Conversion of 7-Dehydrocholesterol to 7-Ketocholesterol Is Catalyzed by Human Cytochrome P450 7A1 and Occurs by Direct Oxidation without an Epoxide Intermediate

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Raku Shinkyo1, Libin Xu1, Keri A. Tallman§, Qian Cheng§, Ned A. Porter†§¶, and F. Peter Guengerich*†

From the Departments of Biochemistry and Chemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

7-Ketocholesterol is a bioactive sterol, a potent competitive inhibitor of cytochrome P450 7A1, and toxic in liver cells. Multiple origins of this compound have been identified, with cholesterol being the presumed precursor. Although routes for formation of the 7-keto compound from cholesterol have been established, we found that 7-dehydrocholesterol (the immediate precursor of cholesterol) is oxidized by P450 7A1 to 7-ketocholesterol (kM/kp = 3 × 10^4 M^-1 s^-1). P450 7A1 converted lathosterol (Δ5-dehydro-7-dehydrocholesterol) to a mixture of the 7-keto and 7α,8α-epoxide products (~1:2 ratio), with the epoxide not rearranging to the keto. The oxidation of 7-dehydrocholesterol occurred with predominant formation of 7-ketocholesterol and with the 7α,8α-epoxide as only a minor product; the synthesized epoxide was stable in the presence of P450 7A1. The mechanism of 7-dehydrocholesterol oxidation to 7-ketocholesterol is proposed to involve a FeIII-O-C-CFeIII intermediate and a 7,8-hydride shift or an alternative closing to yield the epoxide (Liebler, D. C., and Guengerich, F. P. (1983) Biochemistry 22, 5482–5489). Accordingly, reaction of P450 7A1 with 7-[1H]dehydrocholesterol yielded complete migration of deuterium in the product 7-ketocholesterol. The finding that 7-dehydrocholesterol is a precursor of 7-ketocholesterol has relevance to an inborn error of metabolism known as Smith-Lemli-Opitz syndrome (SLOS) caused by defective cholesterol biosynthesis. Mutations within the gene encoding 7-dehydrocholesterol reductase, the last enzyme in the pathway, lead to the accumulation of 7-dehydrocholesterol in tissues and fluids of SLOS patients. Our findings suggest that 7-ketocholesterol levels may also be elevated in SLOS tissue and fluids as a result of P450 7A1 oxidation of 7-dehydrocholesterol.

Cholesterol is an important component of membranes and a precursor for critical signaling molecules. The balance of synthesis and degradation of cholesterol and its conversions to androgens, estrogens, glucocorticoids, and other important steroids are regulated in part by a number of cytochrome P450 enzymes (1, 2). We have been interested in several issues regarding cholesterol metabolism. One is the fate of 7-dehydrocholesterol, the immediate precursor of cholesterol. 7-Dehydrocholesterol is observed at very low levels or is not normally detectable in tissues and fluids of humans except in skin (3–5), where it is converted to previtamin D₃ under UV irradiation (6). Thus, 7-dehydrocholesterol is important aside from its reduction to cholesterol. Some humans are deficient in 7-dehydrocholesterol reductase (DHCR7) and exhibit Smith-Lemli-Opitz syndrome (SLOS), with elevated levels of 7-dehydrocholesterol and attenuated levels of cholesterol (3, 7–10). 7-Dehydrocholesterol is exceptionally susceptible to free radical oxidation (11), and more than one dozen biologically active oxysterol products have been characterized from 7-dehydrocholesterol oxidation in solution (12–14). In particular, we have been interested in the enzymatic conversion of 7-dehydrocholesterol to oxysterols.

With regard to oxysterols, there is considerable interest in this general class of compounds because of their presence in humans and their biological properties (15–19). One oxysterol of interest is 7-ketocholesterol, which is a strong inhibitor of cytochrome P450 7A1, the cholesterol 7α-hydroxylase (IC₅₀ ~ 1 μM) (20). 7-Ketocholesterol can also exert biological activities, e.g. regulation of cholesterol homeostasis, cytotoxicity, and apoptosis and induction of inflammation, growth inhibition, and vascular endothelial growth factor (16, 19, 21–24). The level of 7-ketocholesterol in human plasma is about one-half that of 7α-hydroxycholesterol, the product of the first committed step of bile acid synthesis (16, 25–27). 7-Ketocholesterol is one of the major oxysterols in human atherosclerotic plaques and accumulates in photodamaged rat retina (15, 28). Levels of 7-ketocholesterol are also elevated in patients with some inherited diseases, e.g. cerebrotendinous xanthomatosis (25) and Niemann-Pick C1 disease (29), and in several animal models of diseases (30, 31). Cholesterol has been the presumed precursor of 7-ketocholesterol, but the mechanism of formation of 7-ketocholesterol in the body is unclear. Much of the literature attributes its source to lipid peroxidation and other radical processes acting on cholesterol (16, 28, 32–35). A 7-hydroxycholesterol dehydrogenase was purified from hamster liver microsomes, although the enzyme is apparently not present in rats or (most) humans (36). 7-Ketocholesterol was not

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1 To whom correspondence should be addressed: Dept. of Biochemistry, Vanderbilt University School of Medicine, 638 Robinson Research Bldg., 2200 Pierce Ave., Nashville, TN 37232-0146. Tel: 615-322-2261; Fax: 615-322-3141; E-mail: f.guengerich@vanderbilt.edu.

2 The abbreviations used are: SLOS, Smith-Lemli-Opitz syndrome; HP/BCD, 2-hydroxypropyl-β-cyclodextrin; HRMS, high resolution mass spectrometry; UPLC, ultra performance liquid chromatography; APCI, atmospheric pressure chemical ionization.
among the many identified oxysterols in the free radical oxidation of 7-dehydrocholesterol (12). However, in a recent study, 7-ketocholesterol was one of the major oxysterols observed in a rat model for SLOS, in which 7-dehydrocholesterol is the dominant sterol (37).

We demonstrate the conversion of 7-dehydrocholesterol to 7-ketocholesterol in human liver microsomes. Of several human P450 enzymes examined, only P450 7A1, the classic cholesterol 7α-hydroxylase, catalyzed the reaction. Detailed studies showed what clearly appears to be a direct oxidation (of the 7,8-olefin) to 7-ketocholesterol, with only trace formation of a 7,8-epoxide but not its required intermediacy (see Fig. 1). P450-catalyzed direct oxidation of an olefin to a carbonyl has been reported before (38, 39), but this appears to be the first case of a dominant conversion with only minor epoxide formation and is relevant to a physiological reaction.

EXPERIMENTAL PROCEDURES

Chemicals—Cholesterol, 7-dehydrocholesterol, 2-hydroxypropyl-β-cyclodextrin (HPβCD), 1,α-1,2-dioleoyl-sn-glycero-3-phosphocholine, 1,α-1,2-dilauroyl-sn-glycero-3-phosphoserine, m-chloroperbenzoic acid, and Tween 20 were purchased from Sigma-Aldrich, and 1,α-1,2-dilauroyl-sn-glycero-3-phosphocholine was purchased from Enzo Life Sciences, Inc. (Plymouth Meeting, PA). m-Chloroperbenzoic acid (in CH₂Cl₂) was washed with 100 mM potassium phosphate buffer (pH 7.4) and dried over Na₂SO₄ before use. Lathosterol (Fig. 1), 7-ketocholesterol, 7-ketocholestanol, and 7α-hydroxycholesterol were purchased from Steraloids Inc. (Newport, RI). [25,26,26,26,27,27,27-d₇]-7-dehydrocholesterol was synthesized as described previously (13). [25,26,26,26,27,27,27-d₇]-7-ketocholesterol was purchased from Toronto Research Chemicals (North York, Ontario, Canada). HPLC-grade solvents were purchased from Fisher.

Enzyme Preparations—Recombinant P450 7A1 with a C-terminal His₆ tag was expressed in Escherichia coli using a plasmid originally provided by I. Pikuleva (Case Western Reserve University, Cleveland, OH) and purified as described elsewhere (40). Recombinant P450 3A4 with a C-terminal His₆ tag (41) was expressed in E. coli and purified as described elsewhere (42). Rat NADPH:P450 reductase (43) and human cytochrome b₅ (44) were expressed in E. coli and purified as described elsewhere.

MS—Finnigan™ TSQ Quantum and Orbitrap (high resolution mass spectrometry (HRMS)) mass spectrometers (ThermoFisher Scientific, Sunnydale, CA) connected to a Waters
Measurement of 7-Dehydrocholesterol Oxidation with P450 7A1 and P450 3A4 Reconstituted Systems—Enzyme reaction mixtures for P450 7A1 reactions typically contained 0.2 mM P450 7A1, 3.0 mM NADPH:P450 reductase, 60 mM l-α,1,2-dilauroyl-sn-glycero-3-phosphocholine, 7-dehydrocholesterol in 3.1 mM HPβCD (0.45%, w/v), and 0.41 mM Tween 20 (0.05%, v/v) in 50 mM potassium phosphate buffer (pH 7.4) (40). For P450 3A4 reactions, a “5× P450 protein premix” was prepared with slight modification of a previously described method (45). Briefly, a 5× protein premix including 2.5 mM P450 3A4, 5 mM NADPH:P450 reductase, 2.5 μM cytochrome b5, 150 μg/ml phospholipid mixture (1:1:1 (w/w/w) l-α,1,2-dioleoyl-sn-glycero-3-phosphocholine/l-α,1,2-dilauroyl-sn-glycero-3-phosphoserine/l-α,1,2-dilauroyl-sn-glycero-3-phosphocholine), 0.50 mg/ml potassium CHAPS (0.77 mM), and 3.0 mM GSH in 50 mM potassium HEPES buffer (pH 7.4) was prepared. A typical 500-μl enzyme reaction mixture was prepared by mixing 100 μl of 5× P450 3A4 protein premix, an equal volume of 5× buffer mix, 5 μl of 7-dehydrocholesterol solution in 310 mM HPβCD (45%, w/v), and 220 μl of H2O. Incubations (at 37 °C) were initiated by the addition of an NADPH-generating system (46). Reactions were quenched with 2.0 ml of CH2Cl2 and mixed with a Vortex device. Following centrifugation (2000 g, 5 min), 1.4 ml of the organic layer was transferred and taken to dryness under an N2 stream. The resulting samples were dissolved in 100 μl of the solvent including 70% (v/v) CH3CN, 30% (v/v) CH3OH, 0.5 mM butylated hydroxytoluene (Sigma), and 0.5 mM triphenylphosphine (Sigma) for analysis.

Samples were analyzed by LC-MS using a UPLC system connected to an ACQUITY UPLC BEH C18 octadeysilane column (2.1 × 100 mm, 1.7 μm). LC conditions were as follow: solvent A contained 95% (v/v) H2O and 5% (v/v) CH3CN, and solvent B contained 95% (v/v) CH3CN and 5% (v/v) CH3OH. The column was maintained at an initial condition of 90% (v/v) solvent B for 1.5 min with a flow rate of 350 μl/min, followed by a linear gradient increasing to 100% (v/v) solvent B over 1.5 min. This condition was maintained for 3.5 min and then returned to the initial condition over 0.1 min and maintained until the end of a 9.2-min run. The column temperature was maintained at 40 °C. The injection volume was 15 μl. Absorbance at 236 nm (7-ketocholesterol) and 205 nm (7α-hydroxycholesterol as an internal standard) was monitored. Data collection and quantitative analysis were conducted with Waters MassLynx version 4.1 and QuanLynx version 4.1 software, respectively.

Measurement of 7-Dehydrocholesterol Oxidation Activity with E. coli Bicistronic Membranes Expressing Human P450 1A1 and P450 1B1—Enzyme reaction mixtures for P450 1A1 or P450 1B1 reactions contained 0.1 μM P450 1A1 or P450 1B1 (with a similar level of NADPH:P450 reductase from coexpression) (47) and 7-dehydrocholesterol in 3.1 mM HPβCD in 100 mM potassium phosphate buffer (pH 7.4) in a typical reaction volume of 500 μl. Assays were conducted at 37 °C for up to 60 min, with incubations initiated by the addition of an NADPH-generating system (46). Following extraction, LC-MS analyses (APCI in the positive ion mode) were conducted as described above.

Measurement of [25,26,26,27,27,27-d6]-7-Dehydrocholesterol Oxidation in Human Liver Microsomes—The amount of cholesterol in a pool of 10 human liver microsomal samples (seven males and three females) was first quantified as described (40). Aliquots of the pool of the 10 human liver microsomal samples containing exactly 50 nmol of cholesterol were added to a 1.0-ml reaction mixture containing 100 nmol of [25,26,26,27,27,27-d6]-7-dehydrocholest erol and 3.1 mM HPβCD in 50 mM potassium phosphate buffer (pH 7.4). The assays were conducted at 37 °C for 60 min. Incubations were initiated by the addition of an NADPH-generating system (46). Reactions were quenched with 2.0 ml of CH2Cl2 and mixed with a Vortex device. Following extraction, LC-MS analyses (APCI in the positive ion mode) were conducted as described above.

Measurement of P450 7A1 Oxidation of Lathosterol—Enzyme reaction mixtures typically contained 0.2 μM P450 7A1, 3.0 mM NADPH:P450 reductase, 60 mM l-α,1,2-dilauroyl-sn-glycero-3-phosphocholine, lathosterol in 3.1 mM HPβCD, and 0.41 mM Tween 20 in 50 mM potassium phosphate buffer (pH 7.4). Incubations were conducted at 37 °C for 3 min, initiated by the addition of an NADPH-generating system (46). Reactions were quenched with 2.0 ml of CH2Cl2 and mixed with a Vortex device. Following extraction of samples, LC-MS analyses (APCI in the positive ion mode) were conducted as described above.

ACQUITY UPLC® system were used for analyses of sterol products. Electron impact HRMS was done at the University of Notre Dame facility (South Bend, IN).
7-Ketocholesterol Formation from 7-Dehydrocholesterol in Human Liver Microsomes—7-Ketocholesterol formation activity was measured with the substrate [7-d1]-7-dehydrocholesterol (100 μM) in a pool of 10 human liver microsomal samples in the presence of endogenous cholesterol (50 μM measured). The [d1]-7-dehydrocholesterol substrate was used because of the presence of endogenous 7-ketocholesterol in the microsomes. The product was defined by co-migration with an authentic standard and an identical fragmentation pattern, as elaborated further under studies with P450 7A1 (see below). The measured rate was 0.014 ± 0.002 pmol/min/mg of protein, which is 100-fold less than that of cholesterol 7α-hydroxylation in human liver microsomes (2.5 ± 1.6 pmol/min/mg of protein) (40). The estimated rate of 7-dehydrocholesterol oxidation in liver microsomes may be compromised by the competition of the endogenous cholesterol, in that the measured $K_m$ for (free) cholesterol for P450 7A1 is ~1 μM (40) and an IC$_{50}$ of 1 μM (for 7-ketocholesterol) has been reported for cholesterol 7α-hydroxylation (20).

7-Dehydrocholesterol Oxidation Activities of P450 7A1, P450 1A1, P450 1B1, and P450 3A4—P450 3A4 (known to have cholesterol 4β-hydroxylation activity (48–50)), P450 1A1, and P450 1B1 did not show any catalytic activity toward 7-dehydrocholesterol. On the other hand, P450 7A1, the cholesterol 7α-hydroxylase, catalyzed the conversion of 7-dehydrocholesterol to two products (Fig. 2A). The most intense product peak at $t_R = 4.82$ min showed $m/z$ 401 and 383, and the $t_R$ and mass spectra (including fragmentation) were identical to those of
7-ketocholesterol (Fig. 2, A–D). One minor oxidation product (m/z 401, 383, and 365 at t_R = 2.74 min) was also detected. This compound was subsequently shown to be cholestanol 7α,8α-epoxide by co-chromatography with the synthetic material and HRMS (Fig. 3).

Rates of 7-ketocholesterol formation by P450 7A1 were measured in the presence of 3.1 mM HPβCD plus 0.41 mM Tween 20. Cholesterol binding to Tween 20 and HPβCD has been reported, with K_d values of 2.5 and 1.5 μM, respectively (51). A model including these two equilibria (i.e., 7-dehydrocholesterol + Tween 20 ⇌ 7-dehydrocholesterol-Tween 20 and 7-dehydrocholesterol + HPβCD ⇌ 7-dehydrocholesterol-HPβCD) was used to fit the data using the program Dynafit (52), and we assume here that the K_d values of 7-dehydrocholesterol for both Tween 20 and HPβCD are similar to those of cholesterol. Based on this correction for the free concentration of 7-dehydrocholesterol, the calculated k_cat and K_m(7-dehydrocholesterol) values were 2.2 ± 0.1 min⁻¹ and 1.1 ± 0.1 μM, respectively (Fig. 4), yielding an estimated catalytic efficiency of 2.0 × 10⁶ M⁻¹ min⁻¹ (3 × 10⁴ M⁻¹ s⁻¹).

Oxidation of Lathosterol to 7-Ketocholestanol and Cholesta- 

7α,8α-Epoxide

FIGURE 3. LC-HRMS chromatogram of products formed from 7-dehydrocholesterol by P450 7A1. The incubations and conditions are similar to those described in the legend to Fig. 2, except that the products were analyzed using an Orbitrap mass spectrometer (HRMS) under slightly different UPLC conditions. The t_R values for synthetic cholesterol 7α,8α-epoxide and 7-ketocholesterol were 3.83 and 5.43 min, respectively (corresponding to the peaks at t_R = 2.74 and 4.78 min in Fig. 2). The calculated mass of the MH⁺ ion of both compounds is 401.3420 (1.7 ppm error).

The minor product peak (t_R = 4.49 min) showed m/z 385 and 367 (Fig. 5, A and G), and the structure of this product is unknown.

Rates of 7-ketocholesterol and cholestanol 7α,8α-epoxide formation by P450 7A1 were measured in the presence of 0.41 mM Tween 20 and 3.1 mM HPβCD. The model including these two equilibria (i.e., lathosterol + Tween 20 ⇌ lathosterol-Tween 20 and lathosterol + HPβCD ⇌ lathosterol-HPβCD) was used to fit the data using the program Dynafit (52), assuming that K_d values of lathosterol for both compounds are similar to those of cholesterol. Based on such estimates of the free concentration of lathosterol, the calculated k_cat and K_m(lathosterol) values for 7-ketocholesterol formation were 3.7 ± 0.2 min⁻¹ and 1.8 ± 0.3 μM, respectively (supplemental Fig. S4), yielding an estimated catalytic efficiency of 2.1 × 10⁶ M⁻¹ min⁻¹ (3 × 10⁴ M⁻¹ s⁻¹). The calculated k_cat and K_m(cholestanol) values for cholestanol 7α,8α-epoxide formation were 7.1 ± 0.4 min⁻¹ and 2.1 ± 0.3 μM, respectively (supplemental Fig. S4), yielding an estimated catalytic efficiency of 3.4 × 10⁶ M⁻¹ min⁻¹ (5 × 10⁴ M⁻¹ s⁻¹).

Stability of Cholestanol 7α,8α-Epoxide and 7-Cholesterol 7α,8α-Epoxide in the Absence and Presence of P450 7A1—Cholesterol 7α,8α-epoxide was incubated with or without P450 7A1 in 50 mM potassium phosphate buffer (pH 7.4) for 60 min. No degradation of this compound was detected with or without P450 7A1 (1.0 μM). 7-Ketocholesterol (m/z 403, 385, and 367 at t_R = 4.70 min) was not detected in the sample including P450 7A1 (Fig. 5F and supplemental Fig. S5).

Cholesterol 7α,8α-epoxide was also incubated with or without P450 7A1 in 50 mM potassium phosphate buffer (pH 7.4) for 30 min, and no degradation of this compound was detected with or without P450 7A1 (1.0 μM) using LC-HRMS (supplemental Fig. S3). In particular, no 7-ketocolesterol was formed. These results indicate that cholesterol 7α,8α-epoxide and cholestanol 7α,8α-epoxide are stable under the experimental conditions used and are not converted to 7-ketocholesterol and 7-ketocholesterol, respectively, by P450 7A1.

Oxidation of [7-d1]-7-Dehydrocholesterol by P450 7A1—The oxidation of [7-d1]-7-dehydrocholesterol by P450 7A1 was not detected.

The clogP (calculated logarithm of the octanol-water partition coefficient) value of 7-dehydrocholesterol is 7.93, and that of cholesterol is 7.96 (ChemBioDraw Ultra version 12.0), suggesting similar partitioning with HPβCD and Tween 20.

3 The clogP value of lathosterol is 8.16, and that of cholesterol is 7.96 (ChemBioDraw Ultra version 12.0).
examined. The formation of $d_7$-7-ketocholesterol ($m/z$ 402 and 384 at $t_R = 4.67$ min) was confirmed and indicates a deuterium shift from C-7, presumably to C-8 in the product (Fig. 6).

**DISCUSSION**

In this study, recombinant human P450 7A1 was shown to catalyze the conversion of 7-dehydrocholesterol (the last compound in the pathway to cholesterol) to 7-ketocholesterol. The oxidation proceeds with formation of cholesterol 7,8-epoxide only as a minor product, and an epoxide is not an intermediate in the reaction (Fig. 1B). The conversion of 7-dehydrocholesterol to 7-ketocholesterol was also demonstrated in human liver microsomes.

7-Ketocholesterol and 7-keto bile acids have been known in the literature for some time (15, 16, 25, 32). Cholesterol is generally considered to be the precursor of these 7-keto compounds, but the mechanism of formation has been unclear. Song et al. (36) purified a 7α-hydroxycholesterol dehydrogenase from hamster liver microsomes. This enzyme had catalytic activity toward a number of hydroxysteroids, including bile acids. The catalytic efficiency reported ($\sim 5 \times 10^4 \text{M}^{-1} \text{s}^{-1}$) is similar to the value we found for human P450 7A1 ($3 \times 10^4 \text{M}^{-1} \text{s}^{-1}$) (Fig. 4), although an issue in comparisons is how abundant these enzymes are in the liver. Antibody and activity measurements showed that the dehydrogenase was apparently absent in rat liver and present only at low levels in some humans (36). Although we have not tested the specificity of P450 7A1 with other 7-dehydrosteroids, we would presume that the specificity of P450 7A1 is rather limited, in that it is generally assumed to work only with cholesterol and very related compounds (i.e. 7-dehydrocholesterol; see above).

Another potential source of 7-ketocholesterol is lipid peroxidation. Free radical oxidation of cholesterol occurs by initial hydrogen atom abstraction at C-7, and 7α- and 7β-cholesterol hydroperoxides are the predominant products formed after chain propagation by oxygen addition and hydrogen atom transfer to the peroxyl radical. Secondary hydroperoxides such as 7-hydroperoxycholesterol readily undergo dehydration, pro-
7-Ketocholesterol Formation by Cytochrome P450 7A1

FIGURE 6. LC-MS chromatograms and mass spectra of products formed by P450 7A1 from δ- and [7-d]-7-dehydrocholesterol. Δ, or [7-d]-7-dehydrocholesterol (20 μM) was incubated with a reconstituted system including 0.2 μM P450 7A1, 3 μM NADPH:P450 reductase, 60 μM NAD+ or 1,2-dilauroyl-sn-glycero-3-phosphocholine, 3.1 mM HPβCD (0.45%, w/v), and 0.41 mM Tween 20 (0.05%, v/v) in 50 mM potassium phosphate buffer (pH 7.4) for 3 min. A, extracted ion chromatogram (EIC) of the m/z 401 ion of the products of δ-7-dehydrocholesterol. B, extracted ion chromatogram of the m/z 402 ion of the products of [7-d]-7-dehydrocholesterol. C, mass spectrum of the product of δ-7-dehydrocholesterol (t_R = 4.67 min). D, mass spectrum of the product of [7-d]-7-dehydrocholesterol (t_R = 4.66 min).

viding a route to 7-ketocholesterol (32), and the 7-keto compound can also be formed from 7α- and 7β-hydroperoxycholesterol by dehydration through a radical mechanism by abstracting the remaining hydrogen atom at C-7 (53). The “Russell mechanism” is another reaction pathway that can give rise to 7-ketocholesterol, where a radical chain termination reaction occurs by the reaction of two peroxy radicals, followed by the decomposition of the resulting tetraoxide (54, 55).

7-Ketocholesterol has been studied extensively, in terms of its biological properties, as outlined in the Introduction. Individuals with the rare disease cerebrotendinous xanthomatosis have high blood levels of 7-ketocholesterol (25), which may also be due to elevated levels of 7-dehydrocholesterol in the plasma of these patients (56). Recently, 7-ketocholesterol was reported to be one of the oxysterol biomarkers for Niemann-Pick C1 disease (29). High blood levels of 7-ketocholesterol are also found in rhesus monkeys with hemorrhagic fever (31). 7-Ketocholesterol is a strong inhibitor of P450 7A1 and also up-regulates synthesis of this protein (20). 7-Keto bile acids are also found in humans and may derive from 7-ketocholesterol (57).

Recently, 7-ketocholesterol was found to accumulate in AY9944 (an inhibitor of DHCGR7)-treated rats (37), where 7-dehydrocholesterol is the dominant sterol precursor. Although it seems unusual to observe higher levels of 7-ketocholesterol with decreased levels of cholesterol, our results here on 7-dehydrocholesterol being an alternative precursor to 7-ketocholesterol provide a reasonable explanation for this observation. We have demonstrated that the human liver microsomal enzyme P450 7A1 also converts 7-dehydrocholesterol to 7-ketocholesterol, and accumulation of 7-ketocholesterol in the blood of SLOS patients would be expected.

P450 7A1 was shown here to directly oxidize 7-dehydrocholesterol to 7-ketocholesterol without an intermediate epoxide (Figs. 2 and 3 and supplemental Fig. S6). Mechanisms are proposed in Fig. 1. Such a reaction has precedence in previous work from one of our laboratories, where (rat) P450 2B1 converted trans-1-phenyl-1-butene to two ketones (38). Subsequently, Mansuy et al. (39) showed the conversion of styrene to phenylacetaldehyde in NADPH-fortified rat liver microsomes, apparently catalyzed by a P450. In both cases, the epoxide was formed as the major product, but the epoxides were stable and were shown not to be intermediates in carbonyl formation. A comparison can be made with hydroxylation of aryl compounds and the 1,2-“NIH shift” (i.e. p to m shift of hydrogen due to a 4-keto intermediate) (58), although in the case of aryl compounds, the epoxides are often unstable, and demonstrating a “non-epoxide” mechanism is difficult. Precedent also exists for 1,2-halide migration in the conversion of vinyl halides to carboxyls without the intermediary of epoxides (38, 59).

In the above cases, some epoxide is always formed and is dominant in the case of the hydride shifts. We first examined lathosterol oxidation (Fig. 5) as a model, in that the synthesis of the 7,8-epoxide is more straightforward than in the case of 7-dehydrocholesterol (supplemental Figs. S1 and S2). Choles- tanol 7α,8α-epoxide and 7-ketocholestanol (Fig. 5) were formed in a ratio of ~2:1, respectively (supplemental Fig. S4). The epoxide was stable and did not convert to the 7-keto product, even in the presence of P450 7A1 (supplemental Fig. S5).

Oxidation of 7-dehydrocholesterol yielded a single major product and only trace 7,8-epoxide (Figs. 2 and 3). Again, this 7,8-epoxide was stable and was shown not to rearrange to the 7-keto product (supplemental Fig. S6). This appears to be the only identified example of a dominant conversion of an olefin to a carbonyl (with only minor epoxide formation). Exactly why P450 7A1 senses the shape of 7-dehydrocholesterol to form primarily the carbonyl but gives a mixture of epoxide and 7-keto product with lathosterol is unclear but is undoubtedly due to the structural differences in the sterol A and B rings.

The mechanisms for oxidation (Fig. 1) follow from the mechanism we proposed for trans-1-phenyl-1-butene (38). Beyond this work and that of Mansuy et al. (37), we are unaware of more
7-Ketocholesterol Formation by Cytochrome P450 7A1

precedes or previous searches for substrates that undergo such reactions. Presumably, more P450 reactions exist that involve rather exclusive conversion of an olefin to a carbonyl, although these cannot be predicted.

In summary, we have demonstrated the direct formation of 7-ketocholesterol from 7-dehydrocholesterol. The enzyme mechanism (Fig. 1) is not novel in itself (38), but the formation of only trace epoxide (Figs. 2 and 3) in such a reaction appears to be. In this case, the reaction involves a physiologically important substrate, 7-dehydrocholesterol, which has been shown to accumulate to high levels in some individuals with a deficiency in the 7-dehydrocholesterol reductase, the enzyme that catalyzes the last step in cholesterol synthesis (i.e. SLOS). The product, 7-ketocholesterol, is associated with several disease states and has been shown to accumulate in an animal model for SLOS (37). We propose that, in the absence of other enzymes that form 7-ketocholesterol, the enzyme reaction demonstrated here may be a source of 7-keto bile acids.

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