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Metabolism of Desmosterol in the Prawn, *Penaeus japonicus*

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Abstract

The present study deals with the bioconversion of desmosterol to cholesterol in the prawn, *Penaeus japonicus*. After administration of desmosterol-26-^{14}C, the bioconversion products were investigated by using thin-layer and silver nitrate-impregnated silicic acid column chromatographies. As a bioconversion product, cholesterol-^{14}C was identified. These results suggested that the prawn, *P. japonicus* possesses the enzyme systems for conversion of desmosterol to cholesterol.

In the previous papers [1-3], the authors have shown that the several marine crustaceans are capable of converting some C_{28}- and C_{29}-sterols to cholesterol. These results suggested that a part of cholesterol occurring in the tissues of crustaceans originates from dietary sources of C_{28}- and C_{29}-sterols, because most marine crustaceans probably possess no ability for sterol synthesis [4]. However, there is no report on the metabolic fate of dietary C_{27}-sterols such as 22-dehydrocholesterol and desmosterol. Hence, the authors attempted to clarify the metabolism of desmosterol in marine crustaceans as part of the investigation of sterol metabolism in them. This paper deals with the bioconversion of desmosterol to cholesterol in the prawn, *Penaeus japonicus*.

Materials and Methods

Chemicals. Authentic cholesterol and desmosterol were purchased from Nakarai Chemicals Co., Ltd. and Gas-Chrokogyo Co., Ltd., respectively. Cholesteryl and desmosteryl acetates were prepared by acetylation of free sterols with acetic anhydride-dry pyridine (1 : 1) at room temperature for 48 hours. The purities of these steroids were checked by gas-liquid chromatography (GLC) on 1.5% SE-30 and on 1.5% OV-17 and by thin-layer chromatography (TLC) on Kiesel gel G with benzene-ethyl acetate (4 : 1) and on a silver nitrate-impregnated Kiesel gel G with hexane-benzene (3 : 2). Desmosterol-26-^{14}C (53 mCi/mM) was obtained from the Radiochemical Centre, Amersham (England).

Preparation of the desmosterol-^{14}C-containing diet. To the homogenate (8g) of the short-necked clam, *Tapes philippinarum*, distilled water (2ml) and agar (200mg) were added, mixed, and heated on the water bath at 70-80°C. To this, 2μCi of desmosterol-26-^{14}C dissolved in 0.01ml of ethanol was added, mixed thoroughly, and then the diet was cooled and stored in a refrigerator until used.

Administration of the desmosterol-^{14}C-containing diet to the prawn. The prawn, *P. japonicus*, 4-5g in body weight, spawned in the Subtropical Marine Biological Laboratory,

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Kagoshima, was used in this experiment. The diet was cut into 5 mm cube and supplied to 8 prawns. After supplement of the diet, the prawns were kept in the plastic trough (30×30×60 cm) for 3 days at 20–22°C.

Isolation of sterols. From the prawns fed on the diet, the lipids were extracted and saponified with alcoholic potassium hydroxide, and then the sterols were isolated from the unsaponifiable matters by using column chromatography on alumina with hexane–benzene. The details of the procedures for extraction of lipids and for isolation of sterols have been described in the previous paper [5].

Analytical methods. GLC was carried out by using a Shimadzu GC–3AF gaschromatographic unit with hydrogen flame ionization detectors. The identification of sterols was achieved by using 2 columns, 1.5% SE–30 (non-selective phase) and 1.5% OV–17 (selective phase). In TLC, 2 types of adsorbent, Kiesel gel G (Merck, Germany) and a mixture of Kiesel gel G–silver nitrate (4 : 1) [6], were used. A column chromatography on a silver nitrate–impregnated silicic acid was conducted according to the method of VROMAN and COHEN [6]. The radioactivity was measured with a Beckman liquid scintillation counter LS–230 by using a toluene solution of 0.6% PPO as a scintillator. The efficiency of counting for radioactive materials was approximately 92 per cent.

Results

From the prawns (35.0 g) fed on the desmosterol–^{14}C–containing diet, the sterols (66.8 mg ; 513,000 dpm) were isolated. The GLC on 1.5% SE–30 and on 1.5% OV–17 showed that the sterols were composed of cholesterol (99%), 24–methylenecholesterol (1%) and 22–dehydrocholesterol (trace) as shown in Table 1. The radioactive sterols were acetylated and subjected to TLC on a mixture of Kiesel gel G–silver nitrate (4 : 1) with hexane–benzene (1 : 2) and radioautographed by covering the plate with an X–ray film followed by exposure

Table 1. GLC of the isolated sterols from the prawn fed on the desmosterol–^{14}C–containing diet.

Column				% Composition	Identified as
1.5% SE–30		1.5% OV–17			
Peak	Rt*	Peak	Rt*		
1	16.3	1	25.8	trace	22–Dehydrocholesterol
2	18.0	2	27.8	99	Cholesterol
3	22.8	3	36.8	1	24–Methylenecholesterol

* Retention time (min.)
1.5% SE–30: 200cm×4mm i.d., column temp. 225°C
1.5% OV–17: 300cm×4mm i.d., column temp. 242°C

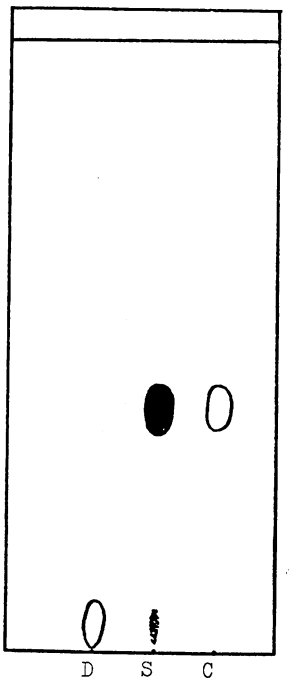

Fig. 1. Radioautography of the isolated sterols (as acetates) from the prawn fed on the desmosterol-^{14}C-containing diet.

The samples were applied to TLC on a mixture of silver nitrate- Kiesel gel G with hexane–benzene (1:2). The reference steryl acetates were detected by spraying conc. sulfuric acid–ethanol (1 : 1). D, desmosteryl acetate; S, radioactive steryl acetate isolated; C, cholesteryl acetate.

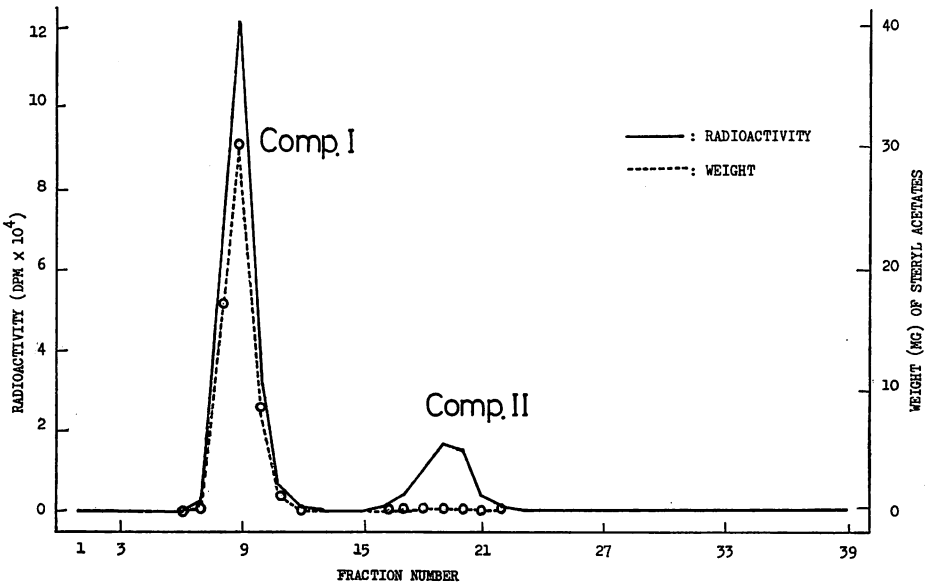

Fig. 2. Column chromatography on a silver nitrate–impregnated silicic acid of the isolated sterols (as acetates) from the prawn fed on the desmosterol-^{14}C-containing diet.

The radioactive steryl acetates (60mg) were chromatographed on a silver nitrate-impregnated silicic acid(10g, 1.5cm×9.0cm) by stepwise-elution with hexane-benzene as follows: 75ml of hexane, 100ml of hexane-benzene (85:15), 100ml of hexane-benzene (80:20), 100ml of hexane-benzene (75:25), 100ml of hexane-benzene (70:30), 100ml of hexane-benzene (65:35), 100ml of hexane-benzene (60:40), 75ml of hexane-benzene (50:50), 100ml of hexane-benzene (40:60), 50ml of hexane-benzene (30:70), 50ml of hexane-benzene (20:80), 50ml of hexane-benzene (10:90). Each 25ml fraction was collected and monitored by GLC and liquid scintillation counter.

for 2 weeks. As shown in Fig. 1, the radioautogram indicated the presence of 2 radioactive steryl acetates corresponding to cholesteryl acetate (Rf, 0.39) and desmosteryl acetate (Rf, 0.05). Furthermore, an aliquot of the radioactive steryl actate was chromatographed on a column of silver nitrate-impregnated silicic acid with hexane-benzene. As shown in Fig. 2, the radiochromatogram of this steryl acetate gave the major peak (Comp. I) corresponding to cholesteryl acetate and the minor peak (Comp. II). When the comp. II was subjected to TLC on a mixture of Kiesel gel G-silver nitrate with hexane-benzene (1 : 2), this compound gave one radioactive spot corresponding to desmosteryl acetate.

To confirm the identity of the comp. I with cholesteryl acetate-^{14}C, about 10mg of non-radioactive cholesteryl acetate was added to the comp. I and recrystallized several times. As shown in Table 2, the crystals of the steryl acetate gave a constant specific activity during the recrystallizations.

On the basis of the above data, it was concluded that the prawn, *P. japonicus*, converts desmosterol to cholesterol.

Table 2. Recrystallization of isolated cholesteryl acetate-^{14}C from the prawn fed on the desmosterol-^{14}C-containing diet.

Crystallization	Solvent system	Specific activity (dpm/mg)
First	Chloroform-methanol	3800
Second	Chloroform-methanol	3830
Third	Methanol	3820
Fourth	Chloroform-methanol	3900

Discussion

In animals, it is generally accepted that desmosterol is a final intermediate in the biosynthesis of cholesterol from lower units [7-9]. In addition, it has been shown that in plants desmosterol or other sterols with the double bond at C-24 are the recipient [10] of a methyl group of S-adenosyl methionine resulting in the formation of 24-methylenecholesterol which is a key intermediate in the biosynthesis of 24-ethylidene-sterols and other C_{29}-sterols [11-17]. On the other hand, IDLER and WISEMAN have reported that the Alaskan king crab, *Paralithodes camtschatica*, contained relatively large amounts of desmosterol (31.1% of total sterols) in the tissues [18]. The authors have also demonstrated the presence of desmosterol (22% of total sterols) in the Amphipoda, *Caprella sp.* [5]. On the marine crustaceans, however, the metabolic

fate of desmosterol has not been clarified yet. In the present study, it was evidently shown that the prawn, *P. japonicus*, is capable of converting desmosterol to cholesterol. This result indicates that the prawn possesses at least $\triangle^{24}$-sterol reductase activity, although this crustacean lacks sterol-synthesizing ability from acetate [4]. In the insects, SVOBODA and coworkers have proved that desmosterol is one of the possible intermediates in the bioconversion of β-sitosterol to cholesterol by the tobacco hornworm, *Manduca sexta* (Johannson)[19-21]. In the previous study [3], the author has also shown that the prawn, *P. japonicus*, is capable of converting β-sitosterol to cholesterol. Accordingly, considering the knowledges on the metabolism of desmosterol in the insects, belonging to the same Arthropoda as the prawn, it may be postulated that desmosterol is one of the intermediates in the dealkylation of β-sitosterol to cholesterol in the prawn, *P. japonicus*, as well as in the tobacco hornworm.

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