CHEMISTRY LETTERS, pp. 539-542, 1982.

SYNTHETIC STUDIES ON TROPONIN I ACTIVE SITE. PREPARATION OF A HEXADECAPEPTIDE WITH BINDING ACTIVITY TOWARD TROPONIN C

Sukekatsu NOZAKI*, Eisaku KATAYAMA†, and Ichiro MURAMATSU††
*Department of Microbiology, Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-02
†Department of Pharmacology, Faculty of Medicine, The University of Tokyo, Hongo, Tokyo 113
††Department of Chemistry, College of Science, Rikkyo University, Nishi-Ikebukuro, Tokyo 171

A hexadecapeptide corresponding to a partial sequence (positions 10 - 25) of troponin I from rabbit fast skeletal muscle was synthesized in a combination of the 'hold-in-solution' method and the usual liquid phase method. The synthesized peptide (1) formed a Ca^{2+}-dependent complex with rabbit skeletal troponin C. The CD spectra of 1 showed that 1 in hydrophobic media contained α-helical peptide bonds.

Troponin I, a constituent protein of troponin complex, is known to be the inhibitory component in the regulation of muscular contraction and to have two functional domains in the molecule: the N-terminal region with binding activity toward troponin C[1] and the central region of the molecule with inhibitory activity toward actomyosin ATPase.[1,2,3] The latter part (positions 96 - 116) is reported to have also binding activity toward troponin C.[1] Although the interaction of troponin I and C plays a key role in the mechanism of the Ca^{2+}-dependent regulation of muscular contraction, the details of the binding sites are not yet understood. Syska et al.[1] observed that a CNBr fragment (positions 1 - 21) of rabbit skeletal troponin I has affinity toward troponin C, while a larger fragment (positions 1 - 48) forms a tighter complex with troponin C. On the basis of this observation and of homology found in the sequences of troponin I from several species, Wilkinson and Grand supposed that the most likely binding site in the N-terminal region of troponin I is located within positions 10 - 25.[4]

In order to investigate in detail the features of the N-terminal binding region of troponin I, we synthesized the hexadecapeptide (1) corresponding to the supposed

```
                  5                  10                  15                  20
Ac-Gly-Asp-Glu-Glu-Lys-Arg-Asn-Arg-Ala-Ile-Thr-Ala-Arg-Arg-Gln-His-Leu-Lys-Ser-Val-
                  25                  30                  35                  40
  -Met-Leu-Gln-Ile-Ala-Ala-Thr-Glu-Leu-Glu-Lys-Glu-Glu-Gly-Arg-Arg-Glu-Ala-Glu-Lys-
                  45          48        178
  -Gln-Asn-Tyr-Leu-Ala-Glu-His-Cys--------Ser-OH : troponin I from rabbit fast skeletal muscle

   10                 15                  20                  25
 H-Ile-Thr-Ala-Arg-Arg-Gln-His-Leu-Lys-Ser-Val-Met-Leu-Gln-Ile-Ala-OH                : 1
```

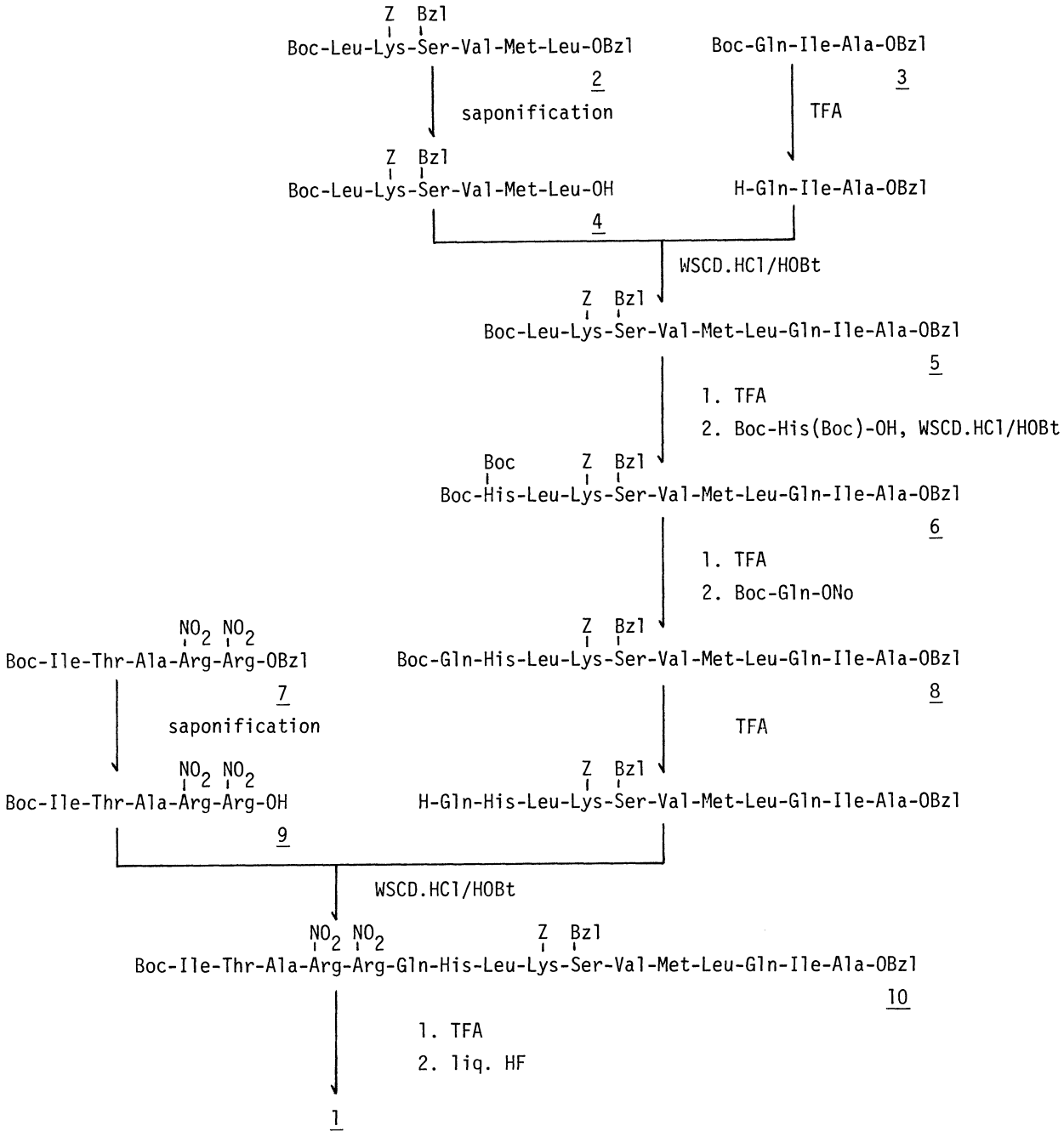

Fig. 1. Synthesis of 1.

binding region.[4] The synthetic route of the peptide is illustrated in Fig. 1.[5] Boc-group was employed for α-amino protection. Side functional groups of arginine, serine, and lysine were protected, while those of threonine and methionine were not. The imidazole ring of histidine was temporarily protected by im-Boc group.[6] Glutamine was incorporated into the peptide chain by the o-nitrophenyl ester method.[7] A segmental peptide (2) was prepared by the 'hold-in-solution' method[8] in an overall yield of 51%. Other segments, 3 and 7, were synthesized exclusively by WSCD.HCl/HOBt procedure in the usual stepwise elongation method. Prior to the fragment condensation,

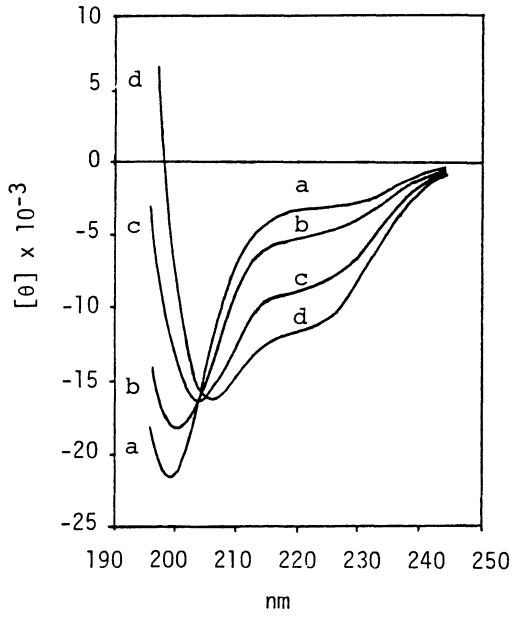

Fig. 2. CD spectra of 1.

the C-terminal protectors of 2 and 7 were removed by saponification to give the corresponding acids, 4 and 9. The purity of these fragment peptides was confirmed by means of their HPLC analyses (LiChrosorb RP-18, methanol/water). The peptides were linked also by the WSCD.HCl/HOBt procedure. Boc-His(Boc)-OH and Boc-Gln-ONo were joined to the peptide chain stepwise. Poor solubility of the subsequent peptides, 5, 6, 8, and 10, made their analyses and purification quite difficult. Peptide 6, 8, and 10 were prepared in dilute solution of DMF and/or DMF/HMPA using excess amounts (5- to 10-fold moles per mole of the amine components) of the acylating reagents for complete acylation. Satisfactory purity of the products was confirmed by means of amino acid analyses of their acid hydrolysates. Free peptide 1 was derived from 10 and was purified by partition chromatography on Sephadex[9] (G-50, 1-butanol/pyridine/acetic acid/water, 16/8/1/12) followed by gel filtration (G-10, 10% acetic acid). TLC (Avicel-cellulose, 1-butanol/pyridine/acetic acid/water, 16/10/3/12) gave a single ninhydrin-positive spot, R_f 0.55. Amino acid composition in the acid hydrolysate (6 M HCl, 110 °C, 16h): Thr 1.00, Ser 0.96, Glu 2.09, Ala 2.04, Val 0.98, Met 0.89, Ile 1.95, Leu 2.02, His 0.92, Lys 0.99, Arg 2.17. Found: C, 47.59; H, 7.66; N, 17.64% (the sample was dried over P_2O_5 in high-vacuum at 43 °C). Calcd for $C_{81}H_{145}O_{21}N_{27}S.3CH_3COOH.8H_2O$: C, 47.72; H, 7.96; N, 17.27%. $[\alpha]_{250}^{16}$ -1090° (c 0.48, 1 M acetic acid).

The binding activity of the synthetic peptide 1 toward rabbit skeletal troponin C was examined by means of a gel electrophoresis method similar to the reported one.[1] In the presence of Ca^{2+}, 1 associated with troponin C to form a stable complex appearing as a new band in the gel, while in the absence of Ca^{2+} the association was not observed. This finding shows that the binding activity of the N-terminal region of troponin I is retained in the hexadecapeptide corresponding to the sequence of the protein positions 10 - 25 as supposed[4]; and moreover the interaction of the binding region and troponin C is Ca^{2+}-sensitive.

The CD spectra of 1 are illustrated in Fig. 2. The peptide in an aqueous medium (50 mM Tris(hydroxymethyl)-aminomethane/HCl, pH 7.2) showed a negative shoulder near 230 nm in addition to a strong negative peak at 199 nm (spectrum a). The spectrum suggests that 1 in the solution contained secondary structure since no negative band is usually observed near 230 nm in peptides in random conformation. Addition of ethanol to 1 in the aqueous medium (25%, b; 50%, c; 75% ethanol, d) produced increases in negative ellipticity near 206 nm and 225 nm, and decreases in that near 200 nm. These results indicate that 1 contained α-helical peptide bonds in the solutions and a hydrophobic environment enhanced the peptide folding. If the environment around the N-terminal region of troponin I in troponin complex is hydrophobic, it is likely

that the N-terminal binding site of troponin I in the complex folds into α-helical conformation.

References

1) H. Syska, J. M. Wilkinson, R. J. A. Grand, and S. V. Perry, Biochem. J., 153, 375 (1976).
2) S. Nozaki, K. Kobayashi, E. Katayama, and I. Muramatsu, Chem. Lett., 1980, 345.
3) J. A. Talbot and R. S. Hodges, J. Biol. Chem., 256, 2798 (1981).
4) J. M. Wilkinson and R. J. A. Grand, Nature, 271, 31 (1978).
5) 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 used are as follows; Boc = t-butoxycarbonyl, Z = benzyloxycarbonyl, Bzl = benzyl ether, OBzl = benzyl ester, NO_2 = nitro, ONo = o-nitrophenyl ester, TFA = trifluoroacetic acid, WSCD.HCl = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole, DMF = N,N-dimethylformamide, HMPA = hexamethylphosphoramide.
6) E. Schnabel, H. Herzog, P. Hoffmann, E. Klaube, and I. Ugi, Liebigs Ann. Chem., 716, 175 (1968).
7) M. Bodanszky and K. W. Funk, J. Org. Chem., 38, 1296 (1973).
8) S. Nozaki, A. Kimura, and I. Muramatsu, Chem. Lett., 1977, 1057.
9) D. Yamashiro, Nature, 201, 76 (1964).

(Received January 22, 1982)