A Human Brain \(1\)-3-Hydroxyacyl-coenzyme A Dehydrogenase Is Identical to an Amyloid \(\beta\)-Peptide-binding Protein Involved in Alzheimer’s Disease

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Xue-Ying He‡, Horst Schulz§, and Song-Yu Yang‡¶

From the ‡Department of Pharmacology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314 and the §Department of Chemistry, City College of the City University of New York, New York, New York 10031

A novel \(1\)-3-hydroxyacyl-CoA dehydrogenase from human brain has been cloned, expressed, purified, and characterized. This enzyme is a homotetramer with a molecular mass of 108 kDa. Its subunit consists of 261 amino acid residues and has structural features characteristic of short chain dehydrogenases. It was found that the amino acid sequence of this human brain enzyme is identical to that of an endoplasmic reticulum amyloid \(\beta\)-peptide-binding protein (ERAB), which mediates neurotoxicity in Alzheimer’s disease (Yan, S. D., Fu, J., Soto, C., Chen, X., Zhu, H., Al-Mohanna, F., Collison, K., Zhu, A., Stern, E., Saito, T., Tohyama, M., Ogawa, S., Roher, A., and Stern, D. (1997) Nature 389, 689–695). The purification of human brain short chain \(1\)-3-hydroxyacyl-CoA dehydrogenase made it possible to characterize the structural and catalytic properties of ERAB. This NAD\(^+\)-dependent dehydrogenase catalyzes the reversible oxidation of \(1\)-3-hydroxyacyl-CoA to form \(3\)-ketocoyl-CoA, but it does not act on the \(D\)-isomers. The catalytic rate constant of the purified enzyme was estimated to be 37 s\(^{-1}\) with apparent \(K_m\) values of 89 and 20 \(\mu\)M for acetoyl-CoA and NADH, respectively. The activity ratio of this enzyme for substrates with chain lengths of \(C_4\), \(C_6\), and \(C_{16}\) was \(1:2:2\). The human short chain \(1\)-3-hydroxyacyl-CoA dehydrogenase gene is organized into six exons and five introns and maps to chromosome Xp11.2. The amino-terminal NAD-binding region of the dehydrogenase is encoded by the first three exons, whereas the other exons code for the carboxyl-terminal substrate-binding region harboring putative catalytic residues. The results of this study lead to the conclusion that ERAB involved in neuronal dysfunction is encoded by the human short chain \(1\)-3-hydroxyacyl-CoA dehydrogenase gene.

\(1\)-3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) catalyzes the third step of the fatty acid \(\beta\)-oxidation pathway: \(1\)-3-hydroxyacyl-CoA + NAD\(^+\) \(\rightarrow\) \(3\)-ketocoyl-CoA + NADH + H\(^+\) (1). This reaction is known to be catalyzed by mitochondrial monofunctional \(1\)-3-hydroxyacyl-CoA dehydrogenase and by prokaryotic and eukaryotic multifunctional \(\beta\)-oxidation enzymes that possess an \(1\)-3-hydroxyacyl-CoA dehydrogenase functional domain (2, 3). The catalytic residue of this kind of dehydrogenase was recently identified to be a conserved histidine (4), and a conserved glutamate residue is also required for high catalytic efficiency (5). The catalytic residue of \(1\)-3-hydroxyacyl-CoA dehydrogenase was proposed to interact with the conserved glutamate, and this electrostatic interaction seemed to be strengthened by the binding of substrate (5). However, we were surprised to see that a catalytic His-Glu pair is not present in the newly isolated bovine liver type II dehydrogenase (6, 7), which is not homologous to any of the known \(1\)-3-hydroxyacyl-CoA dehydrogenases. More interestingly, it was reported that this new type of \(1\)-3-hydroxyacyl-CoA dehydrogenase was not found in human liver by either Northern blot or immuno blot analysis (6, 7). As a result, several important questions emerged. Do humans have an \(1\)-3-hydroxyacyl-CoA dehydrogenase of this kind? If such a dehydrogenase exists in some human organ(s), what are its possible roles in human cells? Where is the human dehydrogenase gene located, and how is the gene organized? To find answers to these questions, we have cloned the cDNA encoding human brain short chain \(1\)-3-hydroxyacyl-CoA dehydrogenase\(^1\) and then mapped its gene and analyzed the gene structure as well. Moreover, the enzyme of interest was purified so that the structural and catalytic properties of this human brain dehydrogenase could be successfully characterized.

During the preparation of this manuscript, a 262-amino acid ERAB\(^2\) protein detected by use of the yeast two-hybrid system to screen human brain and HeLa cell cDNA libraries was reported to bind amyloid \(\beta\)-peptide (8). A fusion protein, ERAB with a polyclonal tag (His-ERAB), was generated and used to study the interaction of ERAB with amyloid \(\beta\)-peptide, and this protein was shown to mediate the cellular toxicity of amyloid \(\beta\)-peptide. ERAB, an important factor contributing to the neuronal dysfunction of Alzheimer’s disease, was proposed to be a hydroxysteroid dehydrogenase (8).

In this paper, we report the structure and organization of the human short chain \(1\)-3-hydroxyacyl-CoA dehydrogenase gene and the structural and catalytic properties of its gene product. The primary structure of human brain \(1\)-3-hydroxyacyl-CoA dehydrogenase was found to be identical to that of ERAB,
and thus, ERAB proved to be an L-3-hydroxyacyl-CoA dehydrogenase.

**EXPERIMENTAL PROCEDURES**

**Materials**—NAD^+^, NADH, CoASH, sodium DL-3-hydroxybutyrate, pig heart L-3-hydroxyacyl-CoA dehydrogenase, and all other standard biochemicals were obtained from Sigma. 2-Octynoic acid was purchased from Aldrich. dl-3-Hydroxybutyryl-CoA was provided by Metabolix Co. Acetoacetyl-CoA (9) and 2-hexadecenoyl acid (10) were synthesized according to published procedures. The CoA derivatives of 2-octynoic acid, 3-hydroxybutyric acid, and 2-hexadecenoic acid were synthesized by the mixed anhydride procedure (11). 3-Ketohexadecanoyl-CoA was enzymatically prepared from 2-hexadecenoyl-CoA as described previously (12). Human fetal brain Marathon-Ready cDNA and the Advantage cDNA PCR kit were purchased from CLONTECH. The pGEM-T vector and the Wizard Mini Prep DNA purification system were from Promega. Escherichia coli strain BL21(DE3)pLysS was obtained from Novagen. Restriction endonucleases and T4 ligase were supplied by New England Biolabs Inc. Oligonucleotides were synthesized by Integrated DNA Technologies, Inc.

**Construction of Expression Plasmid**—A full-length cDNA of the short chain L-3-hydroxyacyl-CoA dehydrogenase was cloned from human fetal brain Marathon-Ready cDNA according to the manufacturer’s procedures. Primer A was synthesized by simulating the active-site sequence of the bovine liver type II enzyme (7), and other primers were designed based on the sequence data obtained in the study. The nucleotide sequences of primers used in the PCR were as follows: primer A, 5'-TGGAGACAGAATATCAGAC-3', primer B, 5'-CTGCCAGATCTATCGGGATCCATATGGCAGCAGCGTGTCGGAGCGTGAAGG-3', and primer C, 5'-CGAATTCCTAGATCAGAGCTATCGAGATATCTG-3'. The resulting PCR products were cloned into the pGEM-T vector according to the manufacturer’s procedures. The DNA nucleotide sequence was determined by the dye-deoxy method (13), and base determination was automated by using an Applied Biosystems Model 370A DNA sequencer. The cDNA insert (Ndel-EcoRI) containing the whole coding region of human brain short chain L-3-hydroxyacyl-CoA dehydrogenase was removed from the recombinant plasmid pGEM-T-HBHAD by digestion with proper restriction enzymes and subcloned into the Ndel-EcoRI site of the vector pSBETA (a kind gift from Dr. H.-H. Steinbüll) to yield an expression plasmid designated pSBET-HBHAD.

**Overexpression and Purification of Human Brain Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase**—The plasmid pSBET-HBHAD was transformed into E. coli BL21(DE3)pLysS by the one-step transformation method (14). The transformants were induced by 0.5 mM IPTG for 6 h. The preparation of cell extract and the purification of the dehydrogenase were performed as described previously (15), except for the following modifications. The hydroxylapatite column (2.5 cm × 4 cm) was equilibrated with 50 mM potassium phosphate (pH 6.6) containing 10% glycerol and 2 mM mercaptoethanol and developed with a gradient made up of 75 ml of this buffer and 75 ml of 0.5 M potassium phosphate (pH 6.6) containing 10% glycerol and 2 mM mercaptoethanol. Fractions (5 ml) were collected and assayed for L-3-hydroxyacyl-CoA dehydrogenase. The recovered enzyme preparation was applied to a smaller hydroxylapatite column (1.5 cm × 5 cm) and eluted with the above-mentioned linear gradient again as a second fractionation step.

**Protein Analysis**—Protein concentrations were determined by the method of Bradford (16). Proteins were separated by SDS-PAGE on a 4–20% gradient gel at pH 8.3 as described previously (17). The molecular mass of the purified L-3-hydroxyacyl-CoA dehydrogenase was determined by gel filtration on a Sephadex G-100 column (1 × 50 cm) according to the standard protocol (18). The purified enzyme was transferred from SDS-polyacrylamide gel to a polyvinylidene difluoride membrane according to the Bio-Rad protocol, and then the N-terminal sequence was analyzed by automated Edman degradation (19) using an Applied Biosystems Model 470A gas-phase sequencer.

**Enzyme Assay**—The activity of L-3-hydroxyacyl-CoA dehydrogenase was determined in the backward direction with acetoacetyl-CoA, 3-ketoacetoacetyl-CoA, or 3-ketocholestenoyl-CoA as substrate according to the procedure described previously (20). The dehydrogenase assay in the forward direction was performed as described previously (21) using L- and dl-3-hydroxybutyryl-CoAs as substrates, respectively. Kinetic parameters of the dehydrogenase were estimated by analysis of the kinetic data with the computer program Leonora (22). The activities of 17β- and 3α,20β-hydroxysteroid dehydrogenases were measured as described previously (23, 24). The enzyme assay was carried out at 25 °C on a Gilford recording spectrophotometer (Model 2600). One unit of activity is defined as the amount of enzyme that catalyzes the conversion of 1 μmol of substrate to product/min.

**RESULTS AND DISCUSSION**

**Molecular Cloning, Expression, and Purification of Human Brain Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase**—The nucleotide sequence of the 5′-RACE PCR product (~0.6 kb) generated from human fetal brain Marathon-Ready cDNA by use of primer A and the adaptor primer 1 was determined by the dideoxy method (13), and then an overlapping 3′-RACE product (~1 kb) was made by PCR using primer B and the adaptor primer 1. The nucleotide sequence of the 3′-RACE fragment was also determined using the dideoxy method (13). Thereafter, a cDNA fragment (~0.8 kb) containing the whole coding region of the gene of interest was created by PCR using a pair of primers B and C that were based on sequence data obtained from the 5′- and 3′-RACE products, respectively. A recombinant plasmid (pSBET-HBHAD carrying this cDNA clone was introduced into E. coli BL21(DE3)pLysS cells. Transformants containing plasmids pSBET-HBHAD and pSBETA were induced by IPTG for 6 h. The specific activity of L-3-hydroxyacyl-CoA dehydrogenase in transformants containing plasmid pSBET-HBHAD was found to be >2 orders of magnitude higher than that in transformants containing a blank vector. Overexpression of the cDNA clone inserted in plasmid pSBET-HBHAD gave rise to a 27-kDa polypeptide band observed on SDS-PAGE (Fig. 1). The human brain dehydrogenase was purified 6-fold with a recovery of 33% of the original activity by chromatography on hydroxylapatite as summarized in Table I. The purified dehydrogenase was homogeneous as judged by SDS-PAGE (Fig. 1). Since the N-terminal sequence of the purified enzyme determined by Edman degradation perfectly matches the amino acid sequence deduced from the

![FIG. 1. SDS-PAGE analysis of E. coli cell extracts and purified human brain short chain L-3-hydroxyacyl-CoA dehydrogenase on a 4–20% gradient gel. Samples were as follows: IPTG-induced cell extracts of BL21(DE3) pLysS transformants containing pSBETA (lane 1) and pSBET-HBHAD (lane 2), purified human brain short chain L-3-hydroxyacyl-CoA dehydrogenase (lane 3), and pig heart L-3-hydroxyacyl-CoA dehydrogenase (lane 4). Protein molecular mass standards were run on the same gel, and their positions are indicated: phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).](image-url)
cDNA sequence (Fig. 2) and since the subunit size determined by SDS-PAGE is very close to the calculated molecular mass of 26,923 Da, it is apparent that the cloned cDNA encodes a novel L-3-hydroxyacyl-CoA dehydrogenase from human brain.

**Structure and Organization of the Human Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase Gene**—Before the human genomic library was screened with a cDNA fragment of the dehydrogenase, the GenBank Data Bank was searched with the Advance Blast program (25) using the human brain L-3-hydroxyacyl-CoA dehydrogenase cDNA sequence as a query sequence. A match with gaps was fortunately found in the human chromosome region Xp11.2. The nucleotide sequence of this 130-kb DNA segment was determined as part of the chromosome X project at the Sanger Center (Hinxton, United Kingdom) and has been submitted by D. Grafham to the GenBank™/EMBL Data Bank (accession no. Z97054). However, it was not known that this piece of DNA contains the human L-3-hydroxyacyl-CoA dehydrogenase gene. The exon locations were determined by closely scrutinizing the nucleotide sequence in the particular region where the human short chain L-3-hydroxyacyl-CoA dehydrogenase gene was mapped. All exon-intron junctions conform to the GT-AG rule (26) (Fig. 2). It was thus found that the direction of transcription of this gene is opposite to that of the KIAA0178 gene and the DNA-binding protein gene, which flank the gene of interest. The human L-3-hydroxyacyl-CoA dehydrogenase gene spans ~4 kb and consists of six exons and five introns (Fig. 3). The N-terminal amino acid sequence of the gene product was determined by Edman degradation. The results demonstrated that the first ATG codon in frame is the initial codon, and the 5'-untranslated region of the transcript contains a Kozak consensus sequence (27). Moreover, the sixth exon contains a stop codon (TGA) and a putative polyadenylation signal (Fig. 2).

**Structural Features of Human Brain Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase**—The molecular mass of the purified dehydrogenase was determined by gel filtration on Sephadex G-100 and found to be 108 kDa (Fig. 4). The subunit of this dehydrogenase consists of 261 amino acid residues (Fig. 2), and its subunit size was estimated to be 27 kDa by SDS-PAGE (Fig. 1). Hence, human brain L-3-hydroxyacyl-CoA dehydrogenase is a homotetramer. In contrast, mitochondrial L-3-hydroxyacyl-CoA dehydrogenase from pig heart is composed of two identical 33-kDa subunits (28) (Figs. 1 and 4).

On the basis of certain common structural features, a number of different dehydrogenases constitute the short chain dehydrogenase family (29). Of 13 largely conserved residues, 6 residues are strictly conserved in all short chain dehydrogenases (29). Sequence analysis revealed that the human brain L-3-hydroxyacyl-CoA dehydrogenase studied here not only has a size similar to other short chain dehydrogenases, but also has all 13 conserved residues (Fig. 5). Therefore, it is concluded that the L-3-hydroxyacyl-CoA dehydrogenase from human brain is a new member of the short chain dehydrogenase family. The primary structure of the human brain dehydrogenase is homologous to bacterial acetoacetyl-CoA reductase (30) and 3α,20β-hydroxysteroid dehydrogenase (31) with ~32% identity. The genes of these dehydrogenases are likely to have evolved from a common ancestor gene. Since the homology extends over the full
The human brain dehydrogenase is structurally distinct from the well documented mitochondrial L-3-hydroxyacyl-CoA dehydrogenases, e.g. pig heart L-3-hydroxyacyl-CoA dehydrogenase, which has a two-domain structure (28). The human brain dehydrogenase does not have the GXXGXXG fingerprint that exists in the NAD-binding domain of pig heart L-3-hydroxyacyl-CoA dehydrogenase and other medium chain dehydrogenases (4, 28, 33), but instead has a GXXXGXG glycine-rich sequence as the marker of the N-terminal NAD-binding region (29, 32). A more remarkable feature of the human brain dehydrogenase is that it does not have the catalytic His-Glu pair identified in other L-3-hydroxyacyl-CoA dehydrogenases and other medium chain dehydrogenases (4, 28, 33), but instead has a GXXXGXG glycine-rich sequence as the marker of the N-terminal NAD-binding region (29, 32). Among the three exons of this gene encode the amino-terminal NAD-binding region of the dehydrogenase, whereas the other exons code for the carboxyl-terminal substrate-binding region harboring putative catalytic residues (Figs. 2 and 3).

### Catalytic Properties of Human Brain Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase

The substrate stereospecificity of this novel enzyme is opposite to that of acetoacetyl-CoA reductase. The human brain dehydrogenase was found to metabolize only L-3-hydroxyacyl-CoA, but not the D-isomer (data not shown). Under the assay conditions used, neither 17β- nor 3α,20β-hydroxysteroid dehydrogenase activity was detected (<0.005 unit/mg). The catalytic properties of the purified human brain dehydrogenase were characterized by steady-state kinetic measurements (35). To simplify the measurements, the enzyme was usually assayed in the backward direction with 3-ketoacyl-CoA as substrate. The catalytic rate constant for the reduction of acetoacetyl-CoA by NADH at pH 7.0 is 37 ± 1.6 s⁻¹. The apparent $K_m$ values for acetoacetyl-CoA and NADH were found to be 89 ± 5.4 and 20 ± 2.8 μM, respectively, and the apparent $K_m$ values of the human brain enzyme are 15 ± 1.7 μM for the medium chain substrate and 16 ± 1.0 μM for the long chain substrate (Table II). The kinetic substrate specificity of this enzyme is quite different from those of other L-3-hydroxyacyl-CoA dehydrogenases. The activity ratio of this dehydrogenase for substrates with C₈, C₁₀, and C₁₆ acyl groups was 1:2:2 under standard assay conditions, whereas the activity of a mitochondrial matrix L-3-hydroxyacyl-CoA dehydrogenase with short chain substrate was usually greater than that with longer chain substrates. For example, the ratios of L-3-hydroxyacyl-CoA dehydrogenase activities with acetoacetyl-CoA to those with 3-ketooctanoyl-CoA were found to be about 20 and 1.2 for the bovine liver type I and II enzymes, respectively (6).

It is noteworthy that when acetoacetyl-CoA was used as substrate, the apparent $K_m$ values of human brain L-3-hydroxyacyl-CoA dehydrogenase for both the substrate and coenzyme were rather high in comparison with those of bovine liver type II 3-hydroxyacyl-CoA dehydrogenase (6). Moreover, in contrast to pig heart L-3-hydroxyacyl-CoA dehydrogenase being inhibited by acetoacetyl-CoA at concentrations above 25 μM (21), no substrate inhibition was observed with human brain short chain L-3-hydroxyacyl-CoA dehydrogenase (data not shown).

### The Human Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase Gene Encodes ERAB

During the preparation of this manuscript, a report appeared describing the involvement of a protein called ERAB in neuronal dysfunction associated with Alzheimer’s disease (8). ERAB was identified by the yeast two-hybrid system and was shown to bind to a fusion protein containing amyloid β-peptide (8). More ERAB antigen was found in the brains of patients with Alzheimer’s disease than in age-matched normal brains by immunoblotting with anti-ERAB peptide antibody (8). The quaternary structure of ERAB is not yet known, and ERAB was said to be a 262-amino acid polypeptide (8, 36). Only a His-ERAB fusion protein, but not the native protein, has been obtained (8). It is clear that the amino acid sequence of human brain short chain L-3-hydroxyacyl-CoA dehydrogenase is identical to that of ERAB and hence...
that ERAB is composed of 261 amino acid residues, but not 262 as reported previously. On the basis of the results presented here, it is concluded that ERAB, which previously was suggested to be a steroid dehydrogenase, is actually human brain short chain L-3-hydroxyacyl-CoA dehydrogenase. Hence, the structural and catalytic properties reported here for this novel L-3-hydroxyacyl-CoA dehydrogenase should apply to ERAB, which mediates neurotoxicity in Alzheimer’s disease (8, 36).

Since the molecular mass of human brain short chain L-3-hydroxyacyl-CoA dehydrogenase determined by SDS-PAGE and gel filtration matches well the value calculated from the amino acid sequence deduced from the cDNA sequence, this enzyme does not seem to undergo post-translational modification. This finding suggests that this novel L-3-hydroxyacyl-CoA dehydrogenase is synthesized in the cytosol, as are many other β-oxidation enzymes. It was reported that ERAB is localized in the endoplasmic reticulum and that the subcellular distribution of ERAB might change in the presence of amyloid β-peptide (8). However, since the N-terminal 16 residues of human brain short chain L-3-hydroxyacyl-CoA dehydrogenase are identical to those of the bovine liver mitochondrial type II enzyme (7), the human brain dehydrogenase of interest may also have a noncleavable mitochondrial targeting signal sequence, as seen in mitochondrial 3-ketoacyl-CoA thiolase (37). It therefore seems likely that this novel L-3-hydroxyacyl-CoA dehydrogenase is transported into mitochondria, as are other β-oxidation enzymes. But it may also be present outside of mitochondria. If so, human brain L-3-hydroxyacyl-CoA dehydrogenase could serve as an intracellular target for amyloid β-peptide (ERAB) and/or function in the elongation of fatty acids at the endoplasmic reticulum. However, we are certain that the newly identified amyloid β-peptide-binding protein is encoded by the human short chain L-3-hydroxyacyl-CoA dehydrogenase gene (Figs. 2 and 3). We have demonstrated in rats that brain mitochondria contain a considerable amount of L-3-hydroxyacyl-CoA dehydrogenase activity (38). We are currently determining the subcellular distribution of human brain short chain L-3-hydroxyacyl-CoA dehydrogenase.

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