ISOLATION OF THE NEW NATURALLY OCCURRING HALOGENATED AMINO ACID MONOCHLOROTYROSINE FROM A MOLLUSCAN SCLEROPROTEIN

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1. Introduction

Although iodinated and brominated amino acids, principally as tyrosine derivatives or related compounds, are well known to occur naturally [1–4] chlorinated amino acids have not until recently been described. Hunt [4] has described the isolation of protein-bound monochloromonobromotyrosine from a molluscan scleroprotein the operculum of the whelk *Buccinum undatum* L. The present communication describes the isolation of monochlorotyrosine from the same source.

2. Experimental

350 mg of opercula protein [5] were hydrolysed, under nitrogen, in 250 ml 6 M HCl at 105° for 24 hr. The hydrolysate was evaporated in vacuo, dissolved in 2 ml 0.02 M acetic acid and chromatographed on a column of Sephadex G-15 (55 × 2.2 cm) eluting with 0.02 M acetic acid. The ultraviolet absorption of the eluate was monitored continuously at 280 nm and 5 ml fractions collected. Fractions containing UV absorbing material were concentrated and rechromatographed on a column of Biogel P2 (50 × 1 cm) eluting with 0.2 M acetic acid. The column effluent was monitored as before. Fractions of interest were concentrated to dryness in vacuo prior to characterization.

Paper chromatography, paper electrophoresis, amino acid analysis and mass spectrometry were carried out as described previously [4].

3. Results and discussion

Fig. 1 shows the separation on Sephadex G-15 of the opercula hydrolysate. Tyrosine is eluted at 210 ml, monobromotyrosine at 360 ml, monochloromonobromotyrosine at 510 ml and dibromotyrosine at 600 ml. The aromatic amino acids experience strong absorption under the conditions used. An unidentified peak is eluted between tyrosine and monobromotyrosine at 320 ml. Preliminary paper chromatography indicated that this peak contained several ninhydrin positive substances. Fig. 2 shows the separation of the 320 ml peak on Biogel P2. Tubes 12–15 were pooled and concentrated. Yield 5.4 mg.

Paper chromatography (*n*-butanol/acetic acid/water = 3:1:1) and paper electrophoresis (pyridine acetate buffer pH 5.2 and formic acid pH 2.2) [4] of the fraction from tubes 12–15 indicated one component only, staining blueish gray with ninhydrin and red-orange with diazotised sulphanilic acid [6]. Authentic standards of 3-chlorotyrosine, 3-bromotyrosine, 3,5-dibromotyrosine, 3,5-monochloromonobromotyrosine, 3-iodotyrosine and 3,5-diiodotyrosine run at the same time as the unidentified compound suggested its identification as monochlorotyrosine. In the amino acid analyser the compound eluted as a single peak 38 min after tyrosine and 35 min before ammonia and in the same position as monochlorotyrosine. 3-Bromotyrosine and 3,5-monochloromonobromotyrosine elute 18 min and 15 min before ammonia respectively, 3,5-dibromotyrosine with ammonia and 3-iodotyrosine and 3,5-diiodotyrosine 24 min and 60 min after ammonia, respectively.

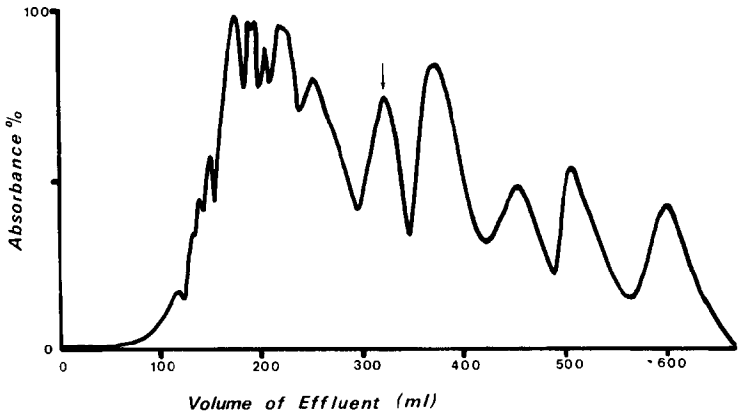

Fig. 1. Separation of an opercula protein hydrolysate on Sephadex G-15. Gel bed 55 × 2.2 cm. Elution with 0.02 M acetic acid. The arrow indicates the position of the unidentified component eluted at 320 ml. Absorbance monitored at 280 nm.

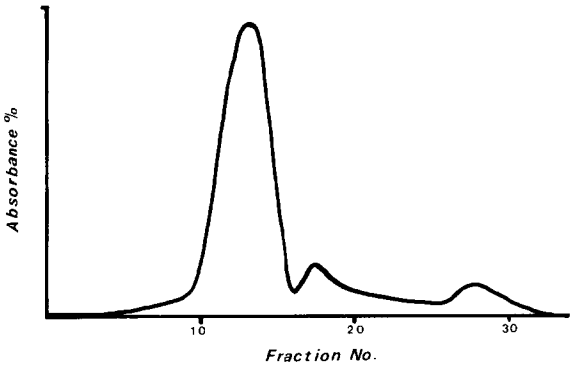

Fig. 2. Separation of the 320 ml peak from the Sephadex G-15 column (fig. 1) on Biogel P2. Gel bed 50 × 1 cm. Elution with 0.2 M acetic acid. The major peak was taken for characterization. Absorbance monitored at 280 nm.

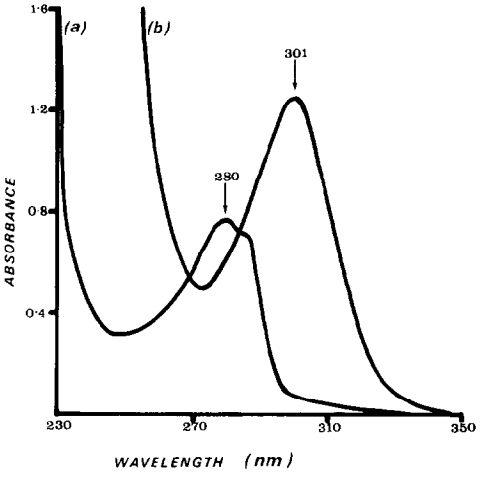

Fig. 3. Ultraviolet absorption spectra of the aromatic compound isolated from opercula protein. (a) In acid and (b) in alkaline solution.

Fig. 3 shows the ultraviolet absorption spectrum of the compound in acid and in alkaline solution and is immediately suggestive of a substituted tyrosine. The spectrum proves to be identical with that of authentic 3-chlorotyrosine. The acid maximum shifts from 280 nm to 301 nm in alkali with a 1.5-fold increase in absorption. The spectra are distinguishable from those of other halogenated tyrosines.

Fig. 4 shows the mass spectrum of the compound, obtained without prior derivatization. The molecular ion occurs at m/e 215, the expected position for monochlorotyrosine, and the isotopic abundance pattern of the cluster is characteristic of a compound containing one chlorine atom [7]. The presence of peaks at m/e 74 $(NH_2{=}CH\text{-}CO_2H)^+$ and m/e 141 (M–74) is consistent with identification of the compound as a primary amino acid [8, 9]. Since M–chlorine is not observed and since M–74 still shows the chlorine pattern it seems likely that the chlorine is sited as a substituent on a benzene ring [8, 9]. Confirmation was obtained from examination of authentic 3-chlorotyrosine which showed an identical mass

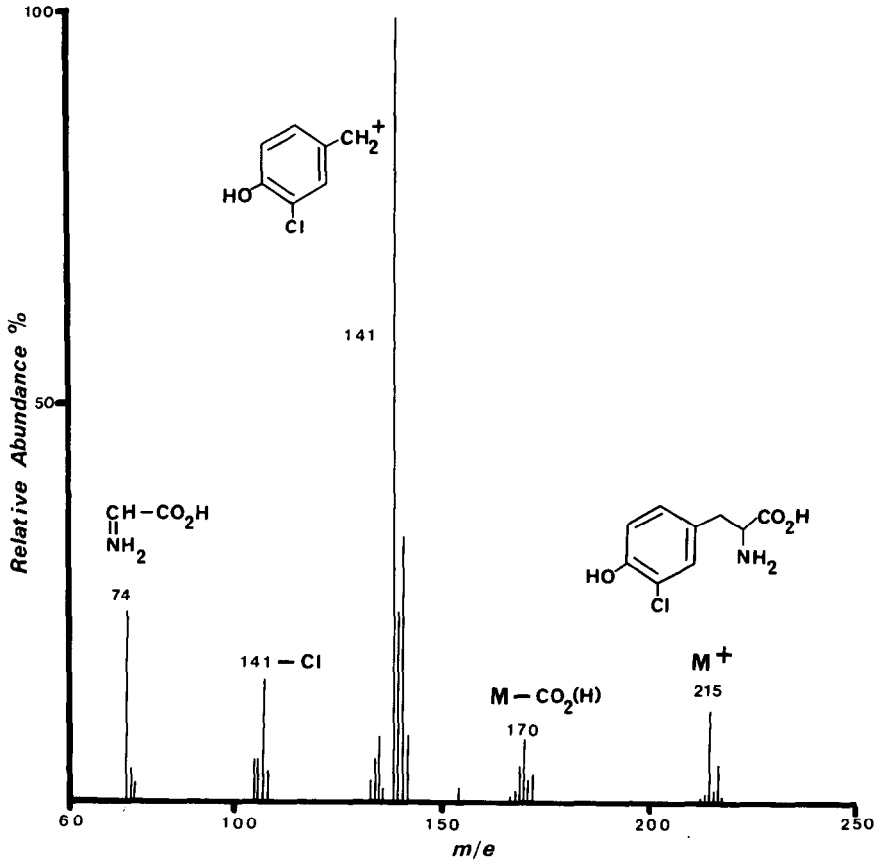

Fig. 4. Mass spectrum of the aromatic compound, isolated from opercula protein, confirming its identity as monochlorotyrosine.

spectrum to the compound. Although it would be possible for substitution in the compound to be at position 2 rather than 3 on the ring the close similarity in all properties to 3-chlorotyrosine renders this unlikely.

Natural monochlorotyrosine appears not to have been reported previously in the literature. However, Professor S.O. Andersen (University of Copenhagen, Zoophysiological Laboratory, August Krogh Institute) has told me that he believes it to be present in insect cuticle. Mollusc operculum and insect cuticle have features in common as tanned protein systems. The function of halogenated amino acids in these situations is uncertain [4]. There are about 5 residues of monochlorotyrosine per 1000 total amino acid residues in the opercula protein, about 30 halogenated tyrosines overall and 53 unsubstituted tyrosines [4, 5]. Preliminary studies of partial hydrolysates suggest a greater proportion of halogenated tyrosines in the stable core of the scleroprotein. This may suggest an association with the cross-linking process [4].

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References

[1] A. Mester, Biochemistry of the Amino Acids, Vol. 1 (Academic Press, New York, 1965) 82.

[2] B. Tschiersch and K. Mothes, in: Comparative Biochemistry, Vol. 5C, eds. M. Florkin and H.S. Mason (Academic Press, New York, 1963) p. 56.
[3] J. Roch, M. Fontaine and J. Leloup, in: Comparative Biochemistry, Vol. 5C, eds. M. Florkin and H.S. Mason (Academic Press, New York, 1963) p. 493.
[4] S. Hunt, Biochim. Biophys. Acta 252 (1971) 401.
[5] S. Hunt, Biochim. Biophys. Acta 207 (1970) 347.
[6] I. Smith, Chromatographic and Electrophoretic Techniques, Vol. 1 (Heinemann, London, 1970) p. 104.
[7] J.H. Beynon, Mass Spectrometry and its Applications to Organic Chemistry (Elsevier, Amsterdam, 1960) p. 297.
[8] H.F. Grützmacher and K. Heyns, Annalen. 698 (1966) 24.
[9] F.W. McLaffery, Anal. Chem. 34 (1962) 16.