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Review

The Short-chain Alcohol Dehydrogenase Superfamily: Variations on a Common Theme

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Multiple alignment of members of the short-chain alcohol dehydrogenase (SCAD) superfamily, according to the conserved domains A–F, has revealed a number of important relationships. It can be shown that the 17β-hydroxysteroid dehydrogenase type 2 enzyme is more closely related to D-β-hydroxybutyrate dehydrogenase than it is to 17β-hydroxysteroid dehydrogenase type 1. Carbonyl reductase, previously considered to be a member of the aldo–keto reductase superfamily, displayed high homology in the conserved domains and is clearly part of the SCAD superfamily despite the insertion of a large peptide between conserved domains. Alignment of the product of the Leishmania methotrexate resistance gene HMTX showed that an internal, highly conserved domain can be substituted by an unrelated sequence without loss of biological activity. Furthermore, comparisons of the chimeric trifunctional enzyme enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase/3-hydroxyacyl-CoA epimerase with other family members suggests that the region between the conserved B and C domains is the last to diverge between closely related enzymes and that the F domain appears to evolve with a different evolutionary clock to the rest of the protein. Finally, a highly conserved pattern of serine and threonine residues in the active site of SCAD enzymes indicates that these residues may play an important role in catalysis. These observations should facilitate alignment of future members of the SCAD superfamily.

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INTRODUCTION

The short-chain alcohol dehydrogenase (SCAD) superfamily of proteins is a phylogenetically related group of enzymes which act on substrates as diverse as sugars, steroids, prostaglandins, aromatic hydrocarbons, antibiotics and compounds involved in nitrogen metabolism. A common theme appears to be the conversion of signalling molecules to either the active or inactive state. In bacteria it is the interconversion of sugars, in plants it is flavonoids and in mammalian cells the action of steroids and prostaglandins is modulated by SCAD family members [1, 2].

Steroid-inactivating enzymes also control target cell sensitivity of glucocorticoids, mineralocorticoids, androgens, and estrogens [3] and they have been implicated in clinical conditions ranging from hypertension [4] to Down's syndrome and Alzheimer's disease [5]. Recently the modulation of glucocorticoid activity has received considerable attention. The inactivation of glucocorticoids by 11β-hydroxysteroid dehydrogenase (11βHSD) is essential for allowing access of aldosterone [6, 7] to the non-selective mineralocorticoid receptor [8] in sodium transporting epithelia. Impaired 11βHSD activity, acquired congenitally or by the consumption of excessive amounts of licorice, leads to glucocorticoid occupation of the mineralocorticoid receptor with concomitant sodium retention and the development of severe hypertension [9]. There is now considerable evidence that there are a number of enzymes with 11βHSD activity. The 11βHSD isoforms can be differentiated on the basis of cofactor dependence, tissue localization and affinity for substrate [10, 11]. Clinical studies have also implicated additional 11βHSD genes and suggest isoform specific diseases, though only one gene has been isolated so

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far [12]. It is opportune that a recent report has described a second 17βHSD isoform [13] as this may prove a harbinger for studies on different 11βHSD species. Thus, it may be fruitful to compare and contrast relationships between recently cloned enzymes and those previously analysed by multiple sequence alignment [14–16].

THE CONSERVED DOMAINS

The SCAD superfamily contains enzymes about 250 residues in length which function independently of metal ions. Several strictly conserved residues were identified in an earlier study when making separate pairwise comparisons of 20 family members [14]. However, sequences having large unrelated regions, or even medium sized insertions or deletions cannot be aligned in this manner. A more recent algorithm [17] locates blocks of similarity between protein sequences and has facilitated the identification of four to six conserved domains in the SCAD superfamily with each enzyme possessing sequences of similar length between domains [15].

Functionality has been ascribed to only two domains so far. The A domain binds the NAD(P) cofactor [18] while the D domain is thought to be part of the active site [19, 20]. While further mutational analyses are needed to define the function of all conserved domains some insight may be gained from elaborating on the context within which these regions are placed in the three-dimensional structure of another member of the family 3α, 20βHSD [21]. In the *Streptomyces hydrogenans* enzyme domains B, C and E are largely made up of β-sheets within the interior of the protein while the F domain appears to be a random coil. The former domains may thus form part of the lattice upon which the protein folds, but the role of the F domain remains more speculative. In 17βHSD1, a protein in which the F domain is not conserved, the corresponding part of the sequence has been shown to bind substrate [22]. In 3α, 20βHSD the conserved F domain appears to fold back onto the residues which bind the cofactor, and it is conceivable that some conserved residues in the F domain may play a role in stabilizing the A domain.

17βHSD TYPE 2 IS MOST SIMILAR TO D-β-HYDROXYBUTYRATE DEHYDROGENASE (BDH)

As new members are added to the family multiple sequence alignment can be used to define their relationship to known family members. When alignment is performed with reference to conserved domains a closer than expected relationship between 17βHSD type II (17βHSD2) and BDH was observed (Fig. 1). BDH is a mitochondrial enzyme which catalyses the reversible oxidation of D-β-hydroxybutyrate to acetoacetate, an intermediate of lipid metabolism. Though initial comparisons showed that 17βHSD1 is phylogenetically related to 17βHSD2 and BDH [13, 23] what is surprising is that 17βHSD2 is more closely related to BDH than it is to 17βHSD1. When the sequences are multiply aligned 17βHSD2 can be seen to have 86 identities with BDH but only 43 with 17βHSD1 (Fig. 1). In addition, 11βHSD2 and BDH contain scattered regions of high homology such as a peptide with 57% identity in a 28 residue stretch between the conserved B and C domains. It is interesting that the homology observed between all three enzymes in this region is a feature also present in enzymes closely related to the ACTIII protein (see below) and suggests that this region is the last to diverge during the evolution of closely related enzymes.

Both 17HSD enzymes display 17β- and 20αHSD activities at a single active site despite having minimal similarity in that domain and exhibiting different intracellular localizations, one cytoplasmic and the other lumenal. These observations suggest that they diverged early in evolution. The subsequent emergence of the BDH enzyme is thus intriguing given that there is only a tenuous link between the three enzymes in that β-hydroxy-β-methylglutaryl CoA, a precursor of acetoacetate, is also a precursor in the steroidogenic pathway. It would not be surprising if BDH is found to have some activity on estradiol and progesterone. Finally, 17βHSD2 and BDH both possess substantial, albeit totally non-homologous amino terminal extensions, a feature seldom observed in this superfamily. In the only other example of this kind the hydrophobic amino terminal extension of 11βHSD was found to be indispensable for enzymatic activity [24–26].

CARBONYL REDUCTASE IS RELATED TO SCADs, NOT ALDO–KETO REDUCTASES

That the SCAD protein structure occasionally allows large insertions is illustrated by two interesting additions to the superfamily. Human carbonyl reductase [27], together with its putative porcine homologue 20βHSD [28], contains a large insertion immediately before the D domain [29]. Historically carbonyl reductase, together with aldehyde reductase and aldose reductase, has been considered to be part of a separate superfamily of oxidoreductases known as the aldo–keto reductases [30]. Functionally these enzymes have been related through a broad and overlapping substrate specificity, but comparisons at the amino acid level suggest convergent evolution of carbonyl reductase with aldose reductase and aldehyde reductase. The latter enzymes exhibit extensive identity at the amino acid level, although the SCAD active site consensus sequence (YCQSK) is present in aldose reductase but absent in aldehyde reductase. However, carbonyl reductase is not significantly homologous to either enzyme, but is instead functionally and structurally related to pig 20βHSD [28] with 85% identity at the

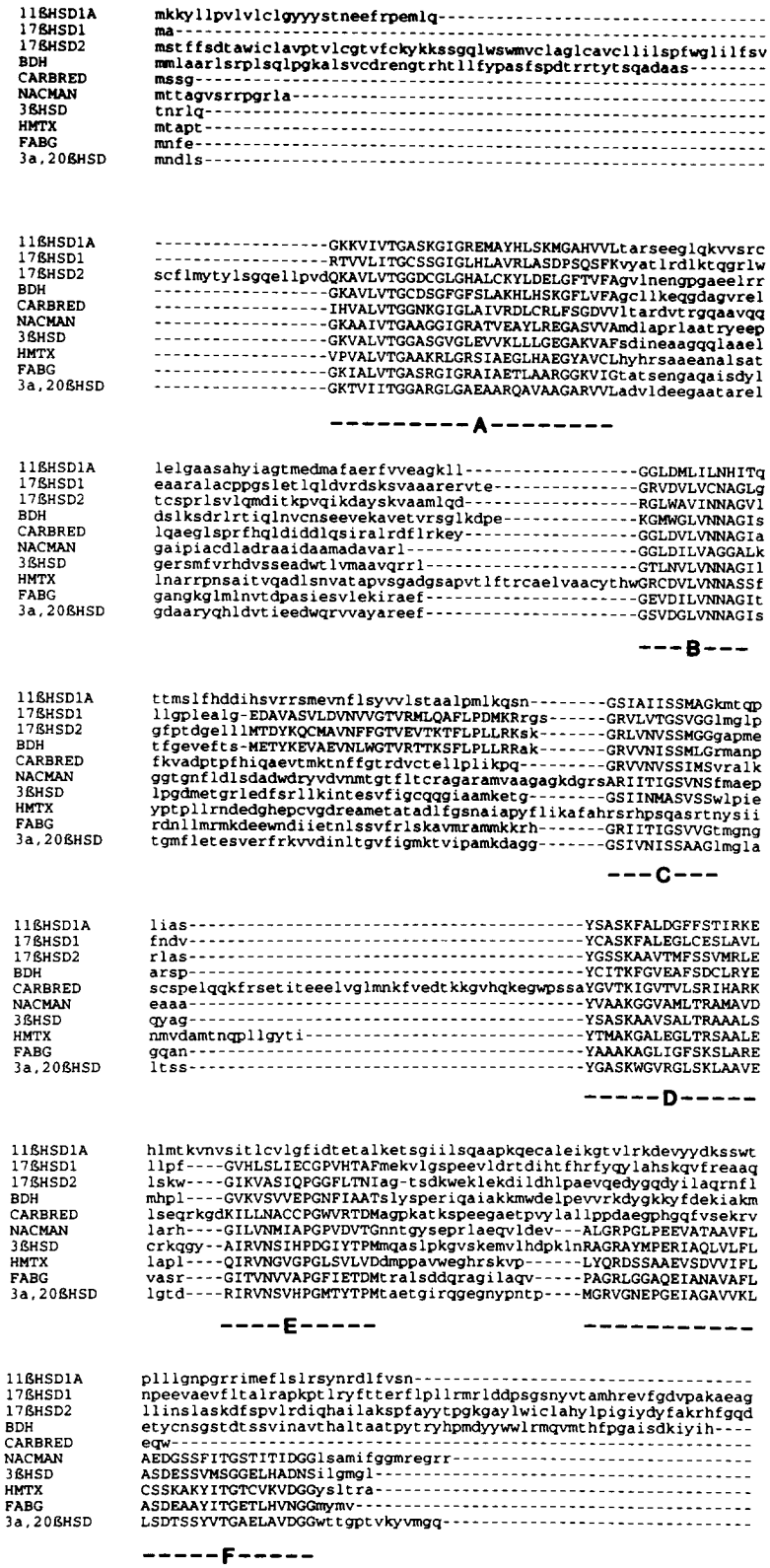

Fig. 1. Multiple alignment of ten SCAD protein sequences. Alignment was performed using the MACAW program [17] followed by manual adjustment of 17βHSD2 and BDH to align obviously similar sequences. Blocks of homology are indicated by residues in capital LETTERS. The positions of conserved domains (A–F) originally identified in Ref. [15] are shown for comparison. A block of significant homology was also observed in the region between the conserved B and C domains in 17βHSD2 and BDH. Abbreviations are as follows: full-length rat 11βHSD, 11βHSD1A [35]; 17βHSD type 1, 17βHSD1 [36]; 17βHSD type 2, 17βHSD2 [13]; D-β-hydroxybutyrate dehydrogenase, BDH [23]; human carbonyl reductase, CARBRED [27]; *N*-acylmannosamine 1-dehydrogenase from Flavobacterium, NACMAN [37]; 3βHSD from *Pseudomonas*, 3βHSD [38]; methotrexate resistance gene product, HMTX [31]; 3-oxoacyl-(acyl-carrier protein) reductase, FABG [39]; 3α, 20βHSD from *Streptomyces hydrogenans*, 3α, 20βHSD [40].

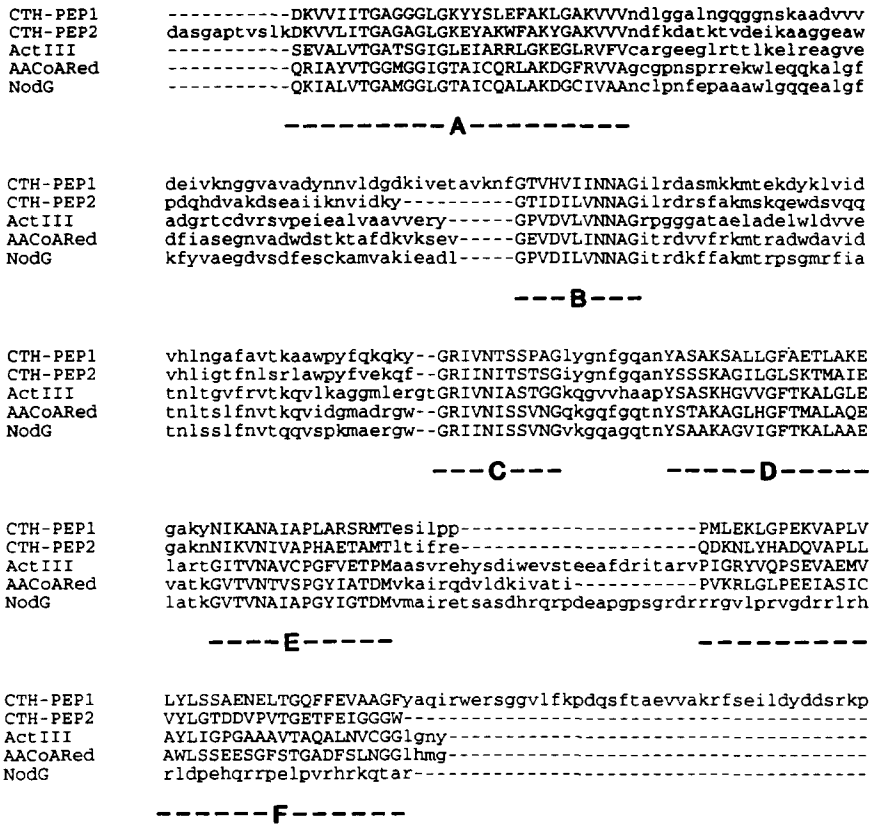

Fig. 2. Alignment of the repetitive domains of the trifunctional enzyme CTHDEG with closely related members of the SCAD superfamily. Two segments of the CTHDEG protein are shown: CTH-PEP1 (CTHDEG amino acid residues 8–283) and CTH-PEP2 (CTHDEG amino acid residues 311–546). The total length of CTHDEG is 906 residues [34]. The following segments of the other SCAD members are shown: ACTIII, residues 6-261 [41]; Acetoacetyl CoA reductase, AACoARed, residues 3–246 [42]; NodG, residues 3–254 [43]. The positions of conserved domains originally identified in Ref. [15] are shown for comparison. The entire segment between domains B and C is also highly conserved in all five peptides ($P < 10^{-8}$), but is not homologous to the corresponding region in the 17βHSD and BDH enzymes.

amino acid level. These similarities make it highly likely that carbonyl reductase is the human homologue of pig 20βHSD. Both these enzymes can be readily aligned with members of the SCAD superfamily with each showing a distinctive insertion of 41 amino acids adjacent to the active site [29]. In addition, the stereo specificity of the carbonyl reductase and 20βHSD enzymes resembles that of the SCAD members in that these enzymes transfer the pro-4S hydrogen atom of NADH while aldehyde and aldose reductase, together with long-chain alcohol dehydrogenases, transfer pro-4R atoms [27]. These observations emphasize the need for a reclassification of the carbonyl reductase enzyme from the aldo–keto reductase to the SCAD superfamily.

A DISPENSABLE CONSERVED DOMAIN

Another departure from the original SCAD structure is the observation that internal conserved domains can be dispensed with in some enzymes. Thus the product of the methotrexate resistance gene (HMTX) in the parasitic protozoan Leishmania [31], lacks a conserved C domain while retaining conservation in the remaining five domains (Fig. 1). The loss of conservation in the C domain is unique to HMTX among the superfamily members and may be the result of a single gene rearrangement under pressure of drug selection. Although the exact mechanism of methotrexate resistance remains unknown it is likely that HMTX is a Leishmania oxidoreductase as it is closely related to 20αHSD, 3βHSD and the product of the FabG gene, a 3-oxoacyl reductase present in bacteria and plants.

The product of HMTX, however, does not contain the currently proposed Prosite [32] consensus sequence for SCADs [the YTMAK motif at the putative active site does not conform to the Y-(PSTAGCV)-(STAGCIV)-(STAGC)-K... family signature]. Since in the present study HMTX can be seen to exhibit conservation with the SCAD family in the A, B, D, E and F domains (Fig. 1) it is clear that this protein belongs to the SCAD superfamily and it is suggested that the family signature should be amended to accommodate this enzyme.

A CHIMERIC ENZYME

The yeast *Candida tropicalis* provides a unique opportunity for us to view a snapshot of the evolution of the SCAD superfamily in progress. An experiment of nature has produced a gene duplication resulting in a chimeric protein containing a tandem repeat [33] highly homologous to the SCAD family members acetoacetyl CoA reductase, NodG and ActIII (Fig. 2). The large resultant 906 residue protein is a trifunctional enzyme with enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-hydroxyacyl-CoA epimerase activity [34]. Each repeat appears to contain all of the domains required for enzymatic activity. The second of the repeats is more closely related to the other enzymes than is the first, suggesting that the first peptide is the progenitor. When the five peptides are aligned, according to previously defined conserved domains, not only are the first five domains highly conserved but there is also considerable identity in the region between the B and C domains, although this region is not related to the corresponding segment in the 17βHSD and BDH enzymes. However, it may be premature to designate this region the status of a separate conserved domain since two pairs of proteins (CTH-PEP1/CTH-PEP2 and AACoARed/NodG, see Fig. 2) are highly homologous over most of their lengths. These observations support the supposition that within a group of closely related enzymes the region between the B and C domains may be the last to diverge.

Despite similarities elsewhere the homology between the F domains of CTHDEG, ActIII, acetoacetyl CoA reductase and NodG is questionable ($P = 1.8 \times 10^{-3}$). However, the C-terminal regions of the CTH-PEP1 and CTH-PEP2 peptides are significantly homologous ($P < 10^{-7}$ and $< 10^{-6}$, respectively) to the F domain of NACMAN, FABG, 3βHSD and HMTX (results not shown). The C-terminal region of SCAD enzymes thus appears to evolve with a different molecular clock to the rest of the protein. An analogous result was obtained when comparing the closely related acetoacetyl CoA reductase and the NodG gene product; while the former enzyme contains an identifiable F domain the latter does not, even though the proteins are 62% identical in the first 200 residues [15].

THE ACTIVE SITE 'S-T' PATTERN

Some SCAD members also tolerate divergence from a highly conserved pattern within the D domain or active site of the protein. Site directed mutagenesis of a number of SCAD proteins has shown that the conserved tyrosine and lysine residues in the putative active site are absolutely required for enzyme activity [19, 20]. Another feature of this domain is the highly conserved presence of a serine or threonine residue immediately after the tyrosine and/or before the lysine residue [14, 15], a motif herein designated the active site 'S-T' pattern. Of the more than 25 SCAD family members identified to date only the bacterial enzymes NACMAN and FABG (Fig. 1) and the yeast CTH-PEP1 (Fig. 2) do not display this pattern, although the highly conserved Ser162 in FabG and Thr177 in NACMAN, and Ser168 in CTH-PEP1 may compensate for the divergence from the 'S-T' pattern. It is likely that the serine and threonine residues comprising the 'S-T' pattern are important for enzymatic activity, though there have not been any studies aimed at answering this question directly. In 3α, 20βHSD, the conserved Tyr152 and Lys156 are located on the same side of an alpha helix with the side-chains of both directed at the active site cleft [21]. The adjacent 'S-T' residues would also be partially directed at the active cleft and may facilitate transfer of the hydride ion to the cofactor.

CONCLUSION

In the present study multiple amino acid alignment of several recently cloned enzymes with members of the SCAD family clearly shows that these enzymes are related and expands membership of the superfamily. With the addition of new members it is now evident that this family of proteins occasionally tolerates considerable divergence in primary sequence structure. These observations should facilitate alignment of future members of the SCAD superfamily.

REFERENCES

1. Baker M. E.: Sequence similarity between Pseudomonas dihydrodiol dehydrogenase, part of the gene cluster that metabolizes polychlorinated biphenyls, and dehydrogenases involved in metabolism of ribitol and glucitol and the synthesis of antibiotics and 17β-oestradiol, testosterone and corticosterone. *Biochem J.* **267** (1990) 839–841.
2. Baker M. E.: Evolution of enzymatic regulation of prostaglandin action: novel connections to regulation of human sex and adrenal function, antibiotic synthesis and nitrogen fixation. *Prostaglandins* **42** (1991) 391–410.
3. Roy A. K.: Regulation of steroid hormone action in target cells by specific hormone-inactivating enzymes. *Proc. Soc. Exp. Biol. Med.* **199** (1992) 265–272.
4. Ulick S., Levine S., Gunczler P., Zanconato G., Ramirez L. C., Rauh W., Rosler A., Bradlow L. H. and New M. I.: A syndrome of apparent mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol. *J. Clin. Endocr. Metab.* **49** (1979) 757–763.
5. Lemieux N., Malfoy B. and Forrest G. L.: Human carbonyl reductase (CBR) localized to band 21q22.1 by high-resolution fluorescence in situ hybridization displays gene dosage effects in trisomy 21 cells. *Genomics* **15** (1993) 169–172.
6. Funder J. W., Pearce P. T., Smith R. and Smith A. I.: Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* **242** (1988) 583–585.
7. Edwards C. R., Stewart P. M., Burt D., Brett L., McIntyre M. A., Sutanto W. S., deKloet E. R. and Monder C.: Localisation of 11 beta-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. *Lancet* **2** (1988) 986–989.
8. Krozowski Z. S. and Funder J. W.: Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Proc. Natn. Acad. Sci. U.S.A.* **80** (1983) 6056–6060.

9. Stewart P. M., Wallace A. M., Valentino R., Burt D., Shakleton C. H. and Edwards C. R. W.: Mineralocorticoid activity of liquorice: 11beta-hydroxysteroid dehydrogenase comes of age. *Lancet* **ii** (1987) 821–824.
10. Mercer W. R. and Krozowski Z. S.: Localization of an 11 beta hydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney. *Endocrinology* **130** (1992) 540–543.
11. Rusvai E. and Naray-Fejes-Toth A.: A new isoform of 11-beta-hydroxysteroid dehydrogenase in aldosterone target cells. *J. Biol. Chem.* **268** (1993) 10717–10720.
12. Nikkila H., Tannin G. M., New M. I., Taylor N. F., Kalaitzoglou G., Monder C. and White P. C.: Defects in the HSD11 gene encoding 11-beta-hydroxysteroid dehydrogenase are not found in patients with apparent mineralocorticoid excess or 11-oxoreductase deficiency. *J. Clin. Endocr. Metab.* 77 (1993) 687–691.
13. Wu L., Einstein M., Geissler W. M., Chan H. K., Elliston K. O. and Andersson S.: Expression cloning and characterization of 17beta-hydroxysteroid dehydrogenase Type 2, a microsomal enzyme possessing 20alpha-hydroxysteroid dehydrogenase activity. *J. Biol. Chem.* **268** (1993) 12964–12969.
14. Persson B., Krook M. and Jornvall H.: Characteristics of short-chain alcohol dehydrogenases and related enzymes. *Eur. J. Biochem.* **200** (1991) 537–543.
15. Krozowski Z.: 11beta-hydroxysteroid dehydrogenase and the short-chain alcohol dehydrogenase (SCAD) superfamily. *Molec. Cell. Endocr.* **84** (1992) C25–C31.
16. Baker M. E.: Sequence analysis of steroid- and prostaglandin-metabolising enzymes: Application to understanding catalysis. *Steroids* **59** (1994) 248–258.
17. Schuler G. D., Altschul S. F. and Lipman D. J.: A workbench for multiple alignment construction and analysis. *Proteins Struct. Funct. Genet.* **9** (1991) 180–190.
18. Coleman J. P., White W. B., Lijewski M. and Hylemon P.: Nucleotide sequence and regulation of a gene involved in bile acid 7-dehydroxylation by Eubacterium sp. strain VPI 12708. *J. Bacteriol.* **170** (1988) 2070–2077.
19. Ensor C. M. and Tai H. H.: Site-directed mutagenesis of the conserved tyrosine 151 of human placental NAD(+)-dependent 15-hydroxyprostaglandin dehydrogenase yields a catalytically inactive enzyme. *Biochem. Biophys. Res. Commun.* **176** (1991) 840–845.
20. Obeid J. and White P. C.: Tyr-179 and Lys-183 are essential for enzymatic activity of 11beta-hydroxysteroid dehydrogenase. *Biochem. Biophys. Res. Commun.* **188** (1992) 222–227.
21. Ghosh D., Weeks C. M., Grochulski P., Duax W. L., Erman M., Rimsay R. L. and Orr J. C.: Three-dimensional structure of holo 3alpha, 20beta-hydroxysteroid dehydrogenase: a member of a short-chain dehydrogenase family. *Proc. natn. Acad. Sci. U.S.A.* **88** (1991) 10064–10068.
22. Murdock G. L., Chin C. C. and Warren J. C.: Human placental estradiol 17β-dehydrogenase: sequence of a histidine-bearing peptide in the catalytic region. *Biochemistry* **25** (1986) 641–646.
23. Churchill P., Hempel J., Romovacek H., Zhang W. W., Brennan M. and Churchill S.: Primary structure of rat liver D-beta-hydroxybutyrate dehydrogenase from cDNA and protein analyses: a short-chain alcohol dehydrogenase. *Biochemistry* **31** (1992) 3793–3799.
24. Obeid J., Curnow K. M., Aisenberg J. and White P. C.: Transcripts originating in Intron-1 of the HSD11 (11beta-hydroxysteroid dehydrogenase) gene encode a truncated polypeptide that is enzymatically inactive. *Molec. Endocr.* 7 (1993) 154–160.
25. Mercer W., Obeyesekere V., Smith R. and Krozowski Z.: Characterization of 11-beta-HSD1B gene expression and enzymatic activity. *Molec. Cell. Endocr.* **92** (1993) 247–251.
26. Krozowski Z., Obeyesekere V., Smith R. and Mercer W.: Tissue-specific expression of an 11 beta-hydroxysteroid dehydrogenase with a truncated N-terminal domain. A potential mechanism for differential intracellular localization within mineralocorticoid target cells. *J. Biol. Chem.* **267** (1992) 2569–2574.
27. Wermuth B., Bohren K. M., Heinemann G. von W. J. and Gabbay K. H.: Human carbonyl reductase. Nucleotide sequence analysis of a cDNA and amino acid sequence of the encoded protein. *J. Biol. Chem.* **263** (1988) 16185–16188.
28. Tanaka M., Ohno S., Adachi S., Nakajin S., Shinoda M. and Nagahama Y.: Pig testicular 20 beta-hydroxysteroid dehydrogenase exhibits carbonyl reductase-like structure and activity. cDNA cloning of pig testicular 20 beta-hydroxysteroid dehydrogenase. *J. Biol. Chem.* **267** (1992) 13451–13455.
29. Krozowski Z. S., Provencher P. H., Smith R. E., Obeyeskere V. R., Mercer W. R. and Albiston A. L.: Isozymes of 11beta-hydroxysteroid dehydrogenase: Which enzyme endows mineralocorticoid specificity? *Steroids* **59** (1994) 116–120.
30. Flynn T. G. and Green N. C.: The aldo-keto reductase: an overview. *Adv. Exp. Med. Biol.* **328** (1993) 251–257.
31. Callahan H. L. and Beverley S. M.: A member of the aldoketo reductase family confers methotrexate resistance in Leishmania. *J. Biol. Chem.* **267** (1992) 24165–24168.
32. Bairoch A.: The prosite dictionary of sites and patterns in proteins. *NAR* **21** (1993) 3097–3103.
33. Baker M. E.: A common ancestor for *Candida tropicalis* and dehydrogenases that synthesize antibiotics and steroids. *Faseb J.* **4** (1990) 3028–3032.
34. Nuttley W. M., Aitchison J. D. and Rachubinski R. A.: cDNA cloning and primary structure determination of the peroxisomal trifunctional enzyme hydratase-dehydrogenase-epimerase from the yeast *Candida tropicalis* pK233. *Gene* **69** (1988) 171–180.
35. Agarwal A. K., Monder C., Eckstein B. and White P. C.: Cloning and expression of rat cDNA encoding corticosteroid 11 beta-dehydrogenase. *J. Biol. Chem.* **264** (1989) 18939–18946.
36. Peltoketo H., Isomaa V., Maentausta O. and Vihko R.: Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Lett.* **239** (1988) 73–77.
37. Yamamoto O. H., Koyama Y., Horiuchi T. and Nakano E.: Cloning, sequencing, and expression of the *N*-acyl-D-mannosamine dehydrogenase gene from Flavobacterium sp. strain 141-8 in *Escherichia coli*. *Appl Environ Microbiol.* **57** (1991) 1418–1422.
38. Abalain J. H., Di S. S., Amet Y., Quemener E., Abalain C. M. and Floch H. H.: Cloning, DNA sequencing and expression of (3-17)beta hydroxysteroid dehydrogenase from Pseudomonas testosteroni. *J. Steroid Biochem. Molec. Biol.* **44** (1993) 133–139.
39. Rawlings M. and Cronan J. J.: The gene encoding Escherichia coli acyl carrier protein lies within a cluster of fatty acid biosynthetic genes. *J. Biol. Chem.* **267** (1992) 5751–5754.
40. Marekov L., Krook M. and Jornvall H.: Prokaryotic 20 beta-hydroxysteroid dehydrogenase is an enzyme of the 'short-chain', non-metalloenzyme' alcohol dehydrogenase type. *FEBS Lett.* **266** (1990) 51–54.
41. Hallam S. E., Malpartida F. and Hopwood D. A.: Nucleotide sequence, transcription and deduced function of a gene involved in polyketide antibiotic synthesis in Streptomyces coelicolor. *Gene* **74** (1988) 305–320.
42. Peoples O. P. and Sinskey A. J.: Poly beta hydroxybutyrate biosynthesis of Alcaligenes eutrophus H16. Characterization of the genes encoding beta-ketothiolase and acetoacetyl-CoA reductase. *J. Biol. Chem.* **264** (1989) 15293–15297.
43. Fisher R. F., Swanson J. A., Mulligan J. T. and Long S. R.: Extended region of nodulation genes in Rhizobium meliloti 1021. II. Nucleotide sequence, transcription start sites and protein products. *Genetics* **117** (1987) 191–201.