マボヤにおけるハロシニンの生合成

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Biosynthesis of Halocynine in the Ascidian *Halocynthia roretzi*[*1]

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In order to know whether halocynine, a novel betaine discovered by us in the muscle of the ascidian *Halocynthia roretzi*, is endogenous or not, the incorporation of radioactivity into it was examined by injecting L-(U-^{14}C)lysine into the muscle of ascidian specimens. Halocynine isolated from the muscle extract by ion-exchange column chromatography and cellulose thin layer chromatography was found to be radioactive in the specimen which had been kept in aerated sea water for 10 days after the administration of radioactive lysine. However, the isolated halocynine exhibited only a very weak activity and no activity in the specimens kept for 90 and 60 h, respectively. These results unequivocally indicate that the ascidian is capable of synthesizing halocynine, although the biosynthetic rate is very slow under the conditions employed.

In a series of our studies on the nitrogenous extractive components in the muscle of the ascidian *Halocynthia roretzi*,[1–4] we discovered a novel betaine named halocynine. We also elucidated its chemical structure to be (*R*)-2-hydroxy-6-trimethylammoniohexanoate,[2] a 2-hydroxylated analogue of laminine (6-*N*-trimethyllysine),[5] and demonstrated that a considerable amount of halocynine was present in the ascidian muscle all the year round.[4] Recently, Park *et al.*[6] have reported its occurrence in the same species of ascidian cultured at the southern coast of Korea.

The present study was undertaken to show that halocynine is derived from L-lysine *in vivo* like laminine[7–9] and its 2-oxo derivative.[10] This paper deals with the results.

Materials and Methods

Radioactive Reagent

L-(U-^{14}C) Lysine hydrochloride (specific radioactivity 12.7 GBq/mmol, radiochemical purity 96.3%) was purchased from Amersham International plc (Buckinghamshire, England).

Ascidian Specimens and Administration of Radioactive Compound

Live ascidians collected from the Bay of Ofunato, Iwate Prefecture, were transported to our laboratory in aerated sea water. Each of 3 specimens was given a muscular injection of L-^{14}C-lysine (370 kBq) in 0.5 m*l* saline. They were kept in aerated sea water tank (about 16 *l*) at 19°C for 60, 90, and 240 h. No feed was given during the experimental period.

Isolation of Halocynine

The muscle was removed from each of the above specimens and extracted with 1% picric acid according to the method of Stein and Moore.[11] The extract was passed through an Amberlite CG 400 column (Type II, OH^- form, 1×10 cm) followed by an Amberlite IRC 50 column (H^+ form, 1×10 cm) and the columns were washed with distilled water. The effluent and washings were combined and concentrated under reduced pressure in a rotary evaporator. The resulting solution was placed on a column of Dowex 50 W-X 4 (200–400 mesh, H^+ form, 1×34 cm). The column was successively rinsed with 200 m*l* each of distilled water and 0.005 N HCl and then developed with 1 N HCl collecting in 10 m*l* cuts. The elution of halocynine was monitored by thin layer chromatography (TLC) as described below. The fractions containing halocynine were combined and concentrated under reduced pressure. For further purification of halocynine, the concentrate was put on a column of Dowex 50 W-X 4 (200–400 mesh,

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1×30 cm) previously equilibrated with 0.1 M pyridine-acetic acid buffer (pH 3.75), and eluted with the same buffer. The eluate was collected in fractions of 5 ml. TLC was employed for identification of halocynine.

TLC

Precoated cellulose plates (Avicel SF, Funakoshi Co., Ltd., Tokyo) were used with the following solvent systems (v/v): (A) 95% ethanol-28% ammonium hydroxide (85 : 15); (B) chloroform-methanol-17% ammonium hydroxide (2 : 2 : 1); (C) 2-propanol-acetic acid-water (20 : 1 : 5); (D) phenol-water (3 : 1). The spot of halocynine was detected by spraying the Dragendorff reagent. Halocynine synthesized from L-lysine as described in the previous report[2] was used as the standard.

Liquid-scintillation Techniques

Samples (0.5 ml) of muscle extracts and fractions from Dowex 50 W-X 4 columns were added to counting vials containing 5 ml of Aquasol L-2 (NEN Research Products, Massachusetts) and the radioactivity measured on an Aloka LSC-670 liquid-scintillation spectrometer. The same counting cocktail was also used for measuring the activity of spots on thin layer plates.

Results and Discussion

The specific radioactivity of the muscle extract from the ascidian specimen kept for 10 days after ^{14}C-lysine administration was 42,000 dpm per 1 g of muscle. A portion of the extract corresponding to 4 g of muscle was passed successively through Amberlite CG 400 and Amberlite IRC 50 columns. When the effluent combined with the washings was concentrated its total radioactivity was 4,250 dpm. The concentrate was then fractionated on Dowex 50 W-X 4 (H^+ form) column with 1 N HCl. Figure 1 shows the elution profile of radioactivity and thin layer chromatogram of some fractions. The highest radioactivity peak was found to correspond precisely with fractions containing halocynine as identified by TLC using the solvent A. The fractions were combined, concentrated, and subjected to TLC as above. A significant radioactivity on TLC was also associated with the migrated position of halocynine, indicating that a part of the radioactivity of ^{14}C-lysine administered to the ascidian was incorporated to halocynine *in vivo*.

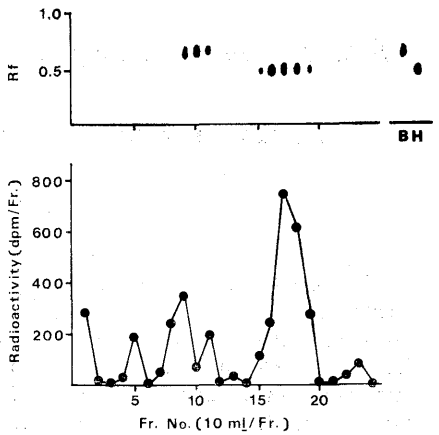

Fig. 1. Elution profile of radioactivity in Dowex 50W (H^+ form) column chromatography with 1 N HCl and TLC of each fraction with cellulose plate and 95% ethanol-28% ammonium hydroxide (85 : 15, v/v). B and H indicate authentic glycinebetaine and halocynine, respectively.

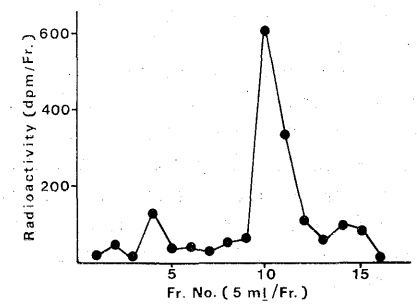

Fig. 2. Elution profile of radioactivity in Dowex 50W-X4 (pyridine form) column chromatography.

In order to confirm the above finding, the second chromatography on Dowex 50 W-X 4 (pyridine form) column was successively carried out. Figure 2 shows the radioactivity profile of the eluate. The major radioactivity peak was observed between Frs. 10 and 12, and halocynine was detected only in those three fractions on TLC with solvent A. They were combined, evaporated, and subjected to TLC with 4 solvent systems. The radioactive area on each chromatogram coincided with the area of halocynine, which was identical in Rf value with the spot of authentic halocynine (Fig. 3). These results unequivocally demonstrate that L-lysine was converted into halocynine.

The same experiments as described above were performed by using the ascidian specimens kept for 60 and 90 h after injection of ^{14}C-lysine.

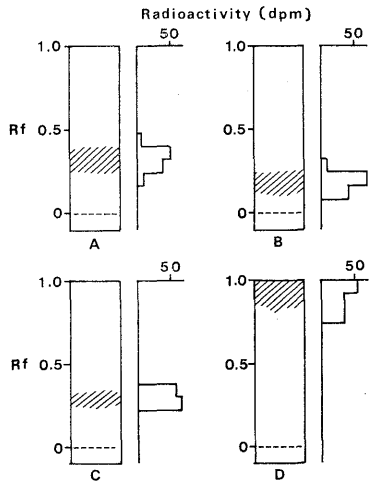

Fig. 3. TLC of halocynine fractions following Dowex 50W-X4 (pyridine form) column chromatography. See text for the stationary and moving (A-D) phases.

Only a trace of radioactivity was incorporated into halocynine after 90 h, but not after 60 h.

It can be concluded from the above results that the ascidian is capable of synthesizing halocynine from L-lysine, although the rate of synthesis is very slow under the condition employed in this study. It has been reported that laminine and its 2-oxo variant, both of which are closely related to halocynine, are derived from L-lysine in mammalian tissues[7-9] and lysine-auxotroph *Neurospora crasa*,[10] respectively, as intermediates in the pathway of carnitine formation. These facts strongly suggest that halocynine is also an intermediate product in the carnitine biosynthesis. A further study is necessary to verify this as well as a study to identify the donor of *N*-trimethyl group of halocynine.

Acknowledgements

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