Comparative Electropherograms of Muscle Proteins of Three Species of Lizard Fishes referable to the Genus *Saurida*

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In 1935, the lizard fishes distributing all over the world were reviewed by NORMAN¹⁾, who recognized nine species in the genus *Saurida*. Of nine species, at least three ones, *S. undosquamis* (RICHARDSON), *S. tumbil* (BLOCH) and *S. elongata* (TEMMINCK et SCHLEGEL) were found from the waters of Japan and its adjacent regions by some ichthyologists such as TATARA²⁾, MATSUBARA and IWAI³⁾. But the classification of this group was not yet so clear so far as the external and internal characters alone were used as criteria.

In the studies of biochemical systematics of fishes, composition of the muscle proteins has often been used as a criterion for identification of some closely related fishes, and for analysis of intra-generic and intra-familial relationships.⁴⁻¹⁵⁾ As to the electrophoretic methods, starch-gel electrophoresis has been often used as a tool for separation of muscle proteins. Although the cellulose acetate electrophoresis was used for the separation of elasmobranch serum proteins by HESS and SMITH¹⁶⁾, it has scarcely been employed to analysis of the composition of teleostean fish muscle proteins, as far as the author's knowledge is concerned. The aim of the present investigation is to describe the differentiation of three Japanese species of the genus *Saurida* based on the property of muscle proteins by means of the handy method of cellulose acetate electrophoresis. The author has found that the fish muscle proteins of the lizard fish stored at -20°C are very stable in composition, showing the species specificity.

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Materials and Methods

The samples of these three species were collected from the waters of Maizuru, Kyoto Prefecture; Yoshimi, Yamaguchi Prefecture; Akashi, Hyogo Prefecture; and East China Sea: *S. undosquamis*; 7 specimens, 26.5–34.5 cm in fork length, collected at Maizuru, in Sept. 1967, 2 specimens, 20.6 and 22.2 cm, at Yoshimi, Nov. 1967. *S. tumbil*; 10 specimens, 27.0–34.0 cm, in East China Sea, July 1967, 2 specimens, 36.6 and 38.0 cm, at Maizuru, June 1967, 1 specimen, 42.5 cm, at Maizuru, Sept. 1967, 1 specimens, 54.5 cm,

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at Maizuru, June 1968. *S. elongata*; 7 specimens, 27.0–38.0 cm, at Maizuru, Aug. 1967, 1 specimen, 34.0 cm, Maizuru, Aug. 1967, 2 specimens, size unknown, at Akashi, Nov. 1967.

The samples were stored at -20°C in a deep freezer for 1 to 45 days after collection. The muscle extract for electrophoresis was prepared by the following methods: a small quantity of muscle, usually 5 gr, obtained from a part of trunk under the first dorsal fin, was homogenized by a Waring blendor at room temperature, with 10 ml of the solution consisting of the same volumes of glycerol and Veronal buffer solution with pH 8.6 and ionic strength of 0.06. The glycerol was added to maintain the stability of the resulting solution. The homogenized material was centrifuged for 15 minutes at 10,000 rev/min (12,000 G), and then the supernatant fraction was used as sample for cellulose acetate electrophoresis. The cellulose acetate electrophoresis was principally operated by OGAWA's method¹⁷⁾. Electrophoretic apparatus manufactured by Jôko Sangyo Co., Ltd., was employed for the separation of muscle extracts. The cellulose acetate membrane used here was a product, "Separax" made by Fuji Shashin Film Co., Ltd. The electrophoresis was carried out for 2 hours with current of 0.3 mA per 1 cm width of sheet. Veronal buffer solution for electrophoresis was changed each time in a series of experiments to compare electropherograms on the same condition. Muscle extracts were introduced carefully on the surface of sheet by means of 0.0008×5 ultra micropipette. Muscle proteins were stained with Ponceau 3R. The sheet of cellulose acetate was made transparent with the liquid paraffin, and traced with a Ozumor 7 Atago self-recording densitometer manufactured by Atago Optical Works Co., Ltd. The subsequent densitometric patterns were analysed for the quantitative and qualitative comparisons of muscle proteins.

Results

The stability of electropherograms of specimens stored at -20°C : The stability of densitometric patterns of muscle proteins of *S. tumbil* was tested by the specimens stored at -20°C for 1 to 45 days (Fig. 1), the muscle extracts being kept at $0-4^{\circ}\text{C}$ for various periods (Table 1). The densitometric pattern has mainly four peaks, which were named Nos. 1–4 respectively from the anode side. The component existing at origin was excluded, because the variation was observed in the percentage of the component which is considered to be affected by the technical factors. There were little differences in the percentages and mobilities among the samples stored for various periods (Fig. 1, Table 1). Therefore, it may be concluded that the specimens stored at -20°C are probably available until at least 45 days after sampling, and the protein samples extracted by the salt solution of low ionic strength also can be used for the electrophoretic investigations until at least 15 days at $0-4^{\circ}\text{C}$ after being extracted.

Table 1. Change of electrophoretic pattern of muscle proteins of *S. tumbil* during storage. Samples were stored at -20°C and protein extract at $0-4^{\circ}\text{C}$.

Date of sampling	Date of extracting	Date of experiment	Percentage in densitometric pattern			
			Comp. 1	Comp. 2	Comp. 3	Comp. 4
15th, June	15th, June	15th, June	4.0	31.7	44.4	19.8
15th, June	22th, June	22th, June	6.3	29.3	41.8	22.6
15th, June	29th, June	29th, June	9.4	27.5	43.3	19.9
15th, June	29th, June	5th, July	7.2	26.4	44.8	21.6
15th, June	29th, June	12th, July	5.7	26.2	44.0	24.1
15th, June	5th, July	5th, July	8.8	26.7	43.0	21.1
15th, June	28th, July	28th, July	8.5	29.7	41.5	20.3

Fig. 1. Densitometric patterns of muscle proteins of *S. tumbil* stored at -20°C for various periods. Arrows show the origin.

Comparison of electropherograms among three species: The electrophoretic patterns were examined here from three aspects, such as the number of components, and the mobility and percentage of each component. The number of components was 8 in *S. elongata*, 7 in *S. tumbil* and 5 in *S. undosquamis* as shown schematically in Fig. 2. In order to compare the percentage of each component, these components appearing in electropherograms were conveniently grouped into three parts which were designated here

Fig. 2. Schematic electrophoretic patterns of muscle proteins of three lizard fishes. Arrow shows the origin.

Fig. 3. Densitometric patterns of muscle proteins of three lizard fishes. Percentages of three parts A, B and C were calculated from those patterns. Arrows show the origin.

as A, B and C respectively from the anode side (Fig. 2). The typical densitometric patterns of three species are shown in Fig. 3. The distinct differences in the electrophoretic patterns were recognized among these three species. The percentage of the parts of each species was useful in comparing the properties of species and in tracing the tendency of inter-specific variations. The percentages of part B ranged from 56.8% to 68.9% in 10 samples of *S. elongata*, from 71.9% to 81.0% in 15 samples of *S. tumbil* and from 85.3% to 95.3% in 9 samples of *S. undosquamis* respectively. The average value of part B was small in *S. elongata* (63.1%), large in *S. undosquamis* (89.9%) and intermediate in *S. tumbil* (76.4%). The percentages of part C ranged from 25.6% to 35.7% in *S.*

elongata, from 12.0% to 20.9% in *S. tumbil* and from 0.4% to 4.2% in *S. undosquamis*. The average value of part C was small in *S. undosquamis* (2.1%), large in *S. elongata* (29.8%) and intermediate in *S. tumbil* (15.6%). In identifying three species of the lizard

fishes, the range of percentages of part B or C is useful because it does not overlap among species treated.

Discussion

Based on their elaborate works, TSUYUKI *et al.*¹¹⁾ stated that muscle protein (myogen) patterns were virtually unaffected by factors other than genetic factor, showing species specificity. YAMASHITA¹⁸⁾ showed that the electrophoretic patterns of serum protein of rock fishes are reproducible within only 3 days after sampling and differentiation of peaks lessened with the prolongation of preservation. The author, however, observed here that the electrophoretic patterns of the lizard fish muscle protein were very stable for at least 45 days when the muscle was kept at -20°C . Judging from the result obtained above, the muscle protein may be stored longer than 45 days after sampling, without showing any change in electrophoretic pattern. Therefore, it is evident that the electrophoretic characters of the muscle proteins, such as the number of components and the percentage and mobility of each component are more reliable than those of the serum protein, and available for the study of biochemical systematics.

The author found the distinct inter-specific differentiation in the electrophoretic patterns of muscle proteins of three lizard fishes. *S. undosquamis* remarkably differs from *S. elongata*, and *S. tumbil* is intermediate between those two species. This tendency well accords to that of divergence of the morphometrical data given by MATSUBARA and IWAI³⁾ (Table 2).

Table 2. Electrophoretic and morphometrical characters, the latter of which were cited from MATSUBARA and IWAI in 1951, of three lizard fishes.

Species	Percentage of part B	No. of components	Scales in lateral line	No. of Vertebrae
<i>S. elongata</i>	63.1	8	65	60
<i>S. tumbil</i>	76.4	7	58	53
<i>S. undosquamis</i>	89.9	5	51	48

According to TATARA²⁾, *S. elongata* is divided into two subpopulations, the one being provided with less vertebrae, 58 in mode and the another with more vertebrae, 63. The specimens examined in this study belong to the former group. Lately, MATSUBARA and OCHIAI¹⁹⁾ expressed an opinion that the difference observed between these two groups is suited at the specific level. In the near future, the author wants to compare these two groups by the electrophoretic method to solve this problem.

Summary

The cellulose acetate electrophoretic patterns of three species of lizard fishes, *Saurida*

undosquamis, *S. tumbil* and *S. elongata* were observed. The property of muscle proteins soluble in salt solution of low ionic strength in each species was analyzed for the mobility and percentage of each component, and the number of components in the electrophoretic pattern. Although the differentiation among these three species was distinct, *S. undosquamis* was more similar to *S. tumbil* than to *S. elongata*, and *S. tumbil* was intermediate between *S. undosquamis* and *S. elongata* in this character. This trend was parallel with that of the morphological evidence.

It is also verified that electrophoretic patterns of muscle proteins were very stable for at least 45 days, when the specimens were stored at -20°C .

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