cDNA and Protein Structure for the α Subunit of Human Liver Alcohol Dehydrogenase†

Hedvig von Bahr-Lindström, Jan-Olov Höög, Lars-Olof Hedén, Rudolf Kaiser, Louise Fleetwood, Kerstin Larsson, Mats Lake, Barton Holmquist, Arne Holmgren, John Hempel, Bert L. Vallee, and Hans Jörnvall*

Department of Chemistry I, Karolinska Institutet, S-104 01 Stockholm, Sweden, KabiGen AB, S-112 87 Stockholm, Sweden, and Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, Massachusetts 02115

Received October 1, 1985

ABSTRACT: Two cDNA clones for human liver alcohol dehydrogenase (ADH) were identified, together covering 1450 nucleotides that contain the cDNA sequence of the ADH_1 locus and include a coding region of 1122 nucleotides for the α subunit of the enzyme. In parallel, direct peptide analyses of the carboxymethylated protein also established most of the amino acid sequence. Nucleotide and peptide data were in complete agreement and show exchanges at 24 positions in the α relative to the β subunit. One of the cDNA clones had a 139-nucleotide internal deletion at a position of possible interest in relation to mRNA processing, ancestral connections, or DNA replication. The structure of the α subunit is homologous to that of the β and γ subunits but has many exchanges, also of functionally important residues, explaining the different enzymatic properties. In total, 35 of 374 amino acid residues differ between the class I isozymes, and the substitutions add an extra SH group in the α subunit. Only in the β-pleated sheet region of the coenzyme-binding domain is almost complete lack of substitutions noted, illustrating the importance of this region. In contrast, the active site region is far less conserved. However, similar exchanges of functional significance have also been found in distantly related alcohol and polyol dehydrogenases.

Human liver alcohol dehydrogenase (ADH; EC 1.1.1.1) has a complex isozyme pattern with forms differing in electrophoretic mobility and kinetic and immunological properties. The isozymes have been divided into three classes: pyrazole sensitive, basic isozymes constituting class I; less pyrazole sensitive, less basic isozymes constituting class II; forms with anodal electrophoretic mobility and low ethanol dehydrogenase activity constituting class III (Strydom & Vallee, 1982).

Class I isozymes consist of homo- and heterodimers of α, β, and γ subunits, which are coded for by three gene loci, ADH_1, ADH_2, and ADH_3 (Smith et al., 1971). Genetic alleles are known to occur at the ADH_2 locus, coding for β_1 and β_2 subunits of known structures (Jörnvall et al., 1984a; Bühler et al., 1984a) and for β-Indianapolis subunits (Bosron et al., 1980). Similarly, γ_1 and γ_2 subunits have been ascribed to the ADH_3 locus (Smith et al., 1971), while allelic variants have not been defined for the ADH_1 locus, which codes for the α subunit.

The α polypeptide is predominant in early fetal liver (Smith et al., 1971), while in adult livers β subunits and, apparently to a lesser extent, γ subunits predominate. Differences in efficiency of ethanol oxidation and in other enzymatic properties are found for the homodimeric class I isozymes (Bosron et al., 1983). The primary structures of β_1, β_2, and γ_1 subunits have been determined (Hempel et al., 1984, 1985; Jörnvall et al., 1948a; Bühler et al., 1984a,b), as well as the nucleotide sequence of the cDNA corresponding to β mRNA (Duester et al., 1984; Ikuta et al., 1985; Hedén et al., 1986). Active site structures and a segment corresponding to a missing region in distantly related alcohol dehydrogenases have also been studied for the α subunit, showing the overall relationships among the class I polypeptides (Hempel et al., 1985). However, no extended structural data have been reported for the ADH_1-derived isozyme.

In this study, we report the nucleotide sequence of a cDNA containing the full length coding region of ADH_1. Peptide data are also given, supporting most regions of the amino acid sequence deduced for the α subunit. The results show that α, β, and γ subunits differ at 35 positions and that α is the form structurally most deviating from the others while β is the one least deviating from the class I average pattern. The subunit structures define all class I relationships and can be correlated with functional differences. Finally, a clone with an internal deletion was recovered that would give a protein

Table I: Synthetic Oligodeoxyribonucleotides Used as Hybridization Probes[a]

	Amino acid	292	297
I	sequence	Val-Gly-Val-Pro-Pro-Ala	
	18-mer	3' CAT CCC CAT GGA GGA CGA 5'	
	Amino acid	345	351
II	sequence	Leu-Ile-Thr-His-Val-Leu-Pro	
	21-mer	5' TTA ATA ACC CAT GTT TTA CCT 3'	
	Amino acid	361	366
III	sequence	Leu-Leu-His-Ser-Gly-Lys	
	18-mer	3' GAC GAA GTG AGA CCC TTT 5'	

[a] Oligonucleotide sequences were selected from a partial cDNA structure for the β isozyme (Duester et al., 1984).

† This work was supported by the Swedish Medical Research Council (Projects 13X-3532, 7148, and 3529), KabiGen AB, the Magn. Bergvall Foundation, and a fellowship (to J.H.) from the Endowment for Research in Human Biology, Boston, and the Samuel Bronfman Foundation, with funds provided by Joseph E. Seagram & Sons.

* Address correspondence to this author at the Department of Chemistry I, Karolinska Institutet.

0006-2960/86/0425-2465$01.50/0

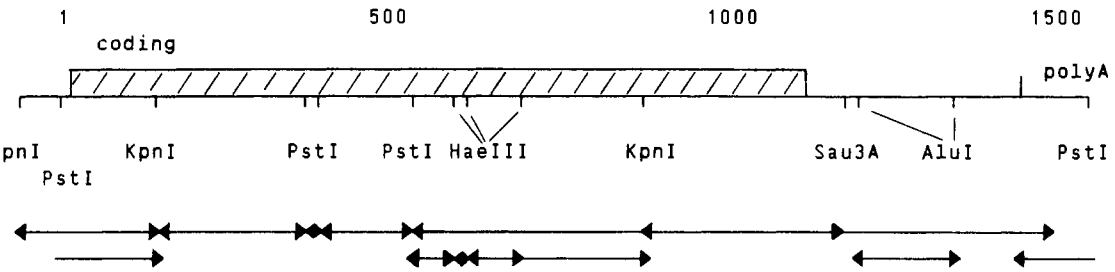

FIGURE 1: Restriction map of the cDNA clones pADH6 and pADH11, corresponding to the α subunit of human liver alcohol dehydrogenase. Only restriction sites used for the dideoxy sequence method are shown. Arrows indicate the direction of sequencing, and the scale at the top refers to base pairs. The hatched region corresponds to the coding sequence.

size and domain subdivision of interest for judgment of evolutionary relations to other dehydrogenases.

Materials and Methods

cDNA Library. Total mRNA was prepared by the guanidinium method (Chirgwin et al., 1979) from adult human liver of healthy Caucasian origin. A cDNA library was prepared by the method of Okayama and Berg (1982, 1983). Plasmid pT4, derived from pBR 322 was used as vector (Hedén et al., 1986).

Oligodeoxyribonucleotides (Table I) were synthesized by the solid-phase phosphoamidite method with an automatic synthesizer (Josephson et al., 1984). The oligodeoxyribonucleotides were labeled at the 5′-end by transfer from [γ-^{32}P]ATP (Amersham) with T4 polynucleotide kinase (Amersham; Sgaramella & Khorana, 1972). Colonies were screened for hybridization with the synthesized oligodeoxyribonucleotides in an unordered fashion and then rescreened after restreaking of putative positive colonies. Hybridization conditions were as described (Wallace et al., 1979); after being washed, the filters were subjected to autoradiography at −70 °C, with intensifying screens.

Plasmid DNA from hybridization-positive clones was cleaved with restriction enzymes *Kpn*I and *Pst*I (Amersham), and the fragments were separated in low melting point agarose (Bio-Rad). Large fragments were recleaved in agarose slices, with *Sau*3AI, *Alu*I, or *Hae*III (Boehringer). All cleavages were performed under the conditions suggested by the manufacturers.

DNA Sequence Analysis. Restriction enzyme fragments in agarose slices were ligated into M13mp8, M13mp9, M13mp18, or M13mp19 vectors (Messing & Vieira, 1982; Norrander et al., 1983). Sequence analysis was carried out by the dideoxy method (Sanger et al., 1977) with single-stranded M13 templates (Schreier & Cortese, 1979) and an M13-specific universal primer (17-mer; Amersham). The labeled nucleotide was [α-^{35}S]dATP (Amersham; 600 Ci/mmol), and the sequence mixture was separated on ultrathin (0.2 mm) urea–polyacrylamide gels.

Protein Analysis. The $\alpha\alpha$ isozyme of human liver alcohol dehydrogenase was isolated as described (Bosron et al., 1983), reduced, carboxymethylated, and fragmented by CNBr or trypsin (Hempel et al., 1984). Peptides were prefractionated by exclusion chromatography on Sephadex G-50 and further purified by reverse-phase high-performance liquid chromatography (Jeffery et al., 1984). Amino acid compositions were determined, after hydrolysis for 24 h at 110 °C in 6 M HCl/0.5% phenol, on a Beckman 121M amino acid analyzer. Amino acid sequence analysis was performed by the manual diaminoazobenzene–isothiocyanate method as described (von Bahr-Lindström et al., 1982) and by liquid-phase sequencer degradations with phenylthiohydantoin identification by high-performance liquid chromatography (Jeffery et al., 1984).

Results

Screening of a cDNA Library. A total of 10000 colonies were screened with three oligodeoxyribonucleotide probes synthesized to correspond to parts of the then known nucleotide sequence for the β subunit (Duester et al., 1984) (Table I). Eleven colonies hybridized with at least one of the probes. On the basis of partial nucleotide and peptide sequence data, two clones, pADH6 and pADH11, were selected for further analysis. Both hybridized with probe I, but only pADH11 gave a positive signal with the two other probes. The total length of the cDNA insert was estimated from agarose gels to be about 1400 nucleotides in pADH6 and about 1200 in pADH11.

Nucleotide Sequence Analysis of pADH6 and pADH11. The DNA sequences of the cDNA inserts in the two plasmids were determined according to the strategy shown in Figure 1. The plasmids were cleaved with *Kpn*I and *Pst*I, and the resulting fragments were separated on agarose gels. Large segments were cleaved in agarose before ligation. All fragments were ligated in both directions into appropriate M13 vectors and subjected to dideoxy sequence analysis (Sanger et al., 1977) on both strands. The 3′-end fragments, however, did not yield interpretable sequences from the poly(A)-tailed ends. Together, the inserts of pADH6 and pADH11 comprise 1450 nucleotides plus a poly(A) tail of about 100 nucleotides, as shown in Figure 2. The coding part of the sequence corresponds to the α subunit of human liver alcohol dehydrogenase, in agreement with the peptide data below.

All corresponding positions of the two cDNA inserts have identical nucleotides, but the difference in length between pADH6 and pADH11 is caused by size differences in two regions. In the 5′-end, the insert in pADH11 starts at nucleotide 249 whereas the insert in pADH6 extends to nucleotide −72. The other difference is that the coding region of pADH6 lacks 139 nucleotides as compared to that of pADH11. This internal deletion covers positions 964–1102. The 3′-noncoding region contains three possible polyadenylation sites as shown in Figure 2.

Direct Amino Acid Sequence Analysis of the α Protein Subunit. Separate samples of the reduced and carboxymethylated $\alpha\alpha$ isozyme were cleaved with CNBr and trypsin, respectively. Peptides obtained were purified by exclusion chromatography and reverse-phase high-performance liquid chromatography, in the same way as for the corresponding peptides from the β_1 and γ_1 subunits (Hempel et al., 1984; Bühler et al., 1984b). Results of structural analyses are given in Table II, and the regions covered by peptide data are shown in Figure 2. All regions analyzed directly are in complete agreement with the amino acid sequence deduced indirectly from the nucleotide sequence. In addition, the total composition from hydrolysis of the protein fits the one inferred from the sequence analyses. The whole structure is homologous to

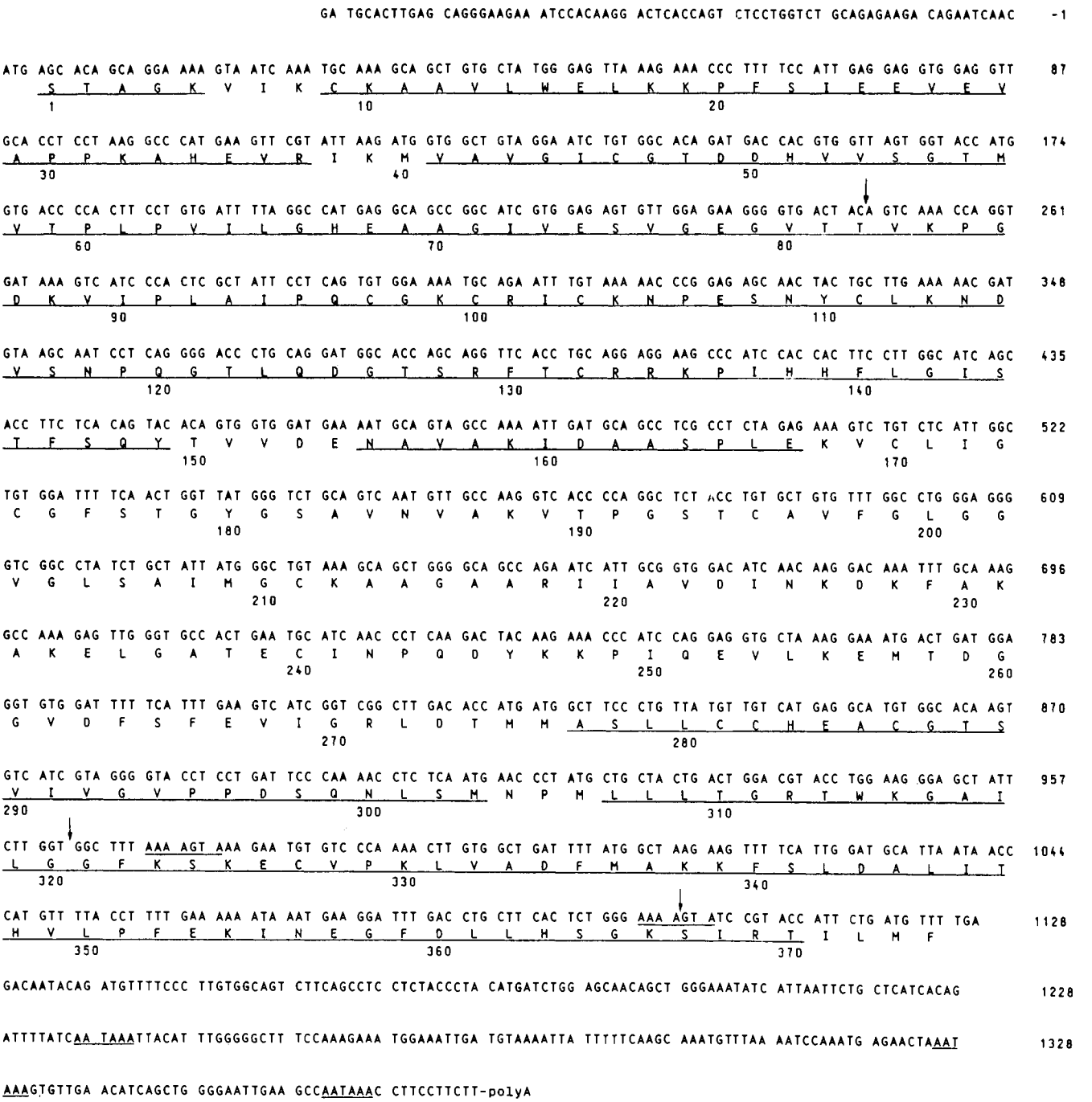

FIGURE 2: Nucleotide sequence (top in each line) of the cDNA clones pADH6 and pADH11, corresponding to the ADH_1 locus, and the amino acid sequence (bottom) of the α subunit of human liver alcohol dehydrogenase. The nucleotides are numbered on the right-hand side in the 5′ to 3′ direction; nucleotide 1 is the A of the ATG start codon; negative numbers refer to the 5′-untranslated region. The amino acids are numbered underneath the sequence. The three putative polyadenylation signals in the 3′-untranslated region are underlined. In the 5′-end, pADH6 extends to nucleotide −72, whereas pADH11 starts at the arrow (nucleotide 249). Internally, pADH6 has a deleted region between the arrows at nucleotide positions 964–1102. The two repeats flanking the deleted region are underlined. Underlining in the amino acid sequence indicates those regions also directly analyzed in peptides from the protein as detailed in Table II. The protein N-terminus is acylated.

that of other class I isozymes, as expected (Hempel et al., 1985), but differs from those of β or γ subunits at 35 positions (Table III). Of the residue differences between the α and β subunits (24 positions), all but three (positions 208, 371, and 373) have been verified also by peptide analyses.

Discussion

Nucleotide Sequence. The total of 1450 nucleotides determined corresponds to a region coding for the entire α subunit of human liver alcohol dehydrogenase plus flanking, untranslated nucleotide segments. Two cDNA clones, pADH6 and pADH11, have been characterized, of which pADH6 shows a 139-nucleotide internal deletion of positions 964–1102 as compared to pADH11.

The 1122 nucleotides of the coding region for the α subunit, as deduced from the two clones analyzed, are in agreement with the amino acid sequence determined directly by peptide data, with the total composition of the α subunit by hydrolysis, and with the entire-chain homology with other class I subunits. Therefore, the coding sequence deduced is concluded to correspond to all exon parts of the ADH_1 locus and not to represent forms with further deletions.

The cDNA sequence starts 72 nucleotides upstream from the initiation codon ATG. Most cellular mRNAs have 5′-

Table II: Structural Data for Peptides from the Carboxymethylated α Subunit[a]

(A) Compositions after Acid Hydrolysis[b]

	peptide[a]						
	T11–18	T19–32	T33–37	E79–107	C277–303	C307–336	CT370–373
Cys(Cm)				2.7 (3)	2.6 (3)	0.7 (1)	
Asx				2.2 (2)	2.1 (2)	1.1 (1)	
Thr				1.8 (2)	0.9 (1)	1.9 (2)	1.0 (1)
Ser		0.9 (1)			3.8 (4)	1.2 (1)	
Glx	0.9 (1)	2.7 (3)	1.0 (1)	2.1 (2)	2.1 (2)	1.2 (1)	
Pro		2.8 (3)		3.6 (4)	2.1 (2)	1.1 (1)	
Gly				2.9 (3)	2.2 (2)	4.1 (4)	
Ala	2.0 (2)	1.0 (1)	1.1 (1)	1.1 (1)	2.1 (2)	2.2 (2)	
Val	0.9 (1)	1.7 (2)	0.9 (1)	2.9 (3)	2.5 (3)	1.6 (2)	
Met					0.5 (1)[d]	0.4 (1)[d]	0.5 (1)[d]
Ile		0.9 (1)		2.6 (3)	0.7 (1)	1.0 (1)	1.0 (1)
Leu	2.2 (2)			1.1 (1)	2.8 (3)	4.8 (5)	0.9 (1)
Phe		1.0 (1)				1.9 (2)	
Trp	0.2 (1)[c]					0.2 (1)[c]	
Lys	1.2 (1)	2.2 (2)		4.2 (4)		3.8 (4)	
His			0.9 (1)		1.0 (1)	1.0 (1)	
Arg			0.8 (1)	1.1 (1)			
sum	8	14	5	29	27	30	4

(B) Results of Sequence Analysis[e]

peptide[a]	sequence
T11–18	Ala-Ala-Val-Leu-Trp-Glu-Leu-Lys 4 4 3 2 1
E79–107	Gly-Val-Thr-Thr-Val-Lys-Pro-Gly-Asp-Lys-Val-Ile-Pro-Leu-Ala-Ile-Pro-Gln-Cys-Gly-Lys-Cys-Arg-Ile-Cys-Lys-Asn-Pro-Glu 16 14 12 9 9 10 9 6
C277–303	Ala-Ser-Leu-Leu-Cys-Cys-His-Glu-Ala-Cys-Gly-Thr-Ser-Val-Ile-Val-Gly-Val-Pro-Pro-Asp-Ser-Gln-Asn-Leu-Ser-Hsl 15 12 13 9 6 5 7 6 1
C307–336	Leu-Leu-Leu-Thr-Gly-Arg-Thr-Trp-Lys-Gly-Ala-Ile-Leu-Gly-Gly-Phe-Lys-Ser-Lys-Glu-Cys-Val-Pro-Lys-Leu-Val-Ala-Asp- 18 19 20 14 13 12 10 10 8 7 5 3 2 2 2 Phe-Hsl <1

[a] Peptides are named by a letter to show the cleavage method (T, trypsin; E, Glu-specific protease; C, CNBr) and numbers to show the positional origins in the final structure (as deduced from the positional numbers in Figure 2 and in the homology-related β subunit; Hempel et al., 1984). Data for positions 41–78 and 108–154 have been given before (Hempel et al., 1985) and are now omitted. [b] Compositions given as molar ratios after acid hydrolysis, without corrections for slow release or destruction; parentheses show values from sequence analysis. Only peptides with differences from the established β structure (Hempel et al., 1984) are given, since fragments covering remaining parts (Figure 2) give compositions identical with the corresponding β peptides. [c] Low value explained by acid hydrolysis. [d] Met recovered as Hse, explaining the low yield. [e] Values give nanomoles of phenylthiohydantoin recovered for residues with stable derivatives. Short peptides analyzed by the manual (dimethylamino)azobenzene–isothiocyanate method are not given, but their compositions are shown in part A and their structures in Figure 2.

noncoding regions in the range from 40 to 80 nucleotides (Kozak, 1983). The sequence determined therefore probably corresponds to a full-length cDNA. Notable in the coding region is that when one type of amino acid occurs in doublets or triplets, different rather than identical codons are often used for adjacent amino acids (18 of 28 cases). The termination codon TGA is followed by a 250-nucleotide noncoding region preceding the poly(A) tail. This region contains three possible polyadenylation signals, AATAAA (Proudfoot & Brownlee, 1976), of which the one at position 1362 is optimally located and therefore probably used. The 3′-noncoding region of the α cDNA is 37 nucleotides longer than the shortest form of the four differently sized known forms for the β cDNA (Duester et al., 1984; Ikuta et al., 1985; Hedén et al., 1986). The potential polyadenylation signal in α at position 1362 is not present in the β cDNA sequence, whereas the one at position 1326 is conserved and probably used in the short form of β cDNA (Hedén et al., 1986). The coding region of the α cDNA sequence differs at 54 positions, of which 27 are silent, as compared to the β cDNA sequence (Hedén et al., 1986).

Clone with Internal Deletion. The 139-nucleotide internal deletion in pADH6 creates a frame shift and would give another termination at position 1118. If expressed, this would correspond to a subunit 49 amino acid residues shorter than the α subunit and with a different C-terminal pentaresidue end. The deletion is not an artifact of the sequence analysis itself, since the size of the cDNA insert as well as the sizes of its fragments agrees with the number of nucleotides in the sequence determined. Neither is the corresponding mRNA, as judged from the sequence data, likely to have a great tendency to form a hairpin loop that could explain the deleted region. However, it is of interest that the deletion coincides with two intron positions of the ADH_2 gene (Duester et al., 1986b) as well as with one border region between the two domains of the ADH protein subunit (Brändén et al., 1975).

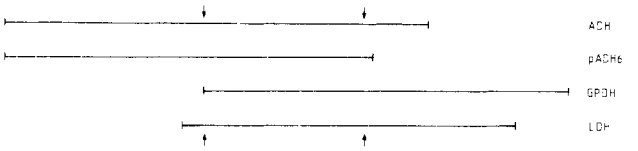

FIGURE 3: Comparison of pADH6 with the previously known linear arrangements of domains in different dehydrogenases. The alignment of alcohol dehydrogenase (ADH), glyceraldehyde-3-phosphate dehydrogenase (GPDH), and lactate dehydrogenase (LDH) is from Rossmann et al. (1975), and the arrows indicate domain borders with the coenzyme-binding domain in each subunit between the arrows. Lines are drawn in scales to show the coenzyme-binding domains in equal size and with remaining parts in correct relative ratios. A hypothetical protein that would be expressed from plasmid pADH6 is shown by the pADH6 line, demonstrating that that protein would have a size, and linear domain arrangement into two parts, like GPDH (and to some extent LDH) rather than ADH [for which the C-terminal end has an extension, as shown, constituting a part of the N-terminal, analytical domain; cf. Rossmann et al. (1975)].

Table III: Positions with Residue Differences between the α, β, and γ Subunits of Class I Type of Human Liver Alcohol Dehydrogenase[a]

Position	α	β	γ	Position	α	β	γ
17	Leu	Val	Leu	141	Leu	Leu	Val
25	Glu	Asp	Glu	143	Ile	Thr	Val
34	His	Tyr	His	185	Asn	Asn	Lys
43	Val	Val	Ala	207	Ala	Ala	Val
47	Gly	Arg	Arg	208	Ile	Val	Val
48	Thr	Thr	Ser	276	Met	Met	Val
50	Asp	Asp	Glu	297	Asp	Ala	Asp
56	Thr	Asn	Asn	303	Met	Ile	Ile
57	Met	Leu	Leu	318	Ile	Val	Ile
93	Ala	Phe	Phe	319	Leu	Tyr	Phe
94	Ile	Thr	Thr	327	Cys	Gly	Ser
102	Ile	Val	Ile	328	Val	Ile	Val
116	Val	Leu	Leu	348	His	His	Asn
117	Ser	Gly	Gly	349	Val	Val	Ile
120	Gln	Arg	Arg	363	His	His	Arg
128	Ser	Arg	Arg	371	Ile	Val	Val
133	Arg	Arg	Ser	373	Met	Thr	Thr
134	Arg	Gly	Gly				
		Positions with unique residues:			17	10	14

Total positions: 35

Differences: α : β 24/35; α : γ 28/35; β : γ 21/35

[a] Structures for α are from the present analysis and those for β and γ from Hempel et al. (1984, 1985; Bühler et al., 1984). In addition, allelic variants have been shown (Jörnvall et al., 1984a; Höög et al., unpublished results), as well as minor differences in cDNA structures reflecting mutations or strain variants (Ikuta et al., 1985; Höög et al., unpublished results). Residues that are circled are those that are unique at each position.

As shown in Figure 3, the deletion would give the corresponding protein a linear domain architecture composed of roughly equal halves rather than the three parts typical for ADH. This would also give the protein a size similar to that of lactate and glyceraldehyde-3-phosphate dehydrogenases in the relationship scheme suggested by Rossmann et al. (1975). Consequently, the deletion in pADH6 may reflect a different mRNA processing that could even be derived from ancestral relationships. However, another possibility is that the short pADH6 reflects a partly deleted ADH_1 gene. The liver cells providing material for the cDNA library would then have been heterozygous for the ADH_1 locus, resulting in pADH11 from the intact form, and to pADH6 from a mutated ADH_1 locus. The cause of such a mutation would be slipped mispairing during DNA replication. Thus, a short repeat, AAAAGTA, is located near the end points of the deletion, at positions 970 and 1099 (underlined in Figure 2), and the presence of short repeats has been suggested to promote deletions by slipped mispairing during DNA replication (Streisinger et al., 1966). This has been exemplified in the *lac* I gene of *Escherichia coli* (Farabaugh & Miller, 1978) and the human β-globin gene (Efstratiadis et al., 1980).

It is not yet possible to prove which of the different alternatives for the origin of the deletion-containing clone is correct. The α subunit is the one studied least among the class I polypeptide chains at the levels of both protein chemistry and population genetics. For the moment, however, pADH6 and the repeat structures indicate that studies of levels and regulations of the α polypeptide are of interest. Clone pADH6 also illustrates risks with indirectly deduced protein structures: had a pADH11 not been found and had the protein not been well-known, pADH6 could for an unknown protein have led to wrong conclusions about the native enzyme structure.

Protein Structure and Enzyme Properties. The structure of the α subunit is strictly homologous to those of the β and γ subunits. In total, only 35 positions of 374 differ, as summarized in Table III. All zinc-liganding residues are conserved (Cys-46, His-67, and Cys-174 to the active site Zn; Cys-97, Cys-100, Cys-103, Cys-111 to the second Zn; Brändén et al., 1975), as well as most of the residues with defined functions. In particular, it is significant that the whole first half of the entire coenzyme-binding domain is largely devoid of substitutions among the class I (α, β, and γ) isozyme subunits. Thus, in the central 153-residue stretch between position 144 and position 296, the α and β subunits differ at only one position (and any class I subunit combination only at four positions), showing that almost half of the structures contain only few exchanges. This fact clearly points to the structural and functional importance of the central strands of β-pleated sheet that are formed from this region. It further includes structurally and functionally important glycine residues (Brändén et al., 1975), in agreement with the general conservation of glycine residues also in distantly related alcohol and polyol dehydrogenases (Jörnvall et al., 1984b).

In contrast, the active site region containing segments adjacent to the Zn-liganding Cys-46 (Eklund et al., 1982) and the coenzyme interacting Arg-47, Ser-48, and His-51 is far less conserved even within the α, β, and γ subunits, of the closely related polypeptides of human class I isozymes. In fact, the pyrophosphate-interacting Arg-47 is itself exchanged in the α subunit (Table III), as is Ser-48. However, in all these cases, the exchanges are similar to those found in other, more distantly related alcohol or polyol dehydrogenases. Thus, compared with the model horse alcohol dehydrogenase of the E type, sorbitol dehydrogenase has also lost Arg-47 (Jörnvall et al., 1984a) and yeast alcohol dehydrogenase Ser-48 (Jörnvall et al., 1978).

Therefore, the variations now detected within class I human alcohol dehydrogenase isozymes are not unique and should be acceptable within largely unaltered conformations, thereby retaining the overall functional properties. Nevertheless, the variations are remarkably large for closely related isozymes with freely hybridizable subunits. Coupled with the conservation of the central part of the coenzyme-binding domain (above), the variations emphasize the importance of the central regions in the conformation rather than of single active site residues, in agreement with previous observations (Hempel et al., 1985). This type of variability is well illustrated by alcohol dehydrogenase and is also reflected in the multiple gene duplications and differential gene activation previously deduced for the isozyme systems (Bühler et al., 1984c).

The Arg/Gly substitution at position 47 should affect enzymatic properties (Hempel et al., 1985) in analogy with other changes (Arg/His) at this position in allelic forms of the β subunit, accounting for their atypical properties (Jörnvall et al., 1984a; Bühler et al., 1984a). The Gln/Arg exchange at position 120 removes a basic residue present in other alcohol dehydrogenases. Among the remainder of the exchanges, all except three (positions 143, 319, and 327) of the alternatives in the α structure also occur in one of the other two class I subunits (Table III). Finally, it may be noticed that the α subunit compared with the β type has an exchange affecting cysteine (position 327), thus increasing the number of SH groups, as in the rat enzyme (though at different positions; Jörnvall, 1974).

Isozyme Divergence. The exchanges found appear to support the suggestion of multiple and separate duplications in the development of alcohol dehydrogenase isozyme systems.

Thus, all class I exchanges now evident (Table III) confirm that α, β, and γ have mixed properties with conserved residues in different parts. They exhibit many differences in any pairwise comparison, suggesting that no line is of considerably more recent origin than the others. However, β appears to be the least unique form (10 positions with exchanges not shared by either α or γ), and α and γ appear to be the ones most divergent (28 differences). Compared with β, the α subunit has several exchanges affecting methionine (positions 57, 303, and 373), explaining altered patterns upon CNBr fragmentation and showing that peptide fingerprinting of proteins when based on single types of residue need not give conserved patterns, even when total conservation is great between isozyme forms (α and β exhibit 94% identity, but size differences in over one-fourth of all CNBr fragments).

Added in Proof

After submission of this paper, we received information on the genomic nucleotide sequence corresponding to the β subunit (Duester et al., 1986a), confirming the present conclusions that pADH6 is a full-length cDNA clone.

Registry No. ADH, 9031-72-5; DNA (human alcohol dehydrogenase α-subunit messenger RNA complementary), 100603-71-2; alcohol dehydrogenase (human α-subunit reduced), 100603-72-3.

References

Bosron, W. F., Li, T.-K., & Vallee, B. L. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5784–5788.

Bosron, W. F., Magnes, L. J., & Li, T.-K. (1983) *Biochemistry 22*, 1852–1857.

Brändén, C.-I., Jörnvall, H., Eklund, H., & Furugren, B. (1975) *Enzymes (3rd Ed.) 11*, 103–190.

Bühler, R., Hempel, J., von Wartburg, J.-P., & Jörnvall, H. (1984a) *FEBS Lett. 173*, 360–366.

Bühler, R., Hempel, J., Kaiser, R., de Zalenski, C., von Wartburg, J.-P., & Jörnvall, H. (1984b) *Eur. J. Biochem. 145*, 447–453.

Bühler, R., Hempel, J., Kaiser, R., von Wartburg, J.-P., Vallee, B. L., & Jörnvall, H. (1984c) *Proc. Natl. Acad. Sci. U.S.A. 81*, 6320–6324.

Duester, G., Hatfield, G. W., Bühler, R., Hempel, J., Jörnvall, H., & Smith, M. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4055–4059.

Duester, G., Smith, M., Bilanchone, V., & Hatfield, G. W. (1986a) *J. Biol. Chem. 261*, 2027–2033.

Duester, G., Jörnvall, H., & Hatfield, G. W. (1986b) *Nucleic Acids Res. 14*, 1931–1941.

Efstratiadis, A., Posakony, J. W., Maniatis, T., Lawn, R. M., O'Connell, C., Spritz, R. A., DeRiel, J. K., Forget, B. G., Weissman, S. M., Slightom, J. L., Blechl, A. E., Smithies, O., Baralle, F. E., Shoulders, C. C., & Proudfoot, N. J. (1980) *Cell (Cambridge, Mass.) 21*, 653–668.

Eklund, H., Plapp, B. V., Samama, J.-P., & Brändén, C.-I. (1982) *J. Biol. Chem. 257*, 14349–14358.

Farabaugh, P. J., & Miller, J. H. (1978) *J. Mol. Biol. 126*, 847–863.

Hedén, L.-O., Höög, J.-O., Larsson, K., Lake, M., Lagerholm, E., Holmgren, A., Vallee, B. L., Jörnvall, H., & von Bahr-Lindström, H. (1986) *FEBS Lett. 194*, 327–332.

Hempel, J., Bühler, R., Kaiser, R., Holmquist, B., de Zalenski, C., von Wartburg, J.-P., Vallee, B. L., & Jörnvall, H. (1984) *Eur. J. Biochem. 145*, 437–445.

Hempel, J., Holmquist, B., Fleetwood, L., Kaiser, R., Barros-Söderling, J., Bühler, R., Vallee, B. L., & Jörnvall, H. (1985) *Biochemistry 24*, 5303–5307.

Ikuta, T., Fujiyosni, T., Kurachi, K., & Yoshida, A. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2703–2707.

Jeffery, J., Cederlund, E., & Jörnvall, H. (1984) *Eur. J. Biochem. 140*, 7–16.

Jörnvall, H. (1974) in *Alcohol and Aldehyde Metabolizing Systems* (Thurman, R. G., Yonetani, T., Williamson, J. R., & Chance, B., Eds.) pp 23–32, Academic Press, New York.

Jörnvall, H., Eklund, H., & Brändén, C.-I. (1978) *J. Biol. Chem. 253*, 8414–8419.

Jörnvall, H., Hempel, J., Vallee, B. L., Bosron, W. F., & Li, T.-K. (1984a) *Proc. Natl. Acad. Sci. U.S.A. 81*, 3024–3028.

Jörnvall, H., von Bahr-Lindström, H., & Jeffery, J. (1984b) *Eur. J. Biochem. 140*, 17–23.

Josephson, S., Lagerholm, E., & Palm, G. (1984) *Acta Chem. Scand., Ser. B B38*, 539–545.

Kozak, M. (1983) *Microbiol. Rev. 47*, 1–45.

Messing, J., & Vieira, J. (1982) *Gene 19*, 269–276.

Norrander, J., Kempe, T., & Messing, J. (1983) *Gene 26*, 101–106.

Okayama, H., & Berg, P. (1982) *Mol. Cell. Biol. 2*, 161–170.

Okayama, H., & Berg, P. (1983) *Mol. Cell. Biol. 3*, 280–289.

Proudfoot, N. J., & Brownlee, G. G. (1976) *Nature (London) 263*, 211–214.

Rossmann, M. G., Liljas, A., Brändén, C.-I., & Banaszak, L. J. (1975) *Enzymes (3rd Ed.) 11*, 103–190.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.

Schreier, P. H., & Cortese, R. (1979) *J. Mol. Biol. 129*, 169–172.

Sgaramella, V., & Khorana, H. G. (1972) *J. Mol. Biol. 72*, 427–444.

Smith, M., Hopkinson, D. A., & Harris, H. (1971) *Ann. Hum. Genet. 34*, 251–271.

Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E., & Inouye, M. (1966) *Cold Spring Harbor Symp. Quant. Biol. 31*, 77–84.

Strydom, D. J., & Vallee, B. L. (1982) *Anal. Biochem. 123*, 422–429.

von Bahr-Lindström, H., Hempel, J., & Jörnvall, H. (1982) *J. Protein Chem. 1*, 257–214.

Wallace, R. B., Schaffer, J., Murphy, R. F., Bonner, J., Hirose, T., & Itakura, K. (1979) *Nucleic Acids Res. 6*, 3543–3557.