ガザミ精巣切片によるプロジェステロンから17a-ハイドロオキシプロジェステロンおよびテストステロンへの変換

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In Vitro Bioconversion of Progesterone to 17α-Hydroxyprogesterone and Testosterone by the Sliced Testes of Crab, *Portunus trituberculatus*

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The present study deals with the *in vitro* bioconversion of progesterone-4-^{14}C by the sliced testes of crab, *Portunus trituberculatus*. After the incubation of the testes with progesterone-4-^{14}C, the metabolites were extracted and investigated by thin-layer and paper chromatography. Final identification of metabolites was based on the specific activity (dpm/mg) during repeated crystallizations with authentic steroids.

As a result, 17α-hydroxyprogesterone and testosterone were identified as the metabolites of progesterone. The results indicated that the testes of the crab, *P. trituberculatus*, contain the enzyme systems for 17α-hydroxylation and side-chain cleavage of progesterone.

From the view of comparative endocrinology, a variety of works have been carried out on the steroidogenesis in lower vertebrates.[1] As far as the present authors know, however, the knowledge on the steroidogenesis in marine invertebrates is relatively few. Recently, a few of workers have taken an interest in this field.[2-5] In the previous paper,[6] the authors reported that the ovaries of crab, *Portunus trituberculatus*, convert progesterone-4-^{14}C to 17α-hydroxyprogesterone, testosterone, and deoxycorticosterone, and suggested the presence of steroid 17α-hydroxylase, steroid 21-hydroxylase, and steroid C_{17}–C_{21} lyase in the ovaries of the crab. On the other hand, it is a well-known fact that testicular tissues play an important role in the biosynthesis of steroid hormones in lower vertebrates as well as in mammalians. However, the steroidogenesis in the testes of marine invertebrates has not been well established yet.

The present paper describes the results of the bioconversion of progesterone by the testes of crab, *Portunus trituberculatus*.

Materials and Methods

Substrate and chemicals. Progesterone-4-^{14}C (29.3 mCi/mM) and non-radioactive steroids were obtained from Daiichi Pure Chemicals Co., Ltd. (Japan), and Sigma Chemical Co. (U.S.A.), respectively. The purities of these steroids were ascertained by thin-layer chromatography (TLC).

Incubation and extraction. The crab, *P. trituberculatus*, 140 g in body weight, was used in this experiment. The details of the procedures for incubation of tissues and for

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extraction of metabolites were described in the previous paper.[6] After the pre-incubation for 30 minutes at 20°C without precursor, the slices of testes (500 mg) were incubated with 1 μ Ci of progesterone-4-^{14}C in 8 ml of the medium containing cofactors (1.0 mg of nicotinamide-adenine dinucleotide phosphate, 2.5 mg of glucose-6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase) and antibiotics (12000 units of penicillin G and 240 μg of aureomycin) for 6 hours successively. After the incubation, the metabolites were extracted twice with 7 volumes of dichloromethane, washed with distilled water, and then the solvent was evaporated under reduced pressure.

Analysis of bioconversion products. To the dichloromethane extract, about 50 μg each of non-radioactive 17α-hydroxyprogesterone, androstenedione, testosterone, 11-ketotestosterone, deoxycorticosterone, corticosterone, 11-deoxycortisol, cortisone, and cortisol were added as a carrier. As mentioned previously, the isolation and tentative identification of metabolites were carried out by TLC and by paper chromatography (PPC). The solvent systems used in TLC were: S1, chloroform-methanol (97:3); S2, benzene-acetone (8:2); S3, chloroform–95% ethanol (95:5); S4, dichloromethane-methanol (9:1); S5, ethyl acetate-cyclohexane-toluene (10:10:1).[7,8] PPC was conducted by using BUSH-type solvent system: heptane-benzene (1:1)–70% methanol (HBM–70). Moreover, a part of the radioactive metabolites purified by chromatography was subjected to acetylation with acetic acid anhydride-pyridine (1:1)[6] and oxidation with 0.5% CrO_3 in 90% acetic acid,[6] and then the chromatographic behaviors of the derivatives were compared with that of authentic compounds. Finally, about 10 mg of authentic steroids was added to the radioactive metabolites, recrystallized from the various solvents, the specific activities of the crystals were investigated. The weights and radioactivities of crystals were measured by a Shimadzu electric balance PMB–1 and by a Beckman liquid scintillation counter LS-150, respectively. The procedure for measurement of radioactivity was described in the previous paper.[9] The criterion of identification was carried out according to the constant specific activity of the crystals in the last three crystallizations.

Results

The dichloromethane extract of metabolites was initially chromatographed on TLC in system S1, and fractionated contiguously into 15 fractions as shown in Fig. 1. The materials (E–8, 9, 10, and 12) revealed relatively high radioactivities were eluted from the plate[10] and subjected to the investigation of metabolites. The materials (E–1, 2, 3, 4, 5, 6, 7, 11, 13, 14, and 15) showed extremely low radioactivities were left-unanalyzed in the present study.

The material (E–8) corresponding to carrier testosterone in the initial TLC was

rechromatographed on TLC in systems S2, S4, S1 and S3, and on PPC in system HBM–70, successively. In these chromatography, the biosynthetic testosterone revealed the same mobilities as authentic testosterone and maintained its radioactivities relatively. Acetylation of this substance gave the radioactive material corresponding to testosterone 17β-mono acetate. When the substance was oxidized with 0.5% CrO_3 in 90% acetic acid, the radioactive compound exhibiting the same mobility as standard androstenedione in TLC using system S2 was obtained.

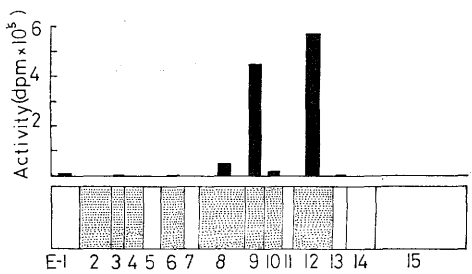

Fig. 1. Bioconversion of progesterone-4-^{14}C by the testes of crab, *Portunus trituberculatus*; radioactivities and UV-absorbing zones in initial TLC.

Table 1. Chromatographic purification steps and the radioactivities recovered from the chromatograms.

Purification steps		Radioactivities (dpm)*	
		Testosterone-^{14}C	17α-Hydroxyprogesterone-^{14}C
1. TLC:	system S1	257000	252000
2. TLC:	system S2	252000	193000
3. TLC:	system S4	94200	140000
4. TLC**:	system S1+S3	47100	———
5. TLC:	system S3	———	122000
6. PPC:	system HBM–70	45100	97100

* The radioactivities of the materials eluted from the zones corresponding to carrier testosterone and 17α-hydroxyprogesterone.

** developed repeatedly by use of system S1 (first) and system S3 (second)

The material (E–9) corresponding to 17α-hydroxyprogesterone was rechromatographed on TLC in systems S2, S4, S3, and on PPC in system HBM–70, successively. Throughout the chromatographic purification steps, the material showed the same mobilities as authentic 17α-hydroxyprogesterone and significant radioactivities. Acetylation and oxidation of the chromatographically purified material gave the radioactive compounds corresponding to 17α-hydroxyprogesterone and androstenedione, respectively.

Finally, to the presumed testosterone-^{14}C and 17α-hydroxyprogesterone-^{14}C, about 10 mg of authentic testosterone and 17α-hydroxyprogesterone were added respectively, and recrystallized several times from the different solvent systems. As shown in Tables 2 and 3, the specific activities of the crystals from the both metabolites were constant in the last three crystallizations.

On the other hand, the materials (E–10 and 12) corresponding to androstenedione

Table 2. Recrystallization of testosterone-^{14}C isolated from incubation of progesterone-4-^{14}C with the testes of crab.

Crystallizations	Solvent systems	Specific activities (dpm/mg)
1 st	hexane-acetone	6300
2 nd	dichloromethane-hexane	6200
3 rd	hexane-acetone	6150

Table 3. Recrystallization of 17α-hydroxyprogesterone-^{14}C isolated from incubation of progesterone-4-^{14}C with the testes of crab.

Crystallizations	Solvent systems	Specific activities (dpm/mg)
1 st	acetone-water	3280
2 nd	hexane-acetone	3650
3 rd	hexane-acetone	3310

and deoxycorticosterone lost the radioactivities during the chromatographic purification steps by TLC in systems S2 and S5. Accordingly, further analysis was not carried out.

Discussion

In fish, androgen, progesterone, and estrogen were isolated from the testes of several species of teleosts and elasmobranches: the dogfish, *Scyliorhinus stellaris*[11], rainbow trout, *Salmo irideus*[12], carp, *Cyprinus carpio*[12], some marine teleost, *Morone labrax*[13], and sockeye salmon, *Oncorhynchus nerka*[14]. Moreover, the *in vitro* bioconversion of steroid hormones by the testes has been demonstrated in the dogfish, *Squalus acanthias*[15,16], Japanese dace, *Tribolodon hakonensis*[17], rainbow trout, *Salmo gairdneri*[18,19], and Atlantic salmon, *Salmo salar*[20].

In marine invertebrates, however, only a few report has been shown on the presence and metabolism of steroid hormones. GILGAN and IDLER reported that the androgenic gland of the American lobster, *Homarus americanus*, transformed androstenedione to testosterone[3]. TCHOLAKIAN and EIK-NES showed the bioconversion of progesterone to deoxycorticosterone by the androgenic gland of the blue crab, *Callinectes sapidus*.[5]

In the present study, it was found that progesterone was transformed to 17α-hydroxyprogesterone and testosterone by the sliced testes of the crab, *Portunus trituberculatus*. The results indicate the presence of the enzyme systems catalyzing the 17α-hydroxylation and side-chain cleavage of progesterone in the testes of this crab. The bioconversions of progesterone to the above two metabolites were shown previously by the ovaries of this carb[6]. However, the transformation of progesterone to deoxycorticosterone was not demonstrated in the case of testes in contrast to the ovaries. In the blue crab, *Callinectes sapidus*, it was reported that the androgenic gland homogenate

converted progesterone to deoxycorticosterone, and that the steroid 21-hydroxylase activitiy was relatively high as compared with that in postrio vas deferens and hepatopancreas homogenates.[5] In addition, steroid 21-hydroxylase has been demonstrated in the testes of many animals. Therefore, the failure of progesterone to deoxycorticosterone in the present experiment may not necessarily imply the lack of steroid 21-hydroxylase in the testes of this crab.

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References

1) R. ARAI and N. EGAMI: *Hormones and Clinics*. **16**, 97–112 (1968).
2) R. R. HATHAWAY: *Gen. Comp. Endocrinol.*, **5**, 504–508 (1965).
3) M. W. GILGAN and D. R. IDLER: *ibid.*, **9**, 319–324 (1967).
4) D. R. IDLER, G. B. SANGALANG, and A. KANAZAWA: *ibid.*, **12**, 222–230 (1969).
5) R. K. TCHOLAKIAN and K. B. EIK-NES: *ibid.*, **12**, 171–173 (1969).
6) S. TESHIMA and A. KANAZAWA: *ibid.*, in press.
7) S. HARA and M. TAKEUCHI: *Endocrinol. Japan*, **10**, 202–207 (1963).
8) R. ARAI: *Annot. zool. Japan.*, **40**, 136–144 (1967).
9) S. TESHIMA and A. KANAZAWA: *Comp. Biochem. Physiol.*, **38**, 597–602 (1971).
10) D. R. IDLER, N. R. KIMBALL, and B. TRUSCOTT: *Steroids*, **8**, 865–876 (1966).
11) G. CHIEFFI and C. LUPO: *Nature*, **190**, 169–170 (1961).
12) L. GALZIGNA and U. D'ANCONA: *Atti Accad. Nazl. Lincei, Rend., Classe. Fis., Mat. Nat.*, **31**, 92–95 (1961).
13) C. LUPO and G. CHIEFFI: *ibid.*, **34**, 443–446 (1963).
14) D. GRAJCER and D. R. IDLER: *Can. J. Biochem. Physiol.*, **41**, 23–30 (1963).
15) T. H. SIMPSON, R. S. WRIGHT, and S. V. HUNT: *J. Endocrinol.*, **31**, 29–38 (1964).
16) T. H. SIMPSON, R. S. WRIGHT, and J. RENFREW: *ibid.*, **31**, 11–20 (1964).
17) R. ARAI, M. SHIKITA, and B. TAMAOKI: *Gen. Comp. Endocrinol.*, **4**, 68–73 (1964).
18) R. ARAI and B. TAMAOKI: *Can. J. Biochem.*, **45**, 1191–1195 (1967).
19) R. ARAI and B. TAMAOKI: *Gen. Comp. Endocrinol.*, **8**, 305–313 (1967).
20) D. R. IDLER and H. C. MACNAB: *Can. J. Biochem.*, **45**, 581–589 (1967).