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METABOLISM OF 24-DIHYDROLANOSTEROL IN *OCHROMONAS MALHAMENSIS* AND *CHLORELLA ELLIPSOIDEA**

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Abstract—24-Dihydrolanosterol-[2-^{3}H] was converted to cholesterol in *Chlorella ellipsoidea* but ergost-5-enol, poriferasterol, and clionasterol were not labelled. The absence of the necessary 24(25) double bond precursor eliminates the possibility of C_{28} and C_{29} sterol synthesis. However, it was confirmed that 24-dihydrolanosterol was metabolized by *Ochromonas malhamensis* to give cholesterol, brassicasterol, and poriferasterol.

INTRODUCTION

Since Nes and coworkers first proposed the double alkylation mechanism for the synthesis of the plant sterol side chain [1], a $\Delta^{24(25)}$ sterol has been considered to be the substrate for the first side chain alkylation. In pea seed homogenates, Russell demonstrated that sterols were alkylated with methyl ^{14}C-labelled methionine only when the side chain contained the 24(25) double bond [2]. However, cholesterol has been reported to be converted to C_{24} alkyl sterols in *Wistaria* [3] and in tobacco [4], and recently the conversion of 24-dihydrolanosterol and cholesterol to poriferasterol has been demonstrated in *Ochromonas* [5]. We decided to repeat the experiment using 24-dihydrolanosterol in *Ochromonas* and compare the result with that obtained with *Chlorella ellipsoidea*, which also synthesizes cholesterol and poriferasterol as well as other sterols [6].

RESULTS AND DISCUSSION

In *C. ellipsoidea*, 24-dihydrolanosterol was converted only into cholesterol (Table 1). Neither the C_{28} nor C_{29} sterols of the alga were labelled.

Table 1. Incubation of *C. ellipsoidea* with 24-dihydrolanosterol-[2-^{3}H]

	Sterol wt (μg)	Radioactivity (dpm)	Sp act (dpm/μg)
24-Dihydrolanosterol added	2221	$4 \cdot 4 \times 10^7$	19810
	(3251)*	($6 \cdot 48 \times 10^7$)	(19932)
4,4-Dimethylsterols	—	$7 \cdot 3 \times 10^6$	—
	—	($9 \cdot 3 \times 10^6$)	—
4α-Methylsterols	—	$2 \cdot 4 \times 10^5$	—
	—	($0 \cdot 8 \times 10^5$)	—
4-Desmethylsterols†	22000	$1 \cdot 4 \times 10^5$	6
	(35700)	($2 \cdot 8 \times 10^5$)	(8)
Cholesterol	20	5733	286
	(23)	(7631)	(332)
Brassicasterol	15	40	3
	(25)	(0)	(0)
Ergost-5-enol	270	111	1
	(2375)	(51)	(< 1)
Poriferasterol	519	15	< 1
	(2640)	(66)	(< 1)
Clionasterol	15	0	0
	(289)	(57)	(< 1)

* Values in parentheses are from a second experiment Total cell dry wt Expt 1, 6·2 g, Expt 2, 13·6 g

† Incorporation into 4-desmethylsterols, Expt 1, 0·3%, Expt 2, 0·4%

Since lanosterol and cycloartenol have been converted into all the sterols of this alga [7], *C. ellipsoidea* is apparently not able to alkylate the sterol side chain in the absence of Δ^{24} bond. In addition, these results also eliminate the possibility

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Table 2 Incubation of *O malhamensis* with 24-dihydrolanosterol-[2-^{3}H]

	Sterol wt (μg)	Radioactivity (dpm)	Sp act (dpm/μg)
24-Dihydrolanosterol added	6580	$1\,46 \times 10^8$	22245
Total sterol isolated	12500	$7\,45 \times 10^7$	5962
from cells*	–	$1\,69 \times 10^7$	
4,4-Di- and 4-mono-methylsterols	-	$2\,37 \times 10^7$	–
4-Desmethylsterols†	12400	$2\,33 \times 10^6$	188
Cholesterol	18 5	$5\,92 \times 10^4$	3199
Brassicasterol	185	$2\,59 \times 10^4$	140
Poriferasterol	12300	$3{\cdot}19 \times 10^5$	26

* Total cell dry wt 1 78 g

† Incorporation into 4-desmethylsterols 1 6%

that the alga can introduce unsaturation at C_{24} for further alkylation.

The conversion of labelled 24-dihydrolanosterol into cholesterol and poriferasterol (Table 2) confirms the earlier work of Beastall *et al* [5] who found that cholesterol and poriferasterol contained about the same total radioactivity In our experiment cholesterol had a much higher specific activity but poriferasterol contained over 50% of the total radioactivity of the desmethylsterol fraction and brassicasterol was also labelled From this work and that of Beastall *et al.* [5] we know that in *O malhamensis* cholesterol is converted to poriferasterol and that 24-dihydrolanosterol is converted to cholesterol, brassicasterol, and poriferasterol. If brassicasterol and other C_{28} sterols were also convertible to poriferasterol in this organism, it would appear that this unusual biochemical capability could account for the fact that *O malhamensis* contains poriferasterol as 98% of its total sterol, while *C. ellipsoidea* and *O danica* contain poriferasterol accompanied by larger relative amounts of other sterols The mechanism by which *O. malhamensis* can convert a saturated C_8 side chain to a C_9 or a C_{10} side chain has not been established, but it seems possible that *Ochromonas* has the ability to introduce the important 24(25) double bond while *Chlorella* does not

EXPERIMENTAL

24-Dihydrolanosterol was isolated and purified from commercial lanosterol It was labelled with tritium at the C_2 position by the method of Thompson and Klein [8] and purity was established by GLC TLC and IR

Labelled sterol was dissolved in 0 1–0 2 ml of 85% EtOH and added to a 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 the carbon source

Ochromonas malhamensis Pringsheim, American type culture collection no 11532, was grown in 500 ml Erlenmeyer flasks containing 250 ml of medium The medium was modified from that of Aaronson and Baker [9] The following components were added to 750 ml of dist H_2O and autoclaved, 3 0 g L-glutamic acid, 0 3 g nitrilotriacetic acid, 0 5 g $MgCO_3$, 0 15 g $CaCO_3$, 0 3 g KH_2PO_4, 0 25 g $MgSO_4$ 0 4 g NH_4Cl, 5 0 g glucose, and 1 ml of a metals mixture The metals mixture contained EDTA-chelated Mn, Ca, Co, Cu and Zn at 1 ppm when made to the final dilution of 1 liter and 5 ppm Fe as the EDTA NaFe and 1 ppm Mo as MoO_3 The following components were added to 250 ml dist H_2O 1 2 g $(NH_4)_2H$ citrate, 0 5 g L-arginine HCl, 0 5 g L-histidine HCl 0 6 g DL-methionine, 2 0 mg thiamine HCl, 4 0 mg biotin and 1 0 mg vitamin B_{12} This soln was sterilized by filtration through a 0 45 μm Millipore filter, then added to the cooled, autoclaved mixture, bringing the final vol to 1 liter The flasks were inoculated with either 5 or 10 ml of inoculum from a fully grown 5-day-old culture The cultures were grown at 26 ± 3 with 3000–5000 lx provided by fluorescent lights The cultures were shaken at least once daily to break up and resuspend any large cell aggregations Labelled sterol was added during the 3rd day after inoculation and the cultures were harvested on the 7th day

Cells of both species were harvested by centrifugation and sterols were extracted from freeze-dried cells with $CHCl_3$ MeOH (2 1)

After saponification the total sterols were separated by TLC [6] into 4,4-dimethylsterols (including unconverted 24-dihydrolanosterol) 4α-methylsterols and 4-desmethylsterols (*C ellipsoidea* sterols cholesterol 1 2%, brassicasterol, 0 8%, ergost-5-enol, 31%, poriferasterol, 61%, and clionasterol, 6% *O malhamensis* sterols cholesterol, <1%, brassicasterol, 1%, and poriferasterol, 98%) The 4-desmethylsterols were further separated and purified as sterol acetates by Anasil B column chromatography and lipophilic Sephadex column chromatography [7] Radioactivity was determined by scintillation counting Quantitation and identifications of sterols in all experiments were made by GLC on a 3% SE-20 column

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