Aphidicolin, a Specific Inhibitor of DNA Polymerase α, Inhibits Conversion of Lanosterol to C-27 Sterols in Mouse L Cells*

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Aphidicolin, a fungal metabolite which is a specific inhibitor of DNA polymerase α, inhibited the incorporation of [14C]acetate into desmosterol in mouse L cells by 50% at a concentration of 8.8 μM. It had no effect on acetate metabolism into fatty acids or CO2. The site of inhibition was determined to be distal to the formation of mevalonic acid since aphidicolin also inhibited the incorporation of [14C]mevalonolactone into desmosterol but had no effect on the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.3.4) or the incorporation of [14C]acetate into total nonsaponifiable lipids. High pressure liquid chromatographic analysis of the distribution of radioactivity among the nonsaponifiable lipids formed from [14C]acetate in the presence of aphidicolin indicated an accumulation of lanosterol accompanied by a proportional decrease in secosterol metabolism. As a consequence of this inhibition was determined to be distal to the formation of mevalonic acid since aphidicolin also inhibited the incorporation of [14C]mevalonolactone into desmosterol but had no effect on the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.3.4) or the incorporation of [14C]acetate into total non-saponifiable lipids. High pressure liquid chromatographic analysis of the distribution of radioactivity among the non-saponifiable lipids formed from [14C]acetate in the presence of aphidicolin indicated an accumulation of lanosterol accompanied by a proportional decrease in the amount of secosterol formed by the conversion of lanosterol to C-27 sterols. Although the exact mechanism of this inhibition has not yet been determined, addition of aphidicolin to 20,000 x g supernatant fractions of mouse liver homogenates inhibited the incorporation of [14C]mevalonolactone into cholesterol in a concentration-dependent manner, suggesting that aphidicolin may act directly on one or more of the enzymatic steps involved in lanosterol demethylation. The ubiquitous occurrence of an aphidicolin binding site on eukaryotic DNA can be demonstrated by immunoprecipitation using high pressure liquid chromatography before use. Aphidicolin was supplied by H. Todd of Imperial Chemical Industries, Ltd.

Aphidicolin is a tetracyclic diterpene tetrasol obtained from certain fungi (1). Recent interest in this steroid-like antibiotic began with the demonstration that it is a specific inhibitor of DNA polymerase α (2), but with no effect on polymerases β and γ. Further studies with aphidicolin showed polymerase α to be the principal polymerase involved in DNA replication (3, 4). Although the exact mechanism of aphidicolin inhibition of polymerase α is not clear (3, 5, 6), it does bind reversibly and with strong affinity to the polymerase (3). The existence of aphidicolin binding sites on all eukaryotic α-like polymerases has been used to argue for the existence of a natural substance, perhaps a steroid, which regulates DNA synthesis via this binding site (1).

There is a correlation between the proliferative state of cells and sterol synthesis (reviewed in Ref. 7). The exact mechanism of this relationship is not understood, although it has been shown that inhibition of sterol synthesis in synchronized hamster cells (8), lectin-stimulated lymphocytes (9), and growth factor-stimulated 3T3 cells and smooth muscle cells (10) results in suppression of subsequent cycles of DNA synthesis. Certain oxysterols, e.g. 25-hydroxycholesterol, are potent and specific inhibitors of HMG-CoA reductase, and, thus, sterol synthesis, as well as inhibitors of cell growth (7). Since there are some structural similarities between aphidicolin and oxysterols, we investigated the possibility that 25-hydroxycholesterol acts as a direct inhibitor of polymerase α. In this study we conclude that the inhibition of DNA synthesis resulting from suppression of HMG-CoA reductase activity and sterol synthesis by 25-hydroxycholesterol is not the result of the oxysterol mimicking the proposed steroid-like regulator of DNA synthesis.

However, we have shown that aphidicolin, in addition to inhibiting DNA polymerase α, is a rapid reversible inhibitor of sterol synthesis in mouse L cells. These studies show that the activity of HMG-CoA reductase, the major rate-limiting enzyme in the cholesterol biosynthetic pathway, is unaffected by aphidicolin. Rather, inhibition of sterol synthesis occurs at a proposed secondary regulatory site (11–13). Conversion of lanosterol to C-27 sterols was not influenced by aphidicolin.

**MATERIALS AND METHODS**

Cells Culture—L cells (a subline of NCTC mouse fibroblast clone 929) were grown in Waymouth's MB752/1 chemically defined medium supplemented as described previously (14).

**Materials**—Sodium [1-14C]acetate (2.4 mCi/mmol), R,S-3-hydroxy-3-methyl[3-14C]glutaryl-CoA (65.1 mCi/mmol), R,S-[15-3H]mevalonolactone (5.7 Ci/mmol), [1,2-3H]cholesterol (54.8 Ci/mmol), [methyl-3H]hydromide (20 Ci/mmol), [methyl-3H]deoxythymidine triphosphate (16.4 Ci/mmol), and 25-hydroxy[25-3H]cholesterol (73 Ci/mmol) were from New England Nuclear. [α-3H]mevalonolactone (58 mCi/mmol) was from American Corp. 25-hydroxycholesterol was from Steraloids and was recrystallized from methanol prior to use. Cholesterol and squalene were from Sigma. Lanosterol (TLC pure) and desmosterol were from Research Plus and were purified by high pressure liquid chromatography before use. Aphidicolin was supplied by A. H. Todd of Imperial Chemical Industries, England, and Dr. Matthew Sausen of National Products Branch,

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‡ The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; PBS, Dulbecco's phosphate-buffered saline, pH 7.2; TLC, thin layer chromatography.
Division of Cancer Treatment, National Cancer Institute. DNA polymerase α (calf thymus) was from Worthington Diagnostics.

DNA Synthesis—DNA polymerase α activity was assayed in nuclei isolated from hydroxyuracil-synchronized L cells according to the method of Oguro et al. (5). Truncated DNA synthesis (dCTP omitted) by purified DNA polymerase α was assayed as described by Yama-guchi et al. (15) using activated calf thymus DNA as template. DNA synthesis in intact cells was measured by [3H]thymidine incorporation as described previously (16).

[14C]Acetate Metabolism—The metabolism of [14C]acetate into sterol, fatty acids, and CO2 and the DNA content of the cells were determined by procedures previously described in detail (14, 17). When lipids were analyzed by thin layer chromatography or high pressure liquid chromatography, the following modifications were incorporated to inhibit oxidation of intermediate sterols. Cells were harvested by digestion with 1.5 N KOH at 37 °C for 1 h, and cell slurries were stored under nitrogen at −20 °C prior to saponification. After removal of an aliquot for DNA determination, KOH and absolute ethanol were added to final concentrations of 15 and 50%, respectively. In addition, 100 μg each of cholesterol, lanosterol, and squelane, 50 μg of vitamin E, 1 mg/ml of butylated hydroxytoluene, and 40,000 ρg of [14C]cholesterol (to monitor recovery) were included in a final volume of 5 ml. After saponification at 80 °C for 1 h, the lipid fractions were extracted with petroleum ether, washed once with 3% NaHCO3 and twice with water, and dried under nitrogen. Thin layer chromatography was on Whatman K636 prechanneled silica thin layer plates in ethyl acetate:hexane (15:85). Sterol standards were visualized under long wave UV light after spraying with rhodamine B. Chromatograms were divided into 12–14 fractions, scraped into scintillation vials, eluted with 0.5 ml of absolute ethanol, and counted in toluene omnifluor.

High Pressure Liquid Chromatography—Samples were solubilized in 4% water in methanol, passed through a 0.45-μ filter, and applied to a μBondapak-C18 reverse phase column (Waters Associates, Inc., Milford, MA). Solvent was 8% water in methanol at a flow rate of 2 ml/min. Retention time of lipid standards was determined by monitoring absorbance at 210 nm. One-mL fractions were collected and counted in HandiFluor (Mallinkrodt). Oxysterol Binding Protein—Affinity for oxysterol binding protein preparations was measured from homogenates of mouse (C57BL/6J x C3H)F1 liver as described by Scallen et al. (18). S9 preparations (5 mg/ml) supplemented with 3 μM ATP, 3 μM glucose 6-phosphate, and 0.1 μM mevalonolactone were incubated for 45 min at 120 rpm at 37 °C with 14C]mevalonolactone (19). Reactions were terminated by the addition of KOH and ethanol to final concentrations of 15 and 50%, respectively. Nonsaponifiable lipids were extracted and analyzed by thin layer chromatography as described above.

HMG-CoA Reductase Assay—Cells were washed twice with Dulbecco’s PBS, harvested with a rubber policeman, and collected by centrifugation. Reductase activity was measured in sonicated cell lysates using a previously published procedure (17).

Oxysterol Binding Protein—Affinity for oxysterol binding protein was assayed by the ability of a compound to displace 7.5 S-bound [3H]25-hydroxycholesterol. L cells were incubated with [3H]25-hydroxycholesterol and a 40-fold excess of unlabeled compound, and the cytosolic fraction was prepared and analyzed on a sucrose density gradient (20).

RESULTS

25-Hydroxycholesterol and DNA Polymerase α Activity—In our studies of 25-hydroxycholesterol as a direct inhibitor of DNA polymerase α, we used two different systems to assay polymerase α activity. The first used calf thymus DNA as a template for commercially purified bovine DNA polymerase α (Worthington Diagnostics). In the second system, DNA synthesis was assayed in S phase nuclei isolated from synchronized cell cultures. These two assay systems were used since it has been shown that the sensitivity of DNA polymerase α to aphidicolin, a known direct inhibitor of polymerase α, is decreased with increasing purification of the enzyme (2). Under our assay conditions, 15 μM aphidicolin inhibited [3H]thymidine incorporation to 50% of control values in the purified polymerase α assay and to 25% of control values in the isolated nuclei assay. These values are comparable to those reported in the literature (2, 5). Under the same conditions, 25-hydroxycholesterol had no effect on DNA synthesis when added at concentrations up to 50 μM, a concentration three orders of magnitude higher than that necessary for 50% inhibition of HMG-CoA reductase in L cells (21). In experiments where 25-hydroxycholesterol was preincubated with an oxysterol-binding protein-containing supernatant fraction (20), its addition to the reaction mixture still had no effect on [3H]thymidine incorporation. We conclude that neither 25-hydroxycholesterol nor the oxysterol-binding protein complex has an inhibitory effect on DNA polymerase α.

Effect of Aphidicolin on Sterol Synthesis—We found aphidicolin to be a rapid inhibitor of sterol synthesis. Fig. 1 shows that within 1 h aphidicolin inhibits the incorporation of [14C]acetate into desmosterol in a concentration-dependent manner. Instead of cholesterol, L cells synthesize desmosterol as the end product of the sterol synthetic pathway (14, 22). Fifty percent inhibition of sterol synthesis occurs at an aphidicolin concentration of approximately 8.8 μM. The specificity of this inhibition for the cholesterogenic pathway is suggested by the fact that under the same conditions [14C]acetate incorporation into fatty acids and CO2 is unaffected, even after 7 h of treatment (data for 1 h shown in Fig. 1).

Since HMG-CoA reductase is generally considered to be the rate-limiting enzyme in the sterol biosynthetic pathway, we determined the level of reductase activity in cell homogenates which had been treated with aphidicolin. As shown in Fig. 2, HMG-CoA reductase activity in cell homogenates is unaffected by incubation of the cells with aphidicolin at concentrations up to 43 μM. DNA synthesis, however, is strongly inhibited. Differences of two orders of magnitude have been reported in the sensitivity of polymerase α to aphidicolin in purified assay systems versus intact cells (2). We found that 0.14 μM aphidicolin inhibited [3H]thymidine incorporation by 50% in L cells, a value similar to those published for other eukaryotic cell lines (4, 23). A 61-fold higher concentration of aphidicolin (8.8 μM) is required to inhibit [14C]acetate incorporation into...
Aphidicolin Inhibits Sterol Synthesis

Fig. 2. Effect of aphidicolin on thymidine incorporation into DNA and on cellular HMG-CoA reductase activity. Culture preparation and aphidicolin treatment were similar to those described in Fig. 1. For measurement of DNA synthesis 0.4 μCi/ml of [3H]thymidine (New England Nuclear, 2 Ci/mmol) was added with aphidicolin to cells for 1 h. The procedure for determining DNA synthesis has been reported (16). The inset to the figure depicts the results with low concentrations of aphidicolin. Values represent mean ± S.E. for triplicate cultures.

Table I

Effect of aphidicolin on incorporation of [14C]mevalonolactone into sterols

Culture preparation and analysis of [14C]mevalonolactone incorporation into lipids were as described in Fig. 1. After 15 min of preincubation of cells with aphidicolin at the indicated concentrations, [14C]mevalonolactone (diluted to 2 μCi/ml) was added to the cell cultures at 2 μCi/ml, and incubation was continued for 1 h. Values represent mean ± S.E. for triplicate cultures.

<table>
<thead>
<tr>
<th>Aphidicolin (μM)</th>
<th>Nonsaponifiable lipids</th>
<th>Desmosterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40.1 ± 0.4</td>
<td>17.1 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>45.6 ± 2.3</td>
<td>14.3 ± 1.9</td>
</tr>
<tr>
<td>18</td>
<td>35.5 ± 7.2</td>
<td>10.3 ± 1.4</td>
</tr>
<tr>
<td>44</td>
<td>45.6 ± 1.5</td>
<td>6.7 ± 1.6</td>
</tr>
</tbody>
</table>

We next examined the possibility that aphidicolin might be inhibiting HMG-CoA reductase directly. Addition of aphidicolin to cell homogenates in the HMG-CoA reductase assay mixture had no significant effect. Specific activity of HMG-CoA reductase in picomoles per min per mg of protein was 100 ± 3 for controls and 120 ± 16 in the presence of 30 μM aphidicolin.

Since aphidicolin inhibits sterol synthesis but does not affect HMG-CoA reductase, we looked at its effect on the steps subsequent to mevalonate formation. When [14C]mevalonolactone was used as precursor, aphidicolin inhibited its incorporation into desmosterol in a concentration-dependent manner without affecting incorporation into total nonsaponifiable lipids (Table I). From these data we conclude that aphidicolin exerts its inhibitory effect on the cholesterol synthetic pathway at a site distal to mevalonate formation.

TLC Analysis of Nonsaponifiable 14C-Lipids—Analysis of nonsaponifiable lipids by thin layer chromatography was used initially to identify the step(s) in the cholesterol biosynthetic pathway where aphidicolin exerts its inhibitory action. As seen in Fig. 3A, after incubation of cells for 1 h in the presence of [14C]acetate, 47% of the radioactivity in total nonsaponifiable lipids co-migrates with desmosterol. During the 3-h chase period (Fig. 3B) conversion of 14C-labeled intermediate sterols into desmosterol occurred. When cells were preincubated for 2 h with 44 μM aphidicolin and labeled for 1 h in the presence of [14C]acetate plus aphidicolin, synthesis of desmosterol was reduced to 36% of the control, with a corresponding increase in 14C-sterols co-migrating with lanosterol (Fig. 3C). Continued incubation for 3 h in the absence of aphidicolin (Fig. 3D) resulted in conversion of these accumulated sterols into desmosterol, indicating a precursor-product relationship. In addition, these results show the inhibitory effect of aphidicolin on sterol synthesis to be rapidly reversible. When the chase was carried out in the presence of aphidicolin (Fig. 3E), there was some conversion to desmosterol, suggesting that the inhibition is not complete. During the chase period, less than 7% of the labeled nonsaponifiable lipid was excreted into the medium in the presence or absence of aphidicolin. Analysis of these lipids by TLC gave an even distribution of counts across the chromatogram (data not shown). In all experiments there was no effect of aphidicolin on the incorporation of [14C]acetate into total nonsaponifiable lipids.

HPLC Analysis—High pressure liquid chromatography was used to further characterize the 14C-sterol(s) accumulating in aphidicolin-treated cells. Cells were incubated with [14C]acetate for 1 h in the presence or absence of 44 μM aphidicolin. Nonsaponifiable lipids were extracted in the absence of carrier sterols and resuspended in methanol. HPLC analysis of the total 14C-labeled nonsaponifiable lipid fraction is shown in Fig. 4. Three peaks of radioactivity (I, II, and III) decreased in response to aphidicolin treatment, while a single peak (IV) increased. Peak I eluted with Δ5,7,24-cholesta trienol which was identified by its unique UV absorption profile (24). Peak II had a retention time identical to that of desmosterol. The relative retention time of Peak III is the same as that predicted for 4α-methyl-Δ8,24-cholestadienol (25), although no authentic standard was available for chromatographic analysis. Peak IV had a retention time identical to that of the lanosterol standard. From these data we conclude that treatment of cells with aphidicolin results in a decrease in [14C]acetate incorporation into desmosterol as well as two intermediates in the lanosterol → desmosterol pathway, 4α-methyl-Δ8,24-cholestadienol and Δ5,7,24-cholesta trienol. These decreases are accompanied by an increase in [14C]lanosterol, suggesting that aphidicolin exerts its inhibitory action on lanosterol metabolism at a site(s) prior to the formation of 4α-methyl-Δ8,24-cholestadienol.

Kinetics of Aphidicolin Inhibition of Sterol Synthesis—A time course experiment was carried out to determine how rapidly aphidicolin exerts its inhibitory effect. After incubation in the presence of aphidicolin for various lengths of time, cells were pulsed with [14C]acetate for 15 min or 1 h. 14C-Labeled nonsaponifiable lipids were analyzed by TLC. These results are summarized in Table II. Inhibition of [14C]acetate incorporation into desmosterol and concomitant accumulation of 14C-labeled lanosterol can be seen as early as 15 min after the addition of aphidicolin to cells.

When cells were pulsed with [14C]acetate for 1 h, the TLC profile of radioactivity was identical with that shown in Fig.
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FIG. 3. Effect of aphidicolin on TLC profile of nonsaponifiable [14C]-lipids. Cells were seeded (3.5 x 10⁶) in 100-mm Corning culture dishes and grown for 1 day. Aphidicolin in ethanol was added to a final concentration of 44 µM. Ethanol concentration was 0.5% in all dishes. Cells were harvested immediately or chased for 3 h at 37°C in 4 ml of fresh media with or without 44 µM aphidicolin. After the chase, media were removed, spun at 800 × g for 5 min, and the supernatant stored at −20°C prior to lipid extraction. Cells were rinsed twice with saline and harvested by addition of 1.6 N KOH and incubation at 37°C for 1 h. Cell slurries were stored under nitrogen at −20°C until analyzed. Saponification, lipid extraction, and thin layer chromatography were as described under "Materials and Methods." Arrows at top of figure indicate migration of sterol standards. D, desmosterol; L, lanosterol; S, squalene. A, pulse− aphidicolin; B, pulse−, chase−; C, pulse+; D, pulse+, chase−; E, pulse+, chase+.

Table II

Kinetics of aphidicolin action on incorporation of [14C]-acetate into sterols

A, cells (1.5 x 10⁶) were seeded in Corning culture dishes (60 mm), grown for 1 day, and treated with 39 µM aphidicolin for the times indicated. Ethanol concentration was 0.7% in all dishes. [14C]-Acetate (7.7 µCi/ml, 1 mCi/mmol) was added during the final 15 min of incubation. B, cells were seeded (3.5 x 10⁶) in 100-mm Corning culture dishes, grown for 1 day, and aphidicolin was added as a final concentration of 44 µM for the times indicated. [14C]-Acetate (5 µCi/ml, 0.5 mCi/mmol) was added during the final hour of incubation. Cells were harvested, extracted, and analyzed by thin layer chromatography as in Fig. 3. Total radioactivity in nonsaponifiable lipids was determined by summing all chromatogram fractions. Values represent the means of duplicate dishes.

<table>
<thead>
<tr>
<th>Ap tidicolin treatment</th>
<th>[14C]-Acetate labeling</th>
<th>Total nonsaponifiable lipids</th>
<th>Desmosterol</th>
<th>Lanosterol</th>
<th>Desmosterol/lanosterol</th>
<th>dpm/µg DNA</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0 min</td>
<td>15 min</td>
<td>54</td>
<td>12</td>
<td>6</td>
<td>2.00</td>
<td>0.39</td>
<td></td>
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<tr>
<td>15</td>
<td></td>
<td>33</td>
<td>9</td>
<td>41</td>
<td>0.23</td>
<td>0.40</td>
<td></td>
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<tr>
<td>30</td>
<td></td>
<td>85</td>
<td>6</td>
<td>57</td>
<td>0.14</td>
<td>0.23</td>
<td></td>
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<tr>
<td>45</td>
<td></td>
<td>77</td>
<td>4</td>
<td>58</td>
<td>0.08</td>
<td>0.39</td>
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<tr>
<td>B</td>
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<tr>
<td>0 h</td>
<td>1 h</td>
<td>128</td>
<td>38</td>
<td>16</td>
<td>2.48</td>
<td>0.40</td>
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<tr>
<td>1</td>
<td></td>
<td>153</td>
<td>8</td>
<td>61</td>
<td>0.13</td>
<td>0.40</td>
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<tr>
<td>2</td>
<td></td>
<td>186</td>
<td>15</td>
<td>39</td>
<td>0.39</td>
<td>0.40</td>
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</tr>
<tr>
<td>3</td>
<td></td>
<td>166</td>
<td>16</td>
<td>41</td>
<td>0.40</td>
<td>0.40</td>
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</tr>
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</table>
enty-two per cent of the 14C radioactivity was in lipids more polar than cholesterol. When cells were labeled for 15 min in the presence of aphidicolin, only 41% of the total nonsaponifiable radioactivity was associated with these more polar lipids, while the percentage of [14C]lanosterol rose from 6 to 41%. This suggests that these more polar lipids may be intermediates in the metabolism of lanosterol to dehydrosterol.

The marked increase in [14C]lanosterol in cells exposed to aphidicolin for only 15 min indicates a rapid inhibitory effect of aphidicolin on lanosterol metabolism.

Mechanism of Aphidicolin Inhibition of Lanosterol Metabolism—Incubation of 20,000 X g supernatants of mouse liver homogenates with [14C]mevalonolactone and increasing concentrations of aphidicolin resulted in a decrease in the incorporation of radioactivity into cholesterol and a corresponding increase in the level of [14C]lanosterol. Data from a representative experiment are shown in Fig. 5. Similar results were obtained in six separate experiments in which the concentration of aphidicolin required to inhibit cholesterol synthesis by 50% ranged from 40 to 80 μM. Aphidicolin thus seems to have a direct inhibitory effect on the in vitro conversion of lanosterol to cholesterol. The concentration of aphidicolin required to inhibit cholesterol synthesis by 50% in vitro is 5- to 7-fold higher than that required in cultured cells. Most probably this discrepancy is due to the complex nature of the enzyme systems involved in the synthesis of cholesterol from mevalonic acid and the difficulty in optimizing all of these reactions in an in vitro system.

The possibility that the oxysterol-binding protein described by Kandutsch et al. (20, 26) might play a role in the inhibition of lanosterol metabolism by aphidicolin was also examined. Affinity for oxysterol binding protein was measured by an assay of a compound to displace 7.5 S-bound [3H]25-hydroxycholesterol (20). While 25 μM 25-hydroxycholesterol could displace 95% of the 7.5 S-bound [3H]25-hydroxycholesterol, addition of 25 μM aphidicolin had no effect. This result is expected since the ability of oxysterols to compete for 7.5 S binding sites shows a correlation with their ability to suppress HMG-CoA reductase activity in cells (26), and aphidicolin treatment has no effect on HMG-CoA reductase activity (Fig. 2). Aphidicolin inhibition of cholesterol synthesis appears not to be mediated by the oxysterol-binding protein.

**Discussion**

In this study we have shown aphidicolin to be a rapid reversible inhibitor of sterol synthesis in cultured cells. Since aphidicolin is a fungal metabolite and its action in mammalian cells only mimics that of a proposed natural regulatory molecule, we feel that the discrepancy in the potency of aphidicolin as an inhibitor of DNA replication and sterol synthesis in L cells does not argue against our hypothesis that a natural regulatory molecule exists which can affect both of these processes.

There is evidence that some regulation of sterol synthesis occurs at the later steps of the cholesterogenic pathway where aphidicolin exerts its inhibitory effect, viz. conversion of lanosterol to C-27 sterols. Although HMG-CoA reductase is considered to be the major rate-limiting enzyme in the sterol biosynthetic pathway, lanosterol metabolism is also a natural regulatory step in a number of different tissues (reviewed in Ref. 27). In wool fat, the substrate from which lanosterol was first isolated, lanosterol and 24-dihydrolanosterol are the major sterol species (28). Lanosterol is also present in significant amounts in skin lipids (29), and its accumulation has been shown to be a characteristic of differentiating embryonic chick skin (30). In human serum lanosterol represents 0.1% of total sterols (28). A high level of lanosterol was found in a tumor of the mouse preputial gland, a sebaceous gland which synthesizes large amounts of lipid (31). Blood platelets and arterial tissue synthesize very low levels of cholesterol and are unable to convert lanosterol to cholesterol even under conditions where lanosterol synthesis is stimulated (32, 33). Rat kidney has been shown to incorporate [14C]mevalonate into lanosterol both in vivo and in vitro, but there is no significant conversion of lanosterol to cholesterol (34). There is also some evidence that lanosterol may serve as a direct precursor for bile acid synthesis in liver (35).

It has been proposed that lanosterol metabolism may play a role in the regulation of HMG-CoA reductase (12, 13). We have shown that inhibition of lanosterol metabolism by aphidicolin has no short term (up to 4 h) effect on HMG-CoA reductase activity (Fig. 2). This result argues against lanosterol *per se* acting as an inhibitor of reductase activity. If, as suggested by Gibbons et al. (12), it is one of the intermediates of lanosterol demethylation (e.g. 32-oxygenated lanostenols) which functions as the natural regulator of reductase activity, incubation of cells with aphidicolin should decrease the concentration of these lanosterol derivatives and result in an increase in reductase activity. We were unable to show such an increase. However, since aphidicolin blocks progression of the cell cycle by inhibiting DNA polymerase activity, long term treatment will result in the accumulation of cells at the G1/S interface. HMG-CoA reductase activity has been shown to fluctuate during the cell cycle in synchronized 3T3 cells (cited in Ref. 36) and baby hamster kidney cells (37) and decreases rapidly as the cells begin to enter S phase. The apparent lack of effect on reductase activity when unsynchronized cells are treated with aphidicolin may be the result of an increase in reductase activity due to depletion of oxylanostenols and a decrease in activity due to cells accumulating at the start of S phase.

Tissue culture and *in vivo* studies of many mammalian tissues and cells have shown that cholesterol is required for cellular proliferation (reviewed in Ref. 7). Although it is known that cholesterol is essential for plasma membrane synthesis, the exact mechanism whereby it influences cellular...
proliferation is unknown. Aphidicolin is a rapid specific inhibitor of replicative DNA synthesis which appears to act via reversible binding to DNA polymerase α (3). It has been proposed that the existence of aphidicolin-binding sites is physiologically significant and that there is a natural regulator of DNA synthesis which has structural similarities to aphidicolin (i.e., steroid-like) (1). We undertook this study to determine if an oxysterol, 25-hydroxycholesterol, a known inhibitor of sterol synthesis (7) could function as a direct regulator of DNA synthesis. Although 25-hydroxycholesterol does not inhibit polymerase α, our observation that aphidicolin is a rapid reversible inhibitor of lanosterol metabolism raises the possibility that there may be a certain kind of regulator of DNA synthesis which appears to act via steroid-like (1).

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