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Primary Structure of Rat Liver D-β-Hydroxybutyrate Dehydrogenase from cDNA and Protein Analyses: A Short-Chain Alcohol Dehydrogenase[†,‡]

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ABSTRACT: The amino acid sequence of D-β-hydroxybutyrate dehydrogenase (BDH), a phosphatidylcholine-dependent enzyme, has been determined for the enzyme from rat liver by a combination of nucleotide sequencing of cDNA clones and amino acid sequencing of the purified protein. This represents the first report of the primary structure of this enzyme. The largest clone contained 1435 base pairs and encoded the entire amino acid sequence of mature BDH and the leader peptide of precursor BDH. Hybridization of poly(A+) rat liver mRNA revealed two bands with estimated sizes of 3.2 and 1.7 kb. A computer-based comparison of the amino acid sequence of BDH with other reported sequences reveals a homology with the superfamily of short-chain alcohol dehydrogenases, which are distinct from the classical zinc-dependent alcohol dehydrogenases. This protein family, initially discerned from *Drosophila* alcohol dehydrogenase and bacterial ribitol dehydrogenase, is now known to include at least 20 enzymes catalyzing oxidations of distinct substrates.

D-β-Hydroxybutyrate dehydrogenase (BDH)[1] is a lipid-requiring enzyme with a specific requirement of phosphatidylcholine for enzymatic activity. The activated enzyme catalyzes the reversible oxidation of D-β-hydroxybutyrate to acetoacetate utilizing NAD as coenzyme and is localized on the matrix side of the inner mitochondrial membrane (McIntyre et al., 1978). BDH is synthesized on cytoplasmic free ribosomes as a larger size precursor protein (Mihara et al., 1982). Precursor BDH must be transported into and processed by mitochondria in

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[‡] The genetic sequence of D-β-hydroxybutyrate dehydrogenase in this paper has been submitted to GenBank under Accession Number M89902.

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[1] Abbreviations: BDH, D-β-hydroxybutyrate dehydrogenase (EC 1.1.1.30); NEM, *N*-ethylmaleimide; DCCD, *N,N'*-dicyclohexylcarbodiimide; NBRF, National Biomedical Research Foundation; PIR, Protein Identificiation Resource; GCG, Genetics Computer Group; 17HSD, 17β-hydroxysteroid dehydrogenase (EC 1.1.1.62).

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order to attain its mature form.

The enzyme has been purified from bovine heart (Bock & Fleischer, 1975), rat liver (Vidal et al., 1977), and rat brain (Zhang & Churchill, 1990) mitochondria. BDH can be activated by phospholipid vesicles containing phosphatidylcholine (Fleischer et al., 1974) or soluble lecithins below the critical micellar concentration (Gazzotti et al., 1975).

The isolation and sequence analysis of two tryptic peptides from bovine heart BDH, one labeled with [^{3}H]NEM at a sulfhydryl and the other labeled with [^{14}C]DCCD at a glutamic acid residue, have been reported (Prasad & Hatefi, 1986). These authors also reported that the [^{3}H]NEM-labeled peptide had the same amino acid sequence as a BDH tryptic peptide which was labeled by (arylazido)-β-[3-^{3}H]alanyl-NAD (Yamaguchi et al., 1985). Despite this earlier progress, a complete primary structure for BDH has heretofore been lacking.

This paper outlines the elucidation of the primary structure of rat liver BDH from sequencing cDNA clones and Edman degradation of the intact protein and isolated BDH peptides and describes the basis for classifying BDH as a member of the short-chain alcohol dehydrogenase family (Jörnvall et al., 1981; Krook et al., 1990).

Experimental Procedures

Materials. Chemicals are reagent grade unless otherwise specified. Goat anti-rabbit IgG–peroxidase conjugate, 4-chloro-1-naphthol, gelatin, and bovine serum albumin were purchased from Sigma. Adjuvant was from RIBI Inc. T4 DNA ligase and restriction endonucleases were obtained from New England Biolabs. [α-^{35}S]dATPαS and [α-^{32}P]dATP were purchased from New England Nuclear. λZAPII rat liver cDNA library, exonuclease III, and mung bean nuclease were from Stratagene. Rat liver BDH was purified as previously described (Zhang & Churchill, 1990). Polyclonal antibodies against purified BDH were raised in rabbits, and the anti-BDH serum was prepared according to standard methods (Kaminsky et al., 1981).

Isolation of BDH cDNA Clones. A rat liver BDH cDNA clone was obtained by screening a λZAPII rat liver cDNA library (Stratagene) using antibodies prepared against purified BDH which had been preadsorbed to an *Escherichia coli* extract (Hoffman et al., 1983). This 1.2-kb clone was then used as a probe to screen the library by plaque hybridization. A positive clone was detected, replated, and rescreened until pure. This positive clone was excised from the vector and isolated in pBluescript KS (Stratagene).

DNA Sequencing. The two isolated clones in the pBluescript KS plasmids were treated with restriction endonucleases to generate a series of subclones for DNA sequencing. Two series of plasmids whose inserts were deleted successively were also constructed as follows. The plasmids were double-digested with *Sac*I and *Bam*HI and subjected to unidirectional deletion by exonuclease III for varying time intervals, and the plasmid ends were blunted with mung bean nuclease. The plasmids were ligated with T4 DNA ligase and isolated following transformation of *E. coli*. Plasmids with inserts in the reverse orientation were double-digested with *Sac*I and *Bgl*II and also subjected to exonuclease III and mung bean nuclease treatment. Single-stranded DNAs for each subclone in both orientations were used for DNA sequencing by the dideoxy chain termination method (Sanger et al., 1977).

Isolation and Sequencing of BDH Peptides. BDH was reduced and S-[^{14}C]carboxymethylated (Hempel et al., 1984). The intact protein was submitted to automated Edman degradation in an updated Beckman 890M sequencer, and PTH derivatives were separated by reversed-phase HPLC on an Altex Ultrasphere C-18 column using the gradient system of Hawke et al. (1982). Tryptic peptides were generated in 100 mM ammonium bicarbonate, pH 8.1, during a 24-h incubation with TPCK–trypsin (BDH:trypsin = 50:1 w/w). The digest was lyophilized three times and applied to a 4.0 × 250 mm sulfoethyl aspartamide column (The Nest Group, Southboro, MA) in 5 mM potassium phosphate/25% CH_3CN, pH 3.0, at 1 mL/min. A gradient of the same solvent containing 0.4 M KCl was applied over 1 h (Alpert & Andrews, 1988). The effluent was monitored at 214 and 280 nm (Waters 440 detector). The effluent was pooled according to UV absorbance and lyophilized; the pools were separately rechromatographed over a 4 × 250 mm Vydac C-18 column in aqueous 0.1% trifluoroacetic acid with linear gradients of acetonitrile. Aliquots from UV-absorbing peaks were analyzed for amino acid content after hydrolysis (24 h, 110 °C, 6 N HCl, 0.5% phenol in vacuo) with a Beckman 6300 amino acid analyzer. Material from selected peaks was submitted to Edman degradation either on the Beckman sequenator or on a Porton Model 2090E Instrument. With the latter instrument, PTH derivatives were identified on-line by HPLC (Hewlett-Packard 1090) using a gradient of acetonitrile against 0.1 M sodium acetate/0.12% triethylamine/3.5% tetrahydrofuran, pH 4.0.

Northern Analysis. Rat liver RNA was isolated according to the procedure of Chirgwin et al. (1979), and poly(A+)-containing mRNA was selected by oligo(dT)–cellulose column chromatography. The mRNA was denatured in the presence of formaldehyde and analyzed by electrophoresis in formaldehyde-containing denaturing agarose gels. The RNA ladder (0.24–9.4/kb) from Bethesda Research Laboratories was used as a size marker. The RNA was transferred to nitrocellulose and hybridized with the longest cDNA.

Sequence Comparisons. The BDH protein sequence was compared against all sequences in the NBRF database (George et al., 1986; PIR, 1, 2, and 3 data bases, March 1991 release) using the FASTA program (Pearson & Lipman, 1988). Selected pairwise alignments were also obtained via the GCG (Madison, WI) Bestfit program (version 7, April 1991).

Results

Sequence of cDNA Clones. The complete 1.2-kb (λBDH_{4-1}) and 1.4-kb (λBDH_{6-2}) cDNAs of rat liver BDH were sequenced on both strands using restriction sites and unidirectional deletions to produce subclones of each insert in both orientations. The sequencing strategy is presented in Figure 1, and the nucleotide sequence is presented in Figure 2. In the leader peptide portion of precursor BDH, two possible start positions exist at nucleotides 96 and 99. Comparison of this cDNA sequence with that determined from N-terminus of the mature protein (see below) indicates 46 or 47 amino acid residues in the leader (mitochondrial targeting) peptide, depending on which ATG actually initiates translation, with six arginine residues and nine serine/threonine residues. The cDNA sequence is supported by sequence data from numerous internal peptides (Figure 2). A 76-base polyadenylation signal for λBDH_{4-1} is seen within its 358 untranslated bases. The only detected sequence variability between the two isolated clones occurs in the untranslated 3′ ends at position 1411. The sequence of λBDH_{6-2} is TAG from 1412 to 1414 followed by a poly(A) tail of 21 residues. The poly(A) tail for λBDH_{4-1} begins at position 1409.

Protein Structural Analysis. The N-terminal sequence of BDH was established by direct protein sequencing. The carboxymethylated protein was subjected to tryptic digestion for further structural analysis. The digest was initially sep-

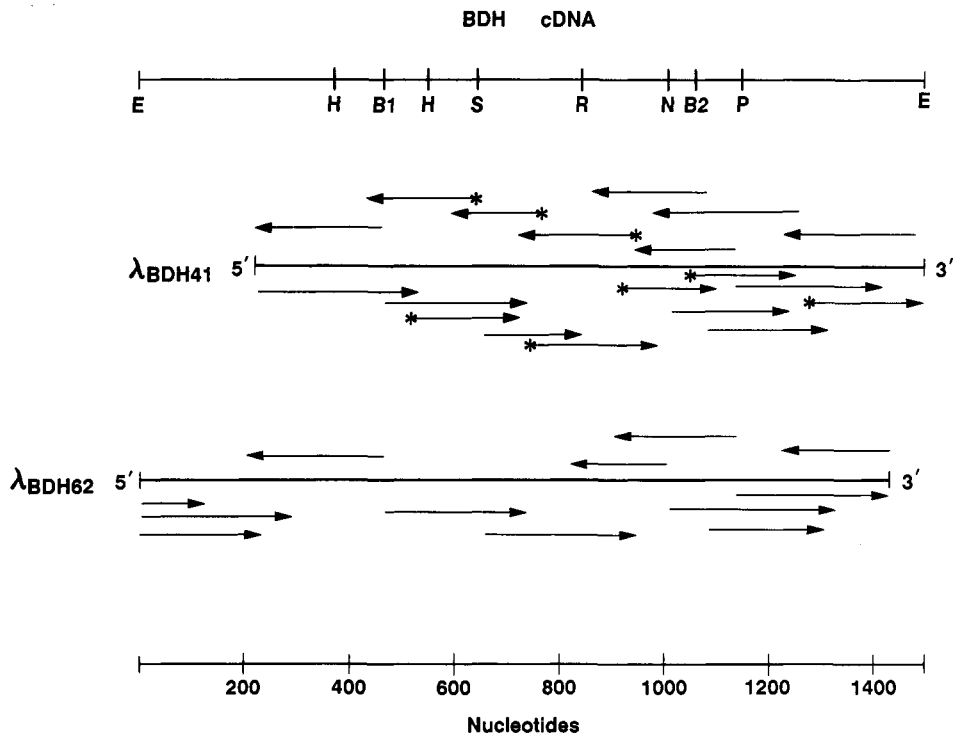

FIGURE 1: Sequencing strategy for BDH cDNA clones. Arrows indicate direction and extent of nucleotide sequence determination from clones obtained from restriction endonuclease cleavage fragments of the cDNAs. Arrows with asterisks indicate sequence clones which were obtained from exonuclease III cleavage of the cDNA. Restriction endonucleases: E, *Eco*RI; H, *Hin*cII; BI, *Bam*HI; S, *Sph*I; R, *Rsa*I; N, *Nco*I; B2, *Bgl*II; P, *Pst*I.

arated into multiple pools using ion-exchange chromatography (Figure 3). These pools were separately rechromatographed, and individual peptide fragments were separated by reversed-phase chromatography. The isolated peptides whose sequences were determined by Edman degradation or whose compositions were elucidated from amino acid analysis are also shown in Figure 2 (see also supplementary material).

Northern Analysis. Hybridization of poly(A+) rat liver mRNA with BDH cDNA revealed two transcripts of 3.2 kb and 1.7 kb on a Northern blot (Figure 4). The 1.7-kb transcript is 3 times more abundant that the larger 3.2-kb transcript. Future studies are required to determine if the sequence variability observed in the 3′ untranslated region is indicative of a difference between the two mRNAs.

Structural Comparisons with Other Dehydrogenases. An alignment of BDH with 17β-hydroxysteroid dehydrogenase (17HSD; PIR Accession S10654)) yielded the highest similarity score (233) using the FASTA program, with 29.7% identity. Excluding hypothetical proteins, of eight other alignments yielding triple-digit optimized scores, all were against members of the short-chain non-zinc-containing alcohol dehydrogenase family (Persson et al., 1991). These are ribitol dehydrogenase (PIR Accession DEKBR; score 110), sorbitol-6-phosphate dehydrogenase (DEESCP; 127), 11β-hydroxysteroid dehydrogenase (A34430; 140), nodulation protein G (C24706; 186), acetoacetyl-CoA reductase (RDALAE; 146), putative ketoacyl reductase (Act III, A28788; 195), nodulation protein C (C24193; 135), and hydroxyacyl-CoA dehydrogenase (S05397; 153).

Discussion

Fidelity of the Sequence. The 297-residue sequence of mature rat liver BDH as deduced from cDNA data is compatible with considerable directly determined protein sequence data (Figure 2) as well as amino acid compositional data on many additional peptides. A peptide sequence published earlier from the bovine enzyme (Yamaguchi et al., 1985) has 14 of 17 residues in common with residues 238–254 in the present mature protein sequence from rat. Another bovine BDH tryptic peptide of 12 residues (Prasad & Hatefi, 1986) is identical to rat liver BDH residues 117–128.

The indicated 46- or 47-residue mitochondrial targeting sequence is also consistent with results from in vitro translations of rat liver RNA followed by immunoprecipitation using anti-BDH serum which indicate that the precursor is approximately 5 kDa greater than the mature enzyme (Mihara et al., 1982). Mitochondrial targeting peptide sequences reported to date have been found to be rich in basic and hydroxylated amino acids. In the BDH signal peptide, seven residues are basic (6 arginine, 1 lysine) and nine are hydroxylated (5 serine, 4 threonine). Recent surveys of the cleavage sites of mitochondrial presursor proteins (Gavel & von Heijne, 1990; Hendrick et al., 1989; von Heijne, 1991) also indicate there are at least three different consensus patterns of amino acids near the cleavage site together with a fourth category that does not contain these patterns. The consensus pattern Arg-X-Tyr↓(Ser/Ala) resembles the BDH cleavage site Arg-X-Tyr↓Thr. Therefore, both the precursor peptide composition and cleavage site sequence of BDH appear consistent with other reported mitochondrial target peptide sequences.

Sequence Comparisons. The proteins from the NBRF database indicated to be most similar to BDH using the FASTA program (see Results) are all members of a short-chain nonmetalloenzyme dehydrogenase family (Persson et al., 1991). These short-chain alcohol dehydrogenases include multiple species of *Drosophila* alcohol dehydrogenase (Thatcher, 1980; Rowan & Dickinson, 1988; Green et al., 1989), bacterial ribitol dehydrogenase (Dothie et al., 1985), bacterial glucose dehydrogenase (Jany et al., 1984), two bacterial steroid dehydrogenases (Coleman et al., 1988; White et al., 1988), placental 17-hydroxysteroid dehydrogenase (Peltoketo et al.,

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CCCTCAATAGCCACACTATTTATTTTATTTCAATTAAAAATTTCTTCCCAAACCTTTCCT        60
GCACCTCCCTCACCCAAAACTATAAACTCGGTGCC ATG ATG CTG GCC GCC             110
                                      M   M   L   A   A

CGT CTT TCC AGA CCC CTG TCA CAG CTC CCA GGA AAA GCT CTA              152
 R   L   S   R   P   L   S   Q   L   P   G   K   A   L

AGT GTC TGT GAT AGA GAA AAT GGG ACA AGA CAC ACA CTG TTG              194
 S   V   C   D   R   E   N   G   T   R   H   T   L   L

TTT TAC CCA GCT TCT TTC AGC CCT GAC ACC CGT CGG ACC TAC              236
 F   Y   P   A   S   F   S   P   D   T   R   R   T   Y

ACC AGC CAG GCA GAT GCG GCT AGT GGC AAA GCT GTC CTG GTT              278
 T   S   Q   A   D   A   A   S   G   K   A   V   L   V
 1                                                  14
ACA GGC TGT GAC TCT GGA TTT GGG TTC TCT TTG GCC AAG CAT              320
 T   G   C   D   S   G   F   G   F   S   L   A   K   H
15                                                  28
CTA CAC TCA AAA GGT TTC CTT GTA TTT GCC GGA TGT TTG TTG              362
 L   H   S   K   G   F   L   V   F   A   G   C   L   L
29                                                  42
AAG GAA CAA GGC GAT GCT GGG GTC AGG GAG CTG GAC AGC CTG              404
 K   E   Q   G   D   A   G   V   R   E   L   D   S   L
43                                                  56
AAG AGT GAC CGG CTG AGA ACC ATC CAG CTC AAT GTC TGC AAC              446
 K   S   D   R   L   R   T   I   Q   L   N   V   C   N
57                                                  70
AGT GAG GAG GTG GAG AAA GCG GTG GAG ACC GTC CGC TCC GGC              488
 S   E   E   V   E   K   A   V   E   T   V   R   S   G
71                                                  84
CTG AAG GAT CCT GAG AAG GGA ATG TGG GGC CTG GTT AAC AAC              530
 L   K   D   P   E   K   G   M   W   G   L   V   N   N
85                                                  98
GCA GGC ATC TCA ACG TTT GGG GAG GTG GAG TTC ACT AGC ATG              572
 A   G   I   S   T   F   G   E   V   E   F   T   S   M
99                                                  112
GAG ACG TAT AAG GAG GTG GCC GAA GTG AAC CTC TGG GGA ACT              614
 E   T   Y   K   E   V   A   E   V   N   L   W   G   T
113                                                 126
GTG CGC ACA ACA AAA TCC TTC CTT CCC CTT CTC CGA AGA GCC              656
 V   R   T   T   K   S   F   L   P   L   L   R   R   A
127                                                 140
AAA GGC CGT GTT GTT AAC ATC AGC AGC ATG CTG GGT CGC ATG              698
 K   G   R   V   V   N   I   S   S   M   L   G   R   M
141                                                 154
GCC AAC CCA GCC CGC TCA CCA TAC TGC ATC ACC AAG TTT GGG              740
 A   N   P   A   R   S   P   Y   C   I   T   K   F   G
155                                                 168
GTA GAG GCT TTC TCG GAC TGC CTA CGC TAT GAG ATG CAC CCT              782
 V   E   A   F   S   D   C   L   R   Y   E   M   H   P
169                                                 182
CTG GGT GTG AAG GTC AGT GTG GTG GAG CCT GGC AAC TTC ATA              824
 L   G   V   K   V   S   V   V   E   P   G   N   F   I

183                                                 196
GCT GCC ACC AGC CTC TAT AGC CCT GAG CGT ATC CAG GCC ATT              866
 A   A   T   S   L   Y   S   P   E   R   I   Q   A   I
197                                                 210
GCC AAG AAG ATG TGG GAT GAG CTG CCA GAG GTC GTC CGC AAA              908
 A   K   K   M   W   D   E   L   P   E   V   V   R   K
211                                                 224
GAC TAT GGC AAG AAG TAC TTC GAT GAA AAG ATT GCC AAG ATG              950
 D   Y   G   K   K   Y   F   D   E   K   I   A   K   M
225                                                 238
GAG ACC TAC TGC AAC AGC GGT TCC ACC GAT ACG TCC TCC GTC              992
 E   T   Y   C   N   S   G   S   T   D   T   S   S   V
239                                                 252
ATC AAC GCT GTC ACC CAT GCC CTG ACT GCT GCC ACC CCT TAT             1034
 I   N   A   V   T   H   A   L   T   A   A   T   P   Y
253                                                 266
ACC CGC TAC CAT CCC ATG GAC TAC TAC TGG TGG CTG CGG ATG             1076
 T   R   Y   H   P   M   D   Y   Y   W   W   L   R   M
267                                                 280
CAG GTC ATG ACC CAT TTT CCT GGA GCC ATC TCT GAC AAG ATC             1118
 Q   V   M   T   H   F   P   G   A   I   S   D   K   I
281                                                 294
TAC ATA CAC TGAAGAGCTGAAGAGGTCCCTGCAGCCTCTGCCAGGGAGCCTGATGGG      1175
 Y   I   H   *
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295     297
AGGGAGTTCATACAGTTATCTTTTGATTAACCATTGTGGGTTGTCCACTGTCTTAGGAAG      1235
ACCTATTTTAACCTTACGTGTTCAATGTGGTGAATGGTTTGGGCCTTCACAAATACAGGG      1295
CACTGGTGGGTGGCCCTAACCCTCAAGGCCAATATGGTGCTTCTATCTGTCTATCTAGAG      1355

TTGATTTTATATAAAGATTTGTGGGAAATACCTTTATATTAAAGACGTTATTAGAA TAG A    1415
                                                          AAA

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA      1475
                  <--- BDH62
AAAAAAAAAA                                                         1485
          <---- BDH41
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FIGURE 2: Nucleotide sequence of the cDNAs for rat liver BDH and the predicted amino acid sequence of the enzyme. The nucleotides are numbered down the right-hand column. Solid underlining denotes regions confirmed by protein sequence analysis. Dashed underlining denotes regions supported by amino acid composition analysis.

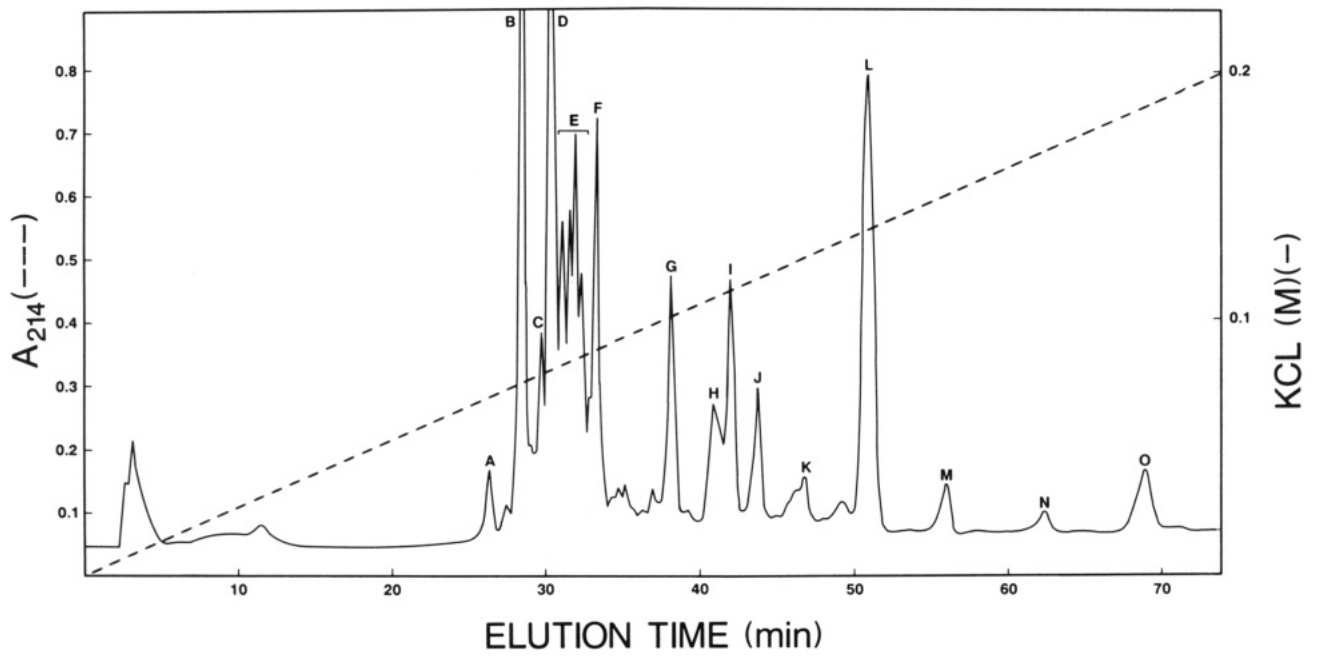

FIGURE 3: Isolation of BDH tryptic peptides. Elution profile of a tryptic digest from 2 mg of carboxymethylated BDH applied to a sulfoethyl aspartamide column in 5 mM potassium phosphate/25% CH_3CN, pH 3.0, and eluted with the same solvent containing 0.4 M KCl applied as a linear gradient, 0–100% over 1 h.

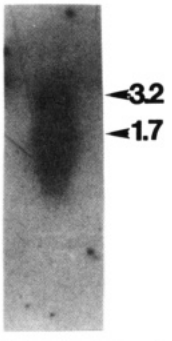

FIGURE 4: Northern analysis of poly(A+)-selected rat liver mRNA. Rat liver mRNA was denatured in the presence of formaldehyde and analyzed by electrophoresis in a denaturing agarose gel. The mRNA was transferred to nitrocellulose and hybridized to λBDH_{6-2}. The sizes of the two mRNAs detected were calculated to be 3.2 and 1.7 kb, on the basis of RNA markers (not shown).

1988), and human 15-hydroxyprostaglandin dehydrogenase (Krook et al., 1990). In pairwise alignments, these enzymes are about 25% identical to one another. Of 6 consensus (three of which are Gly) and 19 highly conserved residues (five are Gly) identified by Persson et al. (1991) from a global alignment of 20 such proteins, all but one of the consensus residues (BDH Asn-67 instead of Asp) and two of the highly conserved residues (Val-144 instead of Ile and Ile-164 instead of Ala) are present in BDH, as indicated in Figure 5. Thus, it seems clear that BDH should now be included in this family. This classification is also compatible with the overall size of the enzyme subunit, ca. 30 kDa, and the approximately 30% identity between 17HSD and BDH.

The structures of short-chain dehydrogenase family members differ significantly from the classical zinc-containing alcohol dehydrogenase family which includes the extensively studied yeast and liver enzymes plus at least six other proteins. The subunit size of classical alcohol dehydrogenases ranges from 350 to 375 residues, while the short-chain family contains approximately 250–300 amino acids (Persson et al., 1991). The classical dehydrogenase family has its catalytic domain in the N-terminal portion of its proteins followed by the coenzyme-binding domain closer to the C-terminal portion. This is reversed in the short-chain family, their coenzyme-binding domains being in the N-terminal segment followed by the catalytic domain. The classical dehydrogenase family frequently, although not always, contains a catalytic zinc atom (Borras et al., 1989). No metal cofactors have been found in any of the short-chain dehydrogenase family members thus far purified and characterized. Furthermore, unlike the extensively studied dehydrogenases isolated from yeast and liver, the reaction mechanism is not known for any short-chain dehydrogenase family member. If the short-chain family members share a common reaction mechanism, then at least

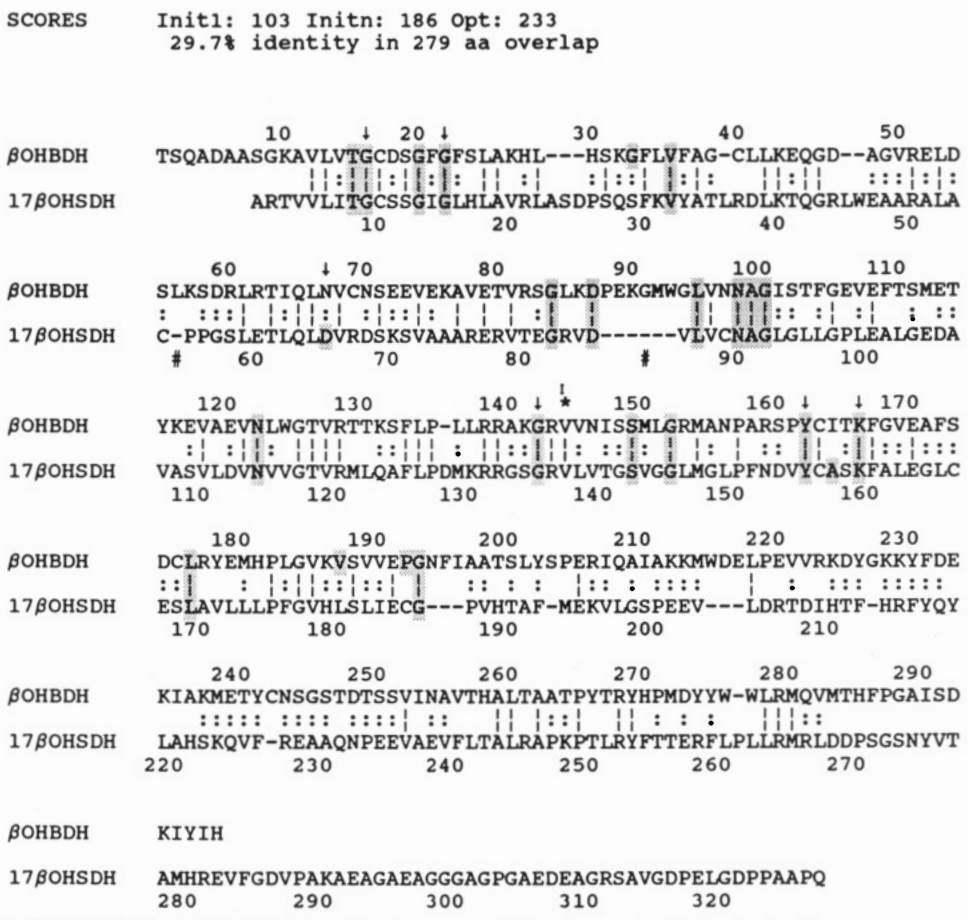

FIGURE 5: Alignment of BDH with 17β-hydroxysteroid dehydrogenase. This alignment, obtained using the FASTA program (ktup = 2) with the NBRF database, yielded the highest similarity score (initial n = 186, optimized = 233). Vertical bars denote matches (29.7% identify in 279 overlapping residues); colons denote substitutions with values ≥ 0 in the PAM 250 matrix (Dayhoff et al., 1983) and are thus not limited only to isofunctional substitutions. Of eight other alignments yielding triple-digit optimized scores, all were against members of the short-chain, non-zinc-containing alcohol dehydrogenase family (Persson et al., 1991) (see text). Residues in this enzyme family which are strongly conserved [identical in at least 15 of 20 sequences as aligned in Persson et al. (1991)] are indicated by a stippled background if they match the pattern. The location of absolutely conserved residues in that alignment are denoted by arrows. The location of a residue which is usually isoleucine in short-chain ADHs is indicated by the asterisk. The location of gaps not included in the short-chain ADH family alignment of Persson et al. is indicated by a pound sign (#).

some active site residues involved in catalysis would be expected to be conserved.

In the N-terminal portion of the BDH molecule, three glycine residues at positions 16, 20, and 22 are highly conserved in short-chain ADHs. This resembles the spacing expected for glycine residues located at the coenzyme-binding domain of a number of dehydrogenases (Rossman et al., 1975; Jörnvall et al., 1984; Wierenga et al., 1985). The second domain of the short-chain dehydrogenases is more variable than the putative coenzyme-binding domain. Previous authors have noted that a short segment from this domain appears to be well conserved in short-chain alcohol dehydrogenases and may be the active site of these enzymes (Jörnvall et al., 1981; Villarroya et al., 1989; Persson et al., 1991). A comparison of BDH with other family members reveals that except for the coenzyme-binding domain, the only conserved polar residues are Tyr-162, Lys-166, and Ser-149. One or more of these amino acids may be functionally important in catalysis. The investigation of the reaction mechanism(s) of the individual enzymes of the short-chain family will be aided by the knowledge and location of common potential catalytic residues within the active site domain. The different substrate utilization by these enzymes coupled with their presence in organisms ranging from bacteria to humans suggests this group of proteins forms a new superfamily of genes encoding dehydrogenases.

It has previously been suggested that similarities between BDH and lactate and malate dehydrogenases (oxidation of a short-chain hydroxy acid to a keto acid and utilization of NAD as cofactor) may be indicative of a common catalytic mechanism (Phelps & Hatefi, 1981; Prasad & Hatefi, 1986). However, no lactate or malate dehydrogenase sequences were found among the 40 best-scoring alignments using the FASTA program. This group included alignment optimization scores as low as 47.

The elucidation of the primary structure of rat liver BDH will prove invaluable to understanding the structure and function of this enzyme and is the first step in elucidating the mechanism of phosphatidylcholine-specific activation. The alignments provide further direction toward this goal. The BDH cDNA obtained will also be useful in studying the expression and regulation of BDH during development (Zhang et al., 1989a), diabetes (Vidal et al., 1983; Churchill et al., 1983), and rat hepatocarcinogenesis (Zhang et al., 1989b).

Supplementary Material Available

One table showing the amino acid compositions of tryptic peptides isolated from rat liver BDH (2 pages). Ordering information is given on any current masthead page.

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