ENZYMATIC OXIDATION OF CYSTEAMINE TO TAURINE IN MARINE MOLLUSC, MYTILUS EDULIS

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Introduction

Taurine is observed by reason of high concentration in the muscles of marine invertebrates, especially marine molluscs. In order to find out from what kinds of substances taurine is formed, changes of the substrate, by using cysteamine or cysteine, were assayed quantitatively when the adductor muscle** of *Mytilus edulis* were used as an enzyme source. Consequently, cysteamine was more consumed than cysteine as detailed in the previous report[1].

In the present report taurine and hypotaurine, which are expected to be enzymatic oxidation products, were separated quantitatively by means of ion exchange chromatography. This method was employed for the analysis of the enzymatic reaction products. After such experiments, cysteamine was found to be oxidized to taurine by the adductor muscle prepared with *Mytilus edulis*.

Experimental

Animals and materials

The marine molluscs, *M. edulis*, were collected at Nanaehama, Kamiisocho, Hokkaido, and horse kidney was obtained from the local slautherhouse. N-ethylmaleimide (NEM) was purchased from the Aldrich Chemical Co. Inc., Milwaukee, Wisconsin, USA. Cysteamine hydrochloride came from the Tokyo Chemical Industry Co., Ltd. All other compounds were commercial products except hypotaurine, which was synthesized as followed.

The synthesis of hypotaurine is due to the procedure of Owen and Wilbraham[2] with some modifications. At first thiosulphonate hydrochloride (0.01 moles) was synthesized from cysteamine hydrochloride (0.02 moles) and hydrogen peroxide (0.03 moles) by the way of Jayson *et al.*[3]. A small amount of sodium iodide (30 mg) was used as catalyst for the reaction according to Cavallini *et al.*[4]. The result solution of thiosulphonate hydrochloride was added to a stirred suspension of Dowex 1-X8 (OH^-, 50–100 mesh) in water (100 ml) during 30 min. Thereupon this basic resin gave an alkaline dismutation of thiosulphonate and at the same time selectively adsorbed hypotaurine split from thiosulphonate. After washing the

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** The "muscle ligaments" in the previous reprot[1] indicates the same muscle that called "adductor muscle" in this paper.

cystamine, the other dismutation product from thiosulphonate, with water (100 ml) the resin bed was passed through with 10% acetic acid (100 ml) during 10 min. Evaporation of the aqueous acetic acid under reduced pressure and addition of ethanol gave crystalline. Recrystalization was performed from aqueous ethanol in a refrigerator. The melting point (175–180°C) and infra-red absoption spectra were in accord with that of hypotaurine as reported by Cavallini *et al.*[5].

Ion exhange chromatography

The chromatographic analysis of taurine and hypotaurine was performed according to the method of De Marco *et al.*[6], with a modification; by using the column (0.5 cm in diameter, 28 cm in height of resin bed) which was designed in such a way that with 20 tubes only (0.5 ml of effluent per tube), taurine and hypotaurine could perfectly be separated. The resin of Dowex 50W-X8 (Na^+, 200–400 mesh) was equilibrated with 0.1 M citrate buffer solution (pH 2.1, 21.01 g of citric acid monohydrate plus 5.84 g of sodium chloride dissolved in 1000 ml of water) and the compounds to be analyzed were dissolved in the same solution.

For the standard chromatogram, 1.0 μ mole of taurine and 1.0 μ mole of hypotaurine were dissolved in 1.0 ml of 0.1 M citrate buffer (pH 2.1) and half a volume of the solution was applied to the top of the column and eluted with the same buffer. Aliquots (0.5 ml) of the effluent were collected in test tubes automatically. On every tube ninhydrine reaction was carried out; to the tubes, 1.0 ml of 0.2 M citrate buffer[7] (pH 5.8, 42.03 g of citric acid monohydrate plus 560 ml of 1 M sodium hydroxide diluted to 1000 ml) and 1.0 ml of ninhydrine reagent[8] (50 ml of 5% ninhydrine in methyl cellosolve plus 250 ml of methyl-cellosolve containing 5 ml of 0.01 M potassium cyanide) were added and mixed. The tubes were capped and heated for 20 min. in a boiling-water bath. After cooling in cold water for 5 min. the samples were diluted with 2.5 ml of 60% ethanol and the absorbance at 570 mμ was determined by the Spectrophotometer, Shimadzu QV 50. A blank value was obtained by using 0.5 ml of 0.1 M citrate buffer (pH 2.1) in place of the effluent.

Under these conditions, the molar extinction coefficient of taurine and hypotaurine are 9.55×10^3 and 21.55×10^3 respectively (Fig. 2). Taurine was found from No. 7 to 9 of fraction number and hypotaurine was found from No. 14 to 16 (Fig. 1). The recoveries of taurine and hypotaurine were 105±1% and 91±4% respectively.

Enzymatic reaction and analysis of the products

Unless stated otherwise, a typical incubation mixture contained 1.0 ml of 0.05 M Tris-HCl buffer, pH 7.4; 0.2 ml of enzyme solution (equivalent to 20 mg of fresh tissue); 5.0 μ moles of substrate, and additional water to a total volume of 2.0 ml. Incubation was carried out in test tubes at 37°C with shaking once every ten minutes, and stopped by the addition of 0.2 ml of 50% trichloroacetic acid.

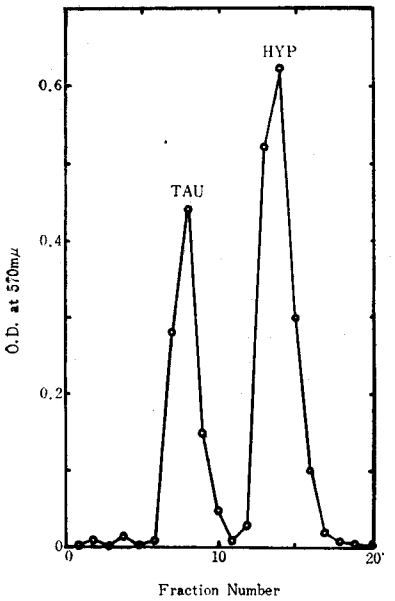

Fig. 1. Ion exchange chromatogram of taurine (TAU) and hypotaurine (HYP)

0.5 μ moles each of taurine and hypotaurine chromatographed on the small column (0.5×28cm) using 0.1M citric acid –0.2M sodium chloride as eluent under room temperature. 0.5ml of effluent per tube.

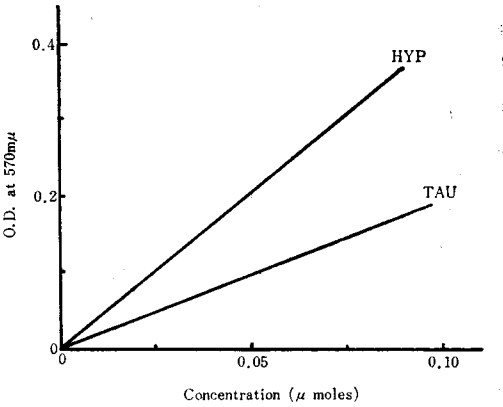

Fig. 2. Standard graph of taurine and hypotaurine

Figures of concentration show μ moles of the compound contained in the etahanol diluted solution (5.0 ml) after ninhydrine reaction.

The precipitate was centrifuged, and the supernatant was treated with ether and after neutralization with ammonia, a large portion of residual substrate was removed with 0.3 g of dry Amberlite IRC–50 (H^+) and the result solution was dried in a desiccator over granular sodium hydroxide and concentrated sulphuric acid. These treatments were carried out quantitatively, and the result products were analyzed by means of ion exhcange chromatography as described above. The chromatograms of the enzymatic reaction products by the adductor muscle preparation of *M. edulis* are shown in Fig. 3.

Changes in cysteamine and taurine during incubation

The amounts of taurine were counted by integral calculus of the square measure of each taurine peaks and plotted against the incubation time as shown in Fig. 4. A. On the other hand the consumption of cysteamine, the substrate, was determined spectrophotometrically with NEM by the same procedure reported previously[1]. The results are shown in Fig. 4. B.

Consequently cysteamine was found to be oxidized to taurine about 30% (against the starting concentration) after 120 min. incubation using adductor

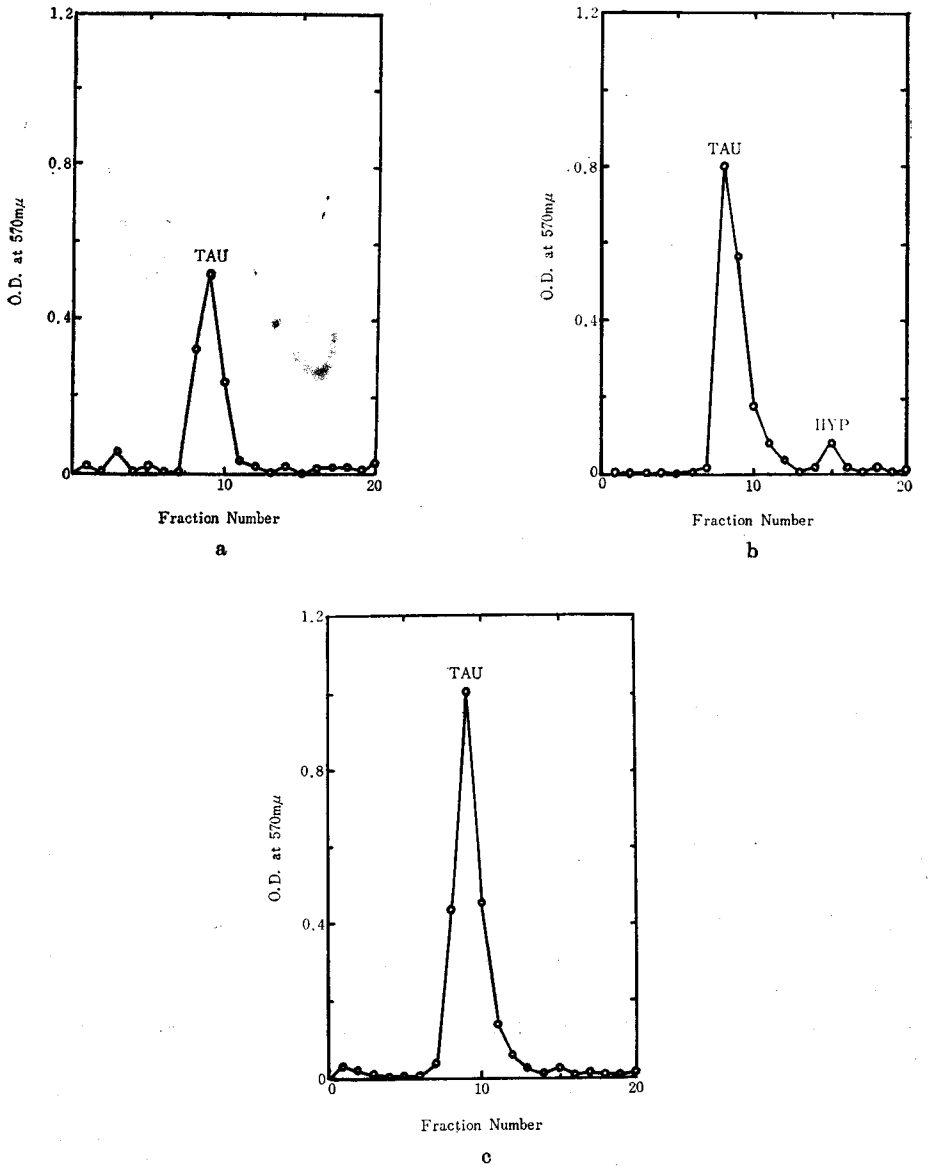

Fig. 3. Ion exchange chromatograms on the enzymatic reaction products through Dowex 50W-X8 (Na^+) column (0.5×28 cm) equilibrated and eluted with 0.1 M citric acid –0.2 M sodium chloride, pH 2.1

Technical details and ninhydrine reaction procedure are reported in the text.

a. Before incubation (the TAU contained in the enzyme solution originally)
b. After 30 min. incubation
c. After 120 min. incubation

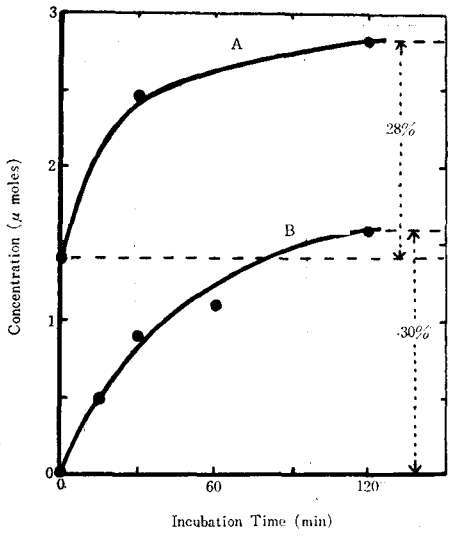

Fig. 4. Taurine production and cysteamine consumption during incubation

The assay system contained 0.2 ml of enzyme solution of fresh adductor muscle of *M. edulis*, 5.0 μ moles of cysteamine at starting and 1.0 ml of 0.05 M Tris-HCl buffer (pH 7.4). Technical details are reported in the text.

A. The amount of taurine production calculated from the ion exchange chromatogram (Fig. 3)

B. The amount of cysteamine consumption determined with NEM; after incubation the same volume of NEM solution (6.0 μ moles NEM in 0.5 M phosphate buffer pH 6.0) was added and calculated from the O.D. at 300 mu in decrease.

muscle preparation of *M. edulis*. But hypotaurine was not produced during incubation, only a small amount of hypotaurine was detected after 30 min. of incubation (Fig. 3. b).

In the same assay system, horse kidney was used as an enzyme source in lieu of adductor muscle of *M. edulis*, in order to make sure whether the system of oxidation of cysteamine to hypotaurine as reported by Duprè *et al.*[9] would exist in live or not; and hypotaurine could not be found increased after incubation for 120 min.

Lastly to make sure that the oxidation system of hypotaurine to taurine by the preparation of *M. edulis* under the same assay condition except the amount of the substrate, 0.25 μ moles of hypotaurine, hypotaurine was slightly decreased but taurine did not change in the amount during incubation for 120 min. as shown in Fig. 5.

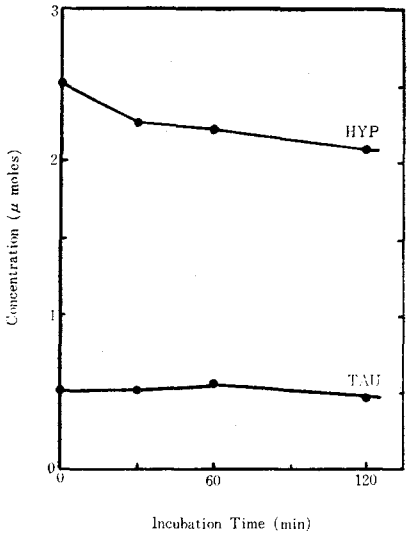

Fig. 5. Unchanges in taurine as reaction product

The incubation mixture contains 0.2 ml of enzyme solution of fresh adductor muscle, 2.5μ moles of hypotaurine, 1.0 ml of 0.05 M Tris-HCl buffer (pH 7.4) and additional water to a total volume of 2 ml. Other technical details are reported in the text.

Discussion

By the quantitative analysis of the enzymatic reaction products by means of ion exchange chromatography using Dowex 50W-X8 (Na^+) column equilibrated and eluted with 0.1 M citric acid–0.2 M sodium chloride, pH 2.1, by which taurine and hypotaurine are separated quantitatively, it appears that the oxidation of cysteamine to taurine is catalyzed by the enzyme solution prepared from the adductor muscle of *M. edulis*.

An enzyme which catalyzes the oxidation of cysteamine to hypotaurine was reported by Cavallini *et al.*[10] and the enzyme source was horse kidney. Under the typical assay system, horse kidney was used in place of adductor muscle of *M. edulis*, but neither hypotaurine nor taurine was detected as being increased during incubation. Moreover hypotaurine was used as the substrate in place of cysteamine but taurine was not produced from this substrate by the preparation of adductor muscle of *M. edulis*.

From these results it can be said that a new enzyme or enzyme system that catalyzes the oxidation of cysteamine to taurine directly or immediately was found in the adductor muscle of *M. edulis*.

Otherwise, about the reducible substance reported in the previous report[1], which has the same mobility as taurine by the electrophoresis at pH 3.1, the

substance seemed to be hypotaurine because of adsorbale characteristic on Dowex 50 (H^+), having the same behavior as hypotaurine synthesized on paper chromatography by the solbents; n-butanol, acetic acid, water=3:1:1 by volume (Rf=0.26) and phenol, water=72.5: 28.5 by volume (Rf=0.56), and the positivity to the iodoplatinate reaction which is very sensitive to sulphinic compound. By the later experiment, one of the thinkable cases is that a small amount of hypotaurine remained in the reaction medium after incubation for 240 min. Nevertheless the constitution of the reaction mixture is different from the typical assay system at the point of concentration of substrate and that of enzyme.

The author is grateful to Professor Tsuneyuki Saito for his kind criticism and for the revision of this paper. Thanks are due to all member of the Laboratory of Biochemistry of our faculty, for the experiments of this report were carried out in the Laboratory under their kind support and helpful advices.

Summary

1. A new enzymatic oxidation of cysteamine to taurine was observed in marine molluscs, *Mytilus edulis*.

2. Taurine was not formed when hypotaurine was used as the substrate under the same assay system.

3. Taurine and hypotaurine were clearly separated by ion exchange chromatography with a small column of Dowex 50.

4. Hypotaurine was synthesized from cysteamine hydrochloride by the modified method of Owen and Wilbraham.

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