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Structure–Activity Relationship of Fluorine-Containing Renin Inhibitory Peptides Based upon the Tertiary Structure of Human Renin

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The structure–activity relationship of acyl-His-trifluorinated leucinol derivatives as inhibitors of human renal renin is discussed based upon the tertiary structure of human renin, which was deduced from the crystal structure of penicillopepsin by assuming structural similarity. The structural requirements for the inhibitors and possible interactions at the renin binding site are discussed.

Keywords——renin inhibitor; computer graphic; model building; structure–activity relationship; human renin tertiary structure; 2-amino-4-trifluoromethylpentanol

Angiotensinogen is selectively cleaved by an acid protease renin to give angiotensin I. Angiotensin I is further converted to the potent pressor substance angiotensin II by the converting enzyme. Many renin inhibitors have been studied as candidate drugs to overcome hypertension related to abnormality of the circulating renin activity. Knowledge of the three-dimensional structure of the renin molecule would provide useful information for the design of potent and selective inhibitors of this enzyme. The amino acid sequence of human renal renin has been determined,[1,2] but its X-ray structure has not yet been reported. It has been suggested that proteins with homologous amino acid sequences have similar three-dimensional structures,[3-6] and the three-dimensional structures of various proteins including blood coagulation factors,[7] mammalian serine proteases,[8] inflammatory protein C5a[9] and cathepsin B[10] have been constructed based upon the concept of structural similarity. We have also deduced a three-dimensional structure of human renal renin[11] based upon the X-ray structure of penicillopepsin[12] and analyzed the mode of interaction between the renin model and renin inhibitors.[11,13] On the basis of this information, many compounds were designed and synthesized, and the renin inhibitory activities of these compounds were measured.[14] We report here the structure–activity relationship of fluorine-containing peptides based upon the renin model.

Z–Phe–His–Leu–OCH_3 (Z = benzyloxycarbonyl) having the P3-P2-P1[15] amino acid residues of renin substrate scarcely inhibited renin activity. Conversion of P1 Leu–OCH_3 to leucinol slightly improved the inhibitory activity (**3** in Table I). Kokubu *et al.*[16] reported the IC_{50} value of **4**, in which the benzene ring of P3 Phe was converted to a 1-naphthyl ring, to be 5.6 μM (see Table I). On the basis of the information obtained from the deduced tertiary structure of human renin, we have modified the inhibitor so as to fit into the binding site more tightly. Compound **2**, which was derived from the (−)-isomer of 2-(1-naphthylmethyl)-3-

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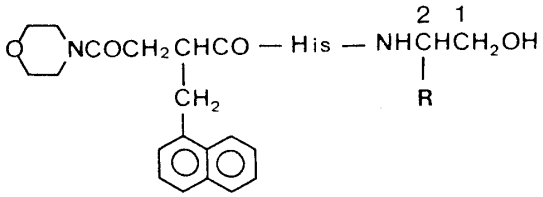
TABLE I. Renin-Inhibitory Activity of Compounds **1**—**4**



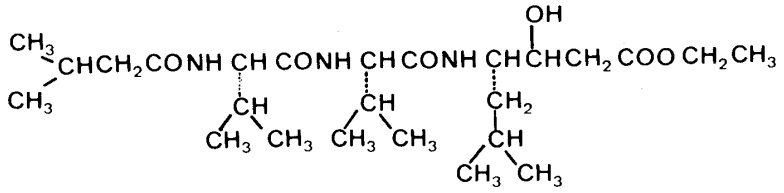
Compound	Structure	IC_{50} (M)
1a	R=(2*S*,4*S*)	5.7×10^{-7}
1b	R=(2*S*,4*R*)	2.6×10^{-6}
1c	R=(2*R*,4*S*)	$>10^{-5}$
1d	R=(2*R*,4*R*)	$>10^{-5}$
2	R=(2*S*)-$CH_2CH(CH_3)_2$	1.3×10^{-6}
3	Z-Phe-His-leucinol	$>10^{-4}$ [a]
4	Z-[3-(1-naphthyl)Ala]-His-leucinol	5.6×10^{-6} [b]

a) 40% inhibiton at 10^{-4} M. *b*) From ref. 16.

(morpholinocarbonyl)propionic acid, was found to be 4.3-fold more potent than **4**. The absolute configuration of this compound has not yet been determined experimentally. However, we assumed[14b,c] that the configuration of the carbon atom having the P3 naphthylmethyl group is *R* type, *i.e.* the same configuration as a naturally occurring amino acid.

In order to elucidate the influence of fluorine on the renin inhibitory activity, we tested compounds **1a**—**d**, which contain a trifluorinated leucinol in the P1 position. As can be seen from Table I, the 2*S* compounds (**1a** and **1b**) were rather more active than the compounds of 2*R* form (**1c** and **1d**). Compounds **1a** and **1b** have the same configuration at the C2 atom as that of P1 Leu of the substrate. It seems that the 2*R* compounds do not tightly fit to the renin model (see the following discussion). Although the trifluoromethyl group with *R* configuration (**1b**) decreased the potency, the trifluoromethyl group with *S* configuration (**1a**) gave a 2.3-fold increase in the inhibitory activity. Thus, the inhibitory activity of the 4*S* isomer was found to be approximately 4.5 times that of the 4*R* isomer.

The deduced active site of human renin with **1a** is shown in Fig. 2. In general, insertions and deletions of amino acid residues in homologous proteins are likely to occur on the surface rather than in the core region of the protein molecule, and hence the three-dimensional structure of the core region including the active site is expected to be highly conserved.[17] Also, in human renin the deletions and insertions seem to have mainly occurred at the surface region.[11] Compound **1a** was builded based on the crystal structure of compound **5**, Iva-Val-Val-Sta-OEt (Iva=isovaleryl; Sta=Statine, 4-amino-3-hydroxy-6-methylheptanoic acid), and fitted into the active site of human renin (see the experimental section). The conformational changes of the backbone of **1a** required to fit it to the enzyme were small.



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The P1 side chain containing the trifluoromethyl group and the P3 naphthylmethyl group

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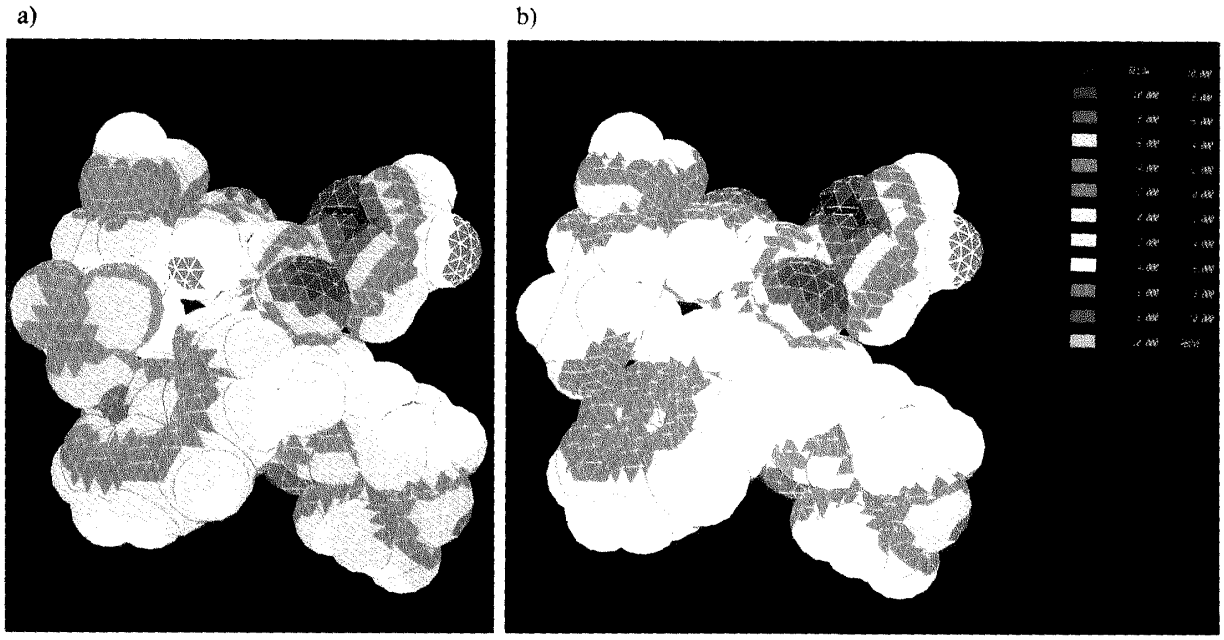

Fig. 1. Electrostatic Character of Compounds **1a** and **2**

a) Electrostatic potential of **1a** (kcal/mol). b) Electrostatic potential of **2** (kcal/mol).

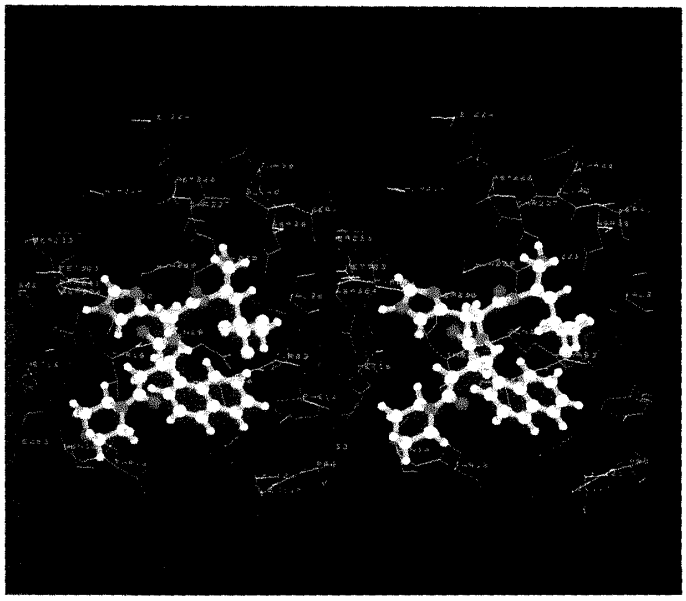

Fig. 2. Stereopair Showing the Active-Site Region of Human Renin with Compound **1a**

The inhibitor is illustrated by a ball and stick model (red = oxygen, blue = nitrogen, white = carbon, yellow = fluorine).

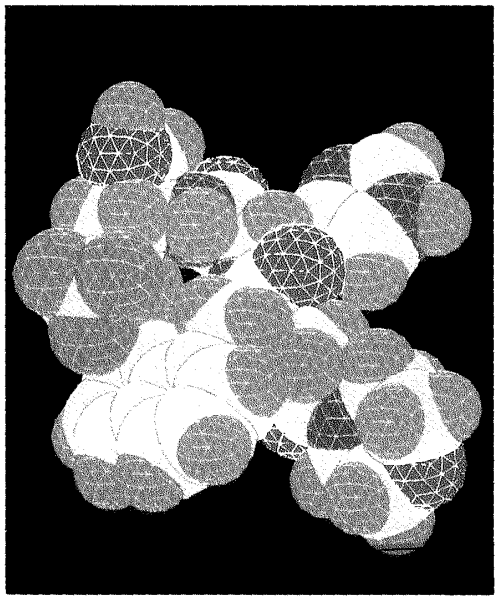

Fig. 3. Space-Filling Display of **1a**

This illustration is represented from the same direction as in Fig. 1a (red = oxygen, blue = nitrogen, white = carbon, pale blue = hydrogen, green = fluorine).

interact with the large and hydrophobic subsites S1 and S3 under the "flap" region, respectively. The P4 morpholine moiety interacts with the S4 site containing Tyr 231 and Phe 253. The morpholine residue also seems to contact solvent water molecules. From Fig. 2, several hydrogen bonds appear to be formed between **1a** and renin; –OH of **1a**↔two –COO(Asp 38 and Asp 226) of renin, –CF3 of **1a**↔–$CONH_2$ of Gln 19, P1 backbone –NH– of **1a**↔–CO– of Gly 228, P3 backbone –CO– of **1a**↔–NH– of Ser 230. The P1 side chain of the

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2*S* isomer (**1a**) can be well fitted into the S1 site as shown in Fig. 2. However, in the case of the 2*R* isomers (**1c** and **1d**), the P1 side chain does not interact with the S1 site unless the essential hydroxy group of the inhibitor is removed from the catalytic site of the renin molecule.

In the crystal structure of compound **5** in penicillopepsin, the methyl group corresponding to the trifluoromethyl group in the isobutyl side chain of **1a**—**d** was found to be directed to the inside of the enzyme pocket.[12] By analogy, it seems possible that the trifluoromethyl group in **1a** is located deep in the S1 subsite of renin. Consequently, when the inhibitor is incorporated into the active site of renin in solution, the degree of desolvation of the trifluoromethyl group would be greater than that of the C6 methyl group.

It is, however, difficult to explain the difference in the inhibitory activity between the stereoisomers **1a** and **1b** only by this hydrophobic energy, because the difference in the transfer free energy from an aqueous phase to an *n*-octanol phase[18] between the trifluoromethyl and methyl groups is calculated to be very small; -1.02 kcal/mol for trifluoromethyl and -0.96 kcal/mol for methyl at 25 °C based upon Rekker and de Kort's compilation.[19] Figures 1a and 1b show electrostatic potential maps of **1a** and **2**, respectively, on each molecular surface. The potential maps indicate that the trifluoromethyl group is more negative than the methyl group as regards electronic charge. The trifluoromethyl group is located close to Gln 19 in our model of the renin–**1a** complex (see Fig. 2). The increment of inhibitory potency of **1a** might be ascribed in part to possible hydrogen bonding[20] between the trifluoromethyl group and the amide group of Gln 19. On the other hand, in the case of **1b**, the C6 methyl group seems to interact with the S1 site, and the trifluoromethyl group would be directed toward the outside of the enzyme. Compound **2**, in which the methyl group interacts with the enzyme, was more active than **1b**, but the reason for this is not clear. Sham *et al.*[21] reported the structurally conserved regions (SCR's) of acid proteases based upon the X-ray structures of penicillopepsin, rhizopuspepsin, endothiapepsin and porcine pepsin. Glu 19 is located in the second region of their SCR's. Therefore, the spatial deviation of Glu 19 from the actual position in the renin molecule would be expected to be small.

It has been reported that a similar mode of binding with some of the same hydrogen bonds is found in the inhibitors in rhizopuspepsin and endothiapepsin.[22] Recently, the X-ray structures of several renin inhibitors bound to the active site of endothiapepsin were determined.[23,24] Similar inhibitor orientations and hydrogen-bonding networks in the complexes were also found. In particular, large differences were not found in the orientations of the more deeply buried moieties such as P1′, P1 and P3 side chains. The hydrogen-bonding network detected in the renin inhibitor backbone and renin molecule was also found in the inhibitor–enzyme complexes of penicillopepsin,[17] rhizopuspepsin[22] and endothiapepsin.[23,24]

The three-dimensional structure of human renin with the renin inhibitor reported here is only a model. The actual three-dimensional structure of renin and the mode of interaction with the inhibitor should be established by crystal structure analysis. Furthermore, it must be kept in mind that the more exposed side chains are reported to show considerable variation in their interactions.[23,24]

Nevertheless, the geometrical information about the inhibitor in the active site and the information about the interacting mode should be valuable in the development of new renin inhibitors.[11,13,14,22–25]

Experimental

Renin Inhibitory Activities——The inhibitory activity of the inhibitor against human renin was measured by radioimmunoassay. To a mixture containing 200 μl of a pyrophosphate buffer (125 mM, pH 7.4), 25 μl of an aqueous solution of Phe–Ala–Pro (20 mM) as a inhibitor of angiotensin converting enzyme, 50 μl of sheep renin substrate (2000 ng angiotensin I/ml), 50 μl of dimethyl sulfoxide (DMSO) solution of the test compound, and 150 μl of deionized water was added 25 μl of human renin (20—30 ng angiotensin I/ml·h). The mixture was incubated for

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15 min on a water bath at 37 °C. Then the reaction mixture was allowed to stand for 5 min on a water bath at 100 °C to stop the reaction. After cooling, 200 μl of the solution were taken up and the amount of angiotensin I produced by renin was determined by radioimmunoassay.

Model Building of Renin–Inhibitor Complexes and Computer Graphics——Three-dimensional models of human renin and human renin–inhibitor complexes were constructed practically according to the method described in the previous paper.[11] In this study, the construction of the models was carried out by using a computer system, BIOCES.[26] Each inhibitor molecule having standard bond lengths and bond angles was fitted into the active site of renin. The values of backbone dihedral angles of the inhibitor were taken from those of Iva–Val–Val–Sta–OEt bound to penicillopepsin.[17] The best position and conformation in the active site of renin were determined in such a way that both the maximum hydrogen bonding and the minimum close contact between the inhibitor and the enzyme were attained by changing the dihedral angles and translating the center of mass. A possible active conformation of **1a** in human renin is shown in Fig. 2. Figure 3 is a space-filling model of **1a**; this model is represented from the same direction as in Fig. 1a.

Electrostatic potentials of the inhibitors were calculated from Mulliken net atomic charges obtained by the semi-empirical modified neglect of diatomic overlap (MNDO) molecular orbital method. The value was visualized by a color code on each spherical triangle[27] defined on the van der Waals surface of the inhibitor (Fig. 1a and b).

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