Determination of β -Alanine Betaine and Glycine Betaine in Some Marine Invertebrates

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A method for the determination of β -alanine betaine and glycine betaine is described. It is based on an ion-exchange chromatographic separation with a Dowex 50-X12 column and subsequent colorimetric determination of both betaines as reineckate. The recovery was satisfactory, ranging from 97 to 100%. In 7 species of mollusc and 2 species of crustacean estimated by this method, β -alanine betaine was found at a fairly high concentration in the fan-mussel, scallop, and krill and at a very low concentration in the oyster. It was not, however, found in the abalone, hard clam, squid, octopus, or prawn. All of these invertebrates were rich in glycine betaine. In the tissues of fan-mussel and scallop, glycine betaine was found to be omnipresent at a fairly high level, whereas β -alanine betaine localized in the adductor muscle.

Since FLÖSSNER¹⁾ pointed out the presence of β -alanine betaine (β -homobetaine) in the cutaneous muscle utricle of the ascidian species as early as in 1932, its distribution in marine animals had been neglected, until we have recently found a considerable amount of it in the adductor muscle of fan-mussel.²⁾ ABE and KANEDA*² have also recognized it in the scallop and oyster. These findings led us to examine a determination method and to investigate its distribution in various marine invertebrates. The present paper describes a method for the determination of β -alanine betaine and glycine betaine and their concentration in some molluscs and crustaceans.

Materials and Methods

Reagents β -Alanine betaine hydrochloride was synthesized from β -bromopropionic acid and trimethylamine after UTSUNOMIYA.³⁾ A commercially available specimen of glycine betaine hydrochloride (reagent-grade) was recrystallized twice from aqueous ethanol.

Materials and preparation of extract Seven species of mollusc and two species of crustacean as listed in Table 1 were analyzed. Live or fresh specimens were obtained at the Tokyo Metropolitan Wholesale Market with the exception of frozen specimens of krill which were supplied by Nippon Suisan Co., Ltd.

For preparation of extract, 50 g of the material as given in Tables 1 and 2 was homo-

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genized and extracted with 75 ml of ethanol. After centrifugation the residue was extracted twice with 100 ml portions of 66% (v/v) ethanol in the same way. The supernatants were combined and left in a cold room at 4°C overnight. The precipitates were filtered off and the filtrate was concentrated under reduced pressure to about 15 ml. The resulting turbid solution was treated five times with 30 ml portions of diethyl ether to remove lipids and made up to an appropriate volume with water.

Separation and determination of β -alanine betaine and glycine betaine After preliminary experiments, the following ion-exchange chromatographic method was adopted for separation of both betaines.

An aliquot of the above extract is introduced to the top of a column of Dowex 50-X12 (H-form, 200–400 mesh, 0.9×105 cm). The column is rinsed with a small amount of water and 0.7 N HCl is allowed to flow through it at a rate of 30 ml/hr. The effluent is collected in 10 ml cuts and the elution of betaine is monitored by thin layer chromatography (TLC) using cellulose plates (Avicel SF) and a solvent mixture of 95% ethanol and 28% ammonium hydroxide (85:15, v/v). The spots are detected by spraying with the Dragendorff reagent, the R_f value being 0.22 for β -alanine betaine and 0.28 for glycine betaine. The column was heated at 30°C in order to attain the complete separation of both betaines. β -Alanine betaine is usually eluted from the column between Fr. Nos. 73 and 76 and glycine betaine between Fr. Nos. 77 and 95. The fractions containing each betaine are combined and the excess HCl is removed *in vacuo*. The residue is taken up in a small amount of water and the solution is passed through a short column of Amberlite CG-400 (OH-form, 200–400 mesh, 1×10 cm) to remove substances which might interfere with the colorimetric determination of betaines. The column is washed with water and the percolate combined with washings is concentrated under reduced pressure to an appropriate volume.

An aliquot of the above solution is subjected to the determination of β -alanine betaine or glycine betaine according to the colorimetric method of FOCHT and SCHMIDT.⁴¹ It was confirmed that the assay method devised for glycine betaine is applicable without any modifications to β -alanine betaine. The recovery of both betaines added to a sample solution from the fan-mussel was satisfactory, ranging from 97 to 100%.

Results and Discussion

The contents of both betaines are given in Table 1. A considerable amount of β -alanine betaine is found in the adductor muscle of both fan-mussel and scallop. The betaine was detected by TLC in the adductor muscle of oyster, but was too small in quantity to be estimated. It was not recognized in the other four species of mollusc. It may be noteworthy that all of the three species in which β -alanine betaine was detected belong to the Order Pseudolamellibranchia. About 30 mg of β -alanine betaine is found in 100 g of the krill. This may be the first time that its presence was demonstrated in a crustacean. All

Table 1. The contents of β -alanine betaine and glycine betaine in some molluscs and crustaceans (mg/100 g)

Species	Date of sampling (1974) Place of catch	No. of specimens Part of the body analyzed	β -Alanine betaine	Glycine betaine
Abalone 'Kuroawabi' <i>Nordotis discus</i>	Sept. Chiba	3 foot	(—)*	668
Fan-mussel 'Tairagi' <i>Atrina pectinata</i>	Mar. Chiba	5 adductor muscle	136	964
Scallop 'Hotategai' <i>Patinopecten yessoensis</i>	July Miyagi	10 adductor muscle	96	211
Oyster 'Magaki' <i>Crassostrea gigas</i>	Dec. Iwate	75 adductor muscle	trace	805
Hard clam 'Hamaguri' <i>Meretrix lusoria</i>	Aug. Kanagawa	90 adductor muscle	(—)	727
Squid 'Surumeika' <i>Todarodes pacificus</i>	Oct. Aomori	3 mantle	(—)	733
Octopus 'Madako' <i>Octopus vulgaris</i>	Sept. Shizuoka	1 arms	(—)	1,434
Krill 'Okiami' <i>Euphausia superba</i>	Jan. Antarctic Ocean	about 100 whole	28	365
Prawn 'Kurumaebi' <i>Penaeus japonicus</i>	Nov. Kanagawa	6 abdominal muscle	(—)	539

* Not detected.

Table 2. Distribution of β -alanine betaine and glycine betaine in the body of fan-mussel and scallop (mg/100 g)

Part of the body	Fan-mussel* ¹		Scallop* ²	
	β -Alanine betaine	Glycine betaine	β -Alanine betaine	Glycine betaine
Adductor muscle				
striated	105	805	27	608
non-striated	21	684	16	1,023
Mantle	trace	748	(—)* ³	71
Gill	trace	751	(—)	123
Mid-gut gland	trace	1,043	trace	603
Gonad				
male			trace	483
female	(—)	952	trace	354
Retractor muscle	(—)	884		
Others	61	796	(—)	262

*¹ Pooled sample of 6 females caught in Chiba in January, 1975.*² Pooled sample of each 10 males and females caught in Hokkaido in January, 1975.*³ Not detected.

of the molluscs and crustaceans examined here are very rich in glycine betaine, as already pointed out by several workers.⁵⁻⁹⁾

The distribution of both betaines in tissues was examined with the fan-mussel and scallop (Table 2). Although glycine betaine is omnipresent at a fairly high level throughout the tissues tested, β -alanine betaine is present in an appreciable quantity only in the adductor

muscle of both species. In the fan-mussel, the other tissues chiefly consisting of siphon, foot, and labial palp also show a fairly high value of β -alanine betaine.

We described in the previous paper²⁾ that the glycine betaine content of marine invertebrates so far reported should be reviewed, since β -alanine betaine might have interfered with the determination of glycine betaine. In the present work, the distribution of β -alanine betaine was found to be not so wide as we supposed before. This means on the one hand that some of the previous data on glycine betaine may be correct and on the other hand that β -alanine betaine should be still taken into consideration in the determination of glycine betaine in some marine organisms.

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