Protein modeling of human prorenin using the molecular dynamics method

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To study the activation–inactivation mechanism of the renin zymogen, prorenin, a tertiary structural model of human prorenin was constructed using computer graphics and molecular dynamics calculations, based on the pepsinogen structure. This prorenin model shows that the folded prosegment polypeptide can fit into the substrate binding cleft of the renin moiety. The three positively charged residues, Arg 10, Arg 15, and Arg 20, in the prosegment make salt bridges with Asp 225, Glu 331, and Asp 60, respectively, in renin. Arg 43, which is in the processing site, forms salt bridges with the catalytic residues of Asp 81 and Asp 269. These ionic interactions between the prosegment and the renin may contribute to keeping the prorenin structure as an inactive form.

Keywords: prorenin, model building, acid activation, molecular dynamics, ionic interactions

INTRODUCTION

Renin (EC 3.4.23.15) catalyzes the first and rate-limiting step of proteolysis to generate a potent vasoactive peptide, as part of the renin–angiotensin system, and plays important roles in the regulation of blood pressure and electrolyte balance. Prorenin is an inactive zymogen of renin,[1] which has an additional 43 amino acid residues of prosegment at the N-terminal.[2,3] Prorenin is the major form of renin in plasma (as much as 90% of renin circulating is prorenin).[4–6] Thus, the activation of the prorenin may be a key step in renin angiotensin system.

Vertebrate aspartic proteinases, such as renin, pepsin, and chymosin, are biosynthesized as inactive zymogens and are then converted to active enzymes through limited proteolysis. These zymogens also have another activation pathway called acid activation.[7–9] In the case of prorenin, this enzymatic activity is expressed by the exposure of the zymogen to acidic condition. The activation is reversible and a unimolecular reaction.[10,11] This suggests that a conformational change that leads to the opening of the substrate binding cleft occurs as a result of acidification.[12]

Site-directed mutagenesis of prorenin indicates that three positively charged residues (Arg 10, Arg 15, and Arg 20) in the prosegment play a significant role in acid activation. When these residues were changed to noncharged residues, the prorenin mutants created were active without acid treatment.[13] A conformational change in the prosegment might have been caused by these mutants, under neutral pH. The hypothesis proposed is that the positively charged residues stabilize the inactive prorenin structure through electrostatic interaction between the prosegment and the active enzyme.[13] To verify this hypothesis of structural interactions, the three-dimensional structure of prorenin is indispensable.

In this study, a structural model of prorenin was constructed using computer graphics and molecular mechanics/dynamics calculations based on the structure of pepsinogen[14] and the model structure of renin[15,16] to investigate the inactivation mechanisms of prorenin. Since the coordinates

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of pepsinogen had not been published then, structural information taken from the reference paper[14] was used. Although those coordinates are now available,[17] structural modeling without using these coordinates may be valuable to establish the protein modeling method used. The pepsinogen coordinates are used to discuss the reliability of these results.

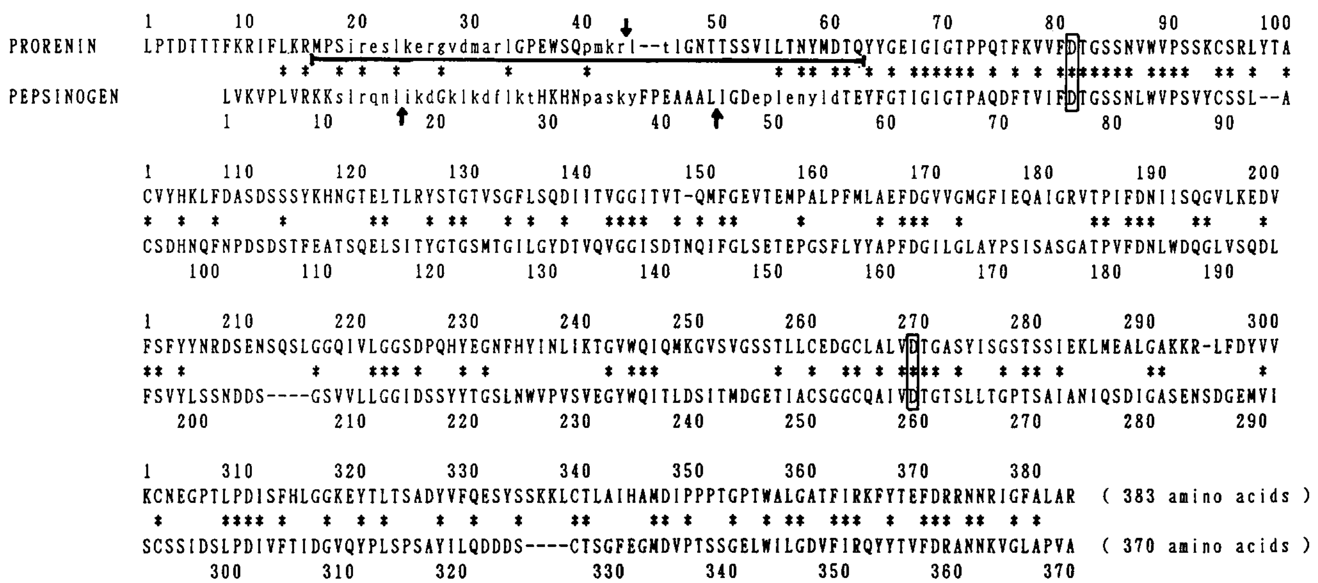
Three-dimensional models of human renin have been constructed by a number of workers based on the x-ray coordinates of other homologous aspartyl proteinases.[15,18,19] As expected from the sequence homology, the three-dimensional structure of rizopuspepsin, endothiapepsin, and penicillopepsin, which all belong to the aspartyl proteinase family, are very similar. Several groups have also used similar methods to construct structural models of various proteins.[20–22]

Recently, the three-dimensional structure of human renin was solved by x-ray crystallography.[23] The structure shows excellent similarity to the renin models. Since the coordinates of human renin were not available from the Protein Databank,[24] a modeled renin structure (constructed from penicillopepsin)[16] was used to construct the prorenin in this study.

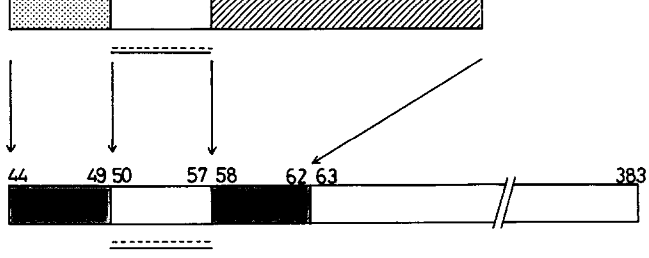
METHODS

Since the inactivation domain of pepsinogen (Lys 9–Glu 57) covers the active site of pepsin,[14] it was expected that the active site of renin is covered in a similar manner.

The primary sequence of human prorenin was aligned with porcine pepsinogen by a hydrophobic core scores algorithm.[25,26] Prorenin and pepsinogen numberings are used for the active enzymes in this paper. The inactivation domain of prorenin was assigned as Met 16–Gln 62 (Figure 1*a*). In pepsinogen, the N-terminal eight residues (Leu 1–Arg 8) form the first strand of six-stranded antiparallel β sheet, displacing the N-terminus of the active enzyme of pepsin (Ile 45–Asn 52). From sequence alignment it was deduced that Phe 8–Arg 15 in prorenin would similarly participate as the first strand to the six-stranded antiparallel β sheet instead of Thr 50–Asn 57 in active renin. Accordingly, model building of the prorenin inactivation domain (Met 16–Gln 62) was done using the pepsinogen model structure, with the remaining parts (Phe 8–Arg 15 and Tyr 63–Arg 383) taken from the renin model structure[16] (Figure 1*b*). The N-terminus seven residues (Leu 1–Thr 7) of prorenin were folded



(a)



(b)

Figure 1. (a) Sequence alignment between prorenin and pepsinogen. Asterisks, equivalenced amino acid; boxes, catalytic residues; arrows, processing sites; underline, the inactivation domain of prorenin; lowercase letters, helical secondary structures in the prosegments. (b) Schematic representation of model-building regions in prorenin. Top bar shows N-terminal 15 residues and inactivation domain in prorenin. Bottom bar shows renin model. The hatched box and shaded box are the region modeled. Hatched box, Leu 1–Thr 7, is formed as an extended β strand. Shaded box, Met 16–Gln 62, is modeled from the pepsinogen inactivation domain. Solid boxes, Leu 44–Thr 49 and Tyr 58–Gln 62, are deleted from the renin model.[16] The other region is based on the renin model. Similar main-chain structures are underlined with (----)

into stable conformation. The model building of prorenin was carried out through following three steps.

1. Model building of pepsinogen prosegment

Since the atomic coordinates of pepsinogen were not available when this study was started, the model building of the inactivation domain of pepsinogen was performed using the data obtained from the published paper[14] as summarized in Table 1. These data include four helical structures, a partial helix topology, and seven hydrogen bonds.

The model building of the pepsinogen inactivation domain was performed using restrained molecular dynamics. This method has been used for the construction of tertiary structures of protein using distance information obtained from two-dimensional NMR.[27] The initial structure for pepsinogen inactivation domain was a completely extended β strand. The dihedral angles of the four α-helix regions were then assigned to the standard values: The dihedral angles of ϕ, ψ, and ω are $-60°$, $-60°$, and $180°$, respectively.[28] The calculations were made in two stages. First, the secondary structures and the overall tertiary fold were formed and second, the folded structure was refined energetically.[27] The two N-terminal α helices were fixed orthogonally to one another according to Ref. 14 in first stage. The distance constraints for the seven hydrogen bonds were kept in both stages. The constraints for hydrogen bonds in the four α helices were kept in the first stage.

The first stage consists of five 5-ps restrained molecular dynamics runs, in which the scaling factors of the energy associated with the distance constraint were increased gradually (0.01,0.05,0.3,1.5,3.0).[27,29] After each run, energy minimization was performed to reduce the structural distortions that accompany the temperature increase of the system. In the second stage, in which the orthogonality of the two N-terminal α helices was released and the scaling factor for the hydrogen bonds was set constant to 3.0, a 10-ps equilibration followed by a 30-ps restrained molecular dynamics run was performed at 300 K. The final snapshot of the molecular dynamics run, which satisfied the conditions shown in Table 1, was used as the reference model for building the prorenin inactivation domain after energy minimization.

Table 1. Structural constraints for pepsin prosegment[a]

Geometry	Residue
α-helical structure	Ser 11–Asp 19
	Lys 21–Thr 28
	Pro 33–Tyr 37
	Glu 48–Asp 55[b]
Helix topology	Helix Ser 11–Asp 19
	orthogonal to helix
	Lys 21–Thr 28
Hydrogen bonding	Arg 8 N^{ϵ}–Glu 57 $O^{\epsilon 1}$ 2.9 Å
(distance)	Arg 8 $N^{\eta 1}$–Glu 57 $O^{\epsilon 1}$ 2.8 Å
	Lys 9 N^{c}–Glu 57 O^{c} 3.0 Å
	Arg 13 N^{ϵ}–Asp 55 $O^{\delta 1}$ 2.8 Å
	Arg 13 $N^{\eta 1}$–Asp 55 $O^{\delta 1}$ 2.8 Å
	His 29 $N^{\epsilon 2}$–Asp 47 $O^{\delta 1}$ 2.8 Å
	His 31 $N^{\epsilon 2}$–Glu 51 $O^{\epsilon 1}$ 2.8 Å

[a]Ref. 14
[b]Irregular α-helix structure
[c]Main-chain atom

2. Construction of prorenin prosegment

According to the sequence alignment between the prorenin and the pepsinogen (Figure 1*a*), the prorenin inactivation domain (Met 16–Glu 62) was constructed by replacing pepsinogen side chains with those of prorenin and by deleting residues Phe 38 and Pro 39 (pepsinogen numbering). The side-chain atomic coordinates of prorenin kept as similar to pepsinogen as possible. Of the prorenin inactivation domain, 36 residues out of the 47 are different from that of pepsinogen. Two 600-step energy minimizations were performed with a weak bond constraint of 1/15 or 1/10 of the standard force constant (420.0 kcal/mol-Å^2)[29] for the C–N bond, which was made artificially by the deletion of the two residues. After the energy minimizations, no irregularity was found for bonds, bond angles, and dihedral angles in the constructed model structure. An additional 400-step energy minimization was performed using a standard force constant for the C–N bond.

3. Docking of the inactivation domain into the active site of renin

The residues of Leu 44–Thr 49 and Tyr 58–Gln 62 were deleted from the model structure of renin[16] because these residues were expected to undergo large conformational change in the activation of prorenin (Figure 1*b*). The side chains of Thr 50–Asn 57 were replaced with Phe 8–Arg 15 because the main chain of these residues forms the first strand of the antiparallel β sheet. Then, the inactivation domain was docked with the modified renin model structure by computer graphics. The N-terminus (Met 16) and C-terminus (Gln 62) of the inactivation domain model were connected to the C-terminus of the β strand (Arg 15) and the N-terminus of the modified renin (Tyr 63), respectively. Avoiding as many bad contacts between the two segments as possible, the inactivation domain was fitted into the active site of the renin model. Since the N-terminal seven residues of prorenin (Leu 1–Thr 7) could not be deduced from the pepsinogen structure, an extended β strand was added to the docked model structure.

To relieve the repulsive contacts and to correct the connected peptide bonds, 900-step energy minimization was performed with weak bond constraints for the connected C–N bonds. An additional 900-step energy minimization was performed with the standard force constant for the C–N bonds. The obtained model structure of prorenin was simulated by molecular dynamics for 10 ps after 10 ps heating and equilibration at 300 K without constraints. The averaged structure was served a final model of prorenin.

The program CHARMM[29] was used for the molecular mechanics/dynamics calculations on a VAX 8810 computer. The program HYDRA[30] was used for the computer graphics on Evance and Southerland PS-390.

RESULTS and DISCUSSION

Sequence alignment

The sequence alignment of prorenin to pepsinogen (Figure 1*a*) starts from the 8th residue of prorenin and 36% sequence identity was found between prorenin and pepsinogen. This is similar to other reports[15,19] that compared the primary sequences of the mature enzyme portions. The catalytic residues, Asp 81 and Asp 269, of renin were matched to those of pepsin (Asp 76 and Asp 259). The sequences around the active site are highly conserved, especially for the 37 residues from Leu 55 to Ser 91 (prorenin numbering) where there are more than 70% identities. From the corresponding regions in the reference pepsinogen this sequence may consist of three β strands, which form the core of the N-terminal domain.

Only 19% of the prosegment residues are identical; however, the predicted helical structures for prorenin from Chou–Fasman algorithm[31] are very similar to those found in the experimental structures of pepsinogen (Figure 1*a*). The deletion of two residues was observed between Tyr 37 and Glu 40 in the prosegment of pepsinogen. From the sequence alignment residues Met 16–Gln 62 were assigned as the inactivation domain of prorenin.

Inactivation domain models of pepsinogen and prorenin

Color Plate 1 shows a superposition of the pepsinogen inactivation domain portions of the computer-modeled and experimental x-ray structures. The main-chain traces and side chains of the amino acid residues which form a hydrophobic core are shown. Both structures are very similar, particularly, in the regions with helical structure (Lys 9–Tyr 37) which have a root mean square deviation (RMSD) of the main-chain atoms of 2.9 Å. The side chains of Leu 12, Leu 16, Leu 22, Phe 25, and Leu 26 of the computational structure contribute to the hydrophobic core as those of experimental structure do (Color Plate 1).

The inactivation domain of prorenin was constructed based on that of the computer-simulated pepsinogen. There was little structural difference between the significant residues, Arg 10, Arg 15, and Arg 20 of prorenin, in the computer-simulated and experimental pepsinogen structures. The overall shape of this structure was a flattened disk with a long N-terminal extension formed by the first 15 residues, as found in the x-ray structure of pepsinogen inactivation domain portion. The hydrophobic core was also conserved in the inactivation domain model of prorenin. The main-chain trace and the hydrophobic core side chains of the constructed model are also shown in Color Plate 1.

Prorenin model structure

In the final model, the shape of the prorenin was more globular than that of the renin, because the substrate binding cleft was filled with the inactivation domain (Color Plate 2). A small conformational change from the mature renin was observed. RMSD was 2.1 Å for the main-chain structure, with N-terminal 62 residues containing the inactivation domain excluded. The polypeptide chain of Thr 45–Val 53 residues formed a large loop structure in the center of the cleft and covered the β hairpin loop of the flap region (Leu 122–Ser 132) (Color Plate 2).

Interactions between renin and its prosegment

It is known that the prosegments of acid proteinases have several positively charged residues at neutral pH.[32] In pepsinogen four positively charged residues form ion pairs with negatively charged residues in the N-terminal 13 residues of the active enzyme, as listed in Table 1, and these ion pairs may contribute to the formation of the inactivation domain.[14] Moreover, Lys 3 in pepsinogen prosegment makes an ion pair with Asp 215 in the mature pepsin portion, and Lys 36 has a pivotal role in the inhibition for the catalytic residues (Asp 76 and Asp 259).[14]

It has been suggested by the site-directed mutagenesis in the prosegment of human prorenin that the positively charged residues such as Arg 10, Arg 15, and Arg 20 (not Lys 9, Lys 14, Arg 32) interact with the negatively charged group(s) in the mature renin portion for maintenance of human prorenin in an inactive form.[13] Possible counterparts in the prorenin model for Arg 10, Arg 15, and Arg 20 are shown in Table 2. The overall structure of prorenin model and the interaction sites are shown in Color Plate 3. Arg 10 makes an ion pair with Asp 225, which corresponds to the ion pair of Lys 3 and Asp 215 in pepsinogen, and has a possibility of an interaction with Glu 230. Arg 15 interacts with Glu 331 and also makes a hydrogen bond with Gln 62. The ion pair corresponds to that of Arg 8 and Glu 57 in pepsinogen. Arg 20 makes an ion pair with Asp 60, corresponding to that between Arg 13 and Asp 55 in pepsinogen. The acid activation of prorenin may be proceeded by the protonation of the anionic residues which are the counterparts of these positively charged residues. If these three Args or their counterparts are substituted by noncharged residues by site-directed mutagenesis, partially active forms of prorenin will probably be obtained. Arg 43 approaches the catalytic residues of Asp 81 and Asp 269 in prorenin (Color Plate 4) in a similar way to the interaction of Lys 36 in pepsinogen. At neutral pH, prorenin is activated by stepwise proteolysis.[33] Arg 43 is one of the process sites for this proteolytic activation.[34] Lys 9, Lys 14, and Arg 32, which are not always conserved in other acid proteinases,[32] are exposed to

Table 2. Interactions between the prorenin prosegment and the renin portion

Positively charged residue	Negatively charged residue	Distance (Å)
Arg 10 $N^{\eta 1}$	Asp 225 $O^{\delta 1}$	2.7
$N^{\eta 2}$	Glu 230 $O^{\epsilon 1}$	5.3[a]
Arg 15 $N^{\eta 1}$	Gln 62 $N^{\epsilon 1}$	3.3
$N^{\eta 1}$	Glu 331 $O^{\epsilon 1}$	2.8
Arg 20 $N^{\eta 1}$	Asp 60 $O^{\delta 1}$	2.7
Arg 43 $N^{\eta 1}$	Asp 81 $O^{\delta 1}$	2.7
$N^{\eta 2}$	Asp 269 $O^{\delta 1}$	2.9

[a]If the dihedral angles of side chain (χ 1 and/or χ 2) are rotated, an ionic interaction can be made

solvent in our model. It is necessary that cleavage sites be exposed to solvent to be recognized and hydrolyzed by trypsin-like proteinases. Since Arg 43 is buried in the intact prorenin, it is suggested that cleavage between Arg 43–Leu 44 occurs only after the cleavage between Lys 9–Arg 10 and Lys 14–Arg 15.[33]

CONCLUSION

A model of human prorenin has been built based on pepsinogen tertiary structure. Inactivation domain of prorenin can fit into the substrate binding cleft of the renin moiety. The model supports the hypothesis that more than three ionic interactions between the prosegment and the renin contribute to keeping the prorenin structure as an inactive form.

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NOTE

The coordinates of prorenin model available upon request from Y. Shiratori.

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