Biochemistry

 Volume 37, Number 17 April 28, 1998

Accelerated Publications

Photoaffinity Labeling of Oxidosqualene Cyclase and Squalene Cyclase by a Benzophenone-Containing Inhibitor†

Ikuro Abe, Yi Feng Zheng, and Glenn D. Prestwich*

Department of Medicinal Chemistry, The University of Utah, 30 South, 2000 East, Room 201, Salt Lake City, Utah 84112-5820

Received February 16, 1998

ABSTRACT: A new orally active oxidosqualene:lanosterol cyclase (OSLC) inhibitor (Ro48-8071; Morand, O. H. et al. (1997) *J. Lipid Res. 38*, 373−390) showed potent noncompetitive inhibition of bacterial squalene:hopene cyclase (SHC) from *Alicyclobacillus acidocaldarius* (IC_{50} = 9.0 nM, K_I = 6.6 nM) and OSLC (IC_{50} = 40 nM, K_I = 22 nM for homogeneous rat liver OSLC). A tritium-labeled isotopomer (18.8 Ci/mmol) of this nonterpenoid inhibitor, which possesses a benzophenone (BP) photophore, was chemically synthesized as a photoaffinity label. Specific, efficient covalent modification of both OSLC and SHC enzymes was observed after UV irradiation at 360 nm. Labeling of both OSLC and SHC by [^{3}H]Ro48-8071 was competitively displaced by coincubation with a 1000-fold molar excess of 18-thia-2,3-oxidosqualene or the nonterpenoid inhibitor BIBX79. Displacement of labeling of OSLC was also achieved with the suicide substrate (3*S*)-29-methylidene-2,3-oxidosqualene. Thus, the nonsubstrate Ro48-8071 and both terpenoid and nonterpenoid inhibitors of these enzymes appear to share a common binding site.

The enzymatic cyclizations of 2,3-oxidosqualene and squalene in the biosynthesis of sterols and nonsteroidal triterpenes comprise a series of template-controlled, stereo- and regiochemically specific, carbon−carbon bond-forming reactions (*1*). Oxidosqualene:lanosterol cyclase (OSLC)[1] (EC 5.4.99.7) and bacterial squalene:hopene cyclase (SHC) (EC 5.4.99.7) bind their respective substrates either in a chair-boat-chair (OSLC) or in an all-chair (SHC) conformation. The bound substrates then undergo electrophile-initiated cyclization via a progression of rigidly held, partially cyclized carbocationic intermediates (Figure 1). In the formation of lanosterol (**3**), the initially formed C-20 protosterol cation (**2**) then undergoes backbone rearrangement; in contrast, the SHC cyclization reaction proceeds without rearrangement of the carbocyclic skeleton. These two membrane-associated 70−85 kDa proteins show 17−27% sequence identity (*2*−*11*). SHC and OSLC contain eight and six repeats, respectively, of a highly conserved α-helix-turn motif rich in

† This work was supported by the National Institutes of Health (Grant GM44836 to G.D.P.) and The University of Utah.

* To whom correspondence should be addressed. Phone: 801 585-9051. Fax: 801 585-9053. E-mail: gprestwich@deans.pharm.utah.edu.

[1] Abbreviations used: 29-MOS, (3*S*)-29-methylidene-2,3-oxidosqualene; BIBB515, 1-(4-chlorobenzoyl)-4-[4-(2-oxazolin-2-yl)benzylidene]piperidine; BIBX79, *trans*-*N*-(4-chlorobenzoyl)-*N*-methyl(4-dimethylaminomethylphenyl)cyclohexylamine; BP, benzophenone; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; LR-MS, low-resolution mass spectrum; HR-MS, high-resolution mass spectrum; NMO, 4-methylmorpholine *N*-oxide; NMR, nuclear magnetic resonance; OSLC, oxidosqualene:lanosterol cyclase; Ro48-8071, [4′-(6-allylmethylaminohexyloxy)-2′-fluorophenyl]-(4-bromophenyl)methanone fumarate; S-18, (3*RS*)-18-thia-2,3-oxidosqualene; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SHC, squalene:hopene cyclase; TPAP, tetrapropylammonium perruthenate; UV, ultraviolet.

S0006-2960(98)00366-3 CCC: $15.00
Published on Web 04/04/1998

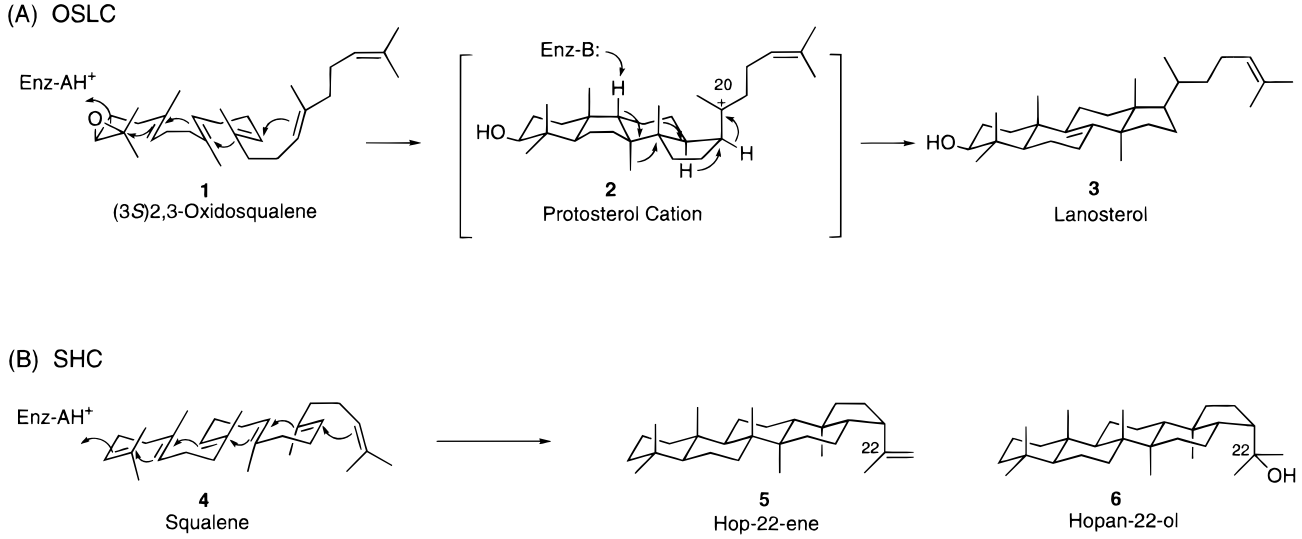

FIGURE 1: Proposed mechanism for the cyclization of (3*S*)2,3-oxidosqualene (**1**) to lanosterol (**3**) by OSLC (A) and squalene (**4**) to hop-22-ene (**5**) and hopan-22-ol (**6**) by SHC (B).

aromatic amino acids (the QW motif), which appear to stabilize the enzyme structure (*12*−*14*). The SHC from the thermoacidophilic bacteria *Alicyclobacillus acidocaldarius* consists of 631 amino acids with a molecular mass of 71 524 Da and has a catalytic optimum at 60 °C and a pH of 6.0 (*9*, *15*). The recently determined three-dimensional structure of the *A. acidocaldarius* SHC complexed to a detergent-like inhibitor reveals an α-helix-rich dumbbell-shapes molecule with a putative active site contained within a large central cavity (*14*).

We previously reported purification (*16*), cloning, and expression (*5*) of rat liver OSLC, an 83 321-Da protein with 733 amino acids. Vertebrate OSLC was specifically labeled with the suicide substrate [^{3}H](3*S*)-29-methylidene-2,3-oxidosqualene (29-MOS) (**9b**) (*16*, *17*), and an aspartate residue (D-456 in rat OSLC) in the highly conserved DCTAEA motif was linked to a partially cyclized inhibitor (*5*, *18*, *19*). This finding implicated the aspartate carboxylate in the stabilization of the C-20 cationic center of the protosterol cation during the lanosterol-forming reaction. Indeed, site-directed mutagenesis experiments have revealed that the D-456 of the DCTAEA motif was essential for catalytic function (*20*, *21*). *A. acidocaldarius* SHC was also covalently modified by [^{3}H](3*S*)29-MOS (**9b**) (*22*), and both D-376 and D-377 of the corresponding DDTAVV motif were shown to be crucial for the enzyme activity (*23*). In addition, **9b** was converted to an unusual dammarene-like derivative by SHC (*22*). We have also recently demonstrated that both [17-^{3}H]- and [22-^{3}H](*RS*)18-thia-2,3-oxidosqualene (S-18) (**8b**) showed potent inhibition of both OSLC and SHC. The labeled S-18 became covalently attached to both pig liver OSLC (*24*) and *A. acidocaldarius* SHC under cyclase reaction conditions (*35*).

The regulation of OSLC levels in vivo has clinical importance and has become a potential target for the design of cholesterol-lowering drugs (*25*). Ro48-8071 (**7a**) is an extremely potent, specific inhibitor of OSLC (IC_{50} = 7 nM for human liver OSLC), recently disclosed by Hoffmann−La Roche (*26*). The two structurally related compounds BIBX79 (**10**) (*27*) and BIBB515 (**11**) (*28*) from Karl Thomae/Boehringer Ingelheim also showed potent inhibition of OSLC (IC_{50} = 6 nM and 9 nM for human Hep G2 OSLC, respectively) as well as oral bioavailability and in vivo activity. Although calculations suggest that these aromatic inhibitors may be similar in shape and size to the protosterol intermediate cation (**2**) (*29*), the molecular mechanism by which the inhibition of (oxido)squalene cyclase occurs is not well understood. To locate the regions of SHC and OSLC interacting with Ro48-8071, we took advantage of the benzophenone (BP) photophore as a potential photoaffinity label (*30*, *31*). This approach was successful, and we describe herein the inhibition kinetics of Ro48-8071 with homogeneous rat liver OSLC and recombinant *A. acidocaldarius* SHC, the synthesis of tritium-labeled Ro48-8071, and the efficient and selective photocovalent modification of the two enzymes.

EXPERIMENTAL PROCEDURES

Chemical Synthesis. Radioinert Ro48-8071 (**7a**) was synthesized by a minor modification of the literature method (*26*). Spectroscopic data (NMR, LR-MS, and HR-MS) were identical to those in the literature. For the radiosynthesis (Figure 3), the intermediate alcohol **13a** was first oxidized to aldehyde **14**. Thus, a mixture of **13a** (0.436 g, 1.1 mmol), 4-methyl morpholine *N*-oxide (NMO) (194 mg, 1.65 mmol), tetrapropylammonium perruthenate (TPAP) (22 mg, 0.06 mmol), and 4-Å molecular sieves (0.55 g) in CH_2Cl_2 (20 mL) was stirred at room temperature for 5 h. After filtration and evaporation, the residue was purified by flash column chromatography on silica gel using 25% ethyl acetate−hexane to give **14** (360 mg, 83% yield).

Next, aldehyde **14** (2 mg) was dissolved in THF (0.1 mL) and reduced with [^{3}H]$NaBH_4$ (25.0 mCi, 75.0 Ci/mmol) in 0.01 N sodium hydroxide solution at room temperature overnight. After evaporation of solvent under N_2, the corresponding alcohol **13b** (18.8 Ci/mmol) was extracted with EtOAc and then purified by chromatography on silica gel using 30% ethyl acetate−hexane.

The labeled alcohol **13b** was converted to the mesylate by using methanesulfonyl chloride (MsCl) (0.14 μL, 1.69 mmol), Et_3N (0.27 μL, 1.90 μmol), and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (3.4 μg) in CH_2Cl_2 (500 μL) at 0

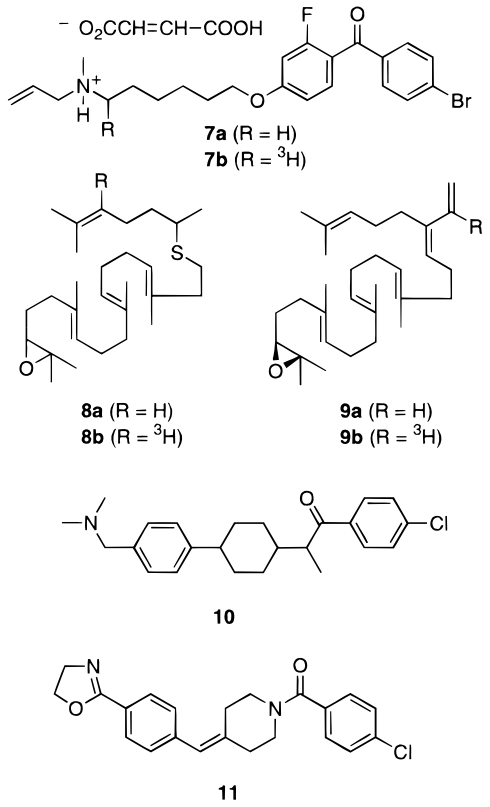

FIGURE 2: Structures of OSLC/SHC enzyme inhibitors: Ro48-8071 (**7**), (*RS*)18-thia-2,3-oxidosqualene (**8**), (3*S*)29-MOS (**9**), BIBX79 (**10**), and BIBB515 (**11**).

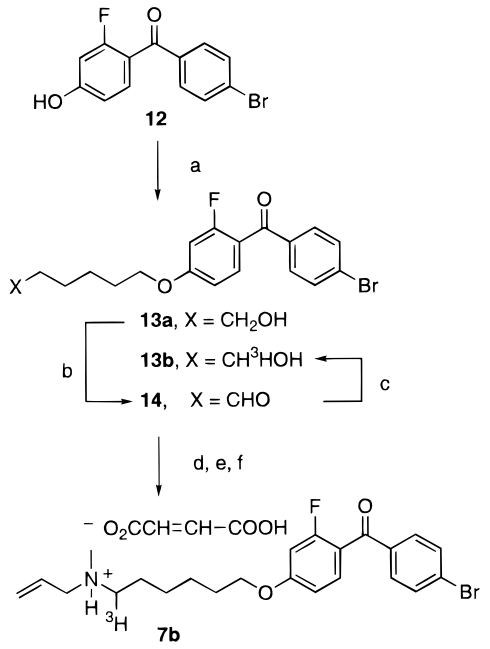

FIGURE 3: Chemical synthesis of [^{3}H]Ro48-8071 (**7b**): reagents, (a) $HO(CH_2)_6Br/K_2CO_3$ in acetone, 75 °C, 8 h, 81%; (b) TPAP, NMO, CH_2Cl_2, rt, 5 h, 83%; (c) NaB^3H_4, EtOH, rt, 12 h, 60%; (d) MsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 5 h, 96%; (e) *N*-allylmethylamine (large excess), *N*,*N*-dimethylacetamide, rt, 22 h, 20%; (f) fumaric acid, EtOH, rt, 1 h.

°C for 3 h. The crude product was dissolved in 200 μL of *N*,*N*-dimethylacetamide and cooled to 0 °C, and 5 drops of *N*-allylmethylamine (large excess) was added. The mixture was stirred at room temperature for 22 h. The free amine was purified on silica gel using CH_2Cl_2/CH_3OH (95/5). Finally, a mixture of the free amine and fumaric acid (1.96 μg, 16.7 μg) in EtOH (200 μL) was stirred at room temperature for 0.5 h to give the fumarate salt, [^{3}H]Ro48-8071 (**7b**) (ca. 500 μCi, 18.8 Ci/mmol).

Enzyme Assays. Rat OSLC was purified from liver, and the OSLC assay was carried out in the presence of 0.1% Triton X-100 in 100 mM Tris HCl, pH 7.4, at 37 °C for 1 h as described previously (*16*). The recombinant *A. acidocaldarius* SHC was expressed in *E. coli* and purified as described previously (*9*). The enzyme converted squalene into a 17:1 mixture of hop-22(29)-ene (**5**) and hopan-22-ol (**6**), and showed an apparent $K_M = 1.6$ μM and $k_{cat} = 2.4$ min^{-1} in the presence of 0.1% Triton X-100 in the assay mixture. For the SHC assay, the reaction mixture contained 100 mM sodium citrate, pH 6.0, 0.1% Triton X-100, 5 μM [^{14}C]squalene (7.0 mCi/mmol), and 0.5 μg of purified recombinant SHC in a final volume of 1 mL. Incubations were carried out at 60 °C for 30 min, and stopped by extraction with 1 mL of CH_2Cl_2. (Note that 60 °C is the optimal temperature for catalytic activity of this SHC.) The extract was concentrated using a Speed-Vac, and subjected to silica gel TLC (Whatmann LK6D). The TLC plates were developed twice, first 5 cm in $CHCl_3$ then 15 cm in hexane. The R_f values of squalene (**4**), hop-22(29)-ene (**5**), and hopan-22-ol (**6**) were 0.45, 0.77, and 0.15, respectively. The conversions were then analyzed by radio-TLC scanner (Bio-Scan, System 500). All assays were carried out in duplicate.

Photoaffinity Labeling. Rat liver OSLC (1 μg of protein in a total volume of 200 μL) was incubated with [^{3}H]Ro48-8071 (**7b**) (100 nM, 18.8 Ci/mmol) in the presence of 0.1% Triton X-100 in 100 mM Tris HCl, pH 7.4, for 15 min at 37 °C and then exposed to UV light (360 nm at 1900 μW/cm^2) for 45 min at 4 °C. For *A. acidocaldarius* SHC (2 μg of protein in a total volume of 200 μL), preincubation with [^{3}H]-Ro48-8071 (100 nM) was carried out in 100 mM sodium citrate buffer, pH 6.0, for 15 min at 60 °C, and the reaction mixture was then exposed to the UV light for 45 min at 4 °C. Samples of each incubation mixture were subjected to SDS−7.5% polyacrylamide gel electrophoresis (PAGE), and proteins were visualized by Coomassie brilliant blue staining. After impregnation with EN^{3}HANCE (NEN Life Science Products) for 1 h, the gels were dried and then exposed to X-ray film (Kodak X-Omat AR film) for 3 days at −80 °C. For competition experiments, the incubations (100 nM of [^{3}H]Ro48-8071) were carried out in the presence of a 1000-fold excess (100 μM) of (*RS*)-18-thia-2,3-oxidosqualene (**8a**), (3*S*)-29-methylidene-2,3-oxidosqualene (**9a**), or BIBX79 (**10**) as enzyme inhibitors or with 100 μM of either (3*S*)-2,3-oxidosqualene (**1**) or squalene (**4**) as substrates.

RESULTS

Inhibition of OSLC activity by Ro48-8071 was reported (*26*) for crude microsomal enzyme preparations ($IC_{50} = 7$ nM for human liver OSLC). In similar preparations, Ro48-8071 was six times less potent toward hamster and Göttingen minipig liver OSLC and about 10 times more potent toward squirrel monkey liver OSLC (*26*). In our assay using homogeneous rat liver OSLC, Ro48-8071 showed an IC_{50} value of 40 nM and a K_I value of 22 nM, with apparent noncompetitive kinetics (Figure 4a). Ro48-8071 is one of the most potent OSLC inhibitors known (*25*).

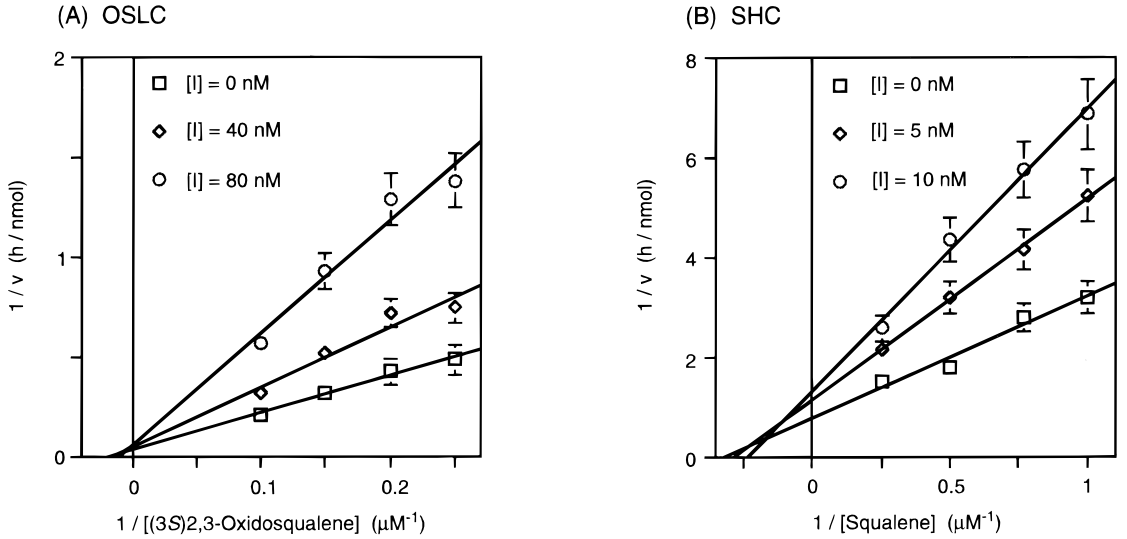

FIGURE 4: Lineweaver−Burk plots of (A) rat liver OSLC and (B) *A. acidocaldarius* SHC inhibition by Ro48-8071 (**7a**). See text for details.

When tested with homogeneous recombinant *A. acidocaldarius* SHC, Ro48-8071 showed even more potent inhibition toward this bacterial SHC (IC_{50} = 9.0 nM, K_I = 6.6 nM), which catalyzes an analogous cyclization from a different substrate in a different conformation relative to that of OSLC. From the Lineweaver−Burk analysis, the inhibition also appeared noncompetitive (Figure 4b); moreover, Ro48-8071 is also the most potent SHC inhibitor known (*15*, *32*).

Ro48-8071 contains a BP moiety that could be employed for photoaffinity labeling of the cyclase enzymes. To test photoaffinity labeling of OSLC and SHC enzymes, we chemically synthesized tritium-labeled Ro48-8071 with high specific activity (Figure 3). Thus, the intermediate alcohol **13a** was first oxidized to aldehyde **14**. Reduction of **14** with [^{3}H]$NaBH_4$ (25.0 mCi, 75.0 Ci/mmol) gave the corresponding alcohol **13b** (18.8 Ci/mmol), which was converted to the corresponding mesylate. The crude product was used to alkylate an excess of *N*-methyl allylamine, and the free amine form of the drug was converted to its fumarate salt to give [^{3}H]Ro48-8071 (**7b**) with a specific activity of 18.8 Ci/mmol.

The two cyclases were photoaffinity labeled using 100 nM [^{3}H]Ro48-8071, that is, 2.5−20-fold the K_I value for each enzyme. After preincubation for 15 min at 37 °C (OSLC) or 15 min at 60 °C (SHC), each reaction mixture was cooled to 4 °C and irradiated with 360-nm light for 45 min at 4 °C. Both OSLC and SHC enzymes were efficiently labeled with [^{3}H]Ro48-8071. As seen in Figures 5 and 6, selective photocovalent modification of the two enzymes was evident from the single radioactive protein band in the fluorogram of the denatured and electrophoretically separated proteins. Both purified and crude enzyme preparations gave a single radioactive band, and labeling of proteins was observed only after UV irradiation.

The specificity of the labeling was further examined by competitive displacement with inhibitors and substrate analogues. Thus, OSLC and SHC were preincubated with a 1000-fold molar excess of several enzyme inhibitors (see Figure 2) prior to the addition of **7b** and UV irradiation. These inhibitors included two mechanism-based inhibitors, S-18 (**8a**) and 29-MOS (**9a**), and a different nonterpenoid

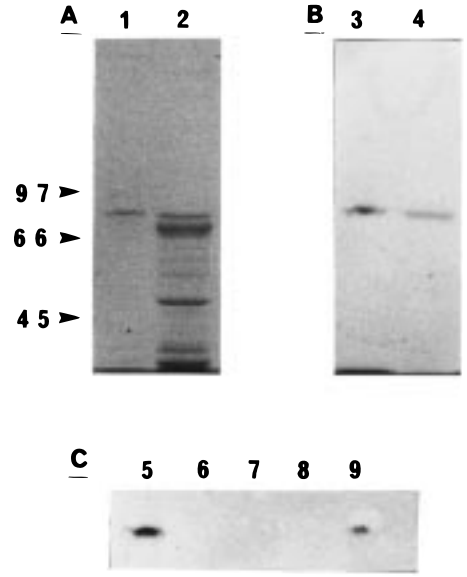

FIGURE 5: Photoaffinity labeling of rat liver OSLC. (A) SDS−PAGE (7.5%) gel stained with Coomassie brilliant blue. (B) Corresponding fluorogram: lane 1 and 3, purified enzyme; lanes 2 and 4, partially purified enzyme. (C) Fluorogram of the competition experiment in the presence of a 1000-fold molar excess (100 μM) of OSLC inhibitor or substrate: lane 5, control; lane 6, **8a**; lane 7, **9a**; lane 8, **10**; lane 9, **1**. Each labeling reaction was carried out at 100 nM **7b** (18.8 Ci/mmol) in a total volume of 200 μL.

inhibitor, BIBX79 (**10**). Competitive displacement was also tested using a 1000-fold molar excess of substrate, that is, (3*S*)-2,3-oxidosqualene (**1**) for rat OSLC and squalene (**4**) for *A. acidocaldarius* SHC. The two mechanism-based inhibitors **8** and **9** covalently modify the catalytic site of both enzymatically active OSLC (*17*, *24*) and SHC (*22*). The nonterpenoid inhibitor **10** is a structural relative of Ro48-8071, developed independently by Boehringer Ingelheim, and also showed potent inhibition of OSLC (IC_{50} = 6 nM for human Hep G2 OSLC) (*27*). Kinetic parameters for these inhibitors with purified enzymes in our cyclase assay systems are summarized in Table 1.

Photocovalent modification of SHC by **7b** was competitively displaced by the presence of a 1000-fold molar excess of **8a** and **10**; for OSLC, labeling by **7b** was also displaced

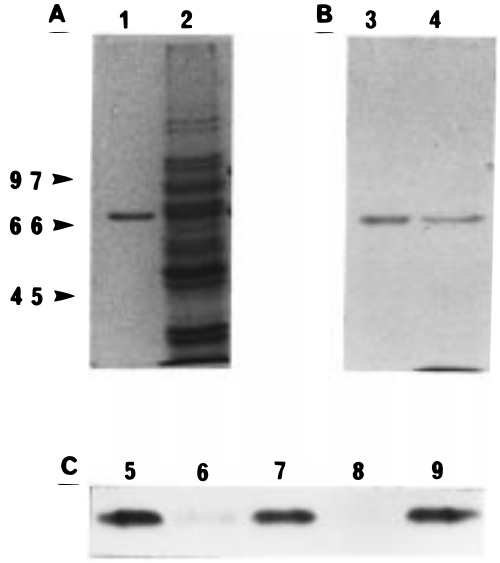

FIGURE 6: Photoaffinity labeling of *A. acidocaldarius* SHC. (A) SDS−PAGE (7.5%) gel stained with Coomassie brilliant blue. (B) Corresponding fluorogram; lane 1 and 3, purified enzyme; lanes 2 and 4, *E. coli* cell-free extract. (C) Fluorogram of the competition experiment in the presence of a 1000-fold molar excess (100 μM) of SHC inhibitor or substrate: lane 5, control; lane 6, **8a**; lane 7, **9b**; lane 8, **10**; lane 9, **4**. Each labeling reaction (UV irradiation) was carried out at a 100 nM concentration of **7b** (18.8 Ci/mmol) in a total volume of 200 μL.

Table 1: Inhibitory Potency for Two Triterpenoid Cyclase Inhibitors[a]

	rat liver OSLC		*A. acidocaldarius* SHC	
inhibitor	IC_{50} (nM)	K_I (nM)	IC_{50} (nM)	K_I (nM)
Ro48-8071 (**7a**)	40	22	9.0	6.6
S-18 (**8a**)	50	37	60	31
29-MOS (**9a**)	300	2500	1200	2100
BIBX79 (**10**)	100	nd	70	nd

[a] Substrate K_M values: **1**, K_M = 86 μM for rat OSLC; **4**, K_M = 1.6 μM for *A. acidocaldarius* SHC.

by suicide substrate **9a** (Figures 5C and 6C). On the other hand, neither substrate **1** nor substrate **4** displaced labeling by **7b**, in accord with their lower binding affinities for their respective cyclases (K_M/K_I = ca. 4000 for OSLC and 200 for SHC). In separate experiments, the mechanism-based covalent labeling of OSLC and SHC enzymes by either [^{3}H]-29-MOS (**9b**) or [^{3}H]S-18 (**8b**) was completely inhibited by the presence of a 100 M excess of Ro48-8071 (**7a**) (data not shown).

DISCUSSION

The Hoffmann−La Roche research group recently reported a series of BP-containing compounds as potent, orally active cholesterol-lowering agents that acted as inhibitors of OSLC (*29*). The inhibitory activity was dependent on the distance between the nitrogen atom of the tertiary amine and the carbonyl group of the BP moiety, and the N to C=O distance is approximately the same for Ro48-8071 (**7**) and BIBX79 (**10**) (Figure 2). The two functions were separated by a lipophilic system: a rigid *trans*-cyclohexylaryl system for **10** and a more flexible aryloxy system for **7**. The optimally active compounds in both series had halogen substitution on the aromatic moiety (*26*). A similarity in shape and size between these inhibitors and the protosterol cation (**2**) was suggested. It was hypothesized (*26*) that the nitrogen atom would interact with the acidic group of the active site of the enzyme, which is involved in the oxirane ring opening, whereas the carbonyl group of the BP would interact with the so-called negative-point charge stabilizing the C-20 cationic center of the protosterol cation. In a sense, **7** would function as a mimic of the late-stage high-energy intermediate in the cyclization pathway. One difficulty with this model is the evidence suggesting that the protosterol cation analogue may not be tightly bound by the enzyme; the relatively modest inhibition of purified yeast OSLC by a 20-aza-protosterol analogue (IC_{50} = 22 μM) seemed to support a low affinity of the catalytic site for a late-state high-energy intermediate (*33*). A second difficulty with this hypothesis is that **7** is a more potent inhibitor of SHC (IC_{50} = 9.0 nM, K_I = 6.6 nM for *A. acidocaldarius* SHC) than it is of OSLC (IC_{50} = 40 nM, K_I = 22 nM for rat liver OSLC). Since the SHC cyclization reaction proceeds via an all pre-chair conformation of squalene, there is no protosterol cation formation involved in this process (Figure 1).

For both OSLC and SHC, the inhibition was both highly potent and noncompetitive (Figure 4). In agreement with the inhibition of SHC by **7**, good inhibition of SHC was observed for *n*-alkyldimethylammonium halides and their *N*-oxides in which the alkyl chain lengths were between 12 and 18 carbon atoms (e.g., dodecyltrimethylammonium bromide, K_I = 320 nM; dodecyldimethylamine *N*-oxide, K_I = 140 nM) (*15*). It is noteworthy that these compounds and **7** all have in common a lipophilic alkylammonium moiety. In addition, OSLC was inhibited by *N*-(1-*n*-dodecyl)imidazole (IC_{50} = 3.9 μM for rat liver OSLC) and its derivatives (*34*). It therefore seems that a common inhibition mechanism for both OSLC and SHC enzyme can be attributed to the alkylammonium side chain or the more rigid hydrophobic arrangement in Ro48-8071 (**7**).

Ro48-8071 is the first potent OSLC or SHC inhibitor that contains a BP moiety and could therefore be employed for photoaffinity labeling. BP derivatives have been found to be excellent photoaffinity probes (*30*), since (i) BPs are chemically more stable than diazo esters, aryl azides, and diazirines, (ii) BPs can be manipulated in ambient light and can be activated at 350−360 nm, avoiding protein-damaging wavelengths, and (iii) BPs react preferentially with otherwise unreactive C−H bonds, even in the presence of solvent water and bulk nucleophiles, frequently with remarkable site specificity (*31*).

As expected, both OSLC and SHC were efficiently labeled with [^{3}H]Ro48-8071 (**7b**, 100 nM, 18.8 Ci/mmol) after UV irradiation at 360 nm. The labeling was specific, and both crude and purified enzyme preparation showed a single band in the fluorogram of the separated proteins. This constitutes the first use of photoaffinity labeling to characterize a triterpenoid cyclase. Importantly, the photoaffinity labeling of SHC by **7b** was prevented by the presence of a 1000-fold molar excess of S-18 (**8a**) or BIBX79 (**10**). Photoaffinity labeling of OSLC by **7b** was blocked by these two inhibitors as well as by (3*S*)29-MOS (**9a**). In the obverse of this experiment, mechanism-based covalent modification of the active site of SHC and OSLC either by [^{3}H](3*S*)29-MOS (**9b**) or by [^{3}H]S-18 (**8b**) was completely inhibited by the presence of a 100 M excess of **7a**, suggesting that **7** and

the mechanism-based cyclase inhibitors share a common binding site, specifically the catalytic active site of the enzyme. Here, as described above, **8** and **10** showed extremely potent inhibition toward both OSLC and SHC at nanomolar levels. Despite the catalytic efficiency with which (3*S*)29-MOS (**9**) inactivated OSLC, **9** had considerably higher K_I values and thus lower binding affinity for OSLC and SHC than either **7** or **10**. Finally, the time dependency of the inhibition of SHC by (3*S*)29-MOS ($k_{inact} = 0.06$ min^{-1}) was much slower than that of vertebrate OSLC ($k_{inact} = 221$ min^{-1}) (*22*); this may explain why **9a** competed for the labeling by **7b** but not for the labeling of SHC.

In summary, Ro48-8071 (**7**) is an extremely potent inhibitor of both OSLC and SHC enzymes, and both enzymes were specifically photocovalently modified by [^{3}H]Ro48-8071 (**7b**). The present studies constitute the first application of photoaffinity labeling for both crude and homogeneous terpenoid cyclases, providing a new useful tool for the analysis of the active sites of OSLC and SHC.

ACKNOWLEDGMENT

The authors are indebted to Professor K. Poralla (Universität Tübingen) for the *A. acidocaldarius* SHC clone, Dr. M. Mark (Boehringer Ingelheim, Biberbach, Germany) for BIBX79, and Dr. D. G. Ahern (NEN Life Science Products, Boston, MA) for providing radiochemical reagents.

REFERENCES

1. Abe, I., Rohmer, M., and Prestwich, G. D. (1993) *Chem. Rev. 93*, 2189−2206.
2. Buntel, C. J., and Griffin, J. H. (1992) *J. Am. Chem. Soc. 114*, 9711−9713.
3. Corey, E. J., Matsuda, S. P. T., and Bartel, B. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 2211−2215.
4. Shi, Z., Buntel, C. J., and Griffin, J. H. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 7370−7374.
5. Abe, I., and Prestwich, G. D. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 9274−9278.
6. Kusano, M., Shibuya, M., Sankawa, U., and Ebizuka, Y. (1995) *Biol. Pharm. Bull. 18*, 195−197.
7. Baker, C. H., Matsuda, S. P. T., Liu, D. R., and Corey, E. J. (1995) *Biochem. Biophys. Res. Commun. 213*, 154−160.
8. Corey, E. J., Matsuda, S. P. T., Baker, C. H., Ting, A. Y., and Cheng, H. (1996) *Biochem. Biophys. Res. Commun. 219*, 327−331.
9. Ochs, D., Kaletta, C., Entian, K.-D., Beck-Sickinger, A., and Poralla, K. (1992) *J. Bacteriol. 174*, 298−302.
10. Reipen, I. G., Poralla, K., Sahm, H., and Sprenger, G. A. (1995) *Microbiology 141*, 155−161.
11. Perzl, M., Müller, P., Poralla, K., and Kannenberg, E. L. (1997) *Microbiology 143*, 1235−1242.
12. Poralla, K. (1994) *Bioorg. Med. Chem. Lett. 4*, 285−290.
13. Poralla, K., Hewelt, A., Prestwich, G. D., Abe, I., Reipen, I., and Sprenger, G. (1994) *Trends Biochem. Sci. 19*, 157−158.
14. Wendt, K. U., Poralla, K., and Schulz, G. E. (1997) *Science 277*, 1811−1815.
15. Ochs, D., Tappe, C. H., Gärtner, P., Kellner, R., and Poralla, K. (1990) *Eur. J. Biochem. 194*, 75−80.
16. Abe, I., Bai, M., Xiao, X.-y., and Prestwich, G. D. (1992) *Biochem. Biophys. Res. Commun. 187*, 32−38.
17. Xiao, X.-y., and Prestwich, G. D. (1991) *J. Am. Chem. Soc. 113*, 9673−9674.
18. Abe, I., and Prestwich, G. D. (1994) *J. Biol. Chem. 269*, 802−804.
19. Abe, I., and Prestwich, G. D. (1995) *Lipids 30*, 231−234.
20. Corey, E. J., Cheng, H., Baker, C. H., Matsuda, S. P. T., Li, D., and Song, X. (1997) *J. Am. Chem. Soc. 119*, 1277−1288.
21. Griffin, J. H., Buntel, C. J., and Siregar, J. J. (1997) *Proc. Natl. Acad. Sci. U.S.A.*, in press.
22. Abe, I., Dang, T., Zheng, Y. F., Madden, B. A., Feil, C., Poralla, K., and Prestwich, G. D. (1997) *J. Am. Chem. Soc. 119*, 11333−11334.
23. Feil, C., Süssmuth, R., Jung, G., and Poralla, K. (1996) *Eur. J. Biochem. 242*, 51−55.
24. Abe, I., Liu, W., Oehlschlager, A. C., and Prestwich, G. D. (1996) *J. Am. Chem. Soc. 118*, 9180−9181.
25. Abe, I., Tomesch, J. C., Wattanasin, S., and Prestwich, G. D. (1994) *Nat. Prod. Rep. 11*, 279−302.
26. Morand, O. H., Aebi, J. D., Dehmlow, H., Ji, Y.-H., Gains, N., Lengsfeld, H., and Himber, J. (1997) *J. Lipid Res. 38*, 373−390.
27. Mark, M., Müller, P., Mainer, R., and Eisele, B. (1996) *J. Lipid Res. 37*, 148−158.
28. Eisele, B., Budzinski, R., Müller, P., Mainer, R., and Mark, M. (1997) *J. Lipid Res. 38*, 564−575.
29. Jolidon, S., Polak-Wyss, A., Hartman, P. G., and Guerry, P. (1993) in *Recent Advances in the Chemistry of Anti-infective Agents* (Bentley, P. H., and Ponsfor, R., Eds.), The Royal Society of Chemistry Letchworth, U.K.
30. Dormán, G., and Prestwich, G. D. (1994) *Biochemistry 33*, 5661−5673.
31. Prestwich, G. D., Dormán, G., Elliott, J. T., Marecak, D. M., and Chaudhary, A. (1997) *Photochem. Photobiol. 65*, 222−234.
32. Flesch, G., and Rohmer, M. (1987) *Arch. Microbiol. 147*, 100−104.
33. Corey, E. J., Daley, D. C., and Cheng, H. (1996) *Tetrahedron Lett. 37*, 3287−3290.
34. Mercer, E. I., Morris, P. K., and Baldwin, B. C. (1985) *Comp. Biochem. Physiol., B 80*, 341−346.
35. Zheng, Y. F., Abe, I., and Prestwich, G. D. (1998) *Biochemistry 37*, (in press).

BI980366C