Purification and Structural Characterization of Placental NAD$^+$-Linked 15-Hydroxyprostaglandin Dehydrogenase. The Primary Structure Reveals the Enzyme To Belong to the Short-Chain Alcohol Dehydrogenase Family†

Maria Krook,‡ Lyuben Marekov,‡,§ and Hans Jörnvall*,‡,∥
Department of Chemistry I and Center for Biotechnology, Karolinska Institutet, S-104 01 Stockholm, Sweden
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ABSTRACT: Human placental NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenase was purified to homogeneity according to a five-step method, with chromatography on DEAE-Sepharose, Blue Sepharose, and Mono-Q FPLC as principal steps. Final yield was 23% and purification about 13 000-fold, with a specific activity of 24 000 milliunits/mg. The subunit molecular weight is about 29 000 as determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, and the native protein molecular weight is about 54 000 as estimated by Sephadex G-100 chromatography, establishing the enzyme to be a dimer of similar-sized protein chains. The subunit N-terminal residue is methionine, and the α-amino group is free. The complete primary structure was determined by peptide analysis, based essentially on four different proteolytic treatments (Lys-specific protease, Glu-specific protease, Asp-specific protease, and CNBr). The protein chain is composed of 266 residues, with C-terminal glutamine. A microheterogeneity was detected at position 217, with both Cys and Tyr, in about equal amounts, from a preparation starting with a single placenta. No other subunit heterogeneities were detected. The protein is clearly but distantly related to insect alcohol dehydrogenases, characterized bacterial dehydrogenases of sugar metabolism, and bacterial and eukaryotic steroid dehydrogenases. Together, these results establish that placental 15-hydroxyprostaglandin dehydrogenase is a member of the short-chain nonmetalloenzyme alcohol dehydrogenase protein family. The protein has four cysteine residues (five with the positional microheterogeneity), but there is no evidence for functional importance of any of these residues. On the other hand, a tyrosine residue at position 151 is conserved in relation to the other enzymes, suggesting that it may be of functional importance. It is close to the middle of the molecule in a region between the N-terminal coenzyme-binding domain that is related to similar structures of other dehydrogenases and the C-terminal domain that is characteristic of prostaglandin dehydrogenase.

NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenases are cytosolic enzymes causing the biological inactivation of prostaglandins (Änggård & Samuelsson, 1964; Änggård, 1966). The enzyme is inhibited by indomethacin, aspirin, and other nonsteroidal antiinflammatory drugs and activated by antidepressants (Mak et al., 1982). Consequently, its biological activity may have far-ranging effects compatible with its key role in prostaglandin inactivation.

In spite of the early detection of the enzyme activity and the importance of the prostaglandin system, the enzyme protein has long been difficult to study. It has widespread occurrence but is labile and often is not present in large amounts. It requires addition of glycerol for stabilization during purification (Jarabak, 1972); dithioerythritol and EDTA are also helpful. Placenta is a fairly rich source, but preparations even from this organ require purification of over 12 000-fold to achieve homogeneity (Jarabak & Watkins, 1988; this work).

Because of long-standing problems with the purification, even those structural properties that are usually easily accessible have been difficult to ascertain. Thus, the molecular weight of the whole enzyme has been variably reported (33 000–55 000), as has the quaternary structure (monomer–dimer) [cf. Hansen (1976), Tanaka et al. (1986), and Nagai et al. (1987)], although the higher value and a dimer seemed most likely (Mak et al., 1982; Bergholte & Okita, 1986; Jarabak & Watkins, 1988). The present work establishes the protein to be a dimer of M_r 28 900 subunits and gives the complete primary structure and subunit relationships to other enzymes. It also reveals that a former report (Mak et al., 1982) was correct in the prediction of the enzyme as being a "short-chain alcohol dehydrogenase" related protein, although the prediction then was based on little evidence, lacking homology proof in the absence of amino acid sequence data.

Human placental NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase was now purified with a method giving fast results and high yield, allowing determination of the primary structure, assignment of the structural relationships, and identification of residues tentatively of functional importance. In addition, a microheterogeneity, compatible with an allelic variation in a heterozygote, was detected in the enzyme from one placenta.

Materials and Methods

The starting material was term human placenta (1–2 per preparation), used directly (from individuals with negative tests for HIV and hepatitis). Different purification protocols were tested, as described under Results. Final methods chosen avoided precipitation steps and introduced FPLC on Mono-Q HR 5/5.

Sephadex G-100, Sephadex G-50, Mono-Q, Blue Sepharose, CM-Sepharose, and DEAE-Sepharose were all products of

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‡ Department of Chemistry I.
§ On sabbatical leave from the Institute of Molecular Biology, 1113 Sofia, Bulgaria.
∥ Center for Biotechnology, Huddinge Hospital.

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Pharmacia (Uppsala, Sweden). Glycerol added at 20% was of pro analysi quality from BDH (Poole, U.K.) and reagents used in the structural analyses were of sequencer quality or equivalent purity from Applied Biosystems (Foster City, CA), Pierce (Rockford, IL). Rathburn (Walkerburn, U.K.), Boehringer (Mannheim, FRG), or Merck (Darmstadt, FRG). Remaining chemicals were of the highest standard purity.

Enzyme Activity. 15-Hydroxyprostaglandin dehydrogenase activity was assayed spectrophotometrically with $PGF_{2\alpha}$ (in the form of a Tris salt, Dinoprost, Upjohn, Puurs, Belgium) as substrate by measurement at 340 nm in a 3-mL cuvette with 0.45 mM NAD^+ in 0.1 M Tris-HCl, pH 9.0, 25 °C, as described (Braithwaite & Jarabak, 1975). One unit of enzyme activity corresponds to the formation of 1 μmol of NADH/min. During purification, protein was determined by the method of Bradford (1976) and the purity by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (Laemmli, 1970).

Structural Analysis. The protein in 6 M guanidine hydrochloride, 0.4 M Tris-HCl, and 2 mM EDTA, pH 8.15, was reduced with dithioerythritol (100 nmol/nmol of protein) for 2 h at 37 °C and carboxymethylated with neutralized ^{14}C-labeled iodoacetic acid (Amersham, U.K.) diluted to 2400 cpm/nmol, 1.5-fold molar excess over total thiols, for 2 h at room temperature in the dark. The reaction was terminated by the addition of 2-mercaptoethanol, and the reagents were removed by chromatography on PD10 columns of Sephadex G-25 M (Pharmacia) in water. Different samples were then treated with *Lysobacter* Lys-specific protease, staphylococcal Glu-specific protease, and *Pseudomonas* Asp-specific protease (all from Boehringer), all used in 0.1 M ammonium bicarbonate and 1 M urea at 37 °C, in most cases for 4 h at protease:substrate ratios of 1:50, but the Asp-specific protease was used at ratios of 1:200 and incubation for 24 h. Cleavage with CNBr was carried out in 70% formic acid for 24 h at room temperature.

Peptides from the enzymatic digests were directly separated by reverse-phase high-performance liquid chromatography (Waters 440 System) on UltroPac TSK ODS-120T, 5 μm, 4.6 × 250 mm (LKB/Pharmacia, Bromma, Sweden) utilizing acetonitrile gradients in 0.1% trifluoroacetic acid, as described (Jeffery et al., 1984). The CNBr fragments were prefractionated on Sephadex G-50 in 30% acetic acid (Jeffery et al., 1984), and large fragments were directly analyzed by sequencer degradations, while remaining peptides were further purified with the high-performance liquid chromatography system.

Amino acid compositions were obtained with a Beckman 121M analyzer after hydrolysis with 6 M HCl/0.5% phenol in evacuated tubes for 24 h at 110 °C. Sequence degradations were performed with Applied Biosystems gas-phase sequencers 470A, fitted with an on-line analyzer 120A or separate phenylthiohydantoin analysis by high-performance liquid chromatography on Hewlett-Packard 1090 systems as described (Kaiser et al., 1988).

Results

15-Hydroxyprostaglandin Dehydrogenase Purification. Different purification methods were tested with batches of one placenta (usually about 300 g) utilizing 20% glycerol (Jarabak, 1972), 10^{-4} M dithiothreitol, and 1 mM EDTA for enzyme stabilization. In the protocol finally adopted, 1–2 placentas constitute an appropriate starting amount. Precipitation steps utilizing ammonium sulfate or acid have previously been used (Braithwaite & Jarabak, 1975; Mak et al., 1982) but can give slurry mixtures, difficult to clarify by centrifugation. We therefore abandoned the precipitation methods.

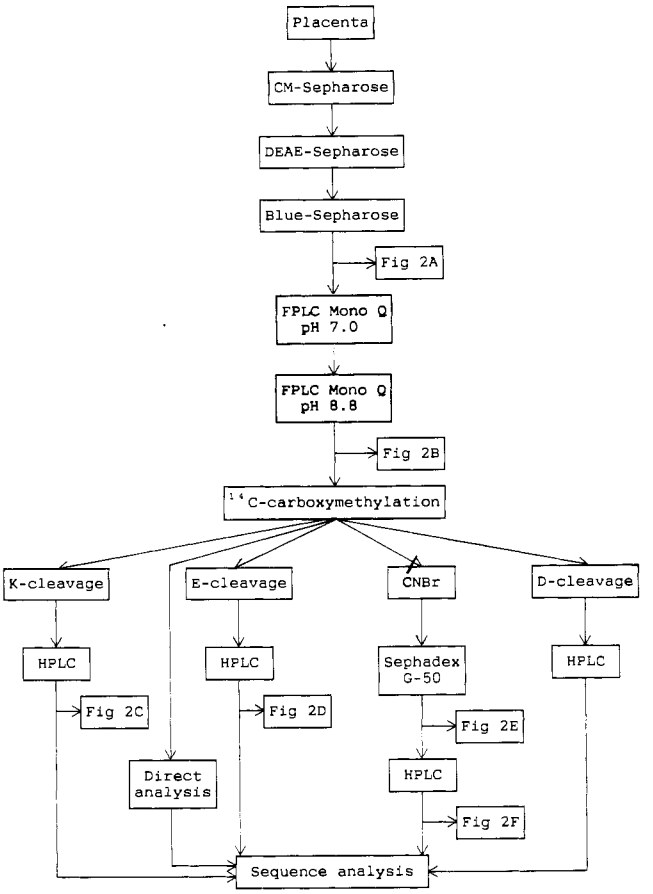

FIGURE 1: Flow scheme showing the purification and the analytical steps utilized in the present work. Elution profiles indicated are given in Figure 2.

DEAE chromatography and affinity chromatography on Blue Sepharose, essentially as described (Braithwaite & Jarabak, 1975; Mak et al., 1982), were useful steps, giving large purifications (over 20-fold and about 10-fold, respectively). CM-Sepharose chromatography (Bergholte & O'cita, 1986) was also tested and utilized as the first step in the preparation leading to the material taken for sequence analysis. However, later preparations gave good results without the initial CM-Sepharose chromatography.

The chromatographic steps above, combined with additional steps of affinity and exclusion chromatography as described (Mak et al., 1982; Braithwaite & Jarabak, 1975; Hansen, 1976; Bergholte & Okita, 1986), yielded preparations with two or three major bands on SDS/polyacrylamide gel electrophoresis. Therefore, FPLC on Mono-Q was tested and produced another 20-fold purification by a two-step procedure utilizing chromatography at pH 7.0 and then at pH 8.8. The resulting material was homogeneous and was obtained with about 60% recovery through the two-step FPLC procedure. Consequently, we now had a purification method overall giving more than 12 000-fold purification with a yield of 23% and a specific activity of about 24 000 milliunits/mg. All purification steps were carried through at 4 °C. The whole scheme is summarized in Table I and Figure 1, and the steps are described below.

Homogenization. Placenta (batches of 300 g in 600 mL of 5 mM potassium phosphate, 20% glycerol, 10^{-4} M dithioerythritol, and 1 mM EDTA, pH 7.0) was homogenized in an MSE ATO mix for 2 min in 400-mL portions. Subsequent centrifugation at 15000*g* produced a clear supernatant with activity as shown in Table I.

CM-Sepharose. Material from the previous step was in-

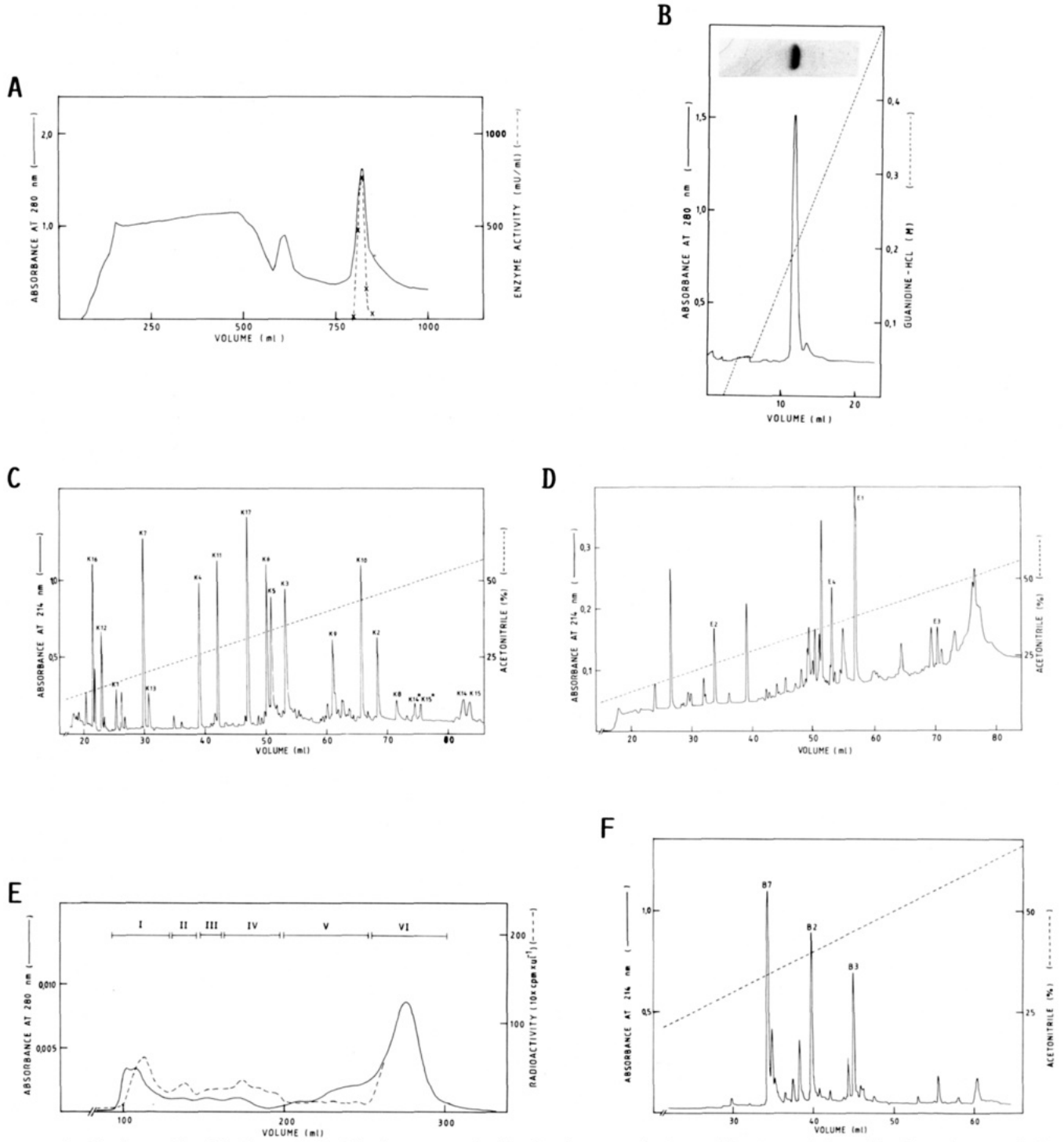

FIGURE 2: Elution profiles (cf. Figure 1) of 15-hydroxyprostaglandin dehydrogenase during purification and of peptides from the proteolytic digests. (A) Blue Sepharose purification; (B) FPLC Mono-Q; the insert shows the electrophoretic pattern of the product, revealing the presence of only one band; (C) high-performance liquid chromatography from Lys-specific cleavage (peptides obtained in additional forms are indicated with an *); (D) high-performance liquid chromatography from Glu-specific cleavage; (E) exclusion chromatography from CNBr cleavage (fractions pooled are indicated in the insert); (F) high-performance liquid chromatography of pool IV from panel E. Peptide nomenclature as in Figure 3.

cubated for 5 min with CM-Sepharose (500 mL) equilibrated in 40 mM potassium phosphate, 20% glycerol, 10^{-4} M dithioerythritol, and 1 mM EDTA, pH 6.0. The mixture was filtered under vacuum, the filtrate was adjusted to pH 7.0 with 0.5 M KH_2PO_4, and the enzyme solution was diluted with 20% glycerol, 10^{-4} M dithioerythritol and 1 mM EDTA to reach the same conductivity as the equilibration buffer below.

DEAE-Sepharose Chromatography. The material from two batches of the previous step was applied to a column (5 × 40 cm) of DEAE-Sepharose, fast flow, equilibrated with 40 mM potassium phosphate, 20% glycerol, 1 mM EDTA, and 10^{-4} M dithioerythritol, pH 7.0. After washing with equilibration buffer, the enzyme was eluted with a gradient of potassium phosphate (40–250 mM).

Blue Sepharose Chromatography. Fractions with the highest specific activity from the previous step were combined and applied directly to a column (2.5 × 20 cm) of Blue Sepharose equilibrated with 10 mM potassium phosphate, 20% glycerol, 1 mM EDTA, and 10^{-4} M dithioerythritol, pH 7.3. After washing with equilibration buffer, the enzyme was eluted

Table I: Purification of Human Placental NAD+-Dependent 15-Hydroxyprostaglandin Dehydrogenase

step	protein (mg)	act. (milliunits)	sp act. (milliunits/mg)	recovery (%)	purification (x-fold)
homogenate	84000[a]	160000[a]	1.9	100	1
CM-Sepharose	23000	150000	6.5	94	3
DEAE-Sepharose	800	115000	144	72	76
Blue Sepharose	45	62000	1400	39	740
Mono-Q	1.5	36000	24000	23	12600

[a] Starting material, 600 g of tissue (1–2 placentas). Activity measurements in initial extracts were nonlinear and difficult to estimate.

with 10 μM NAD+ in 20% glycerol and 10^{-4} M dithioerythritol. The enzyme activity eluted as a peak coinciding with that of the protein material (Figure 2A).

FPLC Mono-Q Chromatography. The material from the previous step was diluted 1:1 with 10 mM imidazole hydrochloride and 20% glycerol, pH 7.0, and applied directly to Mono-Q, equilibrated in the same buffer. Utilizing a gradient of 0–0.5 M NaCl in 20 mL, enzyme activity was eluted in a separate peak slightly before the bulk of the protein material. Narrow pooling and rechromatography in 10 mM Tris-HCl and 20% glycerol, pH 8.8, produced the enzyme activity, again preceding much protein material. The pool of activity thus obtained gave a completely homogeneous fraction on rechromatography (Figure 2B) in 15 mM Tris-HCl, pH 8.15, with a gradient of 0–0.5 M guanidine hydrochloride. The preparation obtained produced a single band on SDS/polyacrylamide gel electrophoresis (insert, Figure 2B).

Enzyme Properties. The enzyme specific activity was high (Table I), and the protein was homogeneous, as judged by the chromatographic and electrophoretic behavior (Figure 2B). The subunit molecular weight, deduced from SDS/polyacrylamide gel electrophoresis, was about 29000, and the native molecule had a molecular weight of about 54000, as estimated by gel exclusion chromatography. Consequently, the protein is deduced to be a dimer with subunits of identical size. This result is in agreement with some of the previous reports (Mak et al., 1982; Bergholte & Okita, 1986; Jarabak & Watkins, 1988). Consequently, quaternary structure, subunit size, and enzyme activity are now established.

Structural Analysis. The carboxymethylated protein was submitted to structural analysis as summarized in Figure 1. Relevant separation schemes are shown in Figure 2C–F. Combined, the different digests give structural information with overlapping peptides from all regions of the molecule. The structure deduced is given in Figure 3. Total compositions of relevant peptides are given in the supplementary material, showing agreement between hydrolytic values and the sum of the sequence. The total composition of the whole protein (Table II) supports the structure deduced, and comparison with the only composition previously reported (Mak et al., 1982) also shows acceptable agreement (Table II), establishing in retrospect a good quality of that preparation. Most of the peptides obtained from the enzymatic cleavages were recovered pure and fully analyzed (cf. Figure 3). However, the digest with the Asp-specific cleavage was performed on an early preparation of the enzyme, which was therefore not completely pure, giving information on 15-hydroxyprostaglandin dehydrogenase and another protein. Remaining digests analyzed were derived from completely pure preparations. The large, N-terminal CNBr fragment covering positions 1–122 was identified and analyzed, but it was not necessary to utilize it for redigestion. Consequently, this fragment is not indicated in Figure 3.

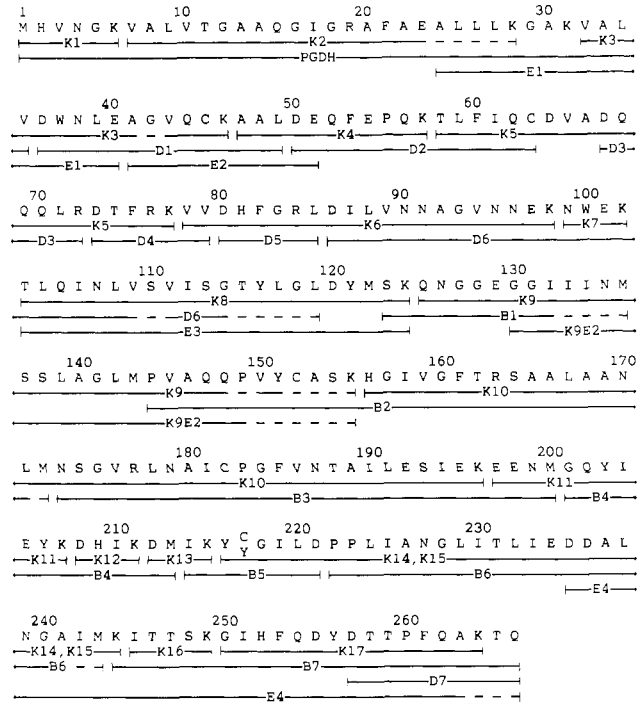

FIGURE 3: Amino acid sequence of human placental NAD+-linked 15-hydroxyprostaglandin dehydrogenase. All peptides analyzed are shown, with continuous lines to indicate regions passed by Edman degradations and with dashed lines to show remaining regions determined by composition only. The doublet at position 217 corresponds to the microheterogeneity detected in peptides K14 and K15. K indicates peptides generated by Lys-specific cleavage, E peptides by Glu-specific cleavage, D peptides by Asp-specific cleavage, B peptides by CNBr, and PGDH the intact protein.

Table II: Total Composition of Human Placental NAD+-Dependent 15-Hydroxyprostaglandin Dehydrogenase[a]

residue	acid hydrolysis: this work (mol/mol)	acid hydrolysis: Mak et al. (mol/mol)	sequence (mol/mol)
Cys	4.3		4
Asx	32.9	35.0	32
Thr	12.7	12.4	13
Ser	10.8	9.0	10
Glx	29.7	30.5	29
Pro	8.5	6.8	7
Gly	24.3	26.0	24
Ala	26.3	27.1	26
Val	17.1	20.3	18
Met	6.0	4.5	8
Ile	19.8	20.3	22
Leu	27.1	26.0	26
Tyr	8.4	<1.0	8
Phe	8.9	9.0	9
Trp			2
Lys	15.5	19.2	17
His	4.6	5.7	5
Arg	6.8	6.8	6
sum			266

[a] The second column gives the values from acid hydrolysis of the present preparation, the third column the only composition previously reported (Mak et al., 1982), and the fourth those from the sum of the sequence (with the form having Tyr-217; cf. text). The second values have been recalculated to the now known subunit size of 266 residues (disregarding Cys and Trp, then not determined).

Direct degradation by gas-phase sequencer revealed that the protein N-terminus is free. The protein N-terminal segment was therefore determined for 35 cycles by direct degradation. Other analyses were as indicated in Figure 3 and presented no special problems, though peptide K9E2 (starting at position 130) gave a bad degradation with considerable cycle overlap.

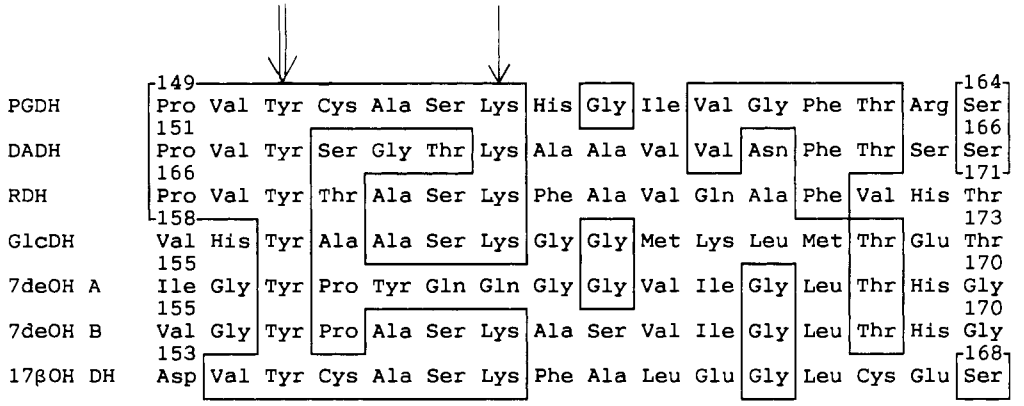

FIGURE 4: Structural comparisons of short-chain alcohol dehydrogenase related enzymes thus far reported, covering the region of maximal similarity previously outlined (Jörnvall et al., 1981; Villarroya et al., 1989). This segment corresponds to positions 149–164 of 15-hydroxyprostaglandin dehydrogenase in Figure 3. A lysine residue previously highlighted as a possible site of conservation (Jörnvall et al., 1981; Villarroyo et al., 1989) is indicated by an arrow and now found not to be strictly conserved. An adjacent tyrosine residue, indicated by a double arrow, is now instead found to be strictly conserved. 15-Hydroxyprostaglandin dehydrogenase (PGDH) now analyzed, *Drosophila melanogaster* alcohol dehydrogenase (DADH) (Thatcher, 1980), ribitol dehydrogenase (RHD) (Dothie et al., 1985), and glucose dehydrogenase (GlcDH) (Jany et al., 1984) all three aligned as before (Jörnvall et al., 1984). 7deOH A and 7deOH B are the two enzymes involved in steroid 7α-dehydroxylation previously aligned (Coleman et al., 1988; White et al., 1988). 17β-Hydroxysteroid dehydrogenase (17βOH DH) (Peltoketo et al., 1988) now is found also to belong to this group. Boxed residues indicate identities between the prostaglandin dehydrogenase and any of the other enzymes.

At one position, a clear microheterogeneity was detected. Peptides K14 and K15 were recovered in equal yield and gave identical structures except for Cys in cycle 2 of K14 and Tyr in cycle 2 of K15. Both peptides were recovered in lower than average yield (as expected for origins from the same segment). Furthermore, both peptides were obtained in additional forms in minor yield (presumably because of deamidation), also in mutually equal amounts. Consequently, the microheterogeneity is established to occur in a single placenta and is compatible with an allelic variation between two residues related by a one-base minimal change in the coding sequence.

In conclusion, the analysis reveals human placental NAD^+-dependent, 15-hydroxyprostaglandin dehydrogenase to have 266-residue subunits with free, N-terminal methionine and C-terminal glutamine. Apparently, the protein chains can be of two types, with Cys or Tyr at position 217, corresponding to hetero- and homodimers with heterozygotes, and homodimers with homozygotes.

Structural Comparisons with Other Dehydrogenases. The availability of the structure for 15-hydroxyprostaglandin dehydrogenase makes direct comparison with other structures possible. It is found that the prostaglandin dehydrogenase is distantly but clearly related (Table III) to the family of short-chain nonmetalloenzyme dehydrogenases initially defined for insect alcohol dehydrogenase, bacterial ribitol dehydrogenase (Jörnvall et al., 1981), and bacterial glucose dehydrogenase (Jörnvall et al., 1984), when each of those structures became available (Thatcher, 1980; Dothie et al., 1985; Jany et al., 1984), and recently extended to two bacterial steroid dehydrogenases involved in 7α-dehydroxylation (presumably 3α-steroid dehydrogenase or 7α-steroid dehydrogenase) (Coleman et al., 1988; White et al., 1988), and now also to another placental dehydrogenase, 17-hydroxysteroid dehydrogenase (Peltoketo et al., 1988) (Table III, bottom line). Residue identities occur throughout much of the molecule, in total at about 25% of all positions (Table III), which, though low, is significant and is similar to the identity between different immunoglobulin classes (Dayhoff, 1978). Structurally, this links one type of alcohol dehydrogenase with enzymes in sugar metabolism, steroid metabolism, and prostaglandin metabolism (Table III) and shows that an enzyme type originally detected in insects and bacteria (Jörnvall et al., 1981) extends to mammalian organisms, including humans.

Table III: Extent of Residue Identities between Human Placental 15-Hydroxyprostaglandin Dehydrogenase and Previously Reported Enzymes Now Known To Belong to the Same Short-Chain Family of Dehydrogenases[a]

PGDH versus	overall residue identities (of 266 residues in PGDH)
DADH	54
RDH	64
GlcDH	57
7deOH A	53
7deOH B	57
17βOH DH	65

[a] Values show extent of residue identities in pairwise comparisons between 15-hydroxyprostaglandin dehydrogenase (PGDH) and insect alcohol dehydrogenase from *Drosophila melanogaster* (DADH) (Thatcher, 1980), ribitol dehydrogenase (RDH) from *Klebsiella aerogenes* (Dothie et al., 1985; Jörnvall et al., 1981), glucose dehydrogenase (GlcDH) from *Bacillus megaterium* (Jany et al., 1984; Jörnvall et al., 1984), two *Eubacterium* enzymes involved in 7α-dehydroxylation of steroids (7deOH A and 7deOH B; Coleman et al., 1988; White et al., 1988), and human placental 17β-hydroxysteroid dehydrogenase (17βOH DH; Peltoketo et al., 1988), all having subunits with about 250 residues. Exact number of residue identities may deviate slightly depending on mode of alignment.

Discussion

Enzyme Purification. The present method gives a pure enzyme in high yield, overcoming a problem that has for a long time prevented proper characterization of 15-hydroxyprostaglandin dehydrogenase. Two reports apparently giving homogeneous material from placenta have appeared (Mak et al., 1982; Jarabak & Watkins, 1988). However, the present purification gives a high yield and a faster preparation, presumably largely because of avoidance of the acid precipitation step, eliminating the complicated separation procedure, and introduction of efficient steps utilizing Mono-Q FPLC (Table I and Figure 2B). The total purification is 13 000-fold and the final specific activity 24 000 milliunits/mg. Recent continuation of this work suggests that the initial step on CM-Sepharose can be omitted unless large batches of starting material are being processed. In this way, speed of purification can be further increased, allowing the complete scheme (Table I and Figure 1, top) to be carried through in about 3.5 days.

Structure Determined. The present purification method provided sufficient material for efficient analysis of the entire structure by allowing work with several digests in parallel

(Figures 1–3). This analysis fully validates the purification scheme, supporting the subunit size and quaternary structure deduced from the electrophoretic and chromatographic mobilities (Figure 2). Furthermore, it establishes subunit homogeneity, except for a single positional microheterogeneity at position 217, corresponding to a probable allelic variation and present presumably only in heterozygotes.

Structural Relationships and Functional Properties. The availability of a structure shows that 15-hydroxyprostaglandin dehydrogenase is a divergent member within the short-chain family of dehydrogenases related to insect alcohol dehydrogenase (Table III and Figure 4). This was predicted long ago on the basis of very little evidence (Mak et al., 1982) and is only now established. It is also shown here (Table III and Figure 4) that the placental 17-hydroxysteroid dehydrogenase is a member of the family of short-chain dehydrogenases, though it was previously considered unique (Peltoketo et al., 1988). Consequently, the family is multiply represented in mammalian sources, and not only in bacteria and insects where it was initially detected.

Finally, the assignment of 15-hydroxyprostaglandin dehydrogenase to the short-chain alcohol dehydrogenase family including the steroid dehydrogenases is of functional interest in another context. Thus, a prostaglandin dehydrogenase activity has quite recently been ascribed to a rat liver 3α-hydroxysteroid dehydrogenase (Penning & Sharp, 1987). Therefore, not only structural but also functional relationships might be expected to be found between alcohol/prostaglandin/steroid and sugar dehydrogenases in relevant organisms.

Essentially nothing is known about the functional mechanism or catalytic properties in structural terms of the short-chain dehydrogenase family. Observations mainly on the *Drosophila* alcohol dehydrogenase have suggested that a central segment containing a lysine residue appears to be fairly well conserved (Jörnvall et al., 1981; Villarroya et al., 1989). Consequently, it is of special interest to observe to what extent this area of maximal similarity in relation to the *Drosophila* alcohol dehydrogenase may also be conserved in the prostaglandin dehydrogenase. This segment is shown in Figure 4 for the enzymes thus far reported. It is clear that this segment does show significant similarities, considering that the six enzymes represent completely different enzyme activities and extremely divergent organisms, ranging from prostaglandin dehydrogenase in humans to sugar dehydrogenase in bacteria via alcohol dehydrogenase in insects! Thus, functional interests should probably still be directed toward this segment inside the middle of the molecules. However, the lysine residue initially considered especially interesting (Jörnvall et al., 1981) is not conserved (Figure 4, position 155). Instead, an adjacent tyrosine residue, then disregarded, is conserved. Tyrosine has also been ascribed a functional role in glucose dehydrogenase (Jany et al., 1984). The possibility therefore remains that the conserved tyrosine residue might be of special functional importance in the whole enzyme group. Interestingly, it is also present in the second mammalian enzyme now assigned to this group, placental 17-hydroxysteroid dehydrogenase (Figure 4).

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL AVAILABLE

A table showing total compositions of peptides relevant to prove the structure of human placental NAD+-linked 15-hydroxyprostaglandin dehydrogenase (1 page). Ordering information is given on any current masthead page.

Registry No. PGDH, 9030-87-9; DADH, 9031-72-5; RDH, 9014-23-7; GlcDH, 9028-53-9; 7deOH, 85130-33-2; 17βOH DH, 9015-81-0; 15-hydroxyprostaglandin dehydrogenase, 217-L-Cys (human placenta reduced), 124041-95-8; 15-hydroxyprostaglandin dehydrogenase, 217-L-Tyr (human placenta reduced), 124041-96-9.

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