containing NADH and NADPH, 0.1 mM each, the enzyme was eluted with the buffer containing 5 μM palmitoyl-CoA. The addition of pyridine nucleotides to the washing buffer was effective in removing several contaminating proteins. The active fractions were pooled and then concentrated on a column of calcium phosphate gel-cellulose (1.4 × 1 cm). This column was equilibrated with 10 mM potassium phosphate and then eluted with 120 mM potassium phosphate. All the buffers used after this purification step contained 20% (v/v) ethylene glycol instead of EDTA. The enzyme was desalted in 10 mM potassium phosphate and then applied to a hydroxyapatite HCA-column (0.76 × 10 cm; Mitsui Toatsu Chemicals) connected to an HPLC system. After the column had been washed with 15 ml of 40 mM potassium phosphate at the flow rate of 0.5 ml/min, the enzyme was eluted with a linear gradient of 40-100 mM potassium phosphate (2 mM/min). The use of ethylene glycol minimized the loss of the enzyme due to the formation of an insoluble aggregate and improved the recovery of the enzyme from the hydroxyapatite column. The active fractions peaked at about 60 mM, and were pooled and concentrated on calcium phosphate gel-cellulose after desalting as described above. The purified enzyme was stored in liquid nitrogen in the presence of 120 mM potassium phosphate (pH 7.5) and 20% ethylene glycol.

Immunotitration—A fixed amount of brain cytosol was incubated at 25°C for 20 min with increasing amounts of anti-rBACH IgG bound to a constant volume of protein A Sepharose (Pierce). The palmitoyl-CoA hydrolase activity was then measured in the supernatant obtained on centrifugation (*16*).

cDNA Cloning and Bacterial Expression—A λgt 11 cDNA library of the human brain (Clontech) was screened by plaque hybridization with an 834-bp cDNA fragment of rBACH, and the insert cDNAs of positive clones were subcloned into pBluescript II SK(+) (Stratagene) at the *Eco*RI site for DNA sequencing, as previously described (*18*). For expression, the coding sequence of hBACH cDNA (nucleotides 59-1072 in Fig. 5) was amplified by PCR using 5′-GAATTCATGTCGGGCCCAGACGTC-3′ and 5′-AAGCTTAGTCTAGGGCTGAGGCTC-3′ as the sense and antisense primers, respectively. The PCR product was digested with *Eco*RI and *Hin*dIII, directionally inserted into the prokaryotic expression vector pKK223-3 (Pharmacia) at the corresponding restriction sites, and then used to transform *Escherichia coli* JM105. The expression of hBACH was induced with isopropyl thiogalactoside.

Genomic Library Screening—A human genomic library constructed in EMBL3 SP6/T7 (Clontech) was screened

with a 1,148-bp hBACH cDNA (nucleotides 1-1148 in Fig. 5) by plaque hybridization. The DNAs of positive clones were isolated, digested with *Sal*I, *Sfi*I, *Sac*I, and *Xho*I, and then subjected to Southern hybridization with the 1,148-bp cDNA probe radiolabeled with [α-^{32}P]dCTP. DNA fragments that hybridized with the probe were subcloned into pBluescript II SK(+) for sequencing.

Other Methods—The concentration of substrate acyl-CoAs was determined using 5,5′-dithio-bis(2-nitrobenzoic acid) after treatment with hydroxylamine (*23*). Protein was determined using a Bio-Rad DC protein assay kit with bovine serum albumin as the standard. Other standard methods were according to Ref. *24*.

RESULTS

Subcellular Distribution and Stability of the Enzyme Activity—Prior to purification, the subcellular distribution and stability of the enzyme activity were examined. When human brain homogenates were centrifuged as described under "MATERIALS AND METHODS," more than 90% of the palmitoyl-CoA hydrolase activity and 20% of the total protein were recovered in the cytosol. Then, the cytosol was diluted 10-fold to 0.5 mg protein/ml and the changes in its palmitoyl-CoA hydrolase activity were followed up to 24 h (Fig. 1). When the diluted cytosol was allowed to stand at 4°C, the hydrolase activity decreased rapidly, with 70% of the initial activity disappearing after 6 h. However, the enzyme activity was stable if only the cytosol was maintained at 25°C, in which 95% of the initial enzyme activity remained after 24 h. When the cytosol was placed at 25°C after incubation at 4°C for 6 h, the enzyme activity which had been lost at the lower temperature was rapidly restored, although not completely. These findings indicate the reversible cold lability of the hydrolase activity. Thus, we decided to purify the acyl-CoA hydrolase (hBACH) at room temperature using the cytosol as the enzyme source.

Purification of hBACH—As summarized in Table I, hBACH was purified 660-fold from the cytosol, giving a specific activity of 198 U/mg with 20 μM palmitoyl-CoA as the substrate. In all the column chromatographies performed, the enzyme activity was eluted as a single peak.

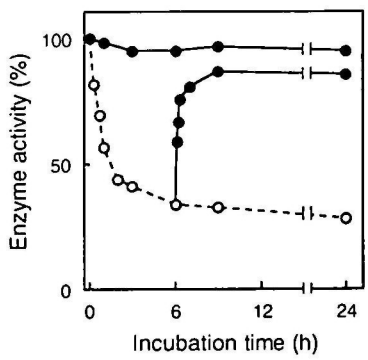

Fig. 1. **Changes in palmitoyl-CoA hydrolase activity in human brain cytosol during incubation.** The cytosol (0.5 mg protein/ml) in 100 mM potassium phosphate (pH 7.5) containing 1 mM EDTA was incubated for up to 24 h at 4 (○) or 25°C (●). A portion of the cytosol was incubated for 6 h at 4°C and thereafter at 25°C. The initial enzyme activity, taken as 100%, was 0.3 U/mg protein.

TABLE I. **Purification of hBACH.**

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)
Cytosol	1,052	3,364	0.3	100
Heat, pH 5.4	907	1,098	0.8	86
$(NH_4)_2SO_4$	762	238	3.2	72
Calcium phosphate gel-cellulose	572	57	10	54
Sephadex G-150	427	14	31	41
Blue-Sepharose CL-6B	228	1.3	175	22
Hydroxyapatite	158	0.8	198	15

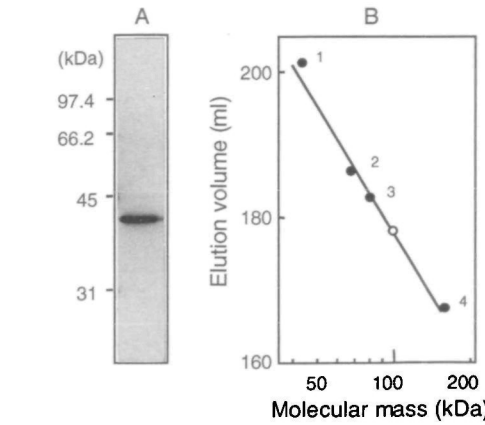

Fig. 2. **Molecular mass estimation of the purified hBACH.** (A) SDS-PAGE (10% gel) of the purified hBACH (0.3 μg) followed by silver staining. The positions of molecular mass markers (Bio-Rad) are indicated: phosphorylase *b* (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), and carbonic anhydrase (31 kDa). (B) Gel filtration on a Sephacryl S-200 HR column (2.6×64 cm) at 25°C with 100 mM potassium phosphate (pH 7.5) containing 20% (v/v) ethylene glycol and 1 mM EDTA, using the following molecular mass markers (●) for calibration: 1, ovalbumin (43 kDa); 2, bovine serum albumin (67 kDa); 3, transferrin (80 kDa); 4, aldolase (158 kDa). The position of the peak of palmitoyl-CoA hydrolase activity is shown (○).

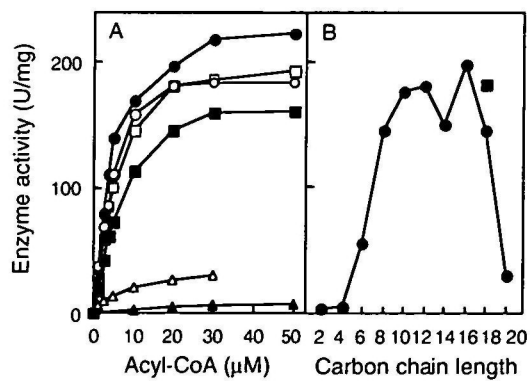

Fig. 3. **Chain length specificity.** (A) Enzyme activity as a function of the concentrations of fatty acyl-CoAs: ▲, butyryl-CoA (C_4); ■, octanoyl-CoA (C_8); ○, lauroyl-CoA (C_{12}); ●, palmitoyl-CoA (C_{16}); △, arachidoyl-CoA (C_{20}); □, oleoyl-CoA ($C_{18:1}$). (B) Profile of chain length specificity at a fixed substrate concentration of 20 μM: ●, saturated fatty acyl-CoAs; ■, oleoyl-CoA. The incubation mixtures included 18 ng of enzyme/assay. Each plot represents the mean for duplicate measurements.

The final preparation consisted of a single polypeptide with a molecular mass of 43 kDa, as determined by SDS-PAGE (Fig. 2A). The molecular mass of hBACH was also estimated to be 100 kDa by gel filtration (Fig. 2B). This was also the case with the brain cytosol, in which palmitoyl-CoA hydrolase activity peaked at a molecular mass of 100 kDa (data not shown). These results suggest that the native enzyme is a dimer, or trimer, of subunits identical in size.

Catalytic Properties—The dependence of the reaction velocity on the concentrations of various fatty acyl-CoAs is shown in Fig. 3A. Acyl-CoAs with carbon chain lengths of C_8, C_{12}, C_{16}, and $C_{18:1}$ gave roughly similar saturation curves, and the kinetic properties fitted the Michaelis-Menten model. Figure 3B summarizes the chain length specificity. The enzyme exhibited a relatively broad specificity, because acyl-CoAs with carbon chains of C_{8-18} were good substrates. Comparable enzyme activities were exhibited with oleoyl-CoA ($C_{18:1}$) and stearoyl-CoA (C_{18}). The kinetic constants are listed in Table II. The K_m values for long-chain acyl-CoAs ($C_{12,16,20}$ and $C_{18:1}$) were similar, being in the range of 6.4-9.2 μM, and the maximal velocities (V_{max}) were 269-295 U/mg for C_8, C_{12}, C_{16}, and $C_{18:1}$.

The enzyme was not affected by the serine-blocking reagents diisopropyl fluorophosphate (10 mM) and bis(*p*-nitrophenyl)phosphate (1 mM), but was completely inhibited by sulfhydryl-blocking *p*-chloromercuribenzoate (0.01 mM).

Immunotitration—A fixed amount of human brain cytosol was incubated with increasing amounts of anti-rBACH IgG (Fig. 4). With saturating amounts of anti-rBACH IgG, 95 ± 1% (mean ± SD for three brain samples) of the palmitoyl-CoA hydrolase activity was titrated, whereas 90 ± 1% of the enzyme activity remained after incubation with control IgG. The anti-rBACH antibody was confirmed to cross-react with the purified hBACH (data not shown), and its monospecificity has been previously shown by immunoblots of human brain cytosol (*17*).

cDNA Cloning and Sequence Analysis—A human brain cDNA library (1×10^6 clones) was screened with an 834-bp rBACH cDNA, 14 positive clones of 1.4-1.6 kbp being isolated. The largest clone, HBACH-7, spanned 1,403 bp, not including the poly(A) tail, and contained 58 bp of the 5′-noncoding sequence and an open reading frame encoding 338 amino acids, followed by a termination codon (TAG). This was followed in turn by a 331-bp 3′-noncoding sequence which contained a putative polyadenylation signal (AATAAA) located 20 bp upstream of a poly(A) tail (Fig. 5).

To verify that the isolated cDNA encoded hBACH, the hBACH cDNA coding sequence (nucleotides 59-1072) was subcloned into the prokaryotic expression vector pKK223-3. This construct, designated as pKK-hBACH, was ex-

TABLE II. **Kinetic constants.**

Substrate	K_m (μM)	V_{max} (U/mg)
Butyryl-CoA	—[a]	—[a]
Octanoyl-CoA	14.2	269
Lauroyl-CoA	7.6	275
Palmitoyl-CoA	6.4	295
Arachidoyl-CoA	9.2	50
Oleoyl-CoA	8.8	270

Values were obtained from double-reciprocal plots of the data shown in Fig. 3A. [a]Not determined.

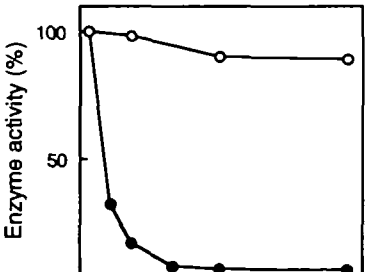

Fig. 4. **Immunotitration of palmitoyl-CoA hydrolase activity in human brain cytosol.** The cytosol from three brain samples, which contained 0.33 ± 0.03 U/mg protein (mean ± SD) of palmitoyl-CoA hydrolase activity, was incubated, respectively, with increasing amounts of anti-rBACH IgG (●) or control IgG (○) prepared from nonimmunized rabbit serum, and then the enzyme activity was measured in the supernatant obtained on centrifugation. Each plot represents the mean for duplicate measurements. Of three independent experiments, a typical result is shown.

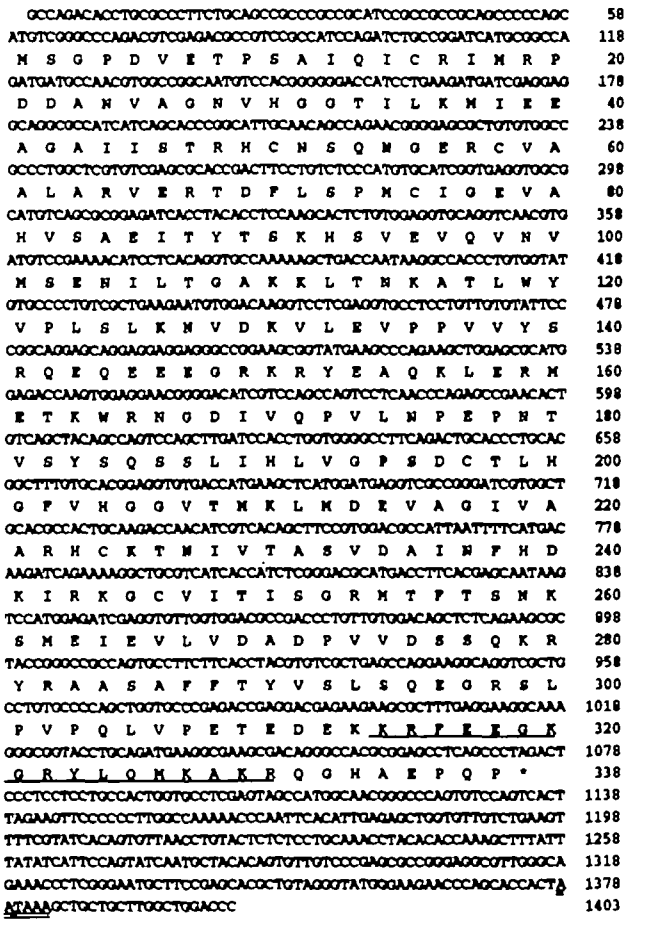

Fig. 5. **Nucleotide and deduced amino acid sequences of the hBACH cDNA.** The termination codon is indicated by an asterisk. The possible nuclear localization signal is underlined. The consensus polyadenylation signal in the 3′-noncoding region is double underlined.

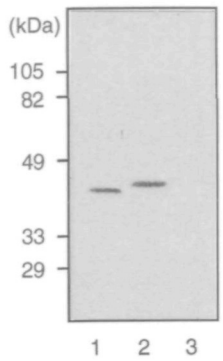

Fig. 6. **Immunoblots of extracts prepared from *E. coli* transformed with hBACH cDNA, probed with an anti-rBACH antibody.** Lane 1, purified hBACH (0.05 μg); lanes 2 and 3, extracts (5 μg) of *E. coli* transformed with pKK-hBACH and pKK223-3, respectively. The positions of molecular mass markers are indicated.

pressed in *E. coli*. As shown by immunoblots of bacterial extracts (Fig. 6), pKK-hBACH directed the expression of a protein immunoreactive to the anti-rBACH antibody (lane 2), whereas the empty vector did not (lane 3). This protein had a molecular mass of 44 kDa, compared to 43 kDa for the purified hBACH (lane 1). The palmitoyl-CoA hydrolase activity of the extract of *E. coli* transformed with pKK-hBACH was two orders of magnitude higher than that in the case of pKK223-3-transformed *E. coli* (3.65 *vs.* 0.04 U/mg protein). In addition, more than 90% of the induced activity in the former extract was neutralized by the anti-rBACH antibody. Therefore, we concluded that the cDNA encoded the correct enzyme. Northern hybridization, with hBACH cDNA as a probe, revealed an apparently single mRNA species of 1.9 kb in size in the human brain autopsy samples (data not shown).

Homology Search—The coding region of the hBACH cDNA was 89 and 95% identical to that of the rBACH cDNA (*18*) at the nucleotide and deduced amino acid levels, respectively. The corresponding values were 87 and 91%, respectively, compared to that of rLACH1, which we previously cloned as an isoform of rBACH (*18*). Of acyl-CoA hydrolases/thioesterases, rat CTE-II cloned by Engberg *et al.* (*25*), and ACT by Broustas *et al.* (*20*) were essentially the same as rBACH, so similar values to above were obtained on comparison with hBACH cDNA for identity. Hajra *et al.* also submitted a cDNA sequence for a human brain acyl-CoA thioester hydrolase (hACT, accession No. U91316), which is 1,492 bp in length and comprised our hBACH cDNA clone with a 91-bp extension at the 5′-end. The 1,403-bp nucleotide sequence of hBACH was 99.5% identical to the corresponding sequence of hACT, and so the deduced amino acid sequences of the first 328 residues completely matched. However, the absence of a nucleotide corresponding to that at 1046 of hBACH cDNA (Fig. 5) in the hACT cDNA caused a shift in the reading frame, resulting in a polypeptide with 372 amino acids, compared with the 338 amino acids of hBACH. Thus, the hBACH cDNA encoded a different enzyme. No other sequence was found in the database that exhibited significant similarity to the hBACH amino acid sequence.

Gene Structure of hBACH—The gene structure of hBACH was analyzed using the genomic sequence data provided by the human genome project, accession Nos. AL031848 and AL031847, which covered exons 1–6 and 7–9, respectively (Table III). Although the data for AL-031847 were in an "unfinished" state according to sequence map criteria, the DNA sequences around exons 7–9 were confirmed by genomic cloning and sequencing, as described under "MATERIALS AND METHODS." The DNA sequences of all the exons completely matched the corresponding sequences of hBACH cDNA. Intron sizes were calculated from the database. As summarized in Table III, the hBACH gene spanned about 130 kb and comprised 9 exons, the intron/exon boundaries of which were consistent with the donor/acceptor splice rule (*26*). Radiation hybrid mapping (*27*) localized the hBACH gene at D1S214–D1S224, which corresponded to 1p36.2 on the cytogenetic ideogram.

TABLE III. **Exon-intron boundaries of the hBACH gene.**

Exon	Exon size (bp)	5′-Splice donor site	Intron size (kb)	3′-Splice acceptor site
1	>105	TGCCG gtaagagagg	43.4	tctgccctag GATCA
2	118	ACGGG gtaagggtct	10.2	gtctccacag GAGCG
3	157	CACAG gtacttctggg	5.9	ttctcttaag GTGCC
4	92	TTGTG gtaaggtgcc	6.1	tttctcgcag TATTC
5	115	CCCAG gtaagagccg	8.7	gcaattccag AGCCG
6	87	CGGAG gtaagaagga	23.5	ttctccgcag GTGTG
7	117	AAAAG gtaccactc	13.6	ttctccacag GCTGC
8	185	TGGTG gtgagtgcgc	16.4	tggtccacag CCCGA
9	427	GACCC		

Exon and intron sequences are indicated in uppercase and lowercase letters, respectively. The size of exon 1 was not determined because the transcription start site was not known.

DISCUSSION

The data presented in this paper demonstrated that the long-chain acyl-CoA hydrolase present in the brain is well conserved in man and the rat. As predicted from the high similarity of the hBACH and rBACH primary structures, hBACH was also highly active toward long-chain acyl-CoAs, with a relatively broad specificity for chain-length, as is the case for rBACH (*17*). The cold lability of the enzyme activity and the molecular size of the native enzyme were also common to the two enzymes. Moreover, both enzymes were cytosolic and accounted for the majority of the palmitoyl-CoA hydrolase activity found in the brain tissue, in which comparable and very high activities of long-chain acyl-CoA hydrolase were detected in the respective species (*17*). These findings suggest a conserved role for BACH in the mammalian brain, although the neuronal localization of hBACH remains unknown.

In this study, the genomic organization of the brain acyl-CoA hydrolase was clarified for the first time (Table III). Previously, we cloned an isoform of rBACH, termed rLACH1, from the rat liver[3], and suggested that it was probably generated through alternative exon usage of the rBACH gene (*18*). The nucleotide sequence of rLACH1 cDNA was 84% identical to that of rBACH cDNA. However, unmatched nucleotides were confined to the 5′-end, which spanned the sequence encoding the N-terminal 21 amino acids of rLACH1. Interestingly, the boundary of the unmatched sequence coincides with that between the sequences corresponding to exons 1 and 2 on hBACH cDNA (between nucleotides 105 and 106 in Fig. 5, see Table III). Therefore, assuming that the structural organization of the

BACH gene is conserved in man and the rat, it is probable that rBACH and rLACH1 are products of a single gene and generated through alternative use of the first exon. Unfortunately, we failed to find the human homolog of rLACH1 on the hBACH genomic sequence. However, several BACH-related sequences have been found among the expressed sequence tags from certain tissues of man and the mouse that could give rise to novel isoforms through replacement of the exon 1 defined here (Table III). Thus, it will be of interest to determine how many kinds of BACH isoforms are generated, what functions they have, and how the expression is regulated, to gain an insight into the physiological role of acyl-CoA hydrolase.

In view of the importance of acyl-CoA in fatty acid metabolism, the potency of acyl-CoA hydrolases as to modulation of the cellular concentrations of acyl-CoAs may provide a regulatory mechanism for various aspects of lipid metabolism in the cell, such as lipid biosynthesis, fatty acid degradation and lipid modification of proteins. Recently, several kinds of novel acyl-CoA hydrolases were cloned (*18, 20, 25, 28-35*), some of which were also suggested to be associated with fatty acid oxidation. However, despite these speculations, the real functions of the enzymes have not yet been established, except for FAS-associated thioesterases (*1-4*), as described above.

In rats, BACH, also designated as CTE-II (*25*) and ACT (*20*), is constitutively expressed in the brain and testes at much higher levels than in other tissues (*16, 18, 20, 25*). However, it is contained in the neuron (*17*) and spermatocyte[4], but apparently not in the supporting cells such as glia and Sertoli cells, which produce various architectural lipid components, *e.g.* myelin lipids, in the respective tissues. In fact, studies by Engberg *et al.* (*25*) involving cell cultures overexpressing CTE-II provided no evidence that this enzyme is involved in membrane phospholipid synthesis. Broustas and Hajra (*19*) also reported that ACT is highly specific to the hydrolysis of acyl-CoA and can not act as an acyltransferase, transacylase or *O*-acyl hydrolase. Thus, the BACH function is enigmatic. BACH may serve as a scavenger of free long-chain acyl-CoAs in the cytosol, which are cytotoxic at high concentrations due to their own property as a detergent (*36, 37*). On the other hand, if the free acyl-CoA has some specific role, for example, as an intracellular signaling molecule, the regulated scavenging activity should be essential for switching off of the signals. In this context, the finding of Hertz *et al.* (*38*) that fatty acyl-CoAs are ligands of nuclear receptor HNF-4α that regulate its transcriptional activity is of interest. Long-chain fatty acids are also known to be ligands of PPARα (*39-41*). Thus, BACH may play a regulatory role by modulating the cellular levels, topologically in some cases, of acyl-CoA ligands for certain transcription factors, as well as the substrates for lipid metabolizing enzymes.

Whether BACH has acyl-protein thioesterase activity like APT1 (*42*) or PPT (*43*), or whether or not it can modulate the FAS product specificity like thioesterase II (*2-4*) remains to be examined. What is the endogenous substrate? Before the real function of brain acyl-CoA hydrolase can be established, many more studies are required. Genetic inactivation of the BACH gene in animal models will provide a useful approach for such investigation. Phenotypic analysis of defects in the hBACH gene will also be very useful. In this context, the present study will make a large contribution.

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