Construction of a Model for the Three-dimensional Structure of Human Renal Renin

WILLIAM CARLSON, MARTIN KARPLUS, AND EDGAR HABER

SUMMARY The aspartyl proteases that have had their complete three-dimensional structures determined by x-ray diffraction techniques exhibit a high degree of structural homology and a correspondingly high degree of sequence homology. Using this homology, we constructed a model for the three-dimensional structure of human renal renin. We then refined and evaluated the model with the energy refinement program called CHARMM. We found that the model for human renin differs from that of mouse submaxillary gland renin in certain features, which may account for the differences in substrate specificities and antibody binding. Amino acid differences between human and mouse renin in the regions that bind the P1' side chain of the substrate appear to change only the shape of the S1' subsite of the enzyme, so that either valine or leucine side chains of the substrate can be accommodated by human renin. Amino acids in the solvent-accessible surface of the 75-85 flap appear to be distinctly different between the two structures and could account for the differences observed in antibody binding to human and mouse renin. (Hypertension 7: 13-26, 1985)

KEY WORDS • human renin • three-dimensional modeling • energy refinement • aspartyl proteases

THE renin-angiotensin system has been implicated in the pathophysiology of several forms of hypertension. In the initial steps of this system, a circulating α2-macroglobulin, angiotensinogen, is converted to the potent vasoconstrictor angiotensin II. The first step, controlled by the enzyme renin, is the cleavage of a tetradecapeptide from the N-terminus of angiotensinogen to produce angiotensin I. Next, angiotensin I is converted to angiotensin II by the removal of the two C-terminal amino acids from angiotensin I. This step is carried out by the angiotensin converting enzyme present in the pulmonary vasculature.

Renin itself has been isolated from several sources, and has been the subject of intense study. Renal renin, a glycoprotein of molecular weight 38,000 to 40,000 is a highly specific enzyme that appears to have binding sites for eight amino acid side chains of its substrate. Its mechanism of action classifies it as an aspartyl protease, characterized by the presence of two essential aspartic acid residues at the active site, and the ability of pepstatin to inhibit catalytic activity. Because of renin's very low concentration in its primary tissue source, the kidney, it has been difficult to obtain adequate quantities for characterization. The submaxillary gland of mice contains an enzyme that appears to be similar to renal renin, which is present in high concentrations that permit sufficient quantities to be isolated. Much of what we know about renal renin was learned from studies of the submaxillary gland enzyme. Its amino acid sequence has been determined directly as well as inferred from a cDNA sequence. More recently, the cDNA sequence of human renin messenger RNA has been determined. These studies demonstrated that human and mouse renins have high sequence homology with one another as well as with the other aspartyl proteases.

The three-dimensional structure of renin is unknown but essential for understanding its specificity. Fortunately, its homology to the other aspartyl proteases permits prediction of its structure. Three aspartyl proteases have had their atomic coordinates determined by x-ray crystallographic procedures. As expected from the sequence homology, the three-dimensional structures of the aspartyl proteases from Rhizopus chinensis (RC) and Endothia parasitica (EP) are very similar. Several groups have used this type of similarity to construct models of the three-dimensional structures of various proteins. Browne and co-workers
were perhaps the first to use comparative model building to construct a model for \( \alpha \)-lactalbumin based on the homology with lysozyme. Subsequently, attempts were made to improve these models by energy refinement with empirical energy potentials. Warme and colleagues applied this procedure to the model of \( \alpha \)-lactalbumin and the final potential energy agreed well with that of the energy-minimized structure of lysozyme. Since then, the technique has been applied to several proteins, including thrombin, and haptoglobin.

As discussed by Blow, an important application of comparative model building is the design of renin inhibitors. With this goal in mind, two laboratories have now constructed models of the three-dimensional structure of mouse submaxillary renin. Although mouse submaxillary gland renin is similar to human renal renin in many ways, important differences in substrate specificity and inhibitor binding have been documented. These functional differences should be reflected in structural differences at the active site. As a first step in interpreting the specificity of renin and as an aid in guiding the synthesis of new inhibitors, a model for the three-dimensional structure of human renal renin is described in this paper. The present approach made use of the known structures and sequences of three aspartyl proteases to determine what parts of the three-dimensional structure are highly conserved and where substantial differences occur. The results of this analysis were then applied in aligning the renin sequence and building its structure. Finally, energy minimization techniques were employed to refine the proposed structure.

Methods

Three-dimensional Structure of the Aspartyl Protease

The aspartyl proteases from \( RC \), \( Penicillium janthinellum (PJ) \), and \( EP \) have all had their three-dimensional structures determined initially by x-ray crystallographic techniques to a resolution of 2.5 Å. Recently, the aspartyl proteases from \( RC \) (rhizopus- pepsin [RHI]), \( EP \) (endothiapepsin [END]), and \( PJ \) (penicillopepsin [PEN]) have had their structures refined to 2.1 Å resolution or better. The renin sequence was aligned not with a single aspartyl protease but with the composite of sequences, aspartic acids. For our purposes, it was necessary to know more precisely the regions of similarity and difference. The atomic coordinates for all the atoms of the three proteases solved to a resolution of 2.1 Å or better and for the \( \alpha \)-carbon atoms of porcine pepsin were obtained from the Brookhaven Protein Data Bank, (Brookhaven National Laboratory, Upton, NY) and individual investigators. A Kendrew-type model for the peptide backbone of each of these proteases was built with a vector graphics system and the FRODO software (as modified in our laboratory). The backbones were superimposed on each other for comparison by taking the backbone atoms of the two essential aspartic acids at the active site and minimizing the root mean square (RMS) differences in positions of these atoms by a least squares procedure. Then the backbone atoms for 20 amino acids that had the smallest RMS deviations in their positions were selected. The RMS differences in their positions were minimized by the same procedure. A three-dimensional drawing of the peptide backbones is presented in Figure 1 (see p. 21).

Sequence Alignment of the Aspartyl Proteases

After the peptide backbones had been oriented, amino acid sequences of the aspartyl proteases from \( RC \), \( PJ \), and \( EP \) were aligned on the basis of structural data. This alignment was accomplished by visually matching the residues that were observed to occupy similar positions in three-dimensional space. First matched were the residues in the core of the molecules: the \( \beta \)-pleated sheets in the N-terminus and C-terminus lobes and the 75–85 flap. Next the cysteines were aligned. Matches for cysteines 254 and 286 in RHI were found in all the aspartyl proteases and pepsin. Matches for cysteines 47 and 52 in RHI, however, were found in pepsin but not in END or PEN. After the remaining portions of the molecules were aligned in this fashion, the RMS differences for all backbone atoms that had matches were calculated. Segments that had RMS differences in their positions of greater than 3 Å were shifted in either direction in the sequence alignment, in steps of one residue, to see if this would decrease the RMS distance between the backbone atoms. With the aid of this technique, a number of additions and deletions were made in the sequences; they occurred at positions that were on the surface of the protein structures and at the ends of loops. Structural homology was maximized instead of the sequence homology with the use of this procedure.

Sequence Alignment of Renin

The renin sequence was aligned not with a single aspartyl protease but with the composite of sequences, derived as described in the previous section. It was thus weighted toward the regions of high structural homology in the aspartyl proteases. In regions of lower structural homology, it was weighted toward residues that were highly conserved in the three aspartyl proteases. The active site aspartic acid residues (37 and 225 in the human sequence) were matched first. This match was followed by the two \( \beta \)-sheet structures, the
α-helices and then the cysteine residues. Additions and
deletions were made in positions that corresponded to
additions or deletions in the alignment of the protease
structures. If further additions and deletions were nec-
escary, they were placed on the surface of the molecule
so as to maximize the sequence homology. In several
instances, the addition or deletion could have been
placed within a range of five adjacent positions without
appreciably altering the sequence homology. In these
instances, they were located at the ends of loops as far
into the solvent as possible. The final alignment is
shown in Table 1.

TABLE 1. Alignment of the Amino Acid Sequences of the Aspartyl
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* The amino acid sequence of RHI has been determined by the 1.8 Å electron density map by Davies DR and Bott R (unpublished data) and has not been determined by protein or DNA sequencing techniques.

Construction of the Renin Model

The renin model was built by constructing the peptide backbone and then adding the amino acid side chains. Coordinates of the RHI backbone, obtained from the 1.8 Å electron density map, were used directly for all residues for which there was overlap between renin and RHI sequences. Additions and deletions were made at positions where they occurred in the sequence alignment. Each addition or deletion was visualized with the molecular graphics system, and an attempt was made to avoid obviously forbidden conformations. Bond distances and bond angles were set to those determined for a model peptide backbone; the backbone was adjusted to avoid unfavorable dihedral angles or violations of Van der Waals radii.

When the peptide backbone was complete, the amino acid side chains were added. If the amino acids were identical for renin and RHI, then the coordinates for the RHI side chains were used directly. If the amino acids were different, then the renin residues were constructed with the χ1 angle for the amino acids in the RHI structure. If there were additional degrees of freedom in the renin amino acid, the χ angle was set as close as possible to that for the RHI side chain. If the RHI side chain was shorter than the corresponding residue in the renin, the χ angle was taken arbitrarily from model compounds. In certain instances, the side chain conformations chosen in this fashion caused steric conflicts. Side chains were then rotated about their Cα to Cβ bond (χ1 angle) to minimize steric overlap.

Empirical Energy Calculation and Refinement

To further evaluate the structure of renin and to rationalize the structure in terms of our knowledge...
of chemical constraints, we used the program CHARMM. This program, described in detail elsewhere, makes use of empirical energy functions consisting of two types of terms. The first set consists of covalent interactions along the polypeptide chain (bond lengths, bond angles, and dihedral angles), and the second set is composed of nonbonded interactions between different parts of the chain (Van der Waals, electrostatic and hydrogen bonding terms). Parameters for these energy terms are obtained from a variety of sources, including model compound studies and quantum mechanical calculations. Given a set of coordinates, the potential energy is calculated. To refine the structure, a convergent energy minimization procedure is used; the one used here — as it is the most efficient — is the adopted base Newton-Raphson algorithm. Constraints were used to keep the structure as close as possible to the original model while incorrect aspects of the stereochemistry or nonbonded interactions were reexamined.

The structure of RHI, solved at 2.8 Å, was refined in parallel to the structure of renin. In the initial stages of refinement, a harmonic constraint was applied to all atoms to limit their displacement owing to bad contacts. This constraint was reduced with each successive run of 25 minimization cycles until it was 0. In total, 200 cycles were run for both the RHI and the renin structures in this manner. The structures were then subjected to another 200 cycles of refinement without harmonic constraints.

Results and Discussion

Structural Homology of the Aspartyl Proteases

Close examination of the three-dimensional structures of the aspartyl proteinases solved by x-ray diffraction techniques indicated that the cores of the structures were almost identical in the conformation of their peptide backbone. The RMS difference in the positions of each atom in the backbone for each pair of aspartyl proteases appeared to confirm this observation. Those regions with the smallest RMS differences (< 1.0 Å) were at the core of the molecules, adjacent to the two aspartic acid residues essential for catalytic activity (37 and 225 in the human renin sequence). The two aspartic acid residues occurred at the edge of β-pleated sheets, which are highly conserved structural features, and showed small RMS differences in positions of the peptide backbone of less than 2.0 Å. The regions that were structurally dissimilar occurred on the surface of the proteins, where the backbone appeared to have a less well-defined secondary structure. These structural dissimilarities generally occurred at the ends of exposed loops, particularly where additions or deletions occurred; all were remote from the active site. These regions had RMS differences in the positions of the peptide backbone atoms of 4 to 10 Å. These regions had somewhat different folds and may be able to assume a range of conformations in a given protein. For comparison, the RMS differences between the positions of the backbone atoms for the RHI structure determined from the 2.8 Å x-ray data and that determined from the 1.8 Å data were calculated and found to be 1.05 Å. James and Sieleck have also found that the average change in the positions of the backbone atoms between the 2.8 Å coordinates and the 1.8 Å coordinates is very close to 1.0 Å. It is therefore probable that the differences between the peptide backbone at the active site of the aspartyl proteinases from different species are on the order of the differences of the x-ray crystallographic determination.

Sequence Homology of Renin and the Aspartyl Proteases

The number of identical amino acids in the sequence alignment has been tabulated for all the possible pairs of sequences and is presented in Table 2. Renin was found to have the greatest sequence identity with pepsin (40%), which was followed by RHI (30%). The greatest sequence identity between pepsin and an aspartyl protease other than renin was with RHI at 40%. Thus, we found that renin most closely resembles pepsin in sequence and probably also in structure. As only the coordinates for the α-carbon atoms of pepsin are available, however, it could not be used for the starting structure in the construction of renin. Therefore, RHI was chosen as the model structure because of its apparent high degree of homology with both renin and pepsin.

Energetic Aspects of the Structure

We expected the initial renin structure to have some energetically unfavorable conformations before energy refinement. The peptide backbone was expected to have a reasonable structure for those portions taken directly from the RHI structure, even though in regions of additions and deletions, high energy conformational

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<td>penicillopepsin</td>
</tr>
<tr>
<td>endothiapepsin</td>
<td>rhizopuspepsin</td>
</tr>
<tr>
<td>mouse renin</td>
<td>rhizopuspepsin</td>
</tr>
<tr>
<td>human renin</td>
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<tr>
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<td>mouse renin</td>
<td>penicillopepsin</td>
</tr>
<tr>
<td>human renin</td>
<td>penicillopepsin</td>
</tr>
</tbody>
</table>

*The amino acid sequence of rhizopuspepsin has been determined from the 1.8 Å electron density map by Davis DR and Bott R (unpublished data) and has not been determined by protein or DNA sequencing techniques.
features are likely to occur. Even where side chains were identical to those of RHI, we anticipated that steric conflicts might arise because of changes in neighboring residues. To evaluate the energetics, the potential energy for the initial structure of renin and the crystallographically determined structure of RHI at 2.8 Å were computed. The results are presented in Table 3. As can be seen, the individual terms for the two proteins are both very high. However, the results are similar with the exception of the energies for Van der Waals forces. Examining the contributions of individual atoms to the Van der Waals term, the high energy appears to come from a relatively small number of atoms in close contact. Some of these were from identical amino acids in both structures and involved mainly backbone atoms. The majority of the close contacts, however, occurred between side chains where the amino acids differed from those of RHI.

Although otherwise similar, the energies of both the model and the crystallographic structure are much higher than expected for a well-refined structure. To improve the structure without large shifts in geometry, 200 cycles of constrained energy refinement were carried out, followed by 200 cycles of unconstrained energy refinement. The results for the individual energy terms are given in Table 4. It is clear from the table that both structures now appear to have satisfactory stereochemistry and nonbonded interactions after refinement. This rationalization of structures was achieved following 200 cycles of unconstrained energy refinement were carried out, followed by 200 cycles of constrained energy refinement. The results for the individual energy terms are given in Table 4. It is clear from the table that both structures now appear to have satisfactory stereochemistry and nonbonded interactions after refinement. This rationalization of structures was achieved following 200 cycles of constrained energy refinement. This finding contrasts with earlier studies that used values for Van der Waals radii that were too small. From other model-building studies, it is evident that obtaining reasonable energies is a necessary, but not sufficient, criterion for a correct structure. Also, further minimization would continue to reduce the energies, but the approximation in the energy functions are such that the resulting changes in the structure are probably not meaningful.

## Accessible Surface of the Renin Structure

The ratio of the accessible surfaces of nonpolar atoms to the total accessible surface of proteins has been shown to be relatively constant — lying between 0.50 to 0.62 for six proteins determined by x-ray diffraction — despite differences in molecular weight over a range of 10,000 to 60,000. To test the renin model, the accessible surfaces of the initial and refined model of renin and RHI were calculated with the programs developed by Lee and Richards and the results are given in Table 5. The ratio for both renin and RHI lies within the normal range of 0.50 to 0.62 and changed only slightly after refinement. The total accessible surface for both proteins decreased slightly during refinement, and the ratio of the nonpolar to total accessible surface increased slightly, although these differences probably are not significant. The fact that there is a slightly greater accessible surface for renin than for RHI may be significant. It has been shown that the total accessible surface of a globular protein can be expressed as a function of the molecular weight, by the formula: accessible surface = 11.1 × (molecular weight).
TABLE 6. Calculated Accessible Surface of Atom Types (Å²) in the Initial and Refined (400 cycles) Structures of the Aspartyl Protease from R. chinensis and Human Renin

<table>
<thead>
<tr>
<th>Structures</th>
<th>Polar</th>
<th>Nonpolar</th>
<th>Total</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopuspepsin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>5723</td>
<td>7370</td>
<td>13,095</td>
<td>0.56</td>
</tr>
<tr>
<td>Refined</td>
<td>6032</td>
<td>6857</td>
<td>12,890</td>
<td>0.54</td>
</tr>
<tr>
<td>Renin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>6312</td>
<td>9037</td>
<td>15,350</td>
<td>0.59</td>
</tr>
<tr>
<td>Refined</td>
<td>6156</td>
<td>8767</td>
<td>14,923</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*Nonpolar to total

weight) With the use of this calculation and the surface area shown in Table 5, RHI should have a molecular weight of 39,400 and renin 47,500. Both of these estimates are higher than what would be calculated from the amino acid sequence, but the difference is greater for renin than for RHI. It is possible that this is an artifact of graphic modeling caused by additional residues in renin, but it may be the result of a need for a larger surface for renin to bind its high-molecular-weight protein substrate angiotensinogen.

Properties of the Renin Structure

Secondary Structure

As the renin structure is based on the RHI structure, it is not surprising that there are similarities, many of which are common to all the aspartyl proteases (Figure 1). The model has two lobes with the active site cleft between them. There are two β-sheet structures in the amino terminal lobe with the peptide backbone in one β-sheet running at right angles to the other. In the carboxy terminal lobe, the corresponding β-sheets make up the bulk of the structure but are less well defined. There are two aspartic acid residues juxtaposed in the active site (positions 37 and 225), and there is a flap at positions 75–85 of the amino terminal lobe that overlays the active site. This flap has been hypothesized to act as a trap door, which swings open to allow access to the active site and then closes when substrate is bound. 41

Posttranslational Cleavage

The aspartyl protease from RC exists as a single polypeptide chain. Evidence from the amino acid sequence of mouse submaxillary gland renin suggests that it has undergone proteolytic cleavage, which has removed arginines 290 and 291 by posttranslational processing. This cleavage results in a structure with two peptide chains held together by a disulfide bond. The cleavage occurs at a point where both renin sequences have an addition of two amino acids that are not present in the other aspartyl proteases. The sequence of human renin has no arginines in this region, and it is uncertain whether or not the same kind of posttranslational processing takes place. In our human renin model there are two lysine residues adjacent to one another that occur in this loop. The model indicates that if a peptide cleavage were to occur in this position, it would be at the surface of the molecule, on the accessible surface of a loop protruding into the solvent. This region would be accessible to a proteolytic enzyme. Our model has been constructed as a single chain polypeptide, and the effect of the presence or absence of these two amino acids on the three-dimensional structure of the active site has not been investigated.

Cystine Residues

One of the most notable differences between the structures of renin and RHI is the presence of a disulfide bond in renin between 216 and 220. Although analogous half-cystine residues do not occur in RHI, 20 they are present in pepsin and are likely to be linked in a disulfide bridge. 22 Misono and colleagues 16 have identified two disulfide bonds and two cystines in mouse renin, but their location is unknown. The half-cystines in the renin structure occur at the same positions in pepsin. As discussed previously (see Table 2), we found that renin most closely resembles pepsin, and thus the three disulfides were included in the model and were located in positions analogous to those in pepsin. The inclusion of these disulfides does not appear to introduce a major distortion nor any localized strain in the renin model.

Figure 1 Stereoscopic drawings of the α-carbon of the aspartyl proteases from R. chinensis, E. parasitica, and P. janthellum, determined by x-ray crystallographic techniques. The α-carbon tracings have been superimposed to show the correspondence of the backbones. The N-terminus lobe is on the right, the C-terminus lobe on the left, and the active site runs vertically between them.
The Active Site

The active site is most important in the modeling of renin-specific inhibitors and in understanding catalytic mechanisms. This region contains the aspartic acid residues that are the most similar among the aspartyl proteases for which atomic coordinates are available. Considerable information about the active site is available from the coordinates of two peptide inhibitors that bind to it. Bott and co-workers reported the structure of pepstatin in the active site of RHI, and James and associates have described the structure of a pepstatin fragment bound to the active site of penicillopepsin. Both inhibitors contain a central statine residue — that is not present in the conventional peptide chain. In both structures, the central statine binds so that its backbone is in close proximity to the two aspartic acid residues at the active site of the enzymes. The introduction of these two carbon atoms elongates the backbone and allows for free rotation around the backbone because the additional atoms are tetrahedral carbons instead of the trigonal carbon of the natural peptide. These changes allow the statine-containing molecule to bind to the aspartyl proteases without being hydrolyzed; however, these changes in the backbone also distort the binding from what would be expected for a productive enzyme-substrate complex. In the model of Bott and colleagues, the side chains of the substrate in the P and P' positions are identified by the isopropyl side chains of statines 4 and 6 of pepstatin. (According to Schechter and Berger, P-Pn refer to the side chain positions of the peptide substrate, whereas S-Sn refer to the subsite on the enzyme that binds the corresponding side chain of the substrate.) The S1 site is formed by residues in the amino terminal lobe, and the S1' site is formed by residues in the carboxyl terminal lobe. These sites are symmetrically related by the dyad axis relating the N-terminus lobe to the C-terminus lobe. The peptide backbone of the substrate in RHI lies in the active site in a manner that places the P and P' side chains on opposite sides of the axis defined by its backbone peptide. When a model of a peptide substrate bound to the active site of RHI was constructed, we found that the P and P' side chains could not reach into the S1 and S1' subsites as far as could the statine side chains of the pepstatin, and the P1' side chain had to be moved out of the S1' subsite.

Models for pepstatin and for two octapeptides (pro-phe-his-leu-leu-val and pro-phe-his-leu-val-ile) reproducing the scissile region of natural substrate for renin were constructed in the active site of renin. It appeared that pepstatin could be accommodated without steric conflicts at the active site of both mouse and human renin, in a conformation identical to that determined for the models of Bott and colleagues and James and co-workers. When the hexapeptide was constructed in the active site of renin, some differences were noted. The isopropyl side chains of the leucines in the P and P' positions had to be moved toward the center of the active site and out of the hydrophobic pockets defined by the isopropyl side chains of statine 4 and statine 6 in the pepstatin model. It is likely that the rotational freedom introduced by the tetrahedral atoms in the backbone of the pepstatin molecule allows the motion of the side chains in the P and P' sites into the hydrophobic pockets on both renin and RHI, which optimizes the hydrophobic interactions and increases the tightness of the binding. In all probability, a similar type of rotational freedom has been introduced in renin inhibitors possessing tetrahedral atoms in place of the trigonal atoms in the peptide backbone at the scissile bond and may account for the tightness of their binding to renin.

Differences Between Mouse and Human Renin

Human renin has a very different and more selective substrate requirement than the other aspartyl proteases. There are substantial differences in substrate requirements between mouse submaxillary renin and human renal renin as well. When we reported a model for the three-dimensional structure of mouse submaxillary renin, differences in active site structures between that enzyme and other aspartyl proteases were noted. These differences were consistent with the specificity of a peptide inhibitor of renal renin (RIP), as well as of inhibitors containing modified peptide bonds at the cleavage site.

The model of human renin presented here differs little from that of the mouse submaxillary enzyme in the topology of the active site (see Figures 4–6). The two aspartic aspartyl residues (37 and 225) at the active site have virtually the identical orientation as in the mouse renin model and the x-ray models of the aspartyl proteases. The adjacent residues (Gly 39, Ser 40, Gly 227) stabilize the carboxyl groups of the aspartic acids by forming hydrogen bonds with them and are fully conserved. We found that there is one residue that is involved in this type of interaction, however, that is invariant in the structures of the other aspartyl proteases yet is different in human renin. In all other aspartyl proteases there is a threonine that forms a hydrogen bond to the essential aspartic acid in the carboxyl terminal lobe (225 in human renin). This residue changes to a serine in the mouse renin and to an alanine in the human renin structure. The change from a threonine to a serine would preserve the hydrogen bonding but change the steric properties by eliminating a methyl group. The change to an alanine would, of course, make hydrogen bonding impossible and thus considerably change the electrostatic potential around the aspartic acids.

The hydrophobic pocket hypothesized to form the S1' site in the RHI protease appears to be well conserved in the models of mouse and human renin. There are, however, some small changes that may be important. Leucine at position 223 in human renin changes to valine. Leucine has a methylene group in its side chain that is not present in valine. The effect on the S1' subsite would be to reduce its size in the human as compared with the mouse enzyme. Because of other relative shifts in the backbones of the two structures, however, this change has little apparent effect on the
MODEL OF HUMAN RENAL RENIN/Carlson et al.

FIGURE 2. Stereoscopic drawings of the active site residues of the human renin model. (See text for details.)

FIGURE 3. Stereoscopic drawings of the active site residues of the human renin model with a hexapeptide that has the sequence of the natural substrate for renin present in its presumed bound conformation. (See text for details.)

FIGURE 4. A. Amino acids in the active site of the mouse renin model. Amino acids that differ between mouse and human are underlined. B. Amino acids in the active site of the human renin model. Amino acids that differ between mouse and human renin are underlined.
size of the pocket but does alter its shape. The pocket is such that in the human renin structure there is sufficient hydrophobic surface available to interact with the smaller valine side chain of the human substrate in the $P_1'$ position and still enough space to accommodate the larger side chains, such as leucine, which are present in the nonprimate renin substrates. This type of interaction could explain how human renin can act on a variety of renin substrates, while the mouse renin cleaves human substrate only poorly. In contrast to this, the residues that line the $S_1$ subsite — identified from the structure of pepstatin at the active site of RHI — appear to be identical in both human and mouse renin. This finding is consistent with the data on the substrate specificity of renns, which demonstrate that the residues on the amino terminal side of the scissile bond in the different renin substrates are identical, while the residues that differ are on the carboxyl terminal side of the scissile bond.

Other notable differences between the human and mouse structures occur in the 75–85 flap. We found that the residues on the protein side of this flap are identical in all the aspartyl proteases and both renins (see Figures 4–6). A tyrosine that was originally thought to be important as a hydrogen bond donor during catalysis but is now thought to form part of the hydrophobic pocket that binds the side chain in the $P_1$ position of the substrate is preserved in both renin structures. On the solvent-accessible surface of the 75–85 flap, however, we found a number of differences between the human and mouse renin structures. At position 81 in the human structure, there is an
arginine that is replaced by a histidine in the mouse structure. There is a threonine at position 86 in the human sequence, while there is an arginine in the mouse structure. These amino acids have quite different properties and could affect antibody specificity by altering the interactions with an antibody that binds to these residues and sterically blocks the active site. This could easily explain the differences in the specificity of the antibodies and enable them to differentiate between the mouse renin and the human renin. It is entirely possible that these same surface residues interact with a portion of the renin substrate (an α₂-macroglobulin with a molecular weight of approximately 65,000) that is remote from the amino terminus decapeptide cleaved by renin. It would thus be possible that the changes in these residues on the accessible surface of the 75–85 flap could account for the differences in substrate specificity and that the alterations in the sequence of the substrate around the scissile bond are fortuitous.

Conclusion

The aspartyl proteases, including renin, exhibit functional homology in that they use a common mechanism for catalysis. They all contain two aspartic acid residues at the active site. Although the detailed mechanism of the hydrolysis of peptides by aspartyl proteases is not fully understood, it is presumably highly dependent on the positioning of the catalytic residues in a precise orientation with respect to the substrate. Thus it is not surprising that these enzymes have very similar three-dimensional structures, so that this enzyme-substrate relationship is conserved. Their secondary structural features of the β-sheets, the α-helices, and the 75–85 flap are all highly conserved. The structural homology is greatest at the core of the molecule, the region that is most important in determining the orientation of residues in the active site. The structural and functional homology of the aspartyl proteases is reflected in their sequence homology. The residues in the core of the molecule and adjacent to the active site are highly conserved in all the aspartyl proteases, including renin.

The homology of the aspartyl proteases and renin has been used to construct a model for the three-dimensional structure of human renin. The model has been evaluated with an empirical energy refinement program and surface accessibility programs, and conclusions have been drawn about the binding of substrate. In the energy refinement evaluation, the model behaved similarly to the aspartyl protease from RHI, which has had its structure determined by x-ray crystallography. The final energy and the accessibility for renin also were similar to those for RHI. The topology of the active site of human renin appears to be very similar to the topology of the active site of mouse renin. We found that changes in the amino acids that line the active site are few and subtle; however, the changes in the active site between mouse and human renin in the S₁' subsite seem to be consistent with the differences in substrate specificity. It is not clear, however, whether these differences or differences in amino acids on the accessible surface of the 75–85 flap account for the obscured differences in specificity. Differences between mouse and human renin in the residues on the solvent-accessible side of the 75–85 flap may also explain the differences in antibody binding between mouse and human renin.

The most notable differences between renin and the other aspartyl proteases probably are determined by changes in amino acid side chains deep in the core of the molecule, which cause subtle shifts throughout its structure and produce topological differences in the active site which in turn alter the Van der Waals, electrostatic, hydrogen bonding, and hydrophobic interactions involved in substrate specificity. Through the use of the energy refinement we hope to have allowed the renin model to adjust to the differences in the amino acids in the core of the structure and adopt a more realistic conformation than the initial model. To interpret the effects of these changes it will be necessary to construct models for the specific inhibitors and then use energy minimization to determine their precise orientation. It is this level of detail that is most likely to yield the most significant insights into the binding of inhibitors and is currently under investigation in our laboratory.

It should be noted that although the structure presented here is a model, it is one that can be tested against experimental data. It is possible to draw some inferences concerning renin’s mechanism of action, which can be tested directly by comparing the experimental behavior of substrates and inhibitors. Other properties of the model that can be tested are the distances between various reactive groups or chromophores on the surface. The final test, of course, will be a comparison of the model to the structure determined by x-ray crystallography.

References

4. Lanzillo JJ, Famburg BL Membrane bound angiotensin converting enzyme from the rat lung J Biol Chem 1974;249 2312–2319
5 Murakami K, Inagami T Isolation of pure and stable renin from hog kidney Biochem Biophys Res Commun 1975,62; 757–763
7 Dzau VJ, Slater EE, Haber E. Complete purification of dog renal renin. Biochemistry 1979;18:5224–5229
26 HYPERTENSION Vol 7, No 1, January-February 1985


12 Fruoton JS The mechanism of the catalytic action of peptic acid and related acid proteinases Adv Enzymology 1976;44:1–36


14 Takahashi K, Chang WJ Specific chemical modifications of acid proteases in the presence and absence of pepstatin J Biochem 1976,73:675–677

15 Burton J, Poulsen K, Haber E Competitive inhibitors of renin and inhibitors effective at physiological pH Biochemistry 1975;14:3892–3898


21 Hsu IN, Delbaere LTJ, James MNG Penicillopepsin from Penicillium janthanellum crystal structure at 2.8 Å and sequence homology with porcine renin Nature 1977,266:140–145

22 Hofmann T Structure and function of acid proteinases In Advances in Chemistry Series, no 136 food related enzymes Washington, DC, American Chemical Society, 1974 146–185


27 Greer J Model for haptoglobin heavy chain based on sequence homology Proc Natl Acad Sci USA 1980,77:3393–3397

28 Blow DM Molecular structure computer cues to combat hypertension Nature 1983,304:213


32 Poul sen K, Haber E, Burton J On the specificity of human renin studies with peptide inhibitors Biochem Biophys Acta 1976,452:533–537

33 James MNG, Sielecki AR Structure and refinement of penicillopepsin at 1.8 Å resolution J Mol Biol 1983;163:299–361


37 Andreeva NS, Bortson VV, Melik-Adamyan VR, Riaz VSH, Trofinova LN, Shutokev NE Determination of the three-dimensional structure of pepsin at a resolution of 5.5–5.0 Å Mol Biol (Mosk) 1972,5:731–742


42 Bott R, Subramanian E, Davies DR. Three-dimensional structure of the complex of the Rhizopus chinensis carboxy proteinase and pepstatin at 2.5 Å resolution Biochemistry 1982,21:6958–6962


45 James MNG, Hsu IN, Delbaere LTJ Mechanism of acid proteinase catalysis based on the crystal structure of penicillopepsin Nature 1977,267:808–813

Construction of a model for the three-dimensional structure of human renal renin.
W Carlson, M Karplus and E Haber

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