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THE STRUCTURE OF ANCOVENIN, A NEW PEPTIDE INHIBITOR OF ANGIOTENSIN I CONVERTING ENZYME

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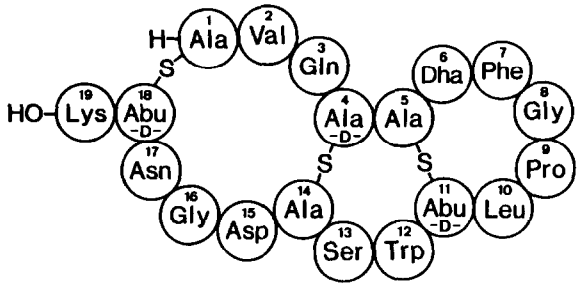
Summary: The structure of ancovenin, a new peptide inhibitor of angiotensin I converting enzyme, was determined to be a unique tricyclic peptide which comprises sixteen amino acid residues including dehydroalanine and three sulfide amino acids as unusual components.

Ancovenin, a new peptide inhibitor of angiotensin I converting enzyme (ACE), was isolated from a culture broth of *Streptomyces* sp. No. A647P-2 in a soil sample collected at Hachioji, Tokyo.[1] The IC_{50} value of ancovenin to rat lung ACE was obtained to be 8.7×10^{-7} M. Ancovenin is more active than potentiator C[2] (2.4×10^{-6} M), one of the known peptide ACE inhibitor, whereas less active than a synthetic antihypertensive agent captopril[3] (2.9×10^{-8} M).



Ancovenin

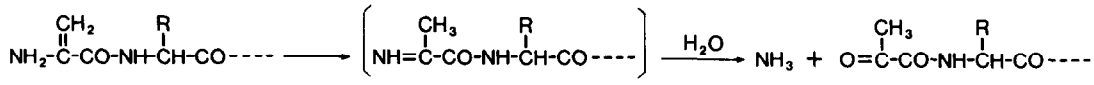
Dha : dehydroalanine
Abu : α-aminobutyric acid (= 3-methylalanine)
Ala–S–Ala : lanthionine*
Ala–S–Abu : β-methyllanthionine*

(*regarded as two amino acid residues for convenience when numbered in the peptide)

Although amino acid composition of ancovenin after acid hydrolysis was already elucidated in a previous paper,[1] we could not decide the original form of three acidic amino acid residues in the peptide. Since an accurate measurement of FAB mass spectra gave a definite molecular weight of 1960,[4] the result indicated that two of three acidic components are of ω-amide form such as asparagine or glutamine. Thus, the constituent amino acids of ancovenin were revealed to be Asp(1 or 0), Asn(1 or 2), Glu or Gln (1), Gly(2), Ser(1), Pro(1), Val(1), Leu(1), Phe(1), Dha(dehydroalanine)(1), *meso*-Lan(*meso*-lanthionine)(1),[5] *threo*-β-MeLan(*threo*-β-methyllanthionine)(2),[5,6] Trp(1), and Lys(1). A total structure of ancovenin will be reported in the present paper.

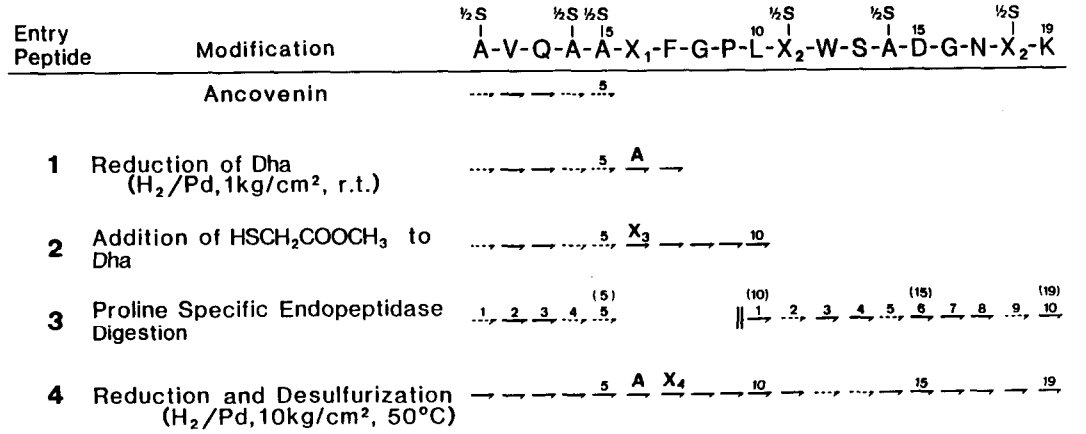
In order to determine the amino acid sequence of ancovenin, Edman degradation was successfully applied to several peptides obtained by chemical or enzymic modification of ancovenin as well as the intact molecule. The results were summarized in Fig. 1. In the Edman degrada-

tion, we could not detect phenylthiohydantoin (PTH) derivative of sulfide amino acid, *i.e.*, *meso*-Lan or *threo*-β-MeLan. Furthermore, the reaction stopped at the position of Dha residue because of an elimination of amino group through its easy decomposition *via* imino intermediate as follows. In fact, the analysis of intact ancovenin could not go on beyond the third amino



acid. This fact suggested that Dha residue may be located at a position near *N*-terminal in the molecule. Indeed, the sixth amino acid from *N*-terminal was assigned to be Dha, which was characterized as Ala in the peptide **1** obtained by catalytic hydrogenation of ancovenin or as methoxycarbonylmethylcysteine in the peptide **2** prepared by an addition of $HSCH_2COOCH_3$ to ancovenin, respectively. However, we could not still clarify the sequence beyond the tenth amino acid residue in this way.

Prolyl peptide bond in ancovenin was then cleaved by digestion with proline specific endopeptidase to obtain a new amino terminal for further development of the sequencing. The digestion resulted in a formation of a single peptide having two amino terminals in the molecule, which indicated the location of Pro residue in one of the rings not in an open chain. Edman degradation of the peptide **3** proceeded well to reveal the sequence from 10 to 19 in ancovenin except three unknown residues. Although the positions of common amino acids and Dha were ascertained from the analyses of ancovenin as well as the modified peptides **1** – **3**, no information about sulfide amino acids was obtained. A possible position of sulfide amino acid was then elucidated on a desulfurized peptide **4** prepared by hydrogenolysis of ancovenin under increased pressure. As a result of the desulfurization, *meso*-Lan was converted into two Ala residues and *threo*-β-MeLan into Ala and Abu (α-aminobutyric acid). The hydrogenation under increased pressure also caused a conversion of Phe to cyclohexylalanine as well as a reduction of Dha to Ala. The whole amino acid sequence of the peptide **4** was clearly determined by Edman



—→: Edman degradation (----: PTH-amino acid was not detected)

X_1= dehydroalanine ; X_2= α-aminobutyric acid=3-methylalanine ;
X_3= S-methoxycarbonylmethylcysteine ; X_4= cyclohexylalanine .

Fig. 1. Sequence analysis of intact and modified ancovenin. Amino acid residues were shown in one-letter notations.

degradation. Thus, the linear sequence of ancovenin was now given as depicted at the upper line in Fig. 1.

In order to elucidate the exact positions of all sulfide bridges in the amino acid sequence, an assignment of sulfide bridge belonging to *meso*-Lan was first attempted as shown in Fig. 2. At least, one of three Ala residues around *N*-terminal is expected to be a part of *meso*-Lan residue. The differentiation of these Ala residues was effectively succeeded by an application of Edman-dansyl method to intact ancovenin. Dansyl (DNS) amino acid obtained by acid hydrolysis of dansylation product after the third cycle of Edman degradation was identical with authentic mono-DNS-*meso*-Lan in HPLC. On the other hand, DNS derivative from DNS-ancovenin or dansylation product after the fourth cycle of Edman degradation of ancovenin was ascertained to be mono-DNS-*threo*-β-MeLan, which was characterized in HPLC to be DNS-Ala—S—Abu by comparison with authentic sample. Based on this investigation, the sulfide bridge belonging to *meso*-Lan was unequivocally revealed to across Ala^4 and Ala^{14}.

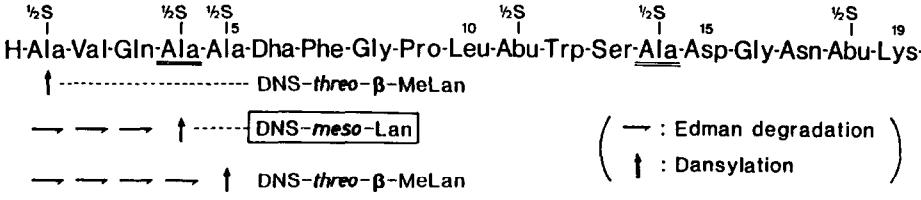


Fig. 2. Determination of the position of *meso*-lanthionine.

The next assignment of sulfide bridge involving in *threo*-β-MeLan was studied as shown in Fig. 3. Ancovenin was first treated with $NaNO_2$ to convert *N*-terminal Ala moiety into 2-hydroxypropionic acid (Hpr) residue, *i.e.*, *threo*-β-MeLan corresponding to *N*-terminal amino acid was changed to *S*-(2-hydroxypropion-3-yl)-3-methylcysteine [3MeCys(Hpr)]. The peptide thus prepared was then digested with proline specific endopeptidase to give peptide **5**. After the second cycle of Edman degradation, we noticed the disappearance of *threo*-β-MeLan and the ex-

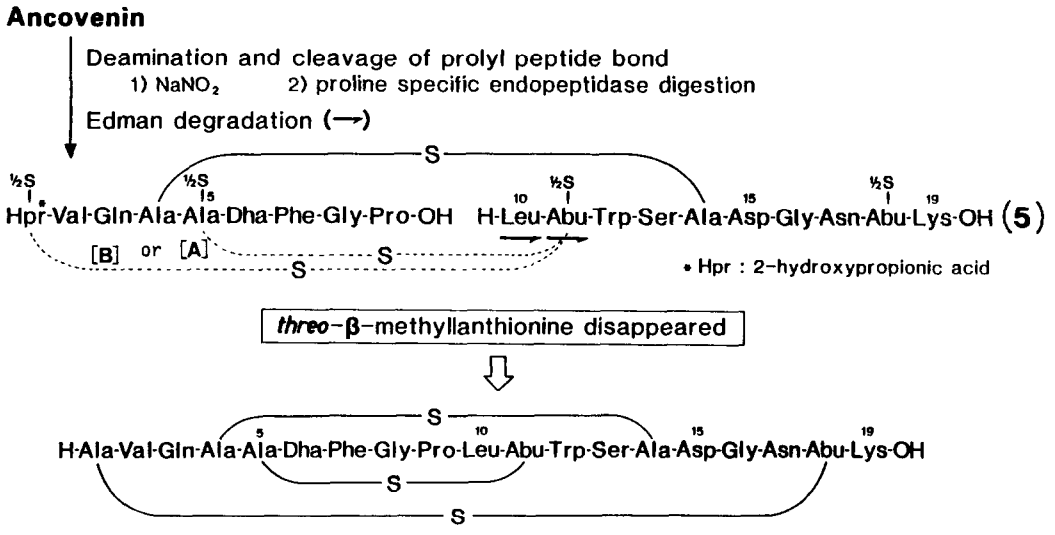


Fig. 3. Assignment of sulfide bridge belonging to *threo*-β-methyllanthionine.

istence of 3MeCys(Hpr) in the hydrolyzate of the product. This result clearly indicated that Abu[11] should be connected with Ala[5] (bridge [A]) and, therefore, Ala[1] with Abu[18]. Thus, the primary structure of ancovenin was decided as depicted in Fig. 3.

Stereochemistry of the constituent amino acids was satisfactorily studied by gas chromatographic determination of the enantiomers of amino acids. Acid hydrolyzate of ancovenin or desulfurized ancovenin **4** was treated with HCl/*i*-PrOH and then $(TFA)_2O$ to convert each amino acid into TFA-amino acid isopropyl ester.[7] The mixture was gas-chromatographed on glass capillary column coated with chiral stationary phase.[8] Based on this experiment, we could deduce that one Ala residue and two Abu residues belonging to *meso*-Lan and *threo*-β-MeLan are of D-form, whereas all other amino acid residues are of L-forms. Therefore, both *threo*-β-MeLan residues must have the form of L-Ala$_S$D-Abu. According to the DNS-diastereomer method,[9] two Cα-configurations in *meso*-Lan were assigned to be D-Ala^4$_S$L-Ala^{14}.

As described above, we could determine the whole structure of new ACE inhibitor ancovenin. Peptide antibiotics nisin and subtilin have been already known as cyclic peptides comprising sulfide ring. Ancovenin is therefore the third cyclic sulfide peptide so far clarified. However, a feature of triply overlapped ring system in ancovenin is very unique and peculiar in comparison with those of nisin[5c,d] and subtilin[5e]. From this point of view, a study of structure and activity relationship of ancovenin seems to be particularly interesting and important.

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8) The column was prepared by Dr. S. Kusumoto *et al.* according to a modified procedure reported in the reference: T. Saeed, P. Sandra, and M. Verzele, J. Chromatogr., 186, 611 (1979).
9) DNS-*meso*-Lan obtained by acid hydrolysis of dansylation product after the third cycle of Edman degradation of ancovenin (Fig. 2) was converted into DNS-Ala^4$_S$L-Leu-Ala^{14}, which was identical with authentic DNS-D-Ala$_S$L-Leu-L-Ala in HPLC.

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