魚皮の生化学的研究II

誌名	日本水産學會誌
ISSN	00215392
著者	林, 征一
巻/号	37巻6号
掲載ページ	p. 508-512
発行年月	1971年6月

Biochemical Studies on the Skin of Fish—II. Seasonal Change of Purine Content of Masu Salmon from Parr to Smolt

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(Received September 9, 1970)

On catadromous group of young *Oncorhynchus masou* the guanine and hypoxanthine in the skin were determined in different seasons. Guanine content was highest in August for one year fish and in March for two years fish being 31.16 and 33.15 μmol/g skin respectively. The silvery coloration was observed on sample fish in either end March or end April being 5.52 μmol/g skin for the latter, not corresponding to the amount of the guanine deposited in the skin.

Guanine and hypoxanthine have been reported from the skin of many fish[1,2]. The author has also found the above two purines in the skin of masu salmon in large quantity alongwith inosine, IMP, ADP and pteridine as minor components.

EALES reported a comparative study on purines in some freshwater fishes in relation to their silvering phenomenon[3,4]. The author reports in this paper the seasonal change of guanine and hypoxanthine content in the skin of masu salmon, *Oncorhynchus masou*, during its biological discoloration from parr to smolt, to put more light on this phenomenon with which have mostly been dealt from biological viewpoint.[5–7]

Experiment

All the samples were caught in the same river near Hakodate. In Table 1 are shown the age, the numbers of fish, the date of their capture, total length, body weight and skin weight of fish used in the experiments.

The skin was torn off immediately after killing by clubbing, and extracted with 4 parts of 0.6 N $HClO_4$ by skin weight. The extraction was repeated by centrifugation of 4000 rpm for 10 minutes. The combined extracts were neutralized with 10 N KOH and made to pH 6–7. The precipitate of $KClO_4$ was removed by centrifugation at 3000 rpm for 10 minutes. The supernatant was separated from contaminants by passing through an activated charcoal column. After washing the column throughly with 1 litre of distilled water, ammoniacal ethanol (ammonia: 95% ethanol: water=2:48:50) was poured onto the column to elute the adsorbed materials. The eluate was concentrated immediately in vacuo at 30° by using a rotary evaporator. Then the concentrate was

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Table 1. The date of capture, age, numbers of fish, total length, body weight and skin weight of fish used in experiments.

Date	'69 7–20	'69 8–21	'68 10–13	'68 12–22	'69 2–25	'69 3–1	'69 3–21	'69 4–30
Age	One-year old (0+)				Two-year old (1+)			
No. of fish sampled	3	4	9	5	4	4	3	2
T.L. (cm)	9.8	12.1	11.5	11.0	12.4	12.0	13.7	15.8
B.W. (g)	9.5	16.8	14.0	9.6	17.2	15.5	23.5	44.9
Skin W. (g)	1.4	2.5	2.4	2.5	2.5	2.0	2.5	2.5

poured onto ion-exchange columns, Dowex 1×8 (100–200 mesh) and Dowex 1×2 (200–400 mesh). The eluting solutions were formic acid and sodium formate for the Dowex 1×8 column to isolate nucleotides and pteridine, and 0.1 N NH_4OH–0.035 N HCl–0.005 N $Na_2B_4O_7$ as A solvent (pH 9.0) and 0.001 N HCl–2×10^{-4}N $Na_2B_4O_7$ as B solvent (pH 2.7) for the Dowex 1×2 column. The effluents were fractionated per 10 ml by tubes using a fraction collector. The optical density at 260 mμ of each fraction was measured by a spectrophotometer (Shimazu QR–50 type). Then the fractions were combined, poured onto an activated charcoal column and eluted with ammoniacal ethanol and the eluate was evaporated to dryness in vacuo at 30°. The dried residue was dissolved either in water or 0.1 N HCl and spotted on a filter paper (Toyo Roshi No51–A, 51 and 3). The solvents for paper chromatography were as follows. The chromatography was performed by one-way descending method at room temperature.

1. 95% ethanol-M ammonium acetate (pH 7.5) (75:30 v/v)
2. 95% ethanol-M ammonium acetate (pH 3.8) (75:30 v/v)
3. 1-butanol-1-propanol-ethanol-25% NH_4OH-water (8:8:2:9:3 v/v/v/v/v)
4. 1-butanol-water-formic acid (38.5:6.5:5.0 v/v/v)
5. 2-propanol-conc. HCl-water (21.2:5.1:4.9 v/v/v)
6. methanol-conc. HCl-water (7:2:1 v/v/v/v)
7. 1-butanol-acetic acid-water (4:1:1 v/v/v)
8. 2-propanol-1% ammonia (2:1 v/v)
9. 2-propanol-2% ammonium acetate (1:1 v/v)

After developing, spots detected by a UV lamp were eluted separately with 4 ml of either water or 0.1 N HCl and their UV spectra, total phosphate and ribose were determined. The total phosphate was measured by FISKE and SUBBAROW's method[8] improved by NAKAJIMA[9]. The ribose was measured by orcinol method[10]. The authentic reagents used were as follows; Hx, 5′–IMP, G and ADP from Sigma Chemical Co. Inosine from Tokyo Kasei Kogyo Co. Ltd. and guanosine from Kanto Kagaku Co. Ltd.

Abbreviation G; guanine, Hx; hypoxanthine, HxR; inoshine, IMP; inosine monophosphate, ADP; adenosine diphosphate.

Results and Discussion

The ion-excahnge chromatogram by Dowex 1×8 and Dowex 1×2 columns are shown in Fig. 1–I and Fig. 1–II, respectively.

Substances responsible for peaks a and c were not identified due to their small amount. The substance responsible for the peak b showed absorption maxima at 270 mμ and 250 mμ, but was not identified. ADP and pteridine were separated by paper chromatography using a solvent system No. 8. Rf values of ADP and pteridine were 0.57 and 0.47, respectively. UV spectra of pteridine is shown in Fig. 2.

The results of analysis are shown in Table 2. The sum of G and Hx and their ratios (G/Hx) are shown in Fig. 3.

A remarkable increase in the sum of guanine and hypoxanthine was observed in August for one year old fish and in March for two years old fish. The former season coincided with an occasion when the fish changed from parr to silvery parr, and the latter season from silvery parr to smolt. The silvery coloration of fish has often been reported to be caused by the deposition of guanine in the skin, but according to the author's present data the silveriness did not necessarily correspond to the amount of gua-

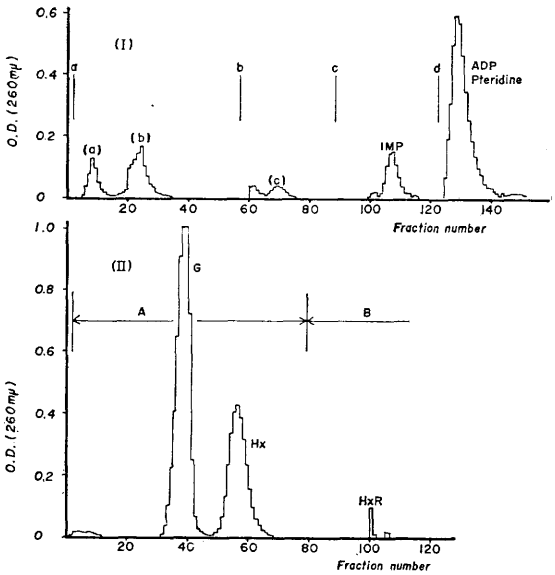

Fig. 1. Diagrams of ion-exchange chromatography of purine basis of samples on Dowex 1×8 column (I) and Dowex 1×2 column (II). The eluting solutions were a: 0,005 M formica cid (F.A.), b: 0.1 M F.A., c: 0.1 M F.A.+0.1 M sodium formate (S.F.) and d: 0.1 M F.A.+0.1 M S.F. for Dowex 1×8 column. The eluting solutions for Dowex 1×2 column are described in the paper.

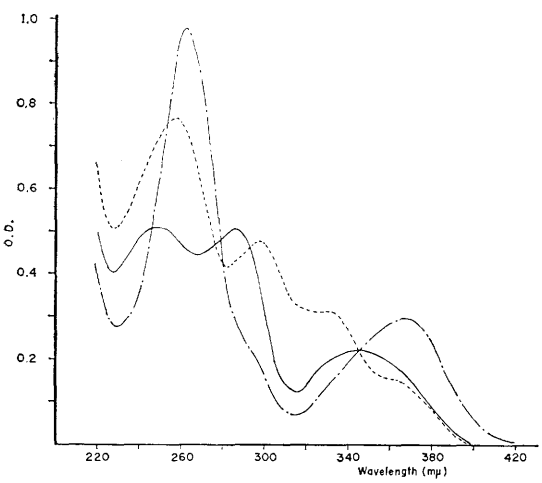

Fig. 2. The UV apsorption spectra of pteridine found in the samples.
——, pH 6–7; ———, pH 10–11; – – –, pH 1–2

Table 2. The analytical data of the acid-soluble fraction in the skin of masu salmon.

Date	'69 7–20	'69 8–21	'68 10–30	'68 12–22	'69 2–25	'69 3–1	'69 3–21	'69 4–30
Hx	8.86	11.84	7.16	4.71	6.02	10.84	10.34	16.82
G	24.32	32.61	12.26	15.49	7.66	30.99	33.15	5.52
Hx+G	33.18	44.45	19.42	20.20	13.68	41.83	43.49	22.34
G/Hx	2.74	2.76	1.71	3.29	1.27	2.87	3.19	0.33
HxR	0.89	0.80	0.74	0.32	0.27	0.33	0.29	0.50
IMP	1.20	0.64	0.74	0.79	0.81	0.54	1.65	1.10
ADP	+	+	+	+	+	+	+	+
Pteridine	+	+	+	+	+	+	+	+

μmoles/g

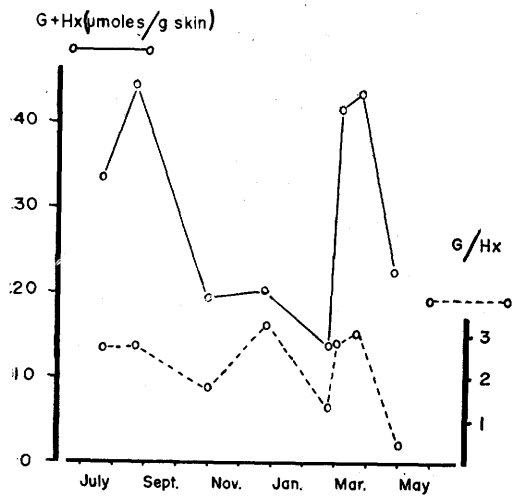

Fig. 3. The seasonal variation of the sum of G and Hx and their ratio (G/Hx).

nine in the skin, as the body colors of the sample fishes on the above two occasions were both with parr marks instead of being silvery, while the guanine content being 32.61, 30.99 μ moles per gram of skin respectively. On the other hand, sample fishes of two years old in end March and end April, the guanine content were 33.15, 5.52 μ moles per gram of skin respectively, the difference of the two being as big as about six times, while the latter samples did presented typical silvery color as the former did. These findings may be supported by an experimental data by the same author that an injection of thyroxine causes the silveriness on the fish about which will be reported in the future paper.

The sum of guanine and hypoxanthine and the ratio G/Hx has positive correlation as is found in Fig. 3. HxR, IMP, ADP and pteridine did not observed to change seasonally.

Acknowledgment

The auther wants to express his sincere gratitude to Professor T. SAITO, Mr. T. KUBO and Mr. J. KOSAKA for their cordial advice.

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