D- α -Iminopropioacetic Acid Isolated from the Adductor Muscle of Scallop*¹

Minoru SATO*², Yoshikazu SATO*², and Yasuhiko TSUCHIYA*²

(Received September 12, 1977)

In the adductor muscle of the scallop, *Patinopecten yessoensis*, two acidic amino acids were detected and isolated by ion exchange chromatography. One, obtained as needles, was identified as *meso*- α -iminodipropionic acid (I), which has recently been isolated from the muscle of the squid *Todarodes pacificus*. The other compound, obtained as prisms, was inferred to be D- α -iminopropioacetic acid (II) by elemental analysis and mass, NMR, ORD, and IR spectrometries; this was confirmed by comparison with data from an authentic specimen. In the liquid chromatography the peak of II appeared between cysteic acid and taurine, overlapping practically with that of I. It is very interesting to note that both I and II have a D-alanine moiety in the molecule, as do octopine and lysopine.

In the preceding report¹⁾, the authors described the presence of *meso*- α -iminodipropionic acid (I) in the muscle of squid, *Todarodes pacificus*. In continuing study on the distribution of I in molluscs, they detected a new acidic amino acid in the extract from the adductor muscle of scallop, *Patinopecten yessoensis*. It was isolated by ion exchange chromatography and analyzed by means of elemental analysis, mass, NMR, ORD and IR spectrometries. The chemical structure of it was confirmed to be D- α -iminopropioacetic acid (II) by comparison with the authentic specimen. The occurrence of II was established in this paper as one of the components in scallop adductor muscle.

The present paper deals with the isolation and identification of II from the adductor muscle of scallop.

Experimental and Results

Material

Living specimens of the scallop, *Patinopecten yessoensis*, were obtained at a shellfish nursery of Sanrikucho, Iwate Prefecture, in June, 1976.

Isolation and Properties of II

The adductor muscle of scallop (2.8 kg) was minced and extracted three times with 5 l of 80% ethanol at room temperature. The combined filtrates were concentrated under reduced pressure. The residues were dissolved in a small volume of water and defatted with ethyl ether. The aqueous

solution thus obtained was dialyzed against water at 5°C for 24 h. Then the outer solution was applied to a column of Dowex 50W-X8 (H⁺ form, 4×50 cm) and washed with 2 l of water. The passings and washings were fractionated in 200 ml portions. Each portion was concentrated and subjected to paper-electrophoresis using Toyo filter paper No. 50, with pyridine-acetate buffer (pH 3.7), at 500 V, for 2 h¹⁾. Amino acids were detected by spraying ninhydrin reagent. The fractions which revealed a spot of R_{CYSO_3H} 0.8 (relative mobility to cysteic acid) were combined, and concentrated *in vacuo*. The residues were dissolved in a small volume of water and charged on a column of Dowex 1-X8 (acetate form, 3×20 cm). The column was washed thoroughly with water and then eluted with each 300 ml of 1 and 2 N acetic acid. The eluate was fractionated in 10 ml portions and checked by paper electrophoresis as stated above. The appropriate fractions showing ninhydrin reaction at R_{CYSO_3H} 0.8 were combined and concentrated to obtain a crude substance. It showed substantially the same R_{CYSO_3H} value as I. But the Rf value of II appeared in the paper chromatography (butanol, acetic acid, water: 12: 3: 5 v/v) was 0.25, and was different from that of I (0.45). Subsequently, it was recrystallized from aqueous ethanol to afford colorless prisms having mp 207–209°C (decomp.). The yield was 810 mg. Molecular weight determined by the mass spectrum (Fig. 1) was 147. Anal. Found: C 40.89, H 6.20, N 9.58%.

*¹ Studies on the Extract from the Muscle of Molluscs—III

*² School of Fisheries Sciences, Kitasato University, Sanriku-cho, Kesen-gun, Iwate Prefecture, 022-01 Japan (佐藤 実・佐藤美和・土屋靖彦: 北里大学水産学部).

Fig. 1. Mass spectrum of D-α-iminopropioacetic acid.

Fig. 2. NMR spectrum of D-α-iminopropioacetic acid.

Calcd. for $C_5H_9O_4N$: C 40.82, H 6.16, N 9.51%. The NMR spectrum of **II** in D_2O is shown in Fig. 2, which indicates the presence of methyl group at 1.57 ppm (doublet, $J=7.0$ Hz), methylene

group at 3.84 ppm (singlet) and methine group at 3.96 ppm (quartet, $J=7.0$ Hz). Owing to the correspondence of coupling constant ($J=7.0$ Hz), the methyl and methine groups give CH_3CH form. The ORD curve (Fig. 3) of this compound in water showed a negative cotton effect with a peak at 217 nm. $[\phi]_{217}^{25} = -1677$, $[\phi]_{230}^{25} = -1083$, $[\phi]_{250}^{25} = -387$, $[\phi]_{300}^{25} = -116$ ($c=0.114$ in water). The strong absorptions at about 1750 and 1220 cm^{-1} in the IR spectrum (Fig. 4) and the electrophoretic behavior readily suggest that **II** is a monoaminodicarboxylic acid. This was also borne out by mass fragmentation (Fig. 1). Based on the foregoing results, a possible structure of **II** can be expressed as D-α-iminopropioacetic acid.

Fig. 3. ORD spectra of isolated and synthesized D-α-iminopropioacetic acid.

Comparison with Synthesized **II**

II was synthesized from D-alanine (2.0 g) and bromoacetic acid (3.1 g) after the method of ABDERHALDEN²¹. The colorless prisms were obtained: yield 1.5 g. Mp 207–209°C (decomp.). Anal. Found: C 40.88, H 6.25, N 9.47%. Calcd.

Fig. 4. IR spectra of isolated and synthesized D- α -iminopropioacetic acid.

Fig. 5. Liquid chromatography of D- α -iminopropioacetic acid (5 μ M/ml). A, cysteic acid; B, *meso*-J-iminodipropionic acid; C, taurine.

for $C_5H_9O_4N$: C 40.82, H 6.16, N 9.51%. The ORD curve showed a negative cotton effect with a peak at 217 nm: $[\phi]_{217}^{25} = -1687$, $[\phi]_{230}^{25} = -1047$, $[\phi]_{250}^{25} = -367$, $[\phi]_{330}^{25} = -95$ ($c=0.108$ in water). Both isolated and synthesized acids agreed closely with each other in melting point, ORD spectra (Fig. 3) and IR spectra (Fig. 4). This substance showed a peak between cysteic acid and taurine, overlapping practically with I in the liquid chromatography (Fig. 5).

Isolation and Identification of I

The mother liquor freed from II was introduced to a column of Dowex 50W-X8 (H^+ form), and the colorless needles melting at 232–234°C were obtained. The yield was 10 mg. The melting point and IR spectrum were quite identical with those of I that was isolated by the authors recently from the squid muscle. The low yield of this compound may be attributable to incomplete separation from the extract.

Discussion

II was isolated from the extract of adductor muscle of scallop, *Patinopecten yessoensis*. The present paper may be the first to report its occurrence in nature, although it was already synthesized by STADNIKOFF³⁾ in 1907. In addition, I was isolated from the mother liquor too. Both I and II are much alike in structure like octopine⁴⁾ and lysopine.⁵⁾ Moreover, it is particularly worthy of notice that these analogous compounds have D-alanine moiety in the molecule. In the later investigations on oyster, *Crassostrea gigas*, and mussel, *Mytilus edulis*, it was clearly established that I coexisted with II*¹. This may suggest a wide distribution of the compounds in molluscs. In this connection, further studies on the distribution in molluscs and physiological role of these analogous compounds will be necessary.

Acknowledgement

The authors wish to express their thanks to Dr. T. Suzuki, Faculty of Agriculture, Tohoku University, for his technical assistance in ORD and mass spectral analyses.

References

- 1) M. SATO, Y. SATO, and Y. TSUCHIYA: *Bull. Japan. Soc. Sci. Fish.*, **43**, 1077–1079 (1977).
- 2) E. ABDERHALDEN and R. HAASE: *Z. physiol. Chem.*, **202**, 49–55 (1931).
- 3) G. STADNIKOFF: *Ber.*, **40**, 1014–1019 (1907).

*1 unpublished data

- 4) K. MORIZAWA: *Acta sch. med. Kioto*, **9**, 285-298 (1927). 5) K. BIEMANN: *Biochim. Biophys. Acta*, **40**, 369-370 (1960).

Addendum; We noticed by courtesy of Dr. A. M. MACKIE that this α -iminopropioacetic acid is identified with strombine that was isolated from *Strombus gigae* by SANGSTER *et. al.* in 1975.