Communications to the editor

CHEMISTRY OF BLEOMYCIN. VI

SELECTIVE CLEAVAGE OF BLEOMYCIN A₂ BY N-BROMOSUCCINIMIDE

Sir:

Recently, we reported the structures of tetrapeptide A and tripeptide S, the major products of a partial acid hydrolyzate of bleomycin A_2^{11} . (Fig. 1). These two peptides include, with no overlapping, all the amino acids and an amine which are obtained by total acid hydrolysis of bleomycin A_2 . In this communication, the results of selective cleavage of bleomycin A_2 by N-bromosuccinimide (NBS) are presented.

Acid hydrolysis of the dinitrophenyl derivative of bleomycin A_2 does not give DNP-threonine. On the other hand, o-methyl threonine is obtained by acid hydrolysis of the methylated bleomycin A_2 , which is derived by treatment with methyl iodide and silver oxide in dimethylformamide². These observations suggest that in bleomycin A_2 the hydroxyl group of the threonine moiety is free and the amino group is connected to one of the carboxyl groups of tetrapeptide A.

We could not get a small fragment which contained the N-masked threonine by partial

acid hydrolysis, perhaps because of preferential cleavage induced by the vicinal free hydroxyl group of threonine. Then we tried NBS oxidation³⁾, expecting a selective cleavage at the carboxyl peptide bond of β -hydroxy histidine⁴⁾.

A solution of bleomycin A₂ in buffer (pyridine, acetic acid, water, 1:10:19 in volume) was treated with 3~10 equivalents of NBS. The reaction mixture was kept at room temperature for 30 minutes and the excess NBS was decomposed by addition of imidazole. Thirty minutes after imidazole addition, the reaction mixture was heated at 100°C for one hour.

Formation of a new peptide with UV absorption similar to tripeptide S was recognized by paper electrophoresis. The mobility of the peptide relative to alanine (Rm value) was 0.97 when a solution of acetic acid, formic acid, and water (75:25:900) was used as the buffer solution for electrophoresis. The Rm value of tripeptide S was 1.13. The peptide, named tetrapeptide S. was isolated by CM Sephadex column chromatography using 0.35 N NaCl followed by desalting with Amberlite CG 50. peptide S gave three amino acid (I, III, VI) and an amine (VII) by total acid hydrolysis. The acid hydrolysis of the dinitrophenyl derivative of tetrapeptide S gave ether-solu-

Fig. 1. The structures of tetrapeptide A and tripeptide S.

* This peptide includes only two peptide bonds, but it gave four amino acids in the process of total acid hydrolysis. Therefore, strictly speaking, it should be named pseudo-tetrapeptide A.

** Names given to the acid hydrolyzates of bleomycin A2 are shown in Roman numbers (I~VII).

Tetrapeptide A*:

Tripeptide S:

Fig. 2. The structure of tetrapeptide S.

ble DNP-III and free I, VI, and VII, which were identified by thin-layer chromatographic comparison with authentic samples.

The DNP-tetrapeptide S dissolved in concentrated hydrochloric acid was kept at 37°C for 3 days. The reaction mixture was diluted with water, and then extracted with ether. The ether extract contained DNP-III, and the aqueous layer contained tripeptide S, a trace of unreacted DNP-tetrapeptide S and a new yellow substance. The yellow substance (Rm 0.92) was more basic than DNP-tetrapeptide S (Rm 0.47) and retransformed to DNP-tetrapeptide S by alkaline treatment. Thus, the yellow substance is suggested to be the N→O acyl shifted product of DNP-tetrapeptide S. Thus, tetrapeptide S can be assigned to the structure shown in Fig. 2.

The recovery yield of tetrapeptide S after CM Sephadex column chromatography was calculated from the UV absorption at 291.5 nm. The molecular extinction coefficient (£ 12,850) of crystalline tripeptide S dihydrobromide at 291.5 nm was used as the basis of calculation. The maximum yield reached 44.8 % when 6 moles of NBS was used for one mole of bleomycin A₂.

The fact that the N-terminus of tetrapeptide S is compound III indicates that tetrapeptide S was formed by NBS cleavage at the carboxyl peptide bond of β -hydroxy histidine (IV), because the peptide bond between IV and III is already shown in

tetrapeptide A. Then, it was established that the C-terminus of III is connected with the N-terminus, I, of tripeptide S.

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