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THE METABOLISM OF CYCLOARTENOL, LANOSTEROL, 24-METHYLENECHOLESTEROL AND FUCOSTEROL IN *CHLORELLA ELLIPSOIDEA**

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Key Word Index—*Chlorella ellipsoidea*; Chlorophyceae; lanosterol, cycloartenol, 24-methylenecholesterol; fucosterol; sterol biosynthesis.

Abstract—Lanosterol and cycloartenol labelled with tritium at C-2, and 24-methylenecholesterol and fucosterol labelled with tritium at C-2 and C-4 were fed to actively growing cultures of *Chlorella ellipsoidea*. Lanosterol and cycloartenol were converted to each of the five desmethyl sterols of *C. ellipsoidea*. Lanosterol was more efficiently incorporated than cycloartenol.

Although there was some evidence for the reduction of the 24-methylene group, it was apparent that 24-methylenecholesterol was converted primarily to the C_{29} sterols, clionasterol and poriferasterol. Labelled fucosterol was reduced at the 24(28) double bond, producing clionasterol.

INTRODUCTION

As in most photosynthetic plants, it is cycloartenol rather than lanosterol that is postulated to be involved in the biosynthesis of phytosterols in *Chlorella* [1–3]. Lanosterol has never been isolated from these algae but sterols with the 9,19-cyclopropane ring have been isolated from several drug-treated cultures of *Chlorella* species [1–4]. Sterols such as lanosterol, cycloartenol, cycloeucalenol, 24-methylene-cycloartanol, 24-methylenelophenol and 24-ethylidenelophenol have been shown to be converted to poriferasterol in *Ochromonas malhamensis* [5, 6]. Cycloartenol and lanosterol have also been converted to 4-desmethylsterols in higher plants [7–11].

24-Methylenesterols have been converted to ergosterol in yeast [12–14] and 24-methylene and 24-ethylidene compounds appear to be intermediates of poriferasterol biosynthesis in *Ochromonas* [5, 6, 15]. However, in the green alga, *Trebouxia*, 24-methylenesterols were not substrates for 24-methylsterols and 24-ethylidenesterols were not precursors of C_{29} sterols [16]. Similar results were obtained with several species of *Chlorella* [17–19]. The reduction of non-specifically labeled fucosterol to clionasterol in *C. ellipsoidea* has been previously reported. It is apparent that the metabolism of $\Delta^{24(25)}$ and $\Delta^{24(28)}$ sterols differs from organism to organism. This study examines the metabolism of specifically labelled cycloartenol, lanosterol, 24-methylenecholesterol and fucosterol in *C. ellipsoidea*.

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RESULTS AND DISCUSSION

Incorporation of cycloartenol-[2-^{3}H] and lanosterol-[2-^{3}H].

The conversion of cycloartenol-[2-^{3}H] and lanosterol-[2-^{3}H] into all phytosterols of *C. ellipsoidea* is shown by the specific activities of the sterols (Table 1). Cycloartenol and 24-methylenecycloartanol have been isolated from *C. ellipsoidea* [24] and cycloartenol is presumed to be the first sterol formed. Although lanosterol has never been isolated in *Chlorella* or any other algae, the utilization of exogenous lanosterol has been demonstrated in several green higher plants and *Ochromonas malhamensis* [5, 8–10, 15] in which cycloartenol has been

Table 1. Specific activity (dpm/μg) of sterols isolated from *Chlorella ellipsoidea* after feeding with labelled sterols

Sterols isolated	Specific and total radioactivity of sterols supplied to culture* Cycloartenol 5.2×10^4 dpm/μg (9×10^7 dpm)	Lanosterol 4.3×10^4 dpm/μg (7×10^7 dpm)	24-Methylene-cholesterol 5.2×10^4 dpm/μg (3×10^7 dpm)	Fucosterol 1.8×10^4 dpm/μg (4×10^6 dpm)
Cholesterol	14	14	5	<1
Brassicasterol	20	42	8	—
Ergost-5-enol	17	45	12	<1
Poriferasterol	16	37	114	<1
Clionasterol	15	43	229	21

* Plant dry weights obtained in the cycloartenol, lanosterol, 24-methylenecholesterol, and fucosterol experiments were 7g, 6g, 5g, and 10g respectively. All data represents an average of two expts. Agreement between expts was excellent.

shown to be the first cyclic precursor of sterol biosynthesis.

Approximately 0.5% of the labelled cycloartenol and 1.5% of labelled lanosterol was incorporated into 4-desmethylsterols of the algae. The higher incorporation of lanosterol compared to cycloartenol found in this study has not been reported in similar studies with other photosynthetic plants. Most fungi synthesize ergosterol as a major sterol via the lanosterol pathway. It appears that most species of *Chlorella* synthesize ergosterol as a major sterol via the cycloartenol pathway [3, 4, 25]. Although *C. ellipsoidea* does not synthesize ergosterol as a major sterol, it is of interest that it utilizes the cycloartenol pathway, but prefers lanosterol to cycloartenol as a substrate. This may be a clue to the evolution of the sterol biosynthetic pathway in thallophytes.

Incorporation of 24-*methylenecholesterol*-[2,4-^{3}H]

24-Methylenecholesterol was efficiently converted into clionasterol and poriferasterol with a little radioactivity found in ergost-5-enol and brassicasterol. The results reveal that the reduction of the 24-methylene side chain is not a major pathway in the formation of C_{28} sterols (ergost-5-enol and brassicasterol) of *C. ellipsoidea*. However, 24-methylene sterols are major substrates in the synthesis of C_{29} sterols of this alga. The small amount of radioactivity retained in both ergost-5-enol and brassicasterol suggests the possibility that formation of these two sterols may occur to a small extent from the reduction of 24-methylenesterols. (Purification of cholesterol and brassicasterol was extremely difficult due to the small amounts of these sterols present).

Incorporation of fucosterol-[2,4-^{3}H]

The alga converted fucosterol only into clionasterol confirming previous work with non-specifically labelled fucosterol [20]. In [CD_3]-methionine experiments using this alga [17], five deuterium atoms were always found in poriferasterol indicating that 24-ethylidene compounds are not precursors of poriferasterol. In addition, there have been no 24-ethylidenesterols isolated from *Chlorella*, even from drug-inhibited cultures [24]. However, in this study, the conversion of a 24-ethylidenesterol into clionasterol reveals that *C. ellipsoidea* contains a $\Delta^{24(28)}$ reductase although this specific substrate is probably never available to the organism. It is interesting to note, that although poriferasterol is the major sterol of *C. ellipsoidea*, it was completely unlabelled in this experiment. Isofucosterol is much more readily converted to poriferasterol than fucosterol in *Ochromonas malhamensis* [15]. Similarly, in the protozoan, *Tetrahymena pyriformis*, 28-isofucosterol is transformed into stigmasta 5,7,22,*Z*-24(28)-tetraen-3β-ol, whereas fucosterol is converted only into stigmasta-5,7,*E*-24(28)-trien-3β-ol [26]. These data indicate that the orientation of the C-29 methyl group is of fundamental importance with respect to the introduction of the 22-double bond. From this study and previous work [20], clionasterol as well as 24-ethylidenesterols have been eliminated as precursors of poriferasterol. Tomita [19] suggests that a $\Delta^{24(25)}$ sterol is an intermediate in poriferasterol biosynthesis, but recent isolations of Δ^{25} sterols from other *Chlorella* species [3, 4] lead us to believe that a $\Delta^{25(26)}$ compound may be a precursor in poriferasterol biosynthesis. The efficient reduction of the 24(28) double bond of fucosterol where C-29 is almost certainly hindering the reaction, is perhaps confusing in comparison to the insignificant reduction of the more accessible 24(28) double bond of 24-methylenecholesterol. Since 24-methylenecholesterol can serve as a substrate for alkylation or reduction of the side chain, an extremely efficient alkylation system could explain our results. Work on this aspect of the problem is in progress.

EXPERIMENTAL

Cycloartenol was isolated and purified from the sterol fraction of the fern *Dennstaedtia punctilobula* Moore. Lanosterol was obtained and purified from commercial products. Sterols were labelled with tritium at the C-2 position by the method of Thompson and Klein [21]. Purity of the labelled sterols was established by GLC, TLC and IR. Fucosterol-[2,4-^{3}H] and 24-methylenecholesterol-[2,4-^{3}H] were kind gifts from M. Thompson. The desired amount of labelled sterol was dissolved in 0.1–0.2 ml of 95% EtOH and added to a growing culture of *C. ellipsoidea*. (Indiana Culture Collection No. 247). Cells were grown autotrophically in the presence of labelled sterol in sterile inorganic medium for 6–8 days. CO_2-in-air (1%) was supplied to the cultures as a carbon source. Cells were harvested by centrifugation and sterols were extracted from freeze-dried cells with $CHCl_3$–MeOH (2:1). In the cycloartenol-[2-^{3}H] and lanosterol-[2-^{3}H] labelling experiments, 4-desmethylsterols (cholesterol, 1.2%; brassicasterol, 0.8%; ergost-5-enol, 31%; poriferasterol, 61%; and clionasterol, 6%) were isolated by thin layer chromatography using Si gel HF 254 + 366. Development of the TLC plate was achieved with the solvent system C_6H_6–EtAc (9:1). 4-Desmethyl sterols were further separated and purified as steryl acetates by Anasil B column chromatography and lipophilic Sephadex column chromatography [22]. In Fucosterol-[2,4-^{3}H] and 24-methylenecholesterol-[2,4-^{3}H] expts, the extracted sterols were partially purified by digitonin precipitation [3] and acetylated. Unconverted fucosterol and 24-methylenecholesterol were separated as acetates from the sterols of *C. ellipsoidea* by column chromatography on 12% $AgNO_3$-impregnated Anasil B [23]. Further separation and purification of each of the *C. ellipsoidea* sterols were achieved by column chromatography on Anasil B and lipophilic Sephadex [22].

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