Crystal Structure of the 10-13 Sequence of Angiotensinogen L-Leucyl-L-leucyl-L-valyl-L-tyrosyl Methyl Ester

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Abstract: The crystal structure of Leu-Leu-Val-Tyr-OMe, a tetrapeptide that is split at the Leu-Leu bond level by the enzyme renin, has been determined by X-ray diffraction. The peptide crystallizes in the orthorhombic space group $P2_12_12_1$ with 4 molecules in a unit cell of dimensions a = 4.979 (1), b = 22.482 (4), and c = 26.559 (5) Å. The structure has been refined to an R value of 0.074 for 1606 observed reflections. The peptide main chain is in a zigzag conformation. In the crystallographic a direction, the molecules are linked by hydrogen bonds to make infinite parallel β-pleated sheets.

Renin is a highly specific enzyme that cleaves the Leu-Leu peptide bond in equine[1] or the Leu-Val bond in human substrates.[2] It plays an important role in the regulation of blood pressure by cleaving angiotensinogen to give angiotensin I, which is subsequently converted into angiotensin II, the potent vasoconstrictor.[3] Specific competitive inhibitors of the first step of the renin-angiotensinogen reaction would be of substantial value as therapeutic agents. Today, in spite of the fact that carboxyl proteinases have been widely studied and several renin inhibitors proposed, their usefulness as pharmacological tools is limited.

This investigation was undertaken to give a precise conformation for the 10-13 sequence of the endogenic substrate, to begin exploration of the substrate requirements for renin, and to plan for the synthesis of specific competitive inhibitors.

Experimental Section

A sample of the title compound was supplied by the Roussel-Uclaf research laboratory. Colorless crystals were obtained from a dimethylformamide/water solution. The crystal used for data collection was a plate of dimensions 0.4 × 0.2 × 0.06 mm elongated along the crystallographic a axis. The preliminary unit cell parameters were evaluated on Weissenberg photographs. The final values were refined from setting angles of 23 reflections ($18 < 2\theta < 57°$) measured on a Nonius CAD4 diffractometer with Cu Kα radiation. The crystallographic data are summarized in Table I. A total of 2237 reflections were collected within the Cu sphere of θ to 55°. The two reference reflections monitored every 50 reflections did not show significant variation in intensity over the data collection period ($\Delta I/I < 0.04$). Among all the reflections, 1606 with $I > 3\sigma(I)$ were considered as observed and were selected for the structural analysis. The intensities were corrected for Lorentz and polarization effects but not for absorption.

The structure was solved by direct methods using the MULTAN 80[4] system of computer programs. An E map ($|E| > 1.58$) calculated from the set of phases having the highest figure of merit revealed all the heavy atoms except the terminal methyls of leucyl residues. The missing atoms were located from a difference Fourier map after two cycles of block-diagonal least-squares refinement [minimization of $(F_c - F_o)^2$]. After a few more cycles of refinement the Cδ1 (Leu-2) atom showed an unreasonable geometry and a relatively large anisotropy; it was presumed to be disordered and was split into two sites with an equal population factor of 0.50 and the corresponding B's were refined as isotropic. Hydrogen atoms were calculated in geometrical positions except those of the methyl groups, hydroxyl OH, and N-terminal end. Due to the very high thermal parameters or the statistical positions of the methyl groups it was not possible to find other hydrogen atoms on a difference Fourier map. The last least-squares refinement including anisotropic thermal parameters for all non-hydrogen atoms having an occupancy equal to 1 converged to R [$=\sum(|F_o| - |F_c|)/\sum|F_o|$] = 0.074. The hydrogen parameters were not refined, and their temperature factors were set equal to B_{eq} of their carrier atoms. The atomic scattering factors for non-hydrogen atoms are taken from ref 5 and for hydrogen atoms from Stewart, Davidson, and Simpson[6]. The atomic coordinates for the non-hydrogen atoms are listed in Table II.

Table I. Crystal Data

chemical formula, $C_{27}H_{44}N_4O_6$
space group, $P2_12_12_1$
$Z = 4$
cell parameters
a = 4.979 (1) Å
b = 22.482 (4) Å
c = 26.559 (5) Å
V = 2973 Å^3
D_{calcd} = 1.16 g cm^{-3}
$R = (\sum\|\|F_o\| - \|F_c\|\|)/\sum\|F_o\| = 0.074$
$R_w = 0.082$

Results and Discussion

The structural formula and the bond lengths and angles are shown in Figure 1. These values are in good agreement with those reported previously for other amino acids and peptides. The atom-labeling scheme is that proposed by the IUPAC-IUB Commission on Biochemical Nomenclature.[7]

As seen in the stereodrawing of the peptide molecule in Figure 2, the main chain is rather extended with the side chains alternately situated on the left and right. There is no intramolecular hydrogen bond. The ω values do not significantly differ from 180°, indicating that the three peptide units have a planar conformation. The Leu-2 and Val residues have the usual ϕ and ψ values for the parallel β-sheet (−129, 124 and −124, 120°, respectively).[8] For such a conformation, the first and the third peptide units are nearly parallel (4.7°).

The dihedral angles χ^{ij} that define the amino acid side-chain conformation are given in Table III. For each amino acid, χ^{11} adopts a value corresponding to a low-energy conformation.[9] For the Leu residues, χ^{11} values are in the region of 180° and in the region of 300° for Val and Tyr. Such values have been observed in other peptides: Leu (172°),[10] Val (291-298°),[11] Tyr (296°).[12] For the Tyr side chain, the χ^2 angles are in the region of ±90°,

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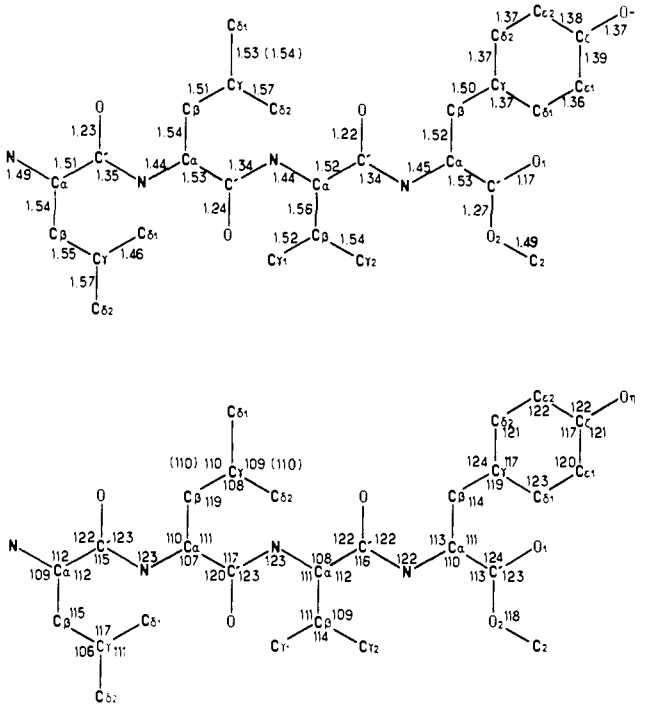

Figure 1. Bond lengths in angstroms. Estimated standard deviations are 0.008–0.015 Å. Bond angles in degrees. Estimated standard deviations are 0.8–1.1°.

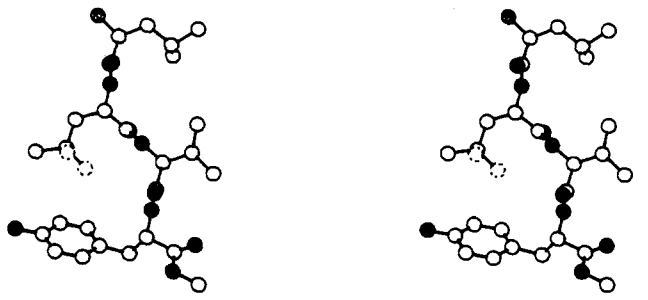

Figure 2. Stereodrawing of the peptide molecule. Thermal ellipsoids are drawn at the 50% probability level. The broken atoms indicate statistical positions.

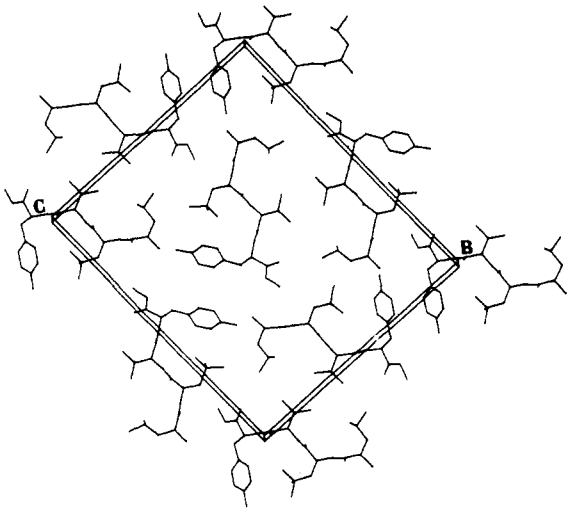

Figure 3. Crystal packing viewed down the *a* axis.

leading the ring in a position parallel to the main chain.

A projection of the crystal packing approximately viewed down the *a* axis is shown in Figure 3. There are two intermolecular hydrogen bonds, O–N terminal, that connect the molecules related by the 2_1 axes parallel to *b*. In the *a* direction, the molecules are

Table II. Positional Parameters ($\times 10^4$) of Non-Hydrogen Atoms

atom	*x*	*y*	*z*
	Leucyl-1		
N	6946 (8)	2918 (2)	3181 (2)
Cα	7596 (8)	2921 (2)	2633 (2)
C′	6191 (7)	3425 (2)	2362 (3)
O	3743 (7)	3487 (2)	2377 (2)
Cβ	6849 (8)	2314 (2)	2407 (2)
Cγ	7440 (8)	2246 (2)	1836 (2)
Cδ1	10168 (9)	2382 (3)	1668 (3)
Cδ2	6654 (8)	1591 (3)	1700 (3)
	Leucyl-2		
N	7807 (8)	3794 (2)	2100 (1)
Cα	6817 (9)	4293 (2)	1814 (2)
C′	8041 (9)	4246 (2)	1289 (2)
O	10510 (8)	4219 (2)	1237 (2)
Cβ	7644 (8)	4878 (2)	2070 (2)
Cγ	6991 (9)	5459 (3)	1812 (3)
Cδ1 (0.5)	8400 (11)	5491 (3)	1299 (3)
Cδ1 (0.5)	3931 (11)	5511 (3)	1739 (3)
Cδ2	8019 (9)	5982 (3)	2156 (3)
	Valyl		
N	6323 (8)	4232 (2)	902 (2)
Cα	7176 (8)	4205 (2)	382 (2)
C	5947 (9)	4731 (2)	105 (2)
O	3532 (8)	4807 (2)	80 (2)
Cβ	6417 (7)	3601 (2)	132 (2)
Cγ1	7477 (8)	3079 (2)	439 (2)
Cγ2	7385 (8)	3601 (2)	−419 (2)
	Tyrosine OMe		
N	7680 (8)	5115 (2)	−106 (2)
Cα	6800 (9)	5615 (2)	−407 (2)
C′	6277 (8)	5414 (2)	−948 (2)
O1	7209 (10)	4983 (3)	−1124 (3)
O2	4578 (9)	5739 (2)	−1177 (2)
C2	3675 (11)	5547 (3)	−1685 (3)
Cβ	8738 (8)	6136 (2)	−390 (2)
Cγ	8719 (9)	6472 (2)	99 (2)
Cδ1	6822 (9)	6907 (2)	171 (2)
Cδ2	10538 (8)	6383 (2)	477 (2)
Cε1	6713 (9)	7244 (2)	598 (2)
Cε2	10477 (9)	6721 (2)	907 (2)
Cξ	8571 (8)	7157 (2)	982 (2)
Oη	8477 (8)	7494 (2)	1411 (2)

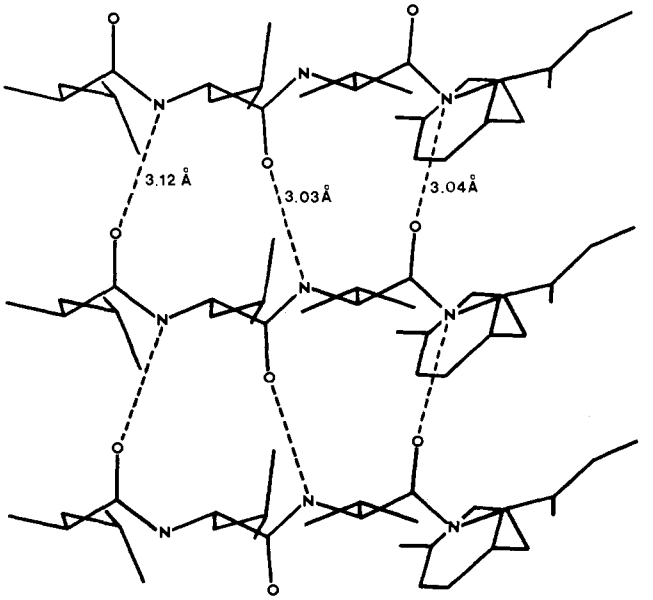

Figure 4. Drawing of the parallel-sheet structure. Hydrogen bonds are represented by dotted lines. The *a* axis points upward.

linked by hydrogen bonds and form parallel β-pleated-sheet-type structures (Figure 4). Every hydrogen bond is built with the oxygen atom of one peptide bond and the nitrogen of the same

Table III. Conformation Angles (Degrees)

	ϕ	ψ	ω	χ^{11}	χ^{12}	χ^{21}	χ^{22}
leucyl-1		125	179	180		53	177
leucyl-2	−129	124	178	176		−43	179
valyl	−124	120	176	−54	180		
tyrosyl	−84	154		−73		−84	98

Table IV. Hydrogen Bonds (Like) in the Structure

bonds		bond length, Å
N(Leu-2)–O(Leu-1)	$1 + x, y, z$	3.12
O(Leu-2)–N(Val)	$1 + x, y, z$	3.03
N(Tyr)–O(Val)	$1 + x, y, z$	3.04
O(Tyr)–N(Leu-1)	$1 - x, 0.5 + y, 0.5 - z$	3.06
O(Tyr)–N(Leu-1)	$2 - x, 0.5 + y, 0.5 - z$	2.70

peptide bond of the next molecule. The mean length of these N—H···O═C hydrogen bonds, 3.06 Å (Table IV), is greater than that proposed by Marsh and Donohue[13] and also longer than that generally observed for the pleated parallel β-sheet in the crystal structures of di- or tripeptides.[14] To our knowledge this is the first structural example showing the exactly parallel (untwisted) β-sheet structure for a tetrapeptide. The fiber axis average period ($C\alpha_{i-1}\cdots C\alpha_{i+1}$ distance in the molecule) is equal to 6.62 Å. The only two other tripeptide structures in which such a β-pleated sheet has been observed appears to be that of glycylphenylalanylglycine[15] and (phenyloxy)acetylleucylvalylphenylalanyl-OMe.[16] As for the tripeptides, the crystal has the characteristic of being elongated along the crystallographic axis that has a period of about 5 Å. This distance corresponds to the one observed between two parallel neighboring molecules in the sheet.

In addition to the hydrogen-bond interactions, there are two intermolecular contacts closer than those of van der Waals interactions. They are observed at the level of the tyrosyl Cδ and Cε atoms: $C\epsilon 2(x,y,z) - C\epsilon 1(1 + x,y,z) = 3.42$ Å and $C\delta 2(x,y,z) - C\delta 1(1 + x,y,z) = 3.44$ Å.

The results obtained from the X-ray crystallographic analysis of the tetrapeptide demonstrate the presence of a β-pleated-sheet conformation in the crystal. Such a geometry must be counted among those accessible to the peptide, and one can speculate that the crystal conformation is closely related to a conformation in another environment, in particular, to one at the level of the renin binding site.

It is interesting to compare this tetrapeptide conformation with that observed for the analogous tripeptide (phenyloxy)acetyl-leucylvalylphenylalanyl-OMe.[16] In spite of chemical modifications and different crystal-lattice forces, the conformations are essentially the same. Finally, one can point out the high frequency of occurrence of Val and Leu residues in the β-pleated sheets observed in protein structures.[17-20] These arguments lead to the conclusion that a β-pleated sheet is probably one of the most stable conformations for the angiotensinogen (10–13) fragment. Such a conclusion agrees with the extended sites described for acidic proteases.[21,22]

The present study shows a model for the 10–13 renin substrate conformation and gives precise information concerning a probable stable geometry. In order to get more definitive insight into the conformational renin substrate requirements, it may be of great use to investigate longer peptides, including the Pro-7 residue, which is known to favor folded conformations.[23]

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Supplementary Material Available: Tables of anisotropic thermal parameters of non-hydrogen atoms and hydrogen coordinates (1 page); tables of observed and calculated structure factors (14 pages). Ordering information is given on any current masthead page.

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