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Biosynthesis of Sterols in the Red Alga, *Porphyridium cruentum*

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Abstract

The biosynthesis of sterols in the red alga, *Porphyridium cruentum*, was investigated. The incubation of resting and growing cells with acetate-1-^{14}C gave the radioactive squalene and sterols. The results indicate that *P. cruentum* is capable of synthesizing sterols from acetate as well as in other plants. The sterols isolated from the resting cells revealed radioactivity only in 22-dehydrocholesterol. In the case of the sterols isolated from the growing cells, radioactivity was present at least in 22-dehydrocholesterol and cholesterol fractions. These results showed that *P. cruentum* synthesizes 22-dehydrocholesterol and cholesterol from acetate.

It is well known that algae contain complex sterol mixtures as compared with higher plants. Advance in analytical techniques for sterols has facilitated the investigation of detail compositions of sterols occurring in algae. The distribution of sterols in algae has been extensively reviewed by PATTERSON [1]. Regarding red algae, the most reasonable conclusion to be drawn from available data is that a number of red algae contain cholesterol and other C_{27}-sterols as a prominent sterol. Literatures have also shown that sterol composition of red algae differs considerably from species to species. Moreover, IDLER *et al.*[2] have demonstrated that a considerable seasonal variation was observed in the sterol composition of some red algae. On the other hand, there are little informations about the origin of sterols in red algae up to the present; it is obscure whether sterols naturally occurring in red algae are synthesized by themselves or derived from exogenous sources.

In a previous study[3], the authors have reported that the red algae, *Meristotheca papulosa*, *Gracilaria textorii*, and *Porphyridium cruentum* which was grown on a chemically defined medium (sterol-free), contained sterols in their cells; with suggestion that *P. cruentum* may be capable of synthesizing sterols from lower units. In the case of *P. cruentum*, the isolated sterols have been composed of 22-dehydrocholesterol (60%), cholesterol (5%), desmosterol (20%), ergosterol (5%), and C_{29}-sterol (10%). On the contrary, AARONSON and BARKER [4] have reported that *P. cruentum*, which was cultured on a chemically defined medium, contained no sterol in the cells. However, it has been obscure whether the discrepancy between the above two investigations is due to any reason. Hence, in the present study, the authors intended to obtain evidence that sterols occurring in *P. cruentum* are formed from lower units. This paper deals with the biosynthesis of sterols from acetate-1-^{14}C in the red alga, *P. cruentum*.

Materials and Methods

The culture method for the red alga, *P. cruentum* was essentially the same as reported previously [3]. The alga was aseptically cultivated in seven 1L-Erlenmeyer flasks containing

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700 ml of the ASP-medium [5] (chemically defined medium) with aeration (flow rate of air : 300 ml/min) under the white fluorescent lamps at 20–22°C. After 10 days, 14.3 μCi of acetate-1-^{14}C (57.0 mCi/m mol) was added to each cultivating flask and *P. cruentum* was cultivated for successive 3 days. After cultivation, the cells were harvested by centrifugation (3000 rpm, 10 min) and stored in a freezer at −20°C until use. In another experiment, the biosynthesis of sterols in *P. cruentum* was investigated by use of the resting cells. The resting cells (52 g wet weight), which were obtained by the same manner as described above, were transferred into two 300ml-flasks containing 30 ml of phosphate buffer (pH 7.2) and 125 μCi of acetate-1-^{14}C and then incubated at 20°C for 12 hours.

From the cells of *P. cruentum* administrated in acetate-1-^{14}C, the lipids were extracted and the incorporation of acetate-1-^{14}C was investigated. Unsaponifiable matters and sterols were isolated according to the same methods as mentioned in the previous paper [3]. Thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and column chromatography on alumina (grade II) and on silver nitrate-impregnated silicic acid were conducted essentially by similar manners as described previously [3,6]. Radioactivity was measured with a Beckman liquid scintillation counter LS-150 using a toluene solution of PPO (0.6%) and POPOP (0.02%) as a scintillator. Radioautogram was obtained by covering a thin-layer plate with X-ray film (SAKURA X-ray film ; Konishiroku Photo Ind. Co., Japan) followed by exposure for 2 weeks.

Results

After administration of acetate-1-^{14}C, the unsaponifiable matters and sterols were isolated from the cells of *P. cruentum*. The radioactivity recovered in the unsaponifiable matter and sterol fractions is shown in Table 1. An aliquot of radioactive unsaponifiable matters was

Table 1. Incorporation of acetate-1-^{14}C into the unsaponifiable matter and sterol fractions by the growing and resting cells of *P. cruentum*.

Lipid fraction		Incorporation of acetate-^{14}C	
		Resting cells*	Growing cells**
Fresh weight of cells (g)		52	22
Unsaponifiable matters	Weight (mg)	324.1	115.0
	%***	0.60	0.52
	dpm	33,100,000	2,340,000
	dpm/mg	102,000	20,300
Pure sterols	Weight (mg)	14.6	2.4
	%***	0.028	0.011
	dpm	1,310,000	203,000
	dpm/mg	89,700	84,600

* In total, 52 g of the resting cells was incubated with 250 μCi of acetate-1-^{14}C in phosphate buffer (pH 7.2) at 20° C for 12 hours.

** In total, 22 g of the cells was obtained from the culture of *P. cruentum* with acetate-1-^{14}C.

*** Per fresh weight of cells

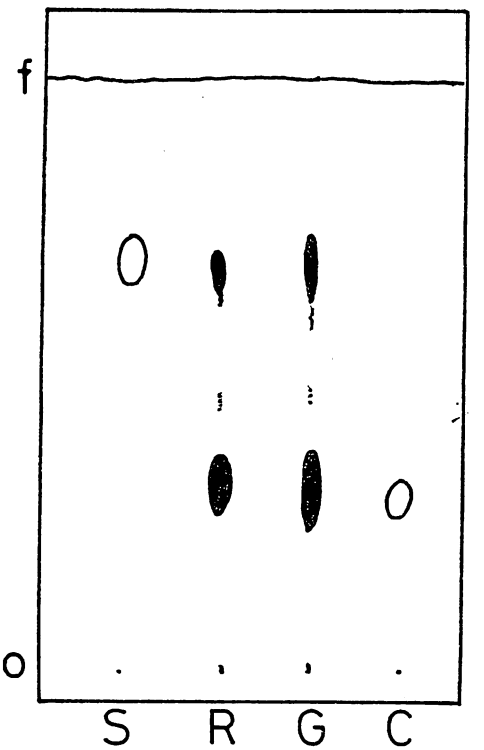

Fig. 1. Radioautography of the unsaponifiable matters isolated from the resting and growing cells of *P. cruentum* incubated with acetate-1-^{14}C.
S and C indicate authentic squalene and cholesterol, respectively. R and G show the unsaponifiable matters isolated from the resting and growing cells, respectively.

subjected to TLC on Kieselgel G with benzene-ethyl acetate (4:1) and then radioautographed. As shown in Fig. 1, the radioautogram showed the presence of radioactive squalene and sterols. The radioactive crude sterols, which were obtained from the unsaponifiable matters by column chromatography on alumina with hexane-benzene, were further purified by recrystallization from methanol. The pure sterols so obtained were subjected to GLC on 1.5% OV-17 and TLC followed by radioautography. In TLC, a significant radioactivity was found to be present in the purified sterols. The results of GLC analyses of the sterols isolated from the growing and resting cells of *P. cruentum* are given in Table 2. The percentage compositions of sterols obtained from the two samples of *P. cruentum* differed from one another, and also differed considerably from that of another sample which was analyzed in the previous study [3]. The variation in the sterol composition of *P. cruentum* was assumed to be due to the discrepancy in physiological conditions of the cells.

In order to clarify the incorporation of acetate-1-^{14}C into the individual sterol components, the sterols were acetylated with acetic anhydride-dry pyridine and then the steryl acetates were subjected to TLC on a silver nitrate-impregnated Kieselgel G with hexane-benzene (5:3). In the case of the steryl acetate mixture from the resting cells, the radioactive spot was detected

Table 2. Percentage composition of the sterols isolated from the red alga, *P. cruentum*.

Sterols	% Composition of sterol		
	Sample of *P. cruentum*		
	Growing cells	Resting cells	KANAZAWA *et al.* (1972)[3]
Unknown	—	trace	<1
22-Dehydrocholesterol	95	39	60
Cholesterol	4	7	5
Brassicasterol	trace	1	—
Desmosterol	1	24	20
Ergosterol	trace	6	5
C_{29}-sterol	—	23	10
Unknown	—	—	<1

only in the position corresponding to 22-dehydrocholesteryl acetate. The zone corresponding to 22-dehydrocholesteryl acetate was scraped off from the plate and the steryl acetate was isolated. To the presumed 22-dehydrocholesteryl acetate-^{14}C, about 10mg of authentic 22-dehydrocholesteryl acetate was added and then recrystallized repeatedly from several solvents. As shown in Table 3, the crystals of steryl acetate gave a constant specific activity in the last three crystallizations.

Table 3. Recrystallization of 22-dehydrocholesteryl acetate-^{14}C isolated from the resting cells incubated with acetate-1-^{14}C.

Crystallization	Solvent system	Specific activity (dpm/mg)
First	Methanol	35,100
Second	Methanol	41,000
Third	Acetone-water	39,700
Fourth	Ethanol	39,200

On the other hand, the radioautogram of steryl acetate mixture from the growing cells showed the presence of definite spots corresponding to cholesteryl acetate and 22-dehydrocholesteryl acetate, and of a dim spot corresponding to ergosteryl acetate. Hence, the radioactive steryl acetate mixture was further analyzed by column chromatography on a silver nitrate-impregnated silicic acid. To an aliquot of the radioactive steryl acetate mixture, about each 3-7 mg of authentic 22-dehydrocholesteryl acetate, cholesteryl acetate, and ergosteryl acetate was added and subjected to column chromatography. The results are given in Fig. 2. The radiochromatogram showed the presence of radioactivity in the fractions corresponding to cholesteryl acetate and 22-dehydrocholesteryl acetate. However, no significant radioactivity could be demonstrated in the fraction corresponding to ergosteryl acetate.

From the above data, it was concluded that the red alga, *P. cruentum*, is capable of synthesizing 22-dehydrocholesterol and probably cholesterol from acetate.

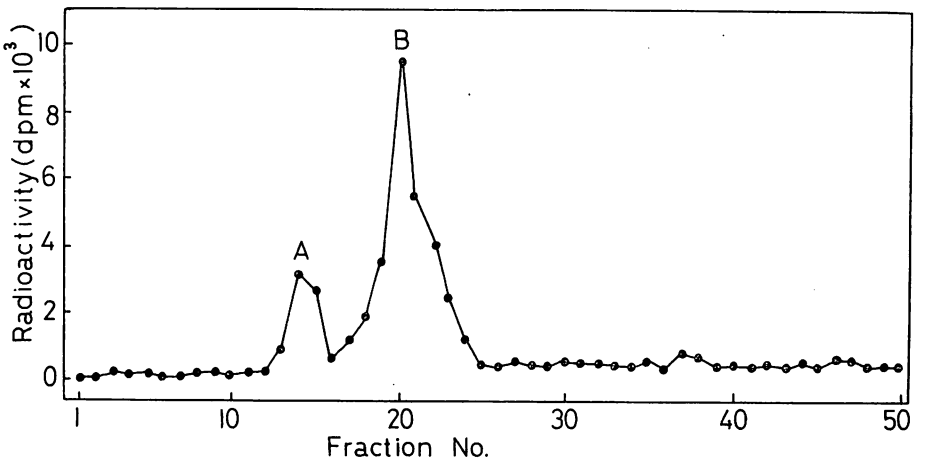

Fig. 2. Chromatography on a silver nitrate-impregnated silicic acid of the steryl acetates isolated from the growing cells of *P. cruentum* incubated with acetate-1-^{14}C.

The column packed with 12.5g of silicic acid-silver nitrate (4 : 1, w/w) was eluted with the following solvents : 150ml hexane, 150ml hexane-benzene (85 : 15), 150ml hexane-benzene (80 : 20), 150ml hexane-benzene (75 : 25), 150ml hexnae-benzene (70:30), 150ml hexane-benzene (65 : 35), 150ml hexane-benzene (60 : 40), 150ml hexane-benzene (55 : 45), 50ml hexane-benzene (40 : 60). Each fraction (25ml) was collected and monitored by GLC. The radioactive peaks A and B were corresponded to cholesteryl acetate and 22-dehydrocholesteryl acetate, respectively. In the fraction corresponded to ergosteryl acetate (fraction No. 37-43), significant radioactivity were not detected.

Discussion

Generally, plants have been recognized to synthesize their sterol from lower units. As an exceptional case, blue-green algae have been long accepted to lack sterols in the cells [7-10]. Recent investigations have shown that the several species of blue-green algae grown on chemically defined media contained sterols [11,12], and also that a species of blue-green alga, *Anabaena cylindrica*, was capable of synthesizing sterols from acetate [13]. Accordingly, most algae have been found to contain sterols in their cells. In the red algae, the earlier studies have revealed that C_{27}-sterols such as cholesterol [2,14-20], 22-dehydrocholesterol [2,18,21], and/or desmosterol [2,18-20] were present as the major sterols and small amounts of C_{28}- and C_{29}-sterols occasionally occurred in some species. However, little is known about the origin of these sterols. KANAZAWA *et al.* [3] have demonstrated that the red alga, *P. cruentum*, grown on a chemically defined medium (the ASP-6 medium [5]) contained 22-dehydrocholesterol, cholesterol, desmosterol, ergosterol, C_{29}-sterols, and unknown sterols. In the present study, it was proved that *P. cruentum* is capable of synthesizing at least 22-dehydrocholesterol and cholesterol from acetate. The results may suggest that in red algae the C_{27}-sterols occurring as the major sterols are probably formed from lower units as well as in other plants.

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