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The Amino Acid Sequence around Cystine Residue in Substance A produced by *Streptomyces Carzinostaticus*

Antibacterial polypeptide substance A[1] produced by *Streptomyces carzinostaticus* var. F-41 was found to contain a disulfide brige which concerned to antibacterial activity. As shown in Table I, by reduction of the disulfide brige in the substance A with high concentration of mercaptoethanol, the activity decreased, and 75% of the activity was regenerated by reoxidation of the sulfhydryl groups after removal of mercaptoethanol by dialysis.

Table I. Change of the Activity of Substance A by Reduction and Reoxidation

Process	Potency[c] (U/ml)	Volume (ml)	Total potency (U)	Recovery (%)
Native	1200	10	12000	100
Reduction[a]	55	11	605	5
Reoxidation[b]	400	24	9600	80

a) Reduction was accomplished by addition of hundred times mole of mercaptoethanol and then stirred at 4° for 5 hr.
b) Reoxidation by air was allowed to stirring for 24 hr. at 4° after dialysis against 0.001M phosphate buffer (pH 7.0).
c) Potency was expressed as the antibacterial activity against *Sarcina lutea*.

The present communication described the separation of a cystine-containing fraction from the partial acid hydrolysate of substance A to elucidate the amino acid sequence around the cystine residue.

Sixhundred and fifty mg (7.7×10^{-5} mole) of substance A was dissolved in 100 ml of 0.25M oxalic acid. The hydrolysis was conducted at 100° for 16 hr and the hydrolysate was neutralized to pH 7.0 with calcium hydroxide. Precipitate of calcium oxalate was removed by filtration and the filtrate was lyophilized.

The lyophilized powder was dissolved in 20 ml of 0.01N HCl and chromatographed on a column of Amberlite CG-120 (3.2×110 cm). The elution was made by the successive change of eluant from 1400 ml of 0.2 M pyridine-formate buffer (pH 3.5) to 2000 ml of 0.5 M pyridine-acetate buffer (pH 5.0) and finally to 2000 ml of 1.0 M pyridine-acetate buffer (pH 5.0). Flow rate was set 1 ml/min, and the effluent was collected in 10 ml fractions. The peptide content of effluent was determined by ninhydrin reaction.

From the result of chromatography eighteen peptide fractions were obtained. The cystine-containing fraction (No. 17) which was eluted with 0.5 M pyridine-acetate buffer (pH 5.0). It was apparent from the result of paper electrophoresis of oxidized peptide with performic acid (Fig. 1) that this fraction was still contaminated. Then the lyophilized powder of the fraction was oxidized with performic acid at

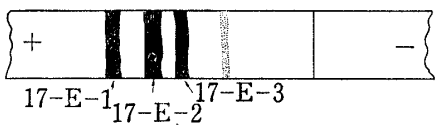

Fig. 1. Paper Electrophoresis of Oxidized Peptide with Performic Acid

Electrophoresis was carried out in pyridine-acetic acid-water (4:1:95) buffer at pH 5.6, Toyo filter paper No. 51A, 20 volts per cm, 3 hr. Peptide fragments were indicated by ninhydrin reaction.

1) H. Sato, T. Tanimura and Z. Tamura, *J. Biochem.*, submitted.

0° according to Hirs.[2] The oxidized peptide was further purified by paper electrophoresis. As shown by the electrophretic pattern of Fig. 1, the three major fragments were preparated.

The amino acid composition and N- and C-terminal amino acid residues of each peptide fragment are shown in Table II.

Table II. Amino Acid Composition and N- and C-terminal Amino Acid

Fragment	Amino acid composition	N-terminal[a]	C-terminal[b]
17-E-1	$CySO_3H$, Glu	Glu	$CySO_3H$
17-E-2	$CySO_3H_2$, Thr, Glu, Gly, Ala_2, Val, Leu	$CySO_3H$	$CySO_3H$
17-E-3	$CySO_3H$, Thr, Gly, Ala_2, Val, Leu	$CySO_3H$	Leu

a) N-terminal amino acid was determined by DNS method.
b) C-terminal amino acid was determined by DNS method after the hydrazinolysis of peptide.

17-E-2 fragment was degraded by the Edman–Dansyl procedure,[3] and the amino acid sequence was established as shown in Fig. 2.

Therefore 17-E-1 and 17-E-3 fragments were supposed to be produced by further partial hydrolysis of 17-E-2, and the structure of the original cystine-containing peptide was established as shown in Fig. 3.

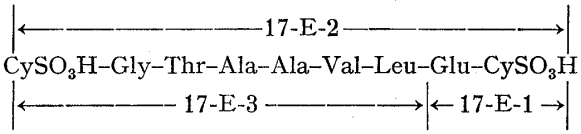

Fig. 2. Amino Acid Sequence of Oxidized Peptide

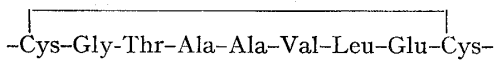

Fig. 3. Amino Acid Sequence of Cystine-Containing Peptide

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