[Chem. Pharm. Bull. 28(3) 973—975 (1980)]

Determination of the Amino- and Carboxyl-terminal Residues of an Immuno-competence Promoting Protein, P-MSY, from Bovine Parotid Glands

IKUKATSU SUZUKI

Faculty of Pharmaceutical Sciences, Nagoya City University[1]

(Received August 9, 1979)

A protein, P-MSY, with immuno-competence promoting activity as well as antitumor activity has been isolated from bovine parotid glands.

To help identify and characterize this protein, the amino-terminal and carboxyl-terminal amino acids were determined by Edman degradation and carboxypeptidase digestion. The amino-terminal was Ser-His- and the carboxyl-terminal was -Leu-His.

Keywords——bovine parotid glands; P-MSY; dansyl-Edman degradation; amino-terminal sequence; carboxypeptidase A; carboxyl-terminal sequence; immuno-competence promoting protein; hypocalcemic activity

We recently isolated a protein (P-MSY) from bovine parotid glands by following the hypocalcemic activity[2] in rabbits.[3] This protein has immuno-competence promoting activities, such as lymphocyte-stimulating activity and activities to increase levels of plaque-forming cells and rosette-forming cells,[4] and also has potent antitumor activity in mice.[5] The protein showed a single band on analytical disc gel electrophoresis, and the molecular weight was estimated to be 66,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[3]

To help identify and characterize this protein, the amino- and carboxyl-terminal amino acids were identified.

Materials and Methods

The protein, P-MSY, was purified from bovine parotid glands as described previously.[3]

The amino-terminal sequence was analyzed by dansyl-Edman degradation following the procedure of Gray and Hartley.[6,7] Standard dansylated amino acids were prepared according to Weiner *et al.*[8] After dansylation of 4.2 mg of P-MSY, the reaction product was hydrolyzed, and the resulting dansylated amino acid was spotted on a polyamide layer sheet (5 × 5 cm) (Seikagaku kogyo Co., Ltd, Tokyo). Known dansylated amino acids were spotted on opposite sides of the plate. Two-dimensional thin-layer chromatography was carried out as follows: the plates were developed with the first solvent (I) 1.5% formic acid in H_2O. For the second dimension, the plates were first developed with (II) C_6H_6–CH_3COOH (9: 1, v/v), then air-dried and developed again with (III) $CH_3COOC_2H_5$–CH_3COOH–MeOH (20: 1: 1, v/v/v). The spots of dansylated amino acids were detected under ultraviolet irradiation (254 nm).

The carboxyl-terminal sequence was analyzed using carboxypeptidase A, essentially as described by Frankel-Conrat *et al.*[9] P-MSY (14.4 mg) was dissolved in 5 ml of H_2O and the pH was adjusted to 8.0 with 0.1 N NaOH. The solution was treated with 12.1 μl of carboxypeptidase A (EC 3.4.2.1) (CPase A-DFP, 16 mg/ml suspension, Sigma) and water was added to make a final volume of 6 ml. The mixture was incubated at 25° and aliquots (1 ml each) were taken at 0, 0.5, 1, 2, 4, and 8 hr. The digestion was

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stopped by adding 0.1 ml of 1 M citric acid and the amino acid fraction was obtained by gel filtration on Sephadex G-10, followed by analysis of the amino acids with a Hitachi KLA-3B automatic amino acid analyzer.

Protein was determined by the method of Lowry *et al.*[10]

Results and Discussion

As shown in Fig. 1 (A), only DNS-Ser was detected after the first Edman degradation step. After the second step, DNS-His was detected, as shown in Fig. 1 (B). These DNS-amino acids were identified by comparison of the mobilities of sample and standard DNS-amino acids spotted on opposite sides of the plates. Based on these results, the sequence of N-terminal amino acids of P-MSY was assigned as Ser-His-.

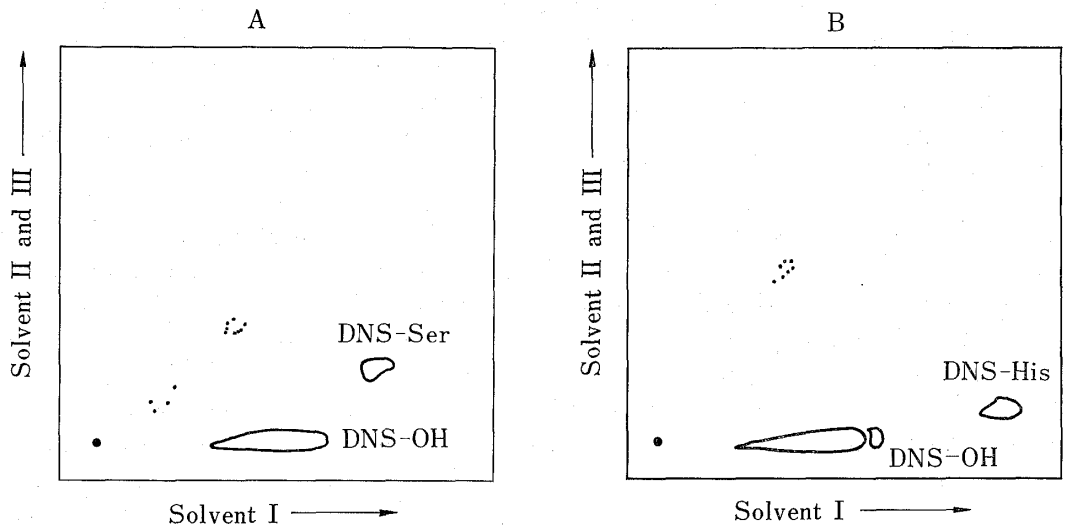

Fig. 1. Chromatography of the Dansylated Amino Acids obtained by Edman Degradation of P-MSY

(A) Chromatography after 1st Edman Degradation.
(B) Chromatography after 2nd Edman Degradation.

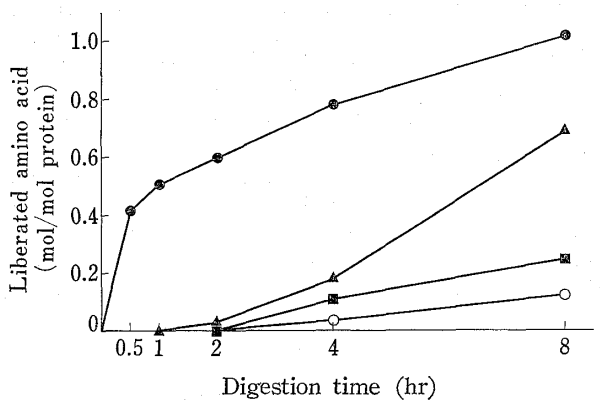

Fig. 2. Time Course of the Release of Amino Acids from P-MSY on Digestion with Carboxypeptidase A

●: histidine, ▲: leucine, ■: tyrosine, ○: alanine.

The time course of the release of amino acids from P-MSY on treatment with carboxypeptidase A is shown in Fig. 2.

His was released first followed by Leu under these conditions. Smaller amounts of Tyr and Ala were detectable after 4 hr. The released amino acids were analyzed by polyamide thin-layer chromatography as described above. As shown in Fig. 3, both DNS-His and DNS-Leu were detected. Thus, the carboxyl terminal sequence was assigned as -Leu-His.

We previously showed that purified parotin from bovine parotid glands has

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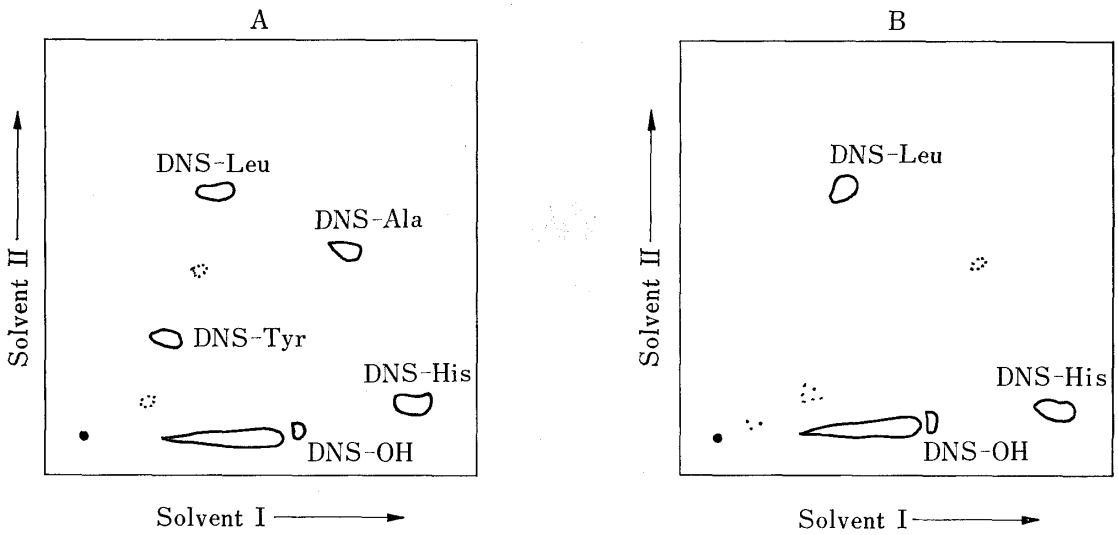

Fig. 3. Chromatography of DNS-amino Acids obtained from P-MSY

A; DNS-amino acids (His, Leu, Tyr, Ala as marker).
B; Sample (P-MSY).

immuno-competence promoting activities similar to those of P-MSY.[11] However, these two proteins were obtained from differect fractions of the parotid gland extracts.[3] Furthermore, the N-terminal and C-terminal amino acids were completely different, the N-terminal and C-terminal amino acids of parotin being Lys-Leu- and -Val-Leu, respectively.[12]

Acknowledgement The author wishes to thank prof. Emeritus Akira Mizutani for valuable discussions during these experiments. Thanks are also due to Mr. Toshikazu Imai for his technical assistance in preparing the orignal materials and to prof. Harumi Okuyama for his help in preparing the manuscript.

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