ムラサキイガイにおけるデスモステロールからステロール類の生合成

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Biosynthesis of Sterols from Desmosterol in a Mussel, *Mytilus edulis*

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The present study deals with the biosynthesis of sterols from desmosterol in a mussel, *Mytilus edulis*. After injection of desmosterol-26-^{14}C, sterols were isolated from the tissues, and then the bioconversion products were investigated by using gas-liquid, thin-layer, and column chromatographic methods. Cholesterol, 22-dehydrocholesterol, and 24-methylenecholesterol were identified as the bioconversion products. The results indicated that the mussel possesses the ability of converting desmosterol to the above three sterols.

It is generally recognized that animals are capable of synthesizing sterols from acetate and mevalonate via squalene, lanosterol and desmosterol, etc. As an exceptional case, insects and crustaceans are known to lack the ability for sterol-synthesis. In the previous papers, the authors have demonstrated that the prawn, *Penaeus japonicus*, possesses no ability for sterol-synthesis and requires sterols of 0.5% as a diet for normal growth and moulting.[1-3]

On the biosynthesis of sterols in mollusks, a few reports have shown that sterols are synthesized from acetate and/or mevalonate in the mollusks, *Saxidomus giganteus*,[4] *Mytilus californianus*,[4] *Helix pomatia*,[5] *Arion rufus*,[6] *Planorbarius corneus*,[7] *Limnaea stagnalis*,[7] *Limnaea peregara*,[7] *Patella coerulea*,[8] *Monodonta turbinata*,[8] *Viviparus fasciatus*,[9] *Littoria littoria*,[9] and *Liolophura japonica*.[10] But, *Ostrea gryphea*,[11] *Buccinum undatum*,[12] and *Sepia officinalis*[13] have been shown to be incapable of synthesizing sterols. The contradictory results mentioned above, perhaps indicate that mollusks differ from one another in the sterol-synthesizing ability. In the culture of mollusks which lack the sterol-synthesizing ability, the sterol content in their diets may be significant for the nutrition of mollusks.

In the previous paper,[14] the authors have reported that the mollusks belonging to the gastropds contain cholesterol as a main sterol, but the pelecypods contain the sterol mixtures composed of a variety of type of Δ^5-sterols. Moreover, it has been shown that the sterols of mussel, *Mytilus edulis*, were composed of 22-dehydrocholesterol (25%), cholesterol (30%), brassicasterol (23%), 24-methylenecholesterol (12%) and minor other sterols, and also that this mussel was capable of incorporating mevalonate-2-^{14}C into sterols.[15] However, there is no evidence that the pelecypods are capable of synthesizing sterols other than cholesterol from lower units, except for the biosynthesis of 24-

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methylenecholesterol in the clam, *Saxidomus giganteus*.[16,17] In plants and microorganisms, the introduction of the substituents such as methylene, methyl, ethylidene, and ethyl groups at C-24 of C_{28}- and C_{29}-sterols has been proposed to occur through desmosterol by successive transmethylations from methionine.[18-28]

Hence, for the purpose of clarifying the origin of sterols other than cholesterol in the pelecypods, the bioconversion of sterols from precursor, desmosterol, was investigated by using the mussel, *M. edulis*. This paper deals with the bioconversion of desmosterol-26-^{14}C to cholesterol, 22-dehydrocholesterol, and 24-methylenecholesterol in the mussel.

Materials and Methods

Mussels and injection of desmosterol-26-^{14}C The mussels were caught at the Kinko-bay, Kagoshima, in October, 1972. Nine mussels were injected with 2.5 μCi of desmosterol-26-^{14}C (53 mCi/m mol; Radiochemical Centre, England) in a small amount of ethanol. The radiochemical purity of desmosterol-26-^{14}C was checked by thin-layer chromatography (TLC) on a silver nitrate-impregnated Kieselgel G. The mussels were kept in a circulatory trough for 11 days at 22–22°C without administration of food.

Investigation of metabolites After 11 days, the mussels were killed by freezing at −20°C. From the whole tissues except the shell, sterols were isolated according to the same method as described previously,[15] and then the metabolites of desmosterol-26-^{14}C were investigated. The detection and isolation of radioactive metabolites were carried out by TLC on a silver nitrate-impregnated Kieselgel G[29] and a paraffin-impregnated Kiesel guhr[30] followed by radioautography and by a column chromatography on a silver nitrate-impregnated silicic acid with hexane-benzene.[31] In column chromatography, sterols were detected by gas-liquid chromatography (GLC) on 1.5% OV-17.[32] The radioactivity of sterols was measured with a Beckman LS-230 using a toluene solution of PPO (0.6%) as a scintillator. The efficiency of counting of radioactive sterols was approximately 90 per cent.

Results

The isolated sterols (25 mg) from the mussels (25 g in wet weight) injected with desmosterol-26-^{14}C gave high radioactivity (788,000 cpm). The GLC on 1.5% OV-17 (Fig. 1) shows that the sterols were composed of 22-*trans*-24-norcholesta-5,22-dien-3β-ol (1%), 22-dehydrocholesterol (18%), cholesterol (35%), brassicasterol (25%), desmosterol (2%), 24-methylenecholesterol (16%), β-sitosterol (1%), and unknown sterols (trace). The sterol composition of mussel was almost similar to that of another sample reported previously.[14]

The radioactive sterols were acetylated with acetic anhydride-dry pyridine (1: 1) at

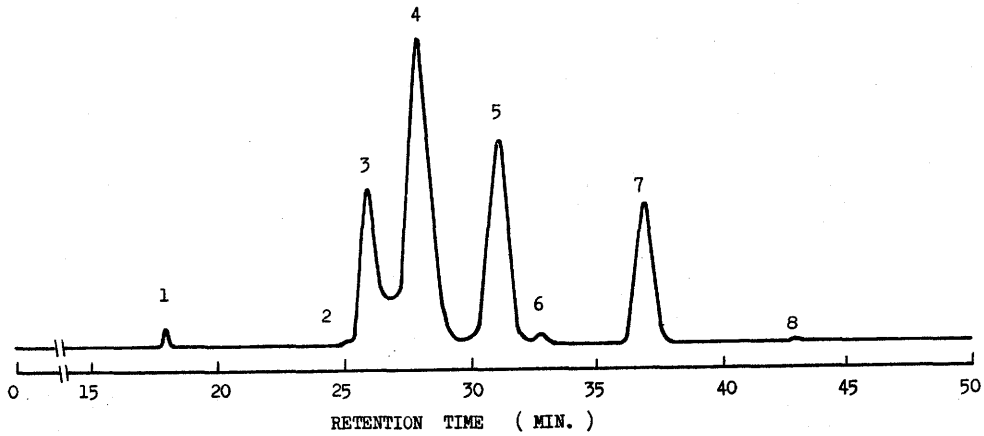

Fig. 1. GLC of the isolated sterols from the mussel injected with desmosterol-^{14}C. The peaks were identified as follows: 1, 22-*trans*-24-norcholesta-5,22-dien-3β-ol (1%); 2, 22-*cis*-22-dehydrocholesterol (trace); 3, 22-trans-22-dehydrocholesterol (18%); 4, cholesterol (35%); 5, brassicasterol (25%); 6, desmosterol (2%); 7, 24-methylenecholesterol (16%); 8, β-sitosterol (1%).

room temperature for 48 hours, and the steryl acetates so obtained were subjected to TLC on a silver nitrate-impregnated Kieselgel G with hexane-benzene (5 : 2) and radioautographed by covering the plate with an X-ray film (Konishiroku Photo Ind. Co., Japan) followed by exposure for 3 weeks. The radioautogram (Fig. 2) indicated the presence of five radioactive spots corresponding to cholesteryl acetate, 22-dehydrocholesteryl acetate, desmosteryl acetate, 24-methylenecholesteryl acetate, and unknown compound.

Also, to an aliquot of radioactive sterols, about 10 mg each of authentic cholesteryl acetate, 22-dehydrocholesteryl acetate, brassicasteryl acetate, and 24-methylenecholesteryl acetate was added and then subjected to column chromatography on a silver nitrate-impregnated silicic acid. As shown in Fig. 3, high radioactivity was recovered in the cholesteryl acetate, 22-dehydrocholesteryl acetate, desmosteryl acetate, and 24-methylene-

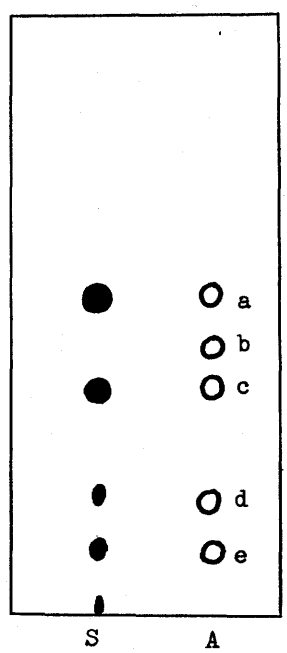

Fig. 2. Radioautogram of the isolated steryl acetates from the mussel injected with desmosterol-^{14}C.

S and A indicate radioactive sample and authentic steryl acetates. a, cholesteryl acetate; b, brassicasteryl acetate; c, 22-dehydrocholesteryl acetate; d, desmosteryl acetate; e, 24-methylenecholesteryl acetate. Adsorbent: Silver nitrate-impregnated Kieselgel G. Solvent system: Hexane-benzene (1 : 2).

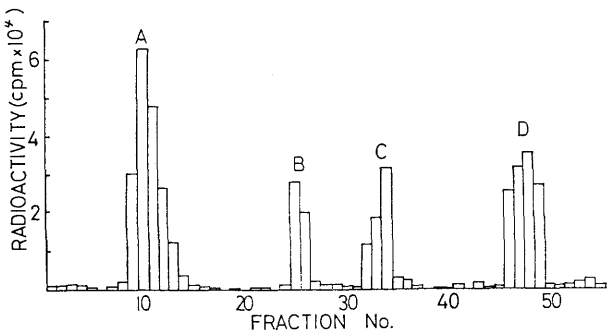

Fig. 3. Column chromatography of the isolated steryl acetates from the mussel injected with desmosterol-26-^{14}C.

Adsorbent: a mixture of silicic acid-silver nitrate (5: 1, w/w). The column (1.5× 30.0 cm) was eluted with the following solvents: 200 m*l* hexane, 100 m*l* hexane-benzene (87: 13), 200 m*l* hexane-benzene (80: 20), 150 m*l* hexane-benzene (78: 22), 100 m*l* hexane-benzene (75: 25), 100 m*l* hexane-benzene (72: 28), 100 m*l* hexane-benzene (69: 31), 100 m*l* hexane-benzene (66: 34), 100 m*l* hexane-benzene (63: 37), 100 m*l* hexane-benzene (60: 40), 100 m*l* hexane-benzene (57: 43), 100 m*l* hexane-benzene (54: 46), 100 m*l* hexane-benzene (51: 49), 100 m*l* hexane-benzene (48: 52), and 200 m*l* hexane-benzene (44: 55). The following volumes of eluate were collected and monitored by GLC: faction 1 (200 m*l*), fractions 2 and 3 (50 m*l*), fractions 4–53 (25 m*l*), fractions 54 and 55 (100 m*l*).

The radioactive peaks A, B, C, and D were found by GLC to correspond to cholesteryl, 22-dehydrocholesteryl, desmosteryl, and 24-methylenecholesteryl acetates, respectively. The fractions 19–23 corresponding to brassicasteryl acetate did not gave significant radioactivity.

cholesteryl acetate fractions. Moreover, the four radioactive steryl acetate fractions were rechromatographed by TLC on a silver nitrate-impregnated Keiselgel G and by column chromatography on a silver nitrate-impregnated silicic acid. In these chromatographies, each steryl ester gave one radioactive spot or peak. However, the chromatography using silver nitrate-impregnated adsorbents has been pointed out not to give a wide separation of sterols differing only in the attachment of alkyl groups such as methyl and ethyl radicals at C–24.[29,31] Hence, in order to further check radioactive metabolites, the four steryl acetates were saponified with 5% alcoholic potassium hydroxide, and then the free sterols so obtained were chromatographed by TLC on a paraffin-impregnated Kiesel guhr with the system (paraffin oil/acetone-water (4: 1)) devised by De Souza and Nes.[30] In this TLC, the four sterols showed one radioactive spot respectively.

The above data indicated that the mussel, *M. edulis*, is capable of converting desmosterol to cholesterol, 22-dehydrocholesterol, and 24-methylenecholesterol. Furthermore, since the mussel is capable of incorporating mevalonate into sterol fraction,[15] it is reasonable to conclude that cholesterol, 22-dehydrocholesterol, and 24-methylenecholesterol occurring in the tissues of mussel is synthesized from lower units via desmosterol.

Discussion

A number of studies have demonstrated that the pelecypods contain complex sterol mixtures. Recently, the sterol composition of mollusks has been extensively reviewed by IDLER and WISEMAN.[33] However, the information about the sterol biosynthesis in pelecypods is relatively little. FAGERLUND and IDLER[4] have shown that the mussel, *Mytilus californianus*, and the clam, *S. giganteus*, are capable of converting acetate-2-^{14}C into the digitonin-precipitable materials. In addition, they have proved that the clam, *S. giganteus*, could synthesize Δ^5-sterol[4] and 24-methylenecholesterol,[16,17] with suggestion that 24-methylenecholesterol is derived from a precursor with a cholesterol-type side chain. Moreover, TAMURA *et al*,[34] have suggested by the nutritional experiment that a part of sterols occurring in the tissues of oyster, *Crassostrea virginica*, may originate from exogenous sources. On the other hand, SALAQUE *et al.*[11] have reported that the oyster, *Ostreas gryphea*, did not incorporate mevalonate-2-^{14}C and L-methionine-^{14}C into both squalene and sterol fractions. Also, WALTON and PENNOCK[35] have shown that the mussel, *M. edulis*, lacks the sterol-synthesizing ability from mevalonate-2-^{14}C, whereas the authors have demonstrated that the same species of mussel is capable of incorporating mevalonate into sterol fraction.[15] As mentioned above, the investigations about the sterol synthesis in mollusks belonging to the class Pelecypoda have given the conflicting results. The inconsistency in the sterol-synthesizing ability among the Pelecypoda cannot be fully explained from the available informations, although the discrepancy in experimental conditions, especially in the maintaining period of mollusks after administration of radioactive precursors, is conceived as one of possible reasons.

In the rat, LONGDON and BLOCH[36] have reported that the cholesterol-synthesizing ability was reduced by addition of a large amount of cholesterol to the diet. Also, VAN DEN OORD[37] has shown that the biosynthesis of sterol in the crabs which were given no food was essentially the same as with the fed crabs. For these reason, during this experimental period no food was given to the mussel.

The present study indicated that the mussel, *M. edulis*, converted desmosterol to cholesterol, 22-dehydrocholesterol, and 24-methylenecholesterol. Since the mussel have been shown to possess the ability for sterol synthesis, the above three sterol occurring in the mussel is conceivable to be formed endogenously. Also, the several pelecypods, *M. edulis*,[14,38] *Arctica islandica*[38], *Mya arenaria*[38], and *Tapes philippinarum*[32] have been reported to contain desmosterol in their tissues. Therefore, it may be assumed that desmosterol plays an important role as intermediates for the biosynthesis of cholesterol and other sterols in some species of pelecypods as well as in the mussel, *M. edulis*.

Considering these facts, if the sterols in mussel are formed endogenously, the supplement of sterols from the diet is assumed to be unessential. However, it is obscure

yet whether endogenous sterols in mussel are sufficient for growth or not. The requirement of mussel for sterols may be clarified by the feeding experiment.

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