3β-Hydroxy-5β-steroid Dehydrogenase Activity of Human Liver Alcohol Dehydrogenase Is Specific to γ-Subunits†

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ABSTRACT: Human liver alcohol dehydrogenase [alcohol:NAD^+ oxidoreductase, EC 1.1.1.1 (ADH)] catalyzes the stereospecific oxidation of different 3β-hydroxy-5β-steroids with ranges of K_m from 46 to 320 μM and values of k_{cat} from 7.0 to 72 min^{-1}, pH 8.5. Only the class I isozymes containing γ-subunits, $\gamma_1\gamma_1$, $\alpha\gamma_1$, $\beta_1\gamma_1$, $\gamma_2\gamma_2$, $\alpha\gamma_2$, and $\beta_1\gamma_2$, catalyze oxidation of these steroids with k_{cat}/K_m ratios 4–10-fold greater than those for ethanol. In marked contrast, class I $\alpha\alpha$, $\alpha\beta_1$, and $\beta_1\beta_1$, class II, and class III isozymes do not oxidize 3β-hydroxy-5β-steroids though they readily oxidize ethanol. 1,10-Phenanthroline and 4-methylpyrazole competitively inhibit both alcohol dehydrogenase catalyzed ethanol and 3β-hydroxy-5β-steroid oxidation demonstrating that the catalysis of both types of substrates occurs at the same active site. The γ-subunit-catalyzed oxidation of 3β-hydroxy-5β-steroids is the most specific catalytic function described thus far for any human liver alcohol dehydrogenase isozyme: there is no other isozyme that catalyzes this reaction. Testosterone, an allosteric inhibitor of ethanol oxidation specific for γ-subunit-containing human liver ADH isozymes [Mårdh, G., Falchuk, K. H., Auld, D. S., & Vallee, B. L. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 2836–2840], also noncompetitively inhibits γ-subunit-catalyzed sterol oxidation. Thus, the γ-isozymes display remarkable stereospecific interactions with certain steroids: 5β-steroids (cis A/B ring fusion) are substrates whereas 5α- and Δ^4-steroids are allosteric inhibitors suggesting that the γ-ADH isozymes are essential in the metabolism of these steroids.

Human liver alcohol dehydrogenase [alcohol:NAD^+ oxidoreductase, EC 1.1.1.1 (ADH)][1] is a dimeric zinc metalloenzyme which is structurally polymorphic (Vallee & Bazzone, 1983; Vallee, 1985). Past studies have focused on the isolation and the electrophoretic and kinetic characteristics of the homo- and heterodimeric isozymes (Lange & Vallee, 1976; Lange et al., 1976; Parês & Vallee, 1981; Wagner et al., 1983; Ditlow et al., 1984). Three classes of ADH isozymes have been recognized and resolved. Among these, three types of subunits, α, β, and γ, comprise the class I isozymes. The allelic variants of the β and γ subunits are designated β_1, β_2, β_3, γ_1, and γ_2 (Jörnvall et al., 1987). Classes II and III consist of the π and χ isozymes, respectively. The primary structures of all human liver ADH isozymes have been determined (Hempel et al., 1984, 1985; Bühler et al., 1984a,b; Jörnvall et al., 1984, 1987).

Recent investigations have revealed characteristic functions of several human liver ADH isozymes whose physiological potential could be significant. Class I isozymes catalyze the interconversion of alcohols and aldehydes in dopamine metabolism (Mårdh & Vallee, 1986); class II exhibits redox-specific activity toward glycol intermediates in norepinephrine metabolism (Mårdh et al., 1985, 1986a); a further role for human liver ADH has recently been demonstrated in the oxidation of 5-hydroxytryptophol, important in the metabolism of serotonin (Consalvi et al., 1986). In all cases, ethanol competes with the physiological metabolites for the active site of ADH.

Catalysis of 3β-hydroxy-5β-steroid oxidation by horse (Reynier et al., 1969; Waller et al., 1965; Ryzewski & Pietruszko, 1977) and rat liver ADH (Mezey & Potter, 1983) has been well documented, and a number of sterols have also been found to be substrates for the unresolved human liver ADH (Pietruszko, 1979). Bulk class I human liver ADH, consisting of a mixture of α, β, and γ homo- and heterodimers, oxidizes the sterol constituents of digitalis, all of them 3β-hydroxy-5β-steroids—digitoxigenin, digoxigenin, and gitoxigenin—to the corresponding 3-ketosteroids (Frey & Vallee, 1980). Further, testosterone and 5α-dihydrotestosterone selectively inhibit ethanol oxidation by class I γ_1 and γ_2 homo- and heterodimers (Mårdh et al., 1986b). The present investigation of individual human liver ADH isozymes shows that only the γ-containing isozymes of ADH specifically catalyze 3β-hydroxy-5β-steroid oxidation.

Materials and Methods

ADH isozymes were purified by the procedures previously described (Ditlow et al., 1984; Wagner et al., 1983, 1984) to which an additional HPLC chromatographic step was added: The individual pools of class I isozymes obtained by CM-52 chromatography were loaded onto a Waters Protein-Pak SP 5PW column (7.5 mm × 7.5 cm) equilibrated with 5 mM Hepes and 0.1 mM DTT, pH 7.7. Elution was performed with a NaCl gradient, 0–0.05 M, in the equilibrating buffer. This additional step is necessary to ensure less than 1% cross-contamination between class I isozymes. For most studies here, $\beta_1\gamma_1$ was used since the γ homodimers are not as abundant in human liver.

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[1] Abbreviations: ADH, alcohol dehydrogenase; NAD^+, nicotinamide adenine dinucleotide (oxidized form); NADH, nicotinamide adenine dinucleotide (reduced form); OP, 1,10-phenanthroline; 4MeP, 4-methylpyrazole; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithiothreitol; 5βA, 3β-hydroxy-5β-androstan-17-one; GRE, glucocorticoid responsive element; HPLC, high-performance liquid chromatography.

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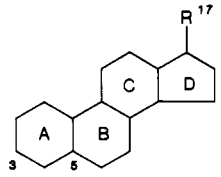
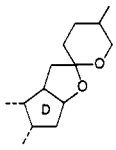
Table I: Kinetic Parameters for Oxidation of Sterols Differing at the 3-, 5-, and 17-Positions by $\beta_1\gamma_1$ Human Liver ADH[a]



steroid[b]	R	K_m	k_{cat}	k_{cat}/K_m
3β-hydroxy-5β-androstan-17-one	=O	160	41	2.6
3α-hydroxy-5β-androstan-17-one	=O	NA[c]		
3α-hydroxy-5α-androstan-17-one	=O	NA[c]		
3β-hydroxy-5α-androstan-17-one	=O	NA[c]		
1,3,5(10)-estratriene-3,17β-diol (17β-estradiol)	—OH	NA[c]		
3β-hydroxy-5β-pregnan-20-one	$CH_3C(O)$—	46	13	2.8
3β-hydroxyetiocholanic acid	—COO^-	320	7	0.22
5β,20(22)-cardenolide-3β,14-diol (digitoxigenin)		300	17	0.57
5β,20α,22α,25D-spirostan-3β-ol (smilagenin)		NA[c]		

[a] Assays performed and kinetic parameters analyzed as described under Materials and Methods. $[\beta_1\gamma_1]$ = 0.1 μM in assay curvette. The units of the kinetic parameters are as follows: K_m, μM; k_{cat}, min^{-1}; k_{cat}/K_m, $M^{-1}\cdot min^{-1} \times 10^{-5}$. [b] Trivial names given in parentheses. [c] No activity detectable.

Steroids were from Sigma (St. Louis, MO), Steraloids, Inc. (Wilton, NH), and Research Plus (Denville, NY) and used as supplied. NAD^+ (grade III) was from Sigma. Sterol and ethanol oxidation was measured in a Varian 219 spectrophotometer at 25 °C by monitoring production of NADH at 340 nm. Assays were performed in 0.1 M sodium pyrophosphate, 2% acetonitrile,[2] and 2.5 mM NAD^+, pH 8.5, unless otherwise noted. The kinetic parameters K_m and k_{cat} were determined from Lineweaver–Burk plots derived from duplicate determinations of initial velocities at 8–10 substrate concentrations ranging from 20 to 400 μM for sterols and from 0.2 and 10 mM for ethanol.

The product of steroid oxidation was identified with a Waters HPLC gradient chromatographic system with a Waters Nova-Pak C_{18} Radial-Pak cartridge under isocratic conditions using an acetonitrile/water (55:45 v/v) solvent system at a flow rate of 2 mL/min. In a typical experiment, 400 μL of a solution of 400 μM sterol, 2.5 mM NAD^+, 5 μM ADH isozyme, 0.1 M sodium phosphate, and 2% acetonitrile, pH 7.4, was incubated at 37 °C for up to 1 h. At the appropriate time, the reaction was stopped by addition of 400 μL of 4 M sodium acetate, pH 4.0, the solution was filtered in a Centricon 10 microconcentrator (Amicon, Danvers, MA), and 200 μL of filtrate (approximately 40 nmol of steroid) was analyzed by HPLC. Peaks were located by means of a Waters R401 differential refractometer. The limit of detection was approximately 2 nmol of product formed per injection. The HPLC retention times of authentic 3β-hydroxy- and 3-keto-steroids were measured as standards and compared to those of the reaction product.

[2] Due to the low solubility of steroid substrates, steroids were dissolved and diluted in acetonitrile and were added to assay solutions to a final acetonitrile concentration of 2% (v/v). This acetonitrile concentration has no effect on the k_{cat} of sterol oxidation but increases K_m by approximately twofold. Acetonitrile has no effect on k_{cat} or K_m for ADH-catalyzed ethanol oxidation under these conditions.

Table II: Kinetic Parameters for Human Liver ADH Catalyzed 5βA and Ethanol Oxidation[a]

		5βA			ethanol		
class	isozyme	K_m	k_{cat}	k_{cat}/K_m	K_m	k_{cat}	k_{cat}/K_m
I	$\alpha\gamma_1$	130	33	2.5	2200	110	0.50
	$\beta_1\gamma_1$	160	41	2.6	2000	150	0.75
	$\gamma_1\gamma_1$	160	72	4.5	1400	180	1.3
	$\alpha\gamma_2$	120	35	2.9	3100	88	0.28
	$\beta_1\gamma_2$	110	43	4.0	2700	110	0.41
	$\gamma_2\gamma_2$	160	66	4.1			
	$\alpha\alpha$			NA[b]			
	$\alpha\beta_1$			NA[b]	1600	110	0.69
	$\beta_1\beta_1$			NA[b]	630	14	0.22
II	π			NA[b]	700000	1200	0.02
III	χ			NA[b]	ND[c]		

[a] K_m, μM; k_{cat}, min^{-1}; k_{cat}/K_m, $M^{-1}\cdot min^{-1} \times 10^{-5}$. Kinetic parameters were determined from Lineweaver–Burk analyses of assays performed in 0.1 M $NaPP_i$ and 2% CH_3CN, pH 8.5; [5βA] = 20–400 μM; [ethanol] = 0.2–10 mM. [b] No activity: these isozymes exhibited ≤1% of the activity of $\gamma_1\gamma_1$ when assayed at 200 μM 5βA and may be the result of slight γ-isozyme contamination. [c] No determination: the χ-isozyme is nonsaturable with ethanol under these conditions.

Inhibition studies were performed with the inhibitors of ADH-catalyzed ethanol oxidation, 1,10-phenanthroline (OP), 4-methylpyrazole (4MeP), and testosterone, in 0.1 M sodium phosphate, 2% acetonitrile, and 2.5 mM NAD^+, pH 7.4.

Results

Sterols with stereochemical variations at the 3- and 5-position and substitutions in the 17-position were tested as substrates for $\beta_1\gamma_1$-ADH-catalyzed oxidation (Table I). Among all the possible isomers only steroids with the 3β-hydroxy-5β-steroid configuration are oxidized; neither the 3α-hydroxy-5β-, the 3α-hydroxy-5α-, nor the 3β-hydroxy-5α-steroid is a substrate. 3β-Hydroxy-5β-androstan-17-one (5βA) proved to be the best substrate and was chosen for subsequent investigations due to its possible metabolic relationship to the ADH inhibitor, testosterone (Dorfman & Ungar, 1965), and its presence as a catabolite in human urine (Kappas et al., 1958; Fukushima & Gallagher, 1957; Baulieu & Emiliozzi, 1960). The product of γ-ADH-catalyzed 5βA oxidation, 5β-androstane-3,17-dione, was identified by HPLC analysis on a Waters C-18 column as described under Materials and Methods. Substitution with bulky or charged groups at the 17-position of the 3β-hydroxy-5β-steroid leads to K_m values ranging from 46 to 320 μM while the k_{cat} values vary from 7.0 to 17 min^{-1}. $\beta_1\gamma_1$-ADH does not exhibit detectable activity toward smilagenin, a 3β-hydroxy-5β-steroid in which the D-ring is fused to a bulky heterocyclic two-ring system.

The individual human liver ADH isozymes were surveyed for catalysis of 5βA and ethanol oxidation at pH 8.5 (Table II). Only class I homo- and heterodimeric γ_1 or γ_2 isozymes catalyze the oxidation of 5βA. The $\alpha\alpha$, $\alpha\beta_1$, $\beta_1\beta_1$, π, and χ isozymes do not catalyze the oxidation of 5βA. In contrast, all three ADH isozyme classes catalyze ethanol oxidation to varying degrees. The K_m values for 5βA are very similar and fall in a narrow range between 110 and 160 μM while for the γ heterodimers k_{cat} varies from 33 to 43 min^{-1} and for the γ homodimers from 66 to 72 min^{-1}. The K_m values for the γ-isozyme-catalyzed 5βA oxidation are 10–30-fold lower and k_{cat}/K_m values are 4–10-fold greater than those for ethanol oxidation.

The competitive inhibitors of ADH-catalyzed ethanol oxidation, 1,10-phenanthroline and 4-methylpyrazole (von Wartburg et al., 1964; Li & Theorell, 1969), competitively inhibit the $\beta_1\gamma_1$-ADH-catalyzed 5βA oxidation with K_i^{OP} and

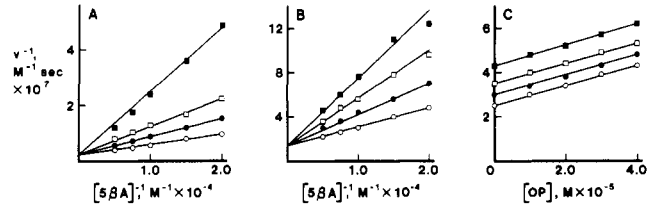


FIGURE 1: Inhibition of $\beta_1\gamma_1$-ADH-catalyzed 3β-hydroxy-5β-androstan-17-one (5βA) oxidation by 4-methylpyrazole (4MeP) and 1,10-phenanthroline (OP). Lineweaver–Burk plots of 4MeP and OP inhibition of 5βA oxidation are presented in panels A and B, respectively. Assays were performed in 0.1 M NaP_i, 2.5 mM NAD^+, and 2% CH_3CN, pH 7.4, at 25 °C with 0 (○), 0.25 (●), 0.5 (□), and 1.0 (■) μM 4MeP and 0 (○), 20 (●), 40 (□), and 60 (■) μM OP. A Dixon plot of the simultaneous inhibition by 4MeP and OP is shown in panel C. Assays were performed at 200 μM 5βA and 0 (○), 0.1 (●), 0.2 (□), and 0.3 (■) μM 4MeP.

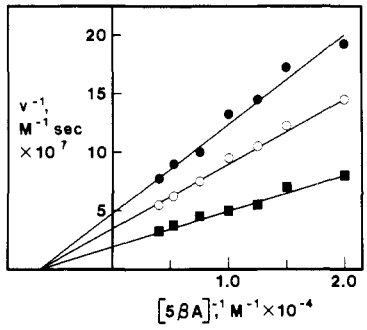


FIGURE 2: Lineweaver–Burk plot of testosterone inhibition of $\beta_1\gamma_1$-ADH-catalyzed 3β-hydroxy-5β-androstan-17-one oxidation. Initial velocities were determined in 0.1 M NaP_i, 2.5 mM NAD^+, and 2% CH_3CN, pH 7.4, at 25 °C with varied 5βA concentration. Assays were carried out at 0 (■), 30 (○), and 60 (●) μM testosterone.

K_i^{4MeP} values of 23 and 0.3 μM, respectively, at pH 7.4 (Figure 1A,B). The degree of interaction between the two inhibitors was determined in a dual inhibitor study (Segel, 1975) (Figure 1C). With 5βA as substrate, the two inhibitors bind in a mutually exclusive manner as is the case with ethanol as substrate (Mårdh et al., 1986b), demonstrating catalysis at the same active site.

Testosterone, an allosteric γ-isozyme-specific inhibitor of ethanol oxidation (Mårdh et al., 1986b), also noncompetitively inhibits $\beta_1\gamma_1$-ADH-catalyzed oxidation of 5βA with a K_i value of 35 μM (Figure 2).

Discussion

The structural polymorphism, substrate specificities, inhibition, and electrophoretic characteristics of the human liver ADH isozymes are becoming increasing well-defined (Vallee, 1985) and describe a complex system of structurally related enzymes whose functions differ quite dramatically. The significant structural and functional differences among these isozymes (Hempel et al., 1984, 1985; Bühler et al., 1984a,b; Jörnvall et al., 1984) call into question the assumption that they are ethanol dehydrogenases functioning solely or primarily in the detoxification of ingested ethanol. Studies of possible physiological roles for the various isozymes so far demonstrate that class I and II isozymes catalyze the interconversion of dopamine, serotonin, and norepinephrine intermediates (Mårdh & Vallee, 1986; Consalvi et al., 1986; Mårdh et al., 1985, 1986a). In all cases, ethanol competes with the metabolite for ADH-catalyzed oxidation.

The present data demonstrate that human liver ADH catalyzes the stereospecific oxidation of 3β-hydroxy-5β-steroids (Table I) with catalysis limited exclusively to the γ-containing isozymes (Table II).[3] The identification of the reaction

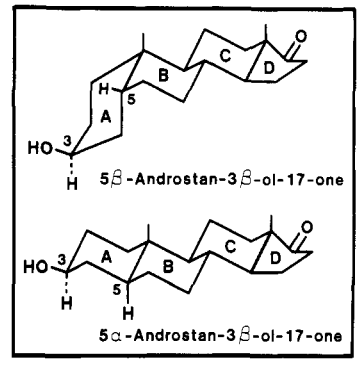


FIGURE 3: Conformations of 3β-hydroxy-5α- and 3β-hydroxy-5β-androstan-17-one as a result of configuration at the 5-carbon. The ring fusions are all trans in the 5α configuration whereas the 5β configuration results in a cis A/B-ring fusion.

product as 5β-androstane-3,17-dione, established by HPLC, agrees with results obtained with digitoxigenin, a 3β-hydroxy-5β-steroid, which undergoes bulk class I ADH catalyzed oxidation to the 3-keto derivative (Frey & Vallee, 1980). The γ-subunit-catalyzed oxidation of 3β-hydroxy-5β-steroids is the most specific catalytic function described for any human liver ADH isozyme: no other isozyme is known thus far to catalyze this reaction. Moreover, testosterone, an allosteric inhibitor of ADH-catalyzed ethanol oxidation specific for the γ-subunit (Mårdh et al., 1986b), noncompetitively inhibits $\beta_1\gamma_1$-catalyzed 5βA oxidation (Figure 2) at concentrations comparable to those for the inhibition of ethanol oxidation. The allosteric inhibition of $\beta_1\gamma_1$-catalyzed steroid oxidation by testosterone suggests feedback regulation of steroid oxidation and the possible importance of these enzymes in steroid metabolism.

Comparison of human liver ADH isozyme sequences with that of the horse enzyme (Hempel et al., 1984, 1985; Bühler et al., 1984a,b; Jörnvall et al., 1984) and computer modeling studies of subunit structure (Eklund et al., 1987) show the overall three-dimensional structure of ADH subunits to be conserved. Though the substrate binding residues of all class I subunits are homologous, in this region the γ-isozyme subunit differs from the others by the replacement of valine-43, threonine-48, and aspartic acid-50 in α and β by alanine, serine, and glutamic acid, respectively. Among these conservative substitutions, that at position 48 may be significant in determining catalytic competence toward 3β-hydroxy-5β-steroids. In the horse enzyme X-ray crystallographic analysis shows that residue 48 is situated at the opening of the hydrophobic substrate barrel (Brändén et al., 1975) where it restricts rotation of bound *p*-bromobenzyl alcohol (Eklund et al., 1982). Threonine-48, present in α, β, π, and χ (Höög et al., 1987; Kaiser et al., 1988), would likely prevent access of bulky substrates, e.g., sterols, to the hydrophobic barrel, as was deduced for substrate binding to yeast ADH (Jörnvall et al., 1978). In γ-subunits the more compact serine-48 would be much less likely to hinder insertion of the steroid A-ring into the active site, permitting productive binding. The exact nature of this interaction may be better understood following X-ray crystallographic studies of the various human ADH isozymes.

[3] Although delineation of the subunit interactions involved in human liver ADH catalysis is beyond the scope of this investigation, it is likely that the individual subunits within the ADH γ_1 and γ_2 hetero- and homodimers catalyze 5βA oxidation in an independent manner. Similar behavior has been observed with respect to cyclohexanol oxidation by β_2-containing isozymes of human liver ADH (Fong & Keung, 1987).

The stereoselectivity of γ-subunits is highly specific for oxidation of 3β-hydroxy-5β-steroids, and changes from this configuration result in total loss of activity (Table I). The configuration at the 5-carbon of the steroid affects the overall shape of the steroid molecule (Figure 3). Steroids with a Δ^4 double bond or a hydrogen substituent in the 5α-position are approximately coplanar throughout the four rings of the steroid due to trans fusion of all rings and are not oxidized via $\beta_1\gamma_1$-ADH catalysis. However, steroids with a hydrogen in the 5β-position exhibit cis fusion of the A- and B-rings and are "bent" at the A–B ring interface, and the A-ring is no longer coplanar. Since the active site zinc atom of human ADH is situated at the distal end of the substrate barrel, this bend in the 5β-steroid may be necessary to position the 3β-hydroxyl group proximal to the catalytic zinc atom. In general, steroids found to be allosteric inhibitors of γ-ADH isozymes are either of the 5α or Δ^4 configuration, i.e., dihydrotestosterone and testosterone, respectively, whereas steroid substrates for the γ-isozyme are limited to those with the 5β configuration.

Evidence for physiological interactions between ADH catalysis and steroid metabolism is accumulating from several avenues of investigation including inhibition of γ-ADH ethanol oxidation by testosterone (Mårdh et al., 1986b), the clinical manifestations of chronic alcoholism such as hypogonadism and feminization (Lieber, 1977), regulation of ADH gene expression at the transcriptional or translational level by testosterone (Ohno et al., 1970a,b), and, as demonstrated here, oxidation of specific steroids.

Testosterone and other steroids have also been demonstrated to regulate ADH gene expression. In adult male mouse kidney ADH activity is fourfold greater than that of adult females but is decreased to that of females by means of castration with concomitant lowering of serum testosterone levels (Ohno et al., 1970a,b). Also, in the female, ovariectomy decreases activity by 50%. Upon adrenalectomy of castrated males or ovariectomized females to abolish remaining synthesis of testosterone and other sex steroids, kidney ADH activity is lost completely within 24 h. Administration of testosterone not only restores the lost kidney ADH activity but increases it to twice that of normal adult male mice. Actinomycin D, a known inhibitor of gene transcription, blocks the testosterone induction of ADH activity, indicating that testosterone regulates the synthesis of kidney ADH at the transcriptional level.

The location of two glucocorticoid responsive elements (GRE) in the 5′-flanking region of the human β-ADH gene is also suggestive of steroid regulation of ADH synthesis (Duester et al., 1986). GRE sequences are found upstream of genes positively activated by glucocorticoid receptor–hormone complexes such as occur in the mouse mammary tumor virus long-terminal repeat (Scheiderit et al., 1983) and the human metallothionein II gene (Karin et al., 1984). The homology among these four GRE sequences is significant, but the function of the β-ADH gene GRE awaits elucidation.

The metabolism of C_{19} and C_{21} steroids is dependent on the hepatic 3-hydroxysteroid dehydrogenases which control the inactivation and excretion of these steroids (Graef et al., 1979). Most investigations concerning mammalian 3β-hydroxysteroid dehydrogenases have been performed on tissue homogenates or extracts; hence, comparatively little is known about the nature of these important enzymes (Hiwatashi et al., 1985). 3-Hydroxysteroid dehydrogenases have been isolated from rat liver (Golf et al., 1980) and bovine adrenocortical microsomes (Hiwatashi et al., 1985), but comparisons among the 3β-hydroxysteroid dehydrogenases and human liver ADH are difficult due to the lack of structural and kinetic data derived from studies performed with the former.

In summary, the selective allosteric inhibition by testosterone and stereospecific oxidation of sterols by γ-isozymes of human liver ADH, together with previously reported findings regarding alcohol dehydrogenase and its interactions with physiologically active steroids, strongly support an essential role for γ-ADH in steroid metabolism.

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Identification of the Site of Acetyl-S-Enzyme Formation on Avian Liver Mitochondrial 3-Hydroxy-3-methylglutaryl-CoA Synthase†

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ABSTRACT: Avian liver mitochondrial hydroxymethylglutaryl-CoA synthase contains an active-site cysteine involved in forming the labile acetyl-S-enzyme intermediate. Identification of and assignment of function to this cysteine have been accomplished by use of an experimental strategy that relies upon generation and rapid purification of the *S*-acetylcysteine-containing active-site peptide under mildly acidic conditions that stabilize the thioester adduct. Automated Edman degradation techniques indicate the peptide's sequence to be Arg-Glu-Ser-Gly-Asn-Thr-Asp-Val-Glu-Gly-Ile-Asp-Thr-Thr-Asn-Ala-Cys-Tyr. The acetylated cysteine corresponds to position 129 in the sequence deduced from cDNA data for the hamster cytosolic enzyme [Gil, G., Goldstein, J. L., Slaughter, C. A., & Brown, M. S. (1986) *J. Biol. Chem. 261*, 3710–3716]. The acetyl-peptide sequence overlaps that reported for a tryptic peptide that contains a cysteine targeted by the affinity label 3-chloropropionyl-CoA [Miziorko, H. M., & Behnke, C. E. (1985) *J. Biol. Chem. 260*, 13513–13516]. Thus, availability of these structural data allows unambiguous assignment of the acetylation site on the protein as well as a refinement of the mechanism explaining the previously observed affinity labeling of the enzyme.

3-Hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA[1] synthase; EC 4.1.3.5) produces a key intermediate in ketogenesis and cholesterogenesis by a three-step reaction sequence:

$$\text{acetyl-SCoA} + \text{HS-Enz} \rightleftharpoons \text{acetyl-S-Enz} + \text{CoASH} \quad (1)$$

$$\text{acetoacetyl-SCoA} + \text{acetyl-S-Enz} \rightleftharpoons \text{CoAS-HMG-S-Enz} \quad (2)$$

$$\text{CoAS-HMG-S-Enz} + \text{H}_2\text{O} \rightarrow \text{HMG-SCoA} + \text{HS-Enz} \quad (3)$$

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