腔腸動物および棘皮動物のステロール生合成能

誌名	日本水産學會誌
ISSN	00215392
著者	金沢, 昭夫 手島, 新一 富田, 茂夫
巻/号	40巻12号
掲載ページ	p. 1257-1262
発行年月	1974年12月

Bulletin of the Japanese Society of Scientific Fisheries 40(12) 1257–1260 (1974)

Sterol Biosynthesis in Some Coelenterates and Echinoderms

Akio KANAZAWA*, Shin-ichi TESHIMA*, and Shigeo TOMITA*

(Received August 21, 1974)

This study deals with the biosynthesis of sterols from mevalonate-2-^{14}C in the coelenterates, *Sarcophyta* sp., *Stereonephthya japonica, Acalycigorgia inermis, Ellisella rubra, Dofleinia armata,* and *Parasicyonis actinostoloides,* and the echinoderms, *Leiaster leachii, Coronaster valsellatus, Protoreaster nodosus, Henricia ohshimai, Clypeaster japonicus,* and *Holothuria leucospilota.* After injection of mevalonate-2-^{14}C into the animals, the sterol-synthesizing ability was evaluated by radioautography in conjunction with the isolation of radioactive sterols.

It was shown that all echinoderms and the four coelenterates, *Sarcophyta* sp., *S. japonica, D. armata,* and *P. actinostoloides,* are more or less capable of synthesizing sterols from mevalonate, but the two species of coelenterates, *A. inermis* and *E. rubra,* lack this ability.

Recently, the sterol constituents of a variety of marine invertebrates have been reinvestigated in detail by using modern techniques such as gas-liquid chromatography (GLC), argentation chromatography, mass spectrometry, and nuclear magnetic resonance spectrometry. AUSTIN[1] has reviewed the recent publications about the sterols of marine invertebrates. On the other hand, several workers have investigated the sterol biosynthesis in the echinoderms[2], mollusks[3,4], crustaceans[5], annelids[6–8], porifera[8], and coelenterates[8–10]. Generally, sterols are conceived to be synthesized from acetate in animals and plants. However, some marine animals appear to be incapable of synthesizing sterols from lower units. The lack of sterol synthesis in some marine invertebrates is of interest in the viewpoint of both chemotaxonomy and animal nutrition. But, the knowledge on the sterol-synthesizing ability in marine invertebrates, especially in coelenterates, is still relatively poor.

Previously, the authors have elucidated the sterol constituents of some echinoderms[11] and coelenterates[12]. The purpose of the present study is to obtain further information about the sterol biosynthesis in the above two phyla of animals. This paper deals with the sterol-synthesizing ability from mevalonate-2-^{14}C in the coelenterates and echinoderms.

Materials and Methods

Animals and injection of mevalonate-2-^{14}C All specimens of the coelenterates, *Sarcophyta* sp., *Stereonephthya japonica, Acalycigorgia inermis, Ellisella rubra, Dofleinia armata,* and *Parasicyonis actinostoloides,* and the echinoderms, *Leiaster leachii, Coronaster valsellatus, Protoreaster nodosus, Henricia ohshimai, Clypeaster japonicus,* and *Holothuria*

* Laboratory of Fisheries Chemistry, Faculty of Fisheries, University of Kagoshima; 4–50–20 Shimoarata, Kagoshima, Japan. (金沢昭夫・手島新一・富田茂夫: 鹿児島大学水産学部)

leucospilota, were collected near Sakurajima, Kagoshima, Japan, during the late autumn-early winter. These animals were injected with mevalonate-2-^{14}C (50 mCi/m mole; Radiochemical Centre, Amersham, England) and kept in a circulatory aquarium. Table 1 shows the taxonomy of tested animals, the dosage of mevalonate-2-^{14}C, and the keeping period after injection of the radioactive precursor.

Table 1. The taxonomy of tested animals, the dosage of mevalonate-2-^{14}C, and keeping period after injection of radioactive precursor

Animals tested	Mevalonate-2-^{14}C injected (μCi)	Keeping period after injection (days)
Phylum Coelenterata		
Class Anthozoa		
Order Alcyonaria : *Sarcophyta* sp. (Umikinoko)*	2	1
Stereonephthya japonica (Kipanatosaka)	2	1
Order Gorgonaria : *Acalycigorgia inermis* (Togenashiyagi)	2	10
Ellisella rubra (Muchiyagi)	2	10
Order Actiniaria : *Dofleinia armata* (Sunaisoginchaku)	2	10
Parasicyonis actinostoloides (Sangoisoginchaku)	2	10
Phylum Echinodermata		
Class Asteroidea : *Leiaster leachii* (Ooakahitode)	2	7
Coronaster valsellatus (Kanmurihitode)	2	7
Protoreaster nodosus (Kobuhitode)	2	7
Henricia ohshimai (Ooshimahimehitode)	2	7
Class Echinoidea : *Clypeaster japonicus* (Takonomakura)	2	7
Class Holothurioidea : *Holothuria leucospilota* (Nisekuronamako)	2	7

* Japanese name.

Isolation of sterol and hydrocarbon fractions The lipids and unsaponifiable matters were obtained from the animals injected with mevalonate-2-^{14}C by the essentially same method as described previously[13]. The hydrocarbon and sterol fractions were isolated from the unsaponifiable matters by column chromatography on alumina (Grade II–III, Merck) with hexane, hexane-benzene, benzene, and benzene-ethyl acetate[13].

Check of radioactivity Radioactivity was checked by scintillation counting and radioautography. In scintillation counting, radioactivity was measured with a Beckman Liquid Scintillation Counter LS–203 by using a toluene solution of 2,5-diphenyloxazole

(0.6%) as a scintillator. An aliquot of the radioactive unsaponifiable matters was subjected to thin-layer chromatography (TLC) on Silicagel G with benzene-ethyl acetate (4: 1, v/v). After completion of TLC, the radioautogram was obtained by covering the thin-layer plate with a X-ray film (Konishiroku Photo Co., Japan) for three weeks.

Results

Table 2 shows the yields of the lipids, unsaponifiable matters, and sterols from the animals examined. The lipid content varied considerably from species to species in the coelenterates and echinoderms. Also, the percentage of the unsaponifiable matters and sterols to lipids differed from each other among the animals. The percentage of the sterols to lipids ranged 0.8–20% and 6.0–15% in the coelenterates and echinoderms, respectively. Table 3 gives the results of the incorporation of mevalonate-2-^{14}C into the lipid fractions in the coelenterates and echinoderms after the injection of mevalonate-2-^{14}C.

The incorporation of mevalonate-2-^{14}C into the lipids was high in the animals such as *Sarcophyta* sp., *D. armata, C. japonicus*, and *H. leucospilota*, but extremely low in *S. japonica* and *H. ohshimai*. It is obscure whether the poor incorporation of mevalonate depended on the slow rate of lipid metabolism in them, the inefficient injection method of precursor, or the other reasons. Irrespective of the animal species tested, the unsaponifiable matters isolated gave a significant radioactivity, suggesting that all the animals are capable of forming some unsaponifiable matters from mevalonate. The hydrocarbon

Table 2. The yields of the lipid fractions isolated from the coelenterates and echinoderms injected with mevalonate-2-^{14}C

Species	Fresh weight	Total lipids		Unsaponifiable matters		Sterols	
	g	mg	(%)*1	mg	(%)*2	mg	(%)*2
Coelenterates							
Sarcophyta sp.	141.0	713	0.51	421	59.0	6	0.8
S. japonica	20.0	111	0.56	65	58.5	12	11
A. inermis	84.0	107	0.13	51	47.7	21	20
E. rubra	65.0	1,110	1.71	831	74.9	58	5.2
D. armata	83.0	5,320	6.41	2,100	39.5	232	4.4
P. actinostoloides	35.0	683	1.95	192	28.1	32	4.7
Echinoderms							
L. leachii	72.5	475	0.66	131	27.7	33	6.9
C. valsellatus	88.0	728	0.83	345	47.4	63	8.7
P. nodosus	70.0	563	0.80	205	36.4	66	12
H. ohshimai	42.5	549	1.29	162	29.5	33	6.0
C. japonicus	169.0	552	0.33	136	24.6	40	7.2
H. leucospilota	41.5	81	0.20	59	72.8	12	15

*1 Percentage of fresh weight.
*2 Percentage of total lipids.

Table 3. Incorporation of mevalonate-2-^{14}C into the lipid fractions in the coelenterates and echinoderms

Species	% Incorporation of mevalonate-^{14}C into the lipids	Unsaponifiable matters (cpm)	Hydrocarbon fraction (cpm)	Sterol fraction		Spots in radioautography*	
				(cpm)	(cpm/mg)	Squalene	Sterols
Coelenterates							
Sarcophyta sp.	17.8	23,800	4,680	970	161	+	+
S. japonica	0.81	15,000	10.0	2,240	188	−	+
A. inermis	2.59	23,000	10.0	1,020	49	−	−
E. rubra	5.11	114,000	32,800	3,670	63	+	−
D. armata	12.3	256,000	8,300	63,000	270	+	+
P. actinostoloides	4.30	100,000	300	5,900	184	+	+
Echinoderms							
L. leachii	2.09	48,000	600	36,000	1,090	+	+
C. valsellatus	8.36	163,000	940	54,200	860	+	+
P. nodosus	6.69	163,000	1,870	7,320	112	+	+
H. ohshimai	0.51	17,500	11.0	6,200	188	−	+
C. japonicus	12.9	409,000	2,040	287,000	7,180	+	+
H. leucospilota	10.9	315,000	145,000	9,860	822	+	+

* An aliquot of the radioactive unsaponifiable matters isolated from the animals injected with mevalonate-2-^{14}C was subjected to TLC on Silicagel G with benzene-ethyl acetate (4: 1, v/v). After TLC, the plate was covered with a X-ray film and left for three weeks for radioautography. Reference squalene and sterols were detected with conc. sulfuric acid-ethanol (1: 1, v/v) followed by heating at 105°C for 10 min. +, detected; −, non-detected.

and sterol fractions gave considerably different radioactivities according to the species of animals. This may suggest the differences in the sterol-synthesizing ability and the speed of steroid metabolism among the animals examined. The sterols isolated from all animals showed a substantial radioactivity, although those of *Sarcophyta* sp. and *A. inermis* revealed low radioactivity. In order to confirm the incorporation of mevalonate-2-^{14}C into both squalene and sterols, an aliquot of the unsaponifiable matters was chromatographed by TLC and radioautographed. Except for the coelenterates, *A. inermis* and *E. rubra*, belonging to the order Gorgonaria, the unsaponifiable matters from all coelenterates and echinoderms showed definitely radioactive spots corresponding to sterols. In the case of *S. japonica, A. inermis,* and *H. ohshimai*, the presence of radioactive

Table 4. Crystallizations of the sterols isolated from the coelenterate, *P. actinostoloides*, and the echinoderm, *C. japonicus*

Crystallization	Solvent	Specific activity (cpm/mg)	
		P. actinostoloides	*C. japonicus*
First	Methanol	184	7,180
Second	Methanol	140	5,330
Third	Methanol	111	4,020
Fourth	Methanol	80	2,900

squalene was not detected. Moreover, the radioactive sterols were subjected to recrystallization followed by the measurement of specific activity (cpm/mg). However, attempts to obtain sterol crystals with constant specific activity by repeated crystallizations failed in every animal. Table 4 shows the results with *P. actinostoloides* and *C. japonicus*. In the present study, the incorporation of mevalonate-2-^{14}C into sterols was judged from the results of radioautography.

The present investigation showed that all echinoderms and the four coelenterates, *Sarcophyta* sp., *S. japonica, D. armata*, and *P. actinostoloides*, are capable of incorporating mevalonate into sterols but the two species of coelenterates, *A. inermis* and *E. rubra*, incapable.

Discussion

Regarding the echinoderms, the sterol biosynthesis from acetate and mevalonate has been investigated in a relatively number of animals, including echinoids (sea-urchins)[8,14,15] holothurians (sea-cucumbers)[16–18], asteroids (starfish)[18–21], and ophiuroid (brittle-star)[8]. Also, the sterols occurring in the echinoderms have been shown to be formed by transformation of exogenous Δ^5-sterols[22–24]. GOAD *et al.*[2] have reviewed the composition and metabolism of sterols in echinoderms. Apart from the results of *Paracentrotus lividus*[14] and *Stichopus japonicus*[16], most echinoderms appear to be capable of synthesizing sterols from lower units such as acetate and mevalonate. The present study also showed that the echinoderms, *L. leachii, C. valsellatus, P. nodosus, H. ohshimai, C. japonicus*, and *H. leucospilota*, possess the ability of sterol synthesis from mevalonate as well as the different species of echinoderms reported by other workers[8,14–21]. Considering these facts, it may be assumed reasonably that all echinoderms form at least some sterols from acetate and mevalonate via a well known pathway.

As to the coelenterates, the sterol-synthesizing ability has so far been studied in only three species of animals, *Rhizostoma* sp. (jellyfish)[9], *Metridium senile* (plumose anemone)[8], and *Calliactis parasitica* (sea anemone)[10]. The above three coelenterates have been pointed out to be incapable of synthesizing sterols from acetate or mevalonate. In the present study, the coelenterates, *A. inermis* and *E. rubra*, were incapable of incorporating mevalonate-2-^{14}C into the sterol fraction. However, *Sarcophyta* sp., *S. japonica, D. armata*, and *P. actinostolodies* incorporated mevalonate-2-^{14}C into the sterol fraction. These results indicated that some coelenterates possess the sterol-synthesizing ability from mevalonate but others do not.

The evaluation of sterol-synthesizing ability in marine invertebrates has been performed by using possible precursors such as radioactive acetate and mevalonate, and the final criterion has been based on the significance of radioactivity of isolated sterols, the presence of radioactive sterols in radioautography, and/or the constant specific activity

of sterols during crystallizations. Considering the complexity of sterols in the echinoderms[2,11] and coelenterates[1,12], however, the criterion based on a constant specific activity during crystallizations were conceived not to be always reasonable as pointed out in some echinoderms by GOAD *et al.*[2]. In the present study, hence, the authors evaluated the sterol-synthesizing ability according to the presence of radioactive spots in the radioautography in conjunction with the significance of radioactivities recovered in the isolated sterols. But, the conclusion should be regarded as tentative. The final decision whether these echinoderms and coelenterates examined possess the sterol-synthesizing ability or not should be done after the investigation of radioactivity in the individual sterol constituents. In the case of the starfish, *L. leachii*, the authors have clarified that this animal is capable of synthesizing only a few sterol constituents from mevalonate-2-^{14}C. This result will be published elsewhere[25]. In the present study, moreover, the low incorporation of mevalonate in some coelenterates and echinoderms suggests that the large portion of sterols found in their tissues is derived from dietary sources of sterols and/or their metabolites.

References

1) J. AUSTIN: in "Advances in Steroid Biochemistry and Pharmacology" (ed. by M. H. BRIGGS), Vol. 1, Academic Press, London and New York, 1970, pp. 73–96.
2) L. J. GOAD, I. RUBINSTEIN, and A. G. SMITH: *Proc. Roy. Soc. Lond.*, **B180**, 223–246 (1972).
3) P. A. VOOGT: in "Experiments in Physiology and Biochemistry" (ed. by G. KERKUT), Vol. 4, Academic Press, New York, 1971, pp. 1–33.
4) D. R. IDLER and P. WISEMAN: *J. Fish. Res. Bd. Can.*, **29**, 385–390 (1972).
5) S. TESHIMA: *Mem. Fac. Fish., Kagoshima Univ.*, **21**, 69–147 (1972).
6) J. M. WOOTON and L. D. WRIGHT: *Nature, Lond.*, **187**, 1027–1028 (1960).
7) J. M. WOOTON and L. D. WRIGHT: *Comp. Biochem. Physiol.*, **5**, 253–264 (1962).
8) M. J. WALTON and J. F. PENNOCK: *Biochem. J.*, **127**, 471–479 (1972).
9) H. E. VAN AAREM, H. J. VONK, and D. I. ZANDEE: *Arch. Int. Physiol. Biochim.*, **72**, 606–614 (1964).
10) J. P. FEREZOU, M. DEVYS, and M. BARBIER: *Experientia*, **24**, 407–408 (1972).
11) A. KANAZAWA, S. TESHIMA, and T. ANDO: *Mem. Fac. Fish., Kagoshima Univ.*, **22**, 21–31 (1973).
12) A. KANAZAWA, S. TESHIMA, and T. ANDO: *Comp. Biochem. Physiol.*, in press.
13) S. TESHIMA and A. KANAZAWA: *ibid.*, **38B**, 597–602 (1971).
14) A. SALAQUE, M. BARBIER, and E. LEDERER: *ibid.*, **19**, 45–51 (1966).
15) P. A. VOOGT: *ibid.*, **43B**, 457–463 (1972).
16) T. NOMURA, Y. TSUCHIYA, D. ANDRE, and M. BARBIER: This Bull., **35**, 299–302 (1969).
17) P. A. VOOGT and J. OVER: *Comp. Biochem. Physiol.*, **45B**, 71–80 (1973).
18) L. J. GOAD, A. G. SMITH, and T. W. GOODWIN: *J. Am. Oil Chem. Soc.*, **47**, 90A (1970).
19) W. W. ALLEN and A. C. GIESE: *Comp. Biochem. Physiol.*, **17**, 23–38 (1966).
20) A. G. SMITH and L. J. GOAD: *Biochem. J.*, **123**, 671–673 (1971).
21) P. A. VOOGT: *Int. J. Biochem.*, **4**, 42–50 (1973).
22) U. H. M. FAGERLUND and D. R. IDLER: *Can. J. Biochem. Physiol.*, **38**, 997–1002 (1960).
23) A. G. SMITH and L. J. GOAD: *FEBS Letters*, **12**, 233–235 (1971).
24) A. G. SMITH, R. GOODFELLOW, and L. J. GOAD: *Biochem. J.*, **128**, 1371–1372 (1972).
25) S. TESHIMA and A. KANAZAWA: *Comp. Biochem. Physiol.*, in press.