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In Vivo Bioconversion of β-Sitosterol to Cholesterol in the Crab, *Portunus trituberculatus*

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Abstract

The present paper deals with the *in vivo* bioconversion of β-sitosterol to cholesterol in the crab, *Portunus trituberculatus*.

After injection of β-sitosterol-4-^{14}C into the crabs, the sterols were isolated from the whole tissues and the metabolites of β-sitosterol were investigated. As a metabolite, a radioactive cholesterol was detected. The identification of cholesterol-^{14}C was performed by using thin-layer chromatography and recrystallization to constant specific activity.

The results indicated that the crab, *P. trituberculatus*, is capable of converting β-sitosterol to cholesterol.

In the previous studies, the authors have demonstrated that the several marine crustaceans possess the ability for conversion of ergosterol[1,2], 24-methylcholesterol[3], β-sitosterol[3] and brassicasterol[4] to cholesterol. Generally, higher animals such as mammals are found to be incapable of converting the above-mentioned C_{28}- and C_{29}-sterols to C_{27}-sterols such as cholesterol. In this respect, the sterol metabolism of marine crustaceans as well as insects appears to be unique as compared with that of other animals.

The purpose of the work described below was to obtain further evidence for bioconversion of exogenous C_{28}- and C_{29}-sterols in crustaceans. This paper deals with the *in vivo* bioconversion of β-sitosterol to cholesterol in the crab, *Portunus trituberculatus*, a carnivorous crustacean.

Materials and Methods

Injecton of β-sitosterol-4-^{14}C. β-Sitosterol-4-^{14}C (61 mCi/mM) was obtained from the Radiochemical Centre, Amersham, England. The crab, *P. trituberculatus*, about 9-11 g in body weight, was caught at the Sakurajima point of Kagoshima-bay, in September, 1970. Three crabs were each injected with 0.5 μ Ci of β-sitosterol-4-^{14}C in 0.01 ml ethanol into the joint of the legs and abdomen, and kept in a circulatory trough (30×30×60 cm) without supplement of food at 20-24°C. Afer 48 hours, the crabs were killed by freezing at −20°C and then the sterols were isolated from the whole tissues.

Isolation and identification of the metabolite of β-sitosterol-4-^{14}C. The sterols were isolated by the essentially similar manner to that described previously.[5] The isolation and identication of metabolite from β-sitosterol-4-^{14}C were mainly performed

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by thin layer chromatography (TLC). However, the conclusive identification of radioactive metabolite was based on the constant specific activity (dpm/mg) of the crystals during crystallizations. The outline of the procedures for isolation and identification of the metabolite from β-sitosterol-4-^{14}C by the crab, ***P. trituberculatus***, is shown in Fig. 1.

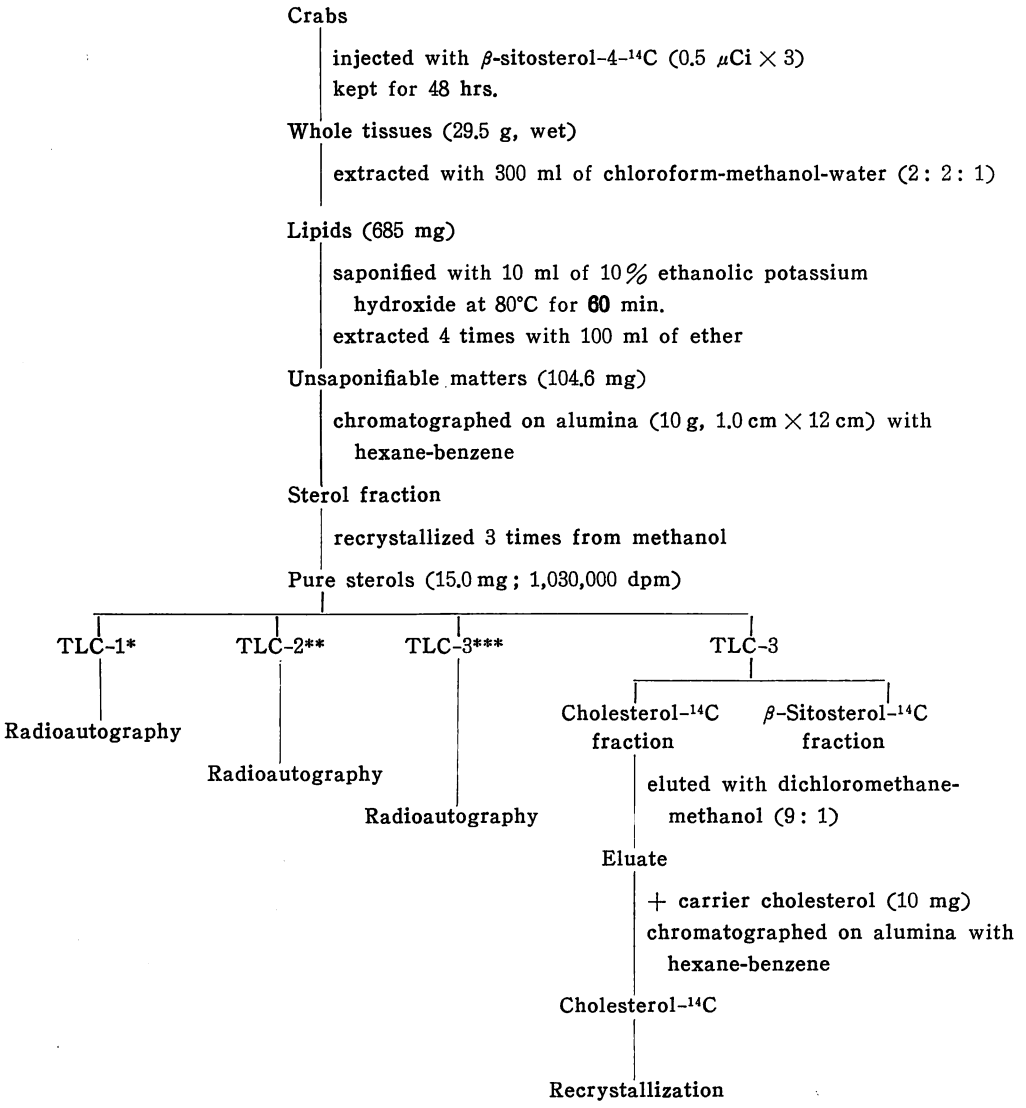

Fig. 1. Outline of isolation and identification of cholesterol-^{14}C.

* TLC-1 : Kiesel gel G, benzene-ethyl acetate (4 : 1)
** TLC-2 : Silver nitrate-impregnated kiesel gel G, hexane-benzene (1 : 2)
*** TLC-3 : Paraffin-impregnated Kiesel-guhr, paraffin oil/acetone-water (4 : 1)

Analytical methods. Gas-liquid chromatography (GLC) was carried out by using a Shimadzu GC-3AF unit. The columns used were 1.5 % SE-30 on 60-80 mesh Chromosorb W (non-selective phase) and 1.5 % OV-17 on 80-100 mesh Shimalite W (selective phase)[5]. In TLC, sterol or steryl acetate was applied to the three types of the plates coated on a Kiesel gel G (Merck), a silver nitrate-impregnated Kiesel gel[6], and a paraffin-impregnated Kiesel-guhr[7]. Steryl acetate was formed by addition of dry pryidine-acetic anhydride (1 : 1) and by standing for 24 hours at room temperatures. Steryl digitonide was prepared by the method of IDLER and BAUMANN[8]. 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.

Results

The sterols isolated from the crabs injected with β-sitosterol-4-^{14}C gave high radioactivity (1,030,000 dpm). Initially, the sterols were subjected to GLC and to TLC on Kiesel gel G followed by radioautography in order to check metabolites formed. In GLC on 1.5 % SE-30 and 1.5 % OV-17, the sterols revealed one peak corresponding to cholesterol. Also, the radioautogram gave only one radioactive spot corresponding to cholesterol.

An aliquot of the sterols was acetylated and subjected to TLC on a silver nitrate-impregnated Kiesel gel G, and then radioautographed. In this TLC, the radioautogram showed the presence of one spot corresponding to cholesteryl acetate. VROMAN and COHEN[6] have shown that $\triangle^5$-sterols (C_{27} and C_{28}) were separable from both the corresponding saturated and diene-sterols such as $\triangle^{5,22}$- and $\triangle^{5,24}$-sterols by TLC on a silver nitrate-impregnated silica gel H performed under the essentially same conditions as those in the present study. Furthermore, they have perceived that the introduction of double bond at C-24 permitted a wide separation from the corresponding monoene- ($\triangle^5$ and $\triangle^7$) and saturated C_{27}-, C_{28}-, and C_{29}-sterols. Accordingly, it may be reasonable to postulate that β-sitosterol was not undergone at least the dehydrogenation at C-24 and reduction of double bond at C-5 by the crab.

When the radioactive sterols were chromatographed on a paraffin-impregnated Kiesel-guhr using the system (paraffin oil/acetone-water (4 : 1)) devised by DE SOUZA and NES[7], the presence of two radioactive sterols which showed the same mobilities as authentic cholesterol and β-sitosterol was detected. The zone corresponding to cholesterol-^{14}C was scraped off from the plate, and cholesterol−^{14}C was eluted with dichloromethane-methanol and then purified by a column chromatography on alumina. Finally, to the presumed cholesterol-^{14}C, about 10 mg of non-radioactive cholesterol was added and recrystallized from the seyeral solvent systems. As shown in Table 1, the resultant crystals gave the constant specific activity in the last three crystallizations. On the basis of the above results, it was concluded that the crab, *P. trituberculatus*, as well as the prawn, *Penaeus japonicus*, is capable of converting β-sitosterol to cholesterol.

Table 1. Recrystallization of cholesterol-^{14}C isolated from the crab, *P. trituberculatus*, injected with β-sitosterol-4-^{14}C.

Crystallization	Solvent system	Specific activity (dpm/mg)
First	Methanol	211
Second	Ethanol	165
Third	Methanol	171
Fourth	Methanol-water	168

Discussion

In the previous paper, one of the authors has shown that the prawn, *P. japonicus* converts β-sitosterol to cholesterol.[3] In addition, the present study showed that the crab, *P. trituberculatus*, also possesses the ability for dealkylation at C-24 of β-sitosterol to cholesterol. In the insects, it has been demonstrated by using tracers that the cockroach, *Blattela germanica*[9], and the tobacco hornworm, *Manduca sexta* (Johannson)[10] convert β-sitotserol to cholesterol. Since most marine crustaceans were conceivable to lack probably cholesterol-synthesizing ability from acetate[11], it may be reasonable to assume that they utilize cholesterol derived from β-sitosterol for normal growth in addition to other dietary source of sterols.

SVOBODA *et al.*[10] have clarified that desmosterol is one of most likely intermediates during the conversion of β-sitosterol to cholesterol in the tobacco hornworm, *M. sexta*. Furthermore, SVOBODA and ROBBINS[12] have proved the intermediary role of desmosterol in the above bioconversion by the fact that the bioconversion of β-sitosterol to cholesterol was blocked by triparanol and 22,25-diazacholesterol, hypocholesterolemic agents in vertebrates, and that desmosterol was accumulated.

Considering these knowledges obtained in the insects, it may be postulated that the bioconversion of β-sitosterol to cholesterol in the crab, *P. trituberculatus*, and prawn, *P. japonicus*, proceeds via several intermediates.

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References

1) TESHIMA, S. and A. KANAZAWA (1971): Bioconversion of the dietary ergosterol in *Artemia salina*. *Comp. Biochem. Physiol.*, **38B**, 603-607.

2) TESHIMA, S. (1971): *In vivo* transformation of ergosterol to cholesterol in crab, *Portunus trituberculatus*. *Bull. Jap. Soc. Sci. Fish.*, **37**, 671-674.

3) TESHIMA, S. (1971): Bioconversion of β-sitosterol and 24-methylcholesterol to cholesterol in marine Crustacea. *Comp. Biochem. Physiol.*, **39B**, 815-822.

4) TESHIMA, S. and A. KANAZAWA (1972): Bioconversion of the dietary brassicasterol to cholesterol in *Artemia salina*. *Bull. Jap. Soc. Sci. Fish.*, **38**, 1305—1310.

5) TESHIMA, S. and A. KANAZAWA, (1971) : Sterol compositions of marine crustaceans. ***Bull. Jap. Soc. Sci. Fish.***, **37**, 63-67.
6) VROMAN, H. E. and C. F. COHEN (1967) : Separation of sterol acetate by column and thin-layer argentation chromatography. ***J. Lipid Res.***, **8**, 150-152.
7) DE SOUZA N. J. and W. R. NES (1969) : Improved separation of sterols by reversed phase thin-layer chromatography. ***J. Lipid Res.***, **10**, 240-243.
8) IDLER D. R. and C. A. BAUMANN (1952) : Skin sterols-II. Isolation of 7-cholestenol. ***J. Biol. Chem.***, **195**, 623-628.
9) ROBBINS, W. E., R. C. DUTKY, R. E. MONROE, and J. N. KAPLANIS (1962) : The metabolism of β-sitosterol-^{3}H by the German cockroach. ***Ann. Entomol. Soc. Am.***, **55**, 102-104.
10) SVOBODA, J. A., M. J. THOMPSON, and W. E. ROBBINS (1967) : Desmosterol, an intermediate in dealkylation of β-sitosterol. ***Life Sci.***, **6**, 395-404.
11) TESHIMA, S. and A. KANAZAWA (1971) : Biosynthesis of sterols in the lobster, ***Panulirus japonica***, the prawn, ***Penaeus japonicus***, and the crab, ***Portunus trituberculatus***. ***Comp. Biochem. Physiol.***, **38B**, 597-602.
12) SVOBODA, J. A. and W. E. ROBBINS (1967) : Conversion of beta-sitosterol to cholesterol blocked in an insect by hypocholemic agents. ***Science***, **156**, 1637-1638.