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SYNTHETIC STUDIES ON TROPONIN I ACTIVE SITE. PREPARATION OF A PENTADECAPEPTIDE WITH INHIBITORY ACTIVITY TOWARD ACTOMYOSIN ADENOSINE TRIPHOSPHATASE

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Three peptides corresponding to the partial sequences of troponin I (positions at 101 - 109, 110 - 115, and 101 - 115) were synthesized in a combination of the 'hold-in-solution' method and the usual liquid phase method. The synthesized pentadecapeptide inhibits actomyosin ATPase in an about 30% activity of the intact troponin I on a molar basis.

Troponin I associates with troponin T and C to form troponin complex.[1] This complex, together with tropomyosin, is responsible for the control of muscular contraction. Troponin I, by itself, inhibits actomyosin adenosine triphosphatase, which plays an essential role in the contraction. Troponin I has been isolated from several species, and their primary structures have been reported.[2] The inhibitory activities of the enzymatic and chemical digests of troponin I from rabbit skeletal muscle were examined, and an active fragment (1) corresponding to the sequence positions at 96 - 116 of the intact protein was obtained from CNBr-cleaved peptides (Fig. 1).[3]

96 100 105 110 115

H-Asn-Gln-Lys-Leu-Phe-Asp-Leu-Arg-Gly-Lys-Phe-Lys-Arg-Pro-Pro-Leu-Arg-Arg-Val-Arg-Hse-OH : 1

H-Asp-Leu-Arg-Gly-Lys-Phe-Lys-Arg-Pro-Pro-Leu-Arg-Arg-Val-Arg-OH : 2

H-Asp-Leu-Arg-Gly-Lys-Phe-Lys-Arg-Pro-OH : 3

H-Pro-Leu-Arg-Arg-Val-Arg-OH : 4

Fig. 1

However it is not clear yet that 1 is the essential fragment required for the inhibitory activity. The C-terminal homoserine is an artificial derivative from methionine. The N-terminal pentapeptide is also contained in a different larger inactive fragment

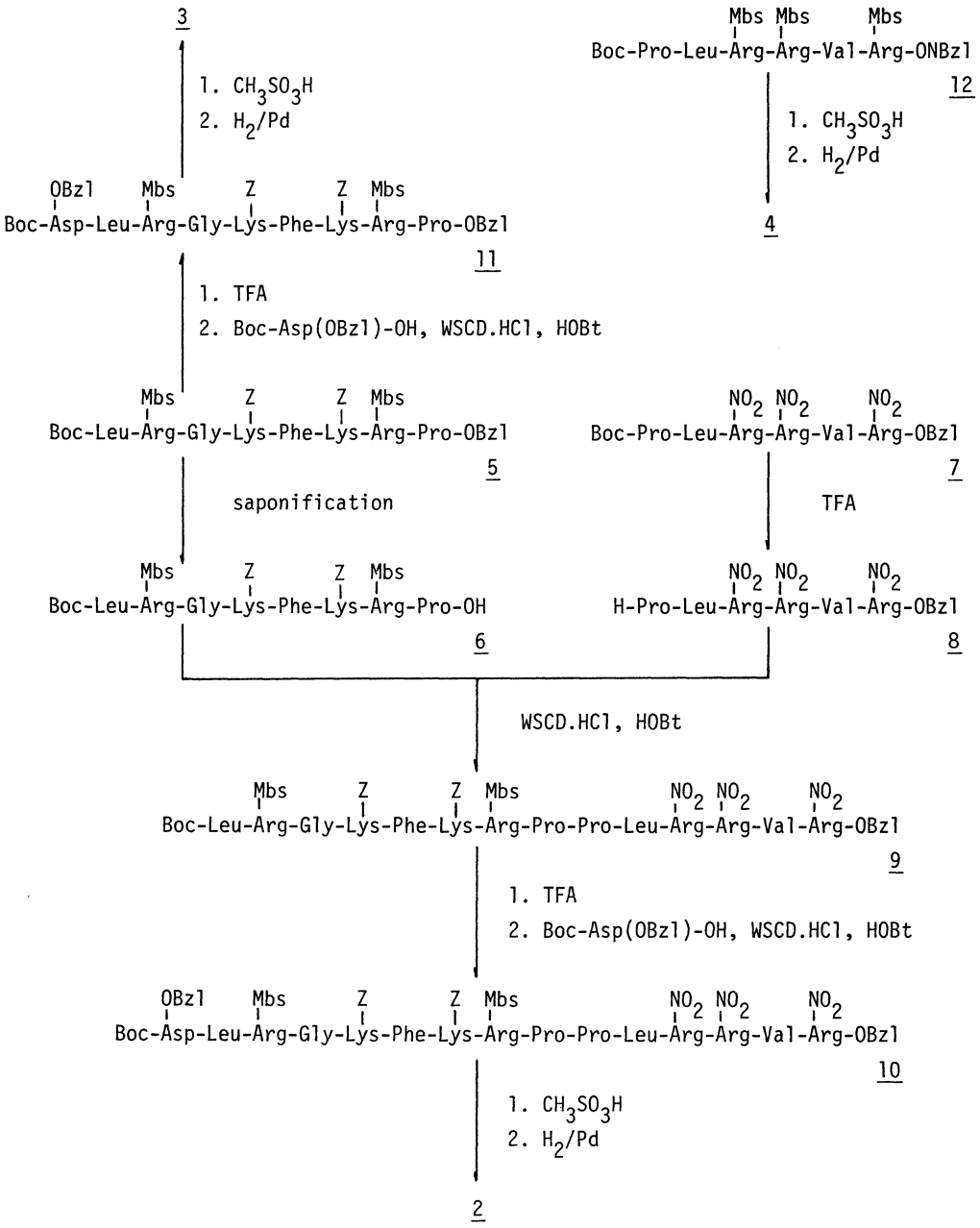

Fig. 2

(positions at 37 - 100)[3] and is regarded as independent of the activity.

In order to investigate further details of the active region, we synthesized the pentadecapeptide (2) excluding the N-terminal pentapeptide sequence and the homoserine of 1. The nonapeptide (3) and the hexapeptide (4), corresponding to the N- and C-terminal moiety of 2 respectively, were also synthesized. These peptides were prepared according to the routes illustrated in Fig. 2.[4] The fully protected octa-

peptide (5) was synthesized by the 'hold-in-solution' method[5] in an overall yield of 60%. To minimize escape of the intermediates to the aqueous phase during the preparation, an Mbs-group was employed as the protector for the guanidine group of arginine.[6] The protected N-terminal nonapeptide (11) was derived from 5. Preparation of the protected C-terminal hexapeptide (12) was tried also by the 'hold-in-solution' method, but failed because of poor solubilities of the intermediates. The peptide 12 was obtained by a conventional stepwise elongation method. The intermediates were oily or amorphous and their purification was considerably difficult. Another protected C-terminal peptide (7) was constructed from Boc-Pro-Leu-OH and H-Arg(NO_2)-Arg(NO_2)-Val-Arg(NO_2)-OBzl prepared by a conventional method. In this case, purification of the intermediates was quite easy. Partially protected peptides, 6 and 8, were coupled to give 9, which was converted to the pentadecapeptide (10). Free peptides, 2, 3, and 4, were obtained from 10, 11, and 12, respectively. They were purified by partition chromatography on Sephadex G-25 or G-50 and/or ion exchange chromatography on SP-Sephadex. Their homogeneities were confirmed by tlc. Elemental analyses of the peptides gave reasonable results. Some of their properties are as follows.

2: $C_{84}H_{148}O_{17}N_{32}.6CH_3COOH.14H_2O$, $[\alpha]_{230}^{27}$ -2405° (c 1, 1 M CH_3COOH), amino acid composition in the hydrolysate (6 M HCl, 110 °C, 16 h); Asp 0.98, Pro 1.88, Gly 1.00, Val 0.94, Leu 1.99, Phe 1.06, Lys 2.03, Arg 5.11.

3: $C_{50}H_{85}O_{11}N_{17}.5CH_3COOH.8H_2O$, $[\alpha]_{230}^{27}$ -1205° (c 1, 1 M CH_3COOH), Asp 0.97, Pro 0.95, Gly 1.00, Leu 1.01, Phe 0.98, Lys 2.08, Arg 2.00.

4: $C_{34}H_{65}O_7N_{15}.3CH_3COOH.3H_2O$, $[\alpha]_{230}^{27}$ -1715° (c 1, 1 M CH_3COOH), Pro 1.02, Val 0.95, Arg 3.03.

The inhibitory activities of the synthetic peptides, 2, 3, and 4, were examined according to the methods reported.[3), 7)] Peptide 2 exhibited activity corresponding to about 30% of that of the intact troponin I on a molar basis. Neither of the peptides 3 and 4 nor their equimolar mixture showed activity. Recently, a 20-residue peptide analog of 1 was synthesized with a solid phase method by J. A. Talbot et. al. and it also exhibited inhibitory activity.[8)] These facts indicate that the function of troponin I, a protein consisting of 178 amino acid residues, is retained in its short portion including a Pro-Pro sequence. It is likely that peptides, shorter than 2, containing the Pro-Pro sequence between the two 'basic sequences' also hold the activity. The detailed results of the assay will be reported elsewhere.

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4) Amino acids used are of L-configuration. The abbreviations for amino acids and peptides are in accordance with the rules of the IUPAC-IBU Commission of Biochemical Nomenclature. Other abbreviations are as follows; Boc = t-butoxycarbonyl, Z = benzyloxycarbonyl, Mbs = p-methoxybenzenesulfonyl, NO_2 = nitro, TFA = trifluoroacetic acid, OBzl = benzyl ester, ONBzl = p-nitrobenzyl ester, WSCD.HCl = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole.
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