Short Paper

Occurrence of Balenine in the Muscle of Eel^{*1}

Balenine (β -alanine-3-methyl-L-histidine), which is contained in the muscles of baleen whales in high concentrations, has been reported to be very scanty, if present, in the muscles of teleosts.¹⁾ During the automatic analysis of free amino acids in the eel muscle, however, we noticed a fairly large peak suspected to be balenine. The present paper reports its isolation and identification.

The eels *Anguilla japonica* used in this experiment were caught at the Lake of Hamana, Shizuoka Prefecture, in January, 1978 and raised on a commercial diet for culturing eel for about 5 months. The muscle was removed from about 40 fish (body weight, 6–15 g), frozen in dry ice, and stored at -20°C until used. Picric acid extract was prepared from 100 g of the muscle according to the method of STEIN and MOORE²⁾, and a half portion of the extract was chromatographed on a column of Dowex 50W-X4 (200–400 mesh, lutidine form, 2.5×26 cm). The column was eluted with 0.1 M lutidine and 10 ml fractions were collected. Each fraction was monitored by TLC (plate, cellulose; solvent, phenol saturated with 6 N HCl; temperature, 30°C ; detection, ninhydrin reagent). The remaining half of the extract was treated similarly. After fractions containing balenine (Fr. No. 22–34) were pooled and evaporated to dryness *in vacuo*, the residue was dissolved in a small amount of water and decolorized by being passed through a column of Amberlite XAD-2 (1×4 cm). The eluate was evaporated to dryness *in vacuo* and the residue was crystallized from aqueous ethanol. Recrystallization from 75% ethanol gave colorless needles (yield, 7 mg).

TLC of this substance carried out as described above gave a spot (Rf, 0.73), which was coincided well with that of authentic balenine. Acid hydrolysis of it with

6 N HCl at 110°C for 16 h afforded β -alanine and 3-methylhistidine, which were identified by TLC [plate, cellulose; solvents, 95% ethanol–28% ammonium hydroxide (85:15, v/v) and 1-butanol–formic acid–water (75:15:10, v/v); Rf, 0.23 and 0.37 for β -alanine, 0.33 and 0.21 for 3-methylhistidine, respectively]. Spectra of ^1H NMR [D_2O ; internal reference, sodium salt of 3-(trimethylsilyl)-propanesulfonic acid; δ 2.64 2H, t, $J=6$ Hz; 2.92 1H, d, $J=7$ Hz; 2.99 1H, d, $J=4$ Hz; 3.21 2H, t, $J=6$ Hz; 3.64 3H, s; 4.41 1H, dd, $J=4, 8$ Hz; 6.89 1H, s; 7.53 1H, s] and IR (KBr, 3460, 3210, 1635, 1560, 1550 cm^{-1}) also coincided well with those of authentic balenine.

Thus the occurrence of balenine in the eel muscle was established for the first time. The content of balenine in the muscle determined by the method of ABE³⁾ was $126\text{ }\mu\text{mol}$ (30 mg)/100 g, the value being considerably higher than those reported so far on a few species of teleosts.¹⁾ The dipeptide may be of endogenous origin, since the diet was deficient in it.

The authors are grateful to Prof. M. SUYAMA of Tokyo University of Fisheries for a gift of balenine, to Dr. S. ARAI of the National Research Institute of Aquaculture for supply of the eel, and to Dr. H. ABE of Kyoritsu Women's University for assistance in determining balenine.

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References

- 1) M. SUYAMA, T. SUZUKI, M. MARUYAMA, and K. SAITO: *Bull. Japan. Soc. Sci. Fish.*, **36**, 1048–1053 (1970).
- 2) W. H. STEIN and S. MOORE: *J. Biol. Chem.*, **211**, 915–926 (1954).
- 3) H. ABE: *Bull. Japan. Soc. Sci. Fish.*, **47**, 139 (1981).

(Received February 18, 1981)

^{*1} ウナギ筋肉中のバレンインの存在について.

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