Crystallographic Studies on the Binding Modes of P2-P3 Butanediamide Renin Inhibitors*

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The binding modes of three peptidomimetic P2-P3 butanediamide renin inhibitors have been determined by x-ray crystallography. The inhibitors are bound with their backbones in an extended conformation, and their side chains occupying the S2 to S1′ pockets. A (2-amino-4-thiazolyl)methyl side chain at the P2 position shows stronger hydrogen-bonding and van der Waals interactions with renin than the His side chain, which is present in the natural substrate. The ACHPA-γ-lactam transition state analog has similar interactions with renin as the dihydroxyethylene transition state analog.

The aspartic protease renin plays an important role in the regulation of blood pressure by catalyzing the release of the decapeptide angiotensin I from angiotensinogen (1). Removal of the two C-terminal residues from angiotensin I, catalyzed by the angiotensin-converting enzyme, produces the physiologically active octapeptide angiotensin II. Inhibitors of ACE have become successful therapeutic antihypertensive agents (1). However, the angiotensin-converting enzyme inhibitors produce unwanted side effects in treatment and have only a 50% response rate in monotherapy (2), clearly indicating the need for other therapeutic agents. The inhibition of renin represents a possible alternative for developing successful antihypertensives.

The cleavage in human angiotensinogen catalyzed by renin occurs between residues 10 and 11 in the sequence His-Pro-Leu10-Val11-Ile12. Compounds containing a butanediamide backbone at the P2-P3 positions (3) are potent peptidomimetic inhibitors of human renin (Fig. 1). They contain either the dihydroxyethylene (4) (inhibitors 1 and 2) or the AChPA-γ-lactam (5) (inhibitor 3) as the transition state analog occupying the P2 and P3′ positions (3). The His side chain in angiotensinogen at the P2 position is replaced with a (2-amino-4-thiazolyl)methyl group.

Crystal structures of free and inhibited human renin have been reported previously at medium resolution (6-8). We recently reported the crystal structure at 1.8-Å resolution of human renin in complex with a polyhydroxymonoamide inhibitor (9). In this paper we describe the binding modes of the P2-P3 butanediamide renin inhibitors as determined by x-ray crystallography and compare their binding interactions with those of other inhibitors (7, 9).

MATERIALS AND METHODS

Data Collection—The purification (10) of recombinant human renin and crystallization (11, 12) of renin-inhibitor complexes has been described earlier. The crystals belong to space group P21 with a = b = c = 143 Å at 4 °C (11). There are two renin molecules in the asymmetric unit. X-ray diffraction data to 2.4-Å resolution of renin in complex with inhibitor 1 were collected at 4°C on the F1 beamline at the Cornell High Energy Synchrotron Source (CHESS). The diffraction data for renin in complex with inhibitors 2 and 3 were collected at cryotemperature (120 K) and 4°C respectively, on an R-axis Axis imaging plate detector mounted on a Rigaku RU-200 x-ray generator operated at 50 kV and 100 milliamps. The data-processing statistics are summarized in Table I.

Structure Determination—The crystal structure of renin in complex with inhibitor 1 was determined by the molecular replacement method, using as the search model a 2.8-Å structure of human renin (8). The orientations of the two renin molecules were determined by rotation function calculations using reflection data between 10- and 5.5-Å resolution with the program GLRF (13). The positions of the two molecules were then determined with the Patterson correlation translation function using the program TF (14).

Structure Refinement—The atomic model for renin in complex with inhibitor 1 derived from the molecular replacement calculations was subjected to rigid body refinement against reflection data between 5- and 3.3-Å resolution. This was followed by slow cooling simulated annealing refinement of the atomic positions using the program X-PLOR (15). The atomic model was examined on an Evans & Sutherland workstation with the program Frodo (16). The inhibitor molecules were clearly visible in the 2Fo - Fo electron density map and were included in further refinement. A parameter file containing the ideal bond lengths and bond angles for the inhibitor, estimated based on values for similar bond types in amino acid residues, was created manually.

At this point reflection data to 1.8-Å resolution became available for human renin in complex with a polyhydroxymonoamide inhibitor, in an isomorphous crystal form (9). The refined model of renin in complex with 1 was used as the initial model for the refinement at 1.8-Å resolution. This produced a more accurate atomic model for renin (9). Consequently, the structure refinement of the inhibitor 1 complex was restarted using the 1.8-Å renin model. This 1.8-Å structure of renin was also used as the starting model in the structure refinement of renin in complex with inhibitors 2 and 3. The final atomic models were obtained after one cycle of refinement with the program TNT (Fig. 2) (17). The refinement statistics of the three structures are summarized in Table I.

RESULTS AND DISCUSSION

The crystal structures of recombinant human renin in complex with three different P2-P3 butanediamide inhibitors are presented in this paper. The crystallographic data and the
aspartic proteinases (18). Conformational differences have also been observed in other renin complexes at high resolution showed much closer overlap of the inhibitors for the P$_2$P$_3$ and P$_4$ residues (9).

The inhibitor molecules are bound in a groove between the N- and C-terminal domains of renin. The backbones of the inhibitors are in an extended conformation. The side chains occupy the S$_5$ to S$_1$' substrate binding pockets. The pattern of hydrogen bonding between the polar atoms in the backbone of the inhibitors and renin is similar to that reported for other peptidomimetic inhibitors (7-9). The absence of a P$_2$ amido nitrogen in the butanediamide backbone results in the loss of a hydrogen bond to the side chain of Thr$^{77}$ (7). The two carbonyl oxygen atoms of the butanediamide backbone are located at similar positions and maintain similar hydrogen bonding interactions to renin as the carbonyl oxygen atoms of the P$_2$ and P$_3$ residues in a peptide substrate (see below).

The pyridylethyl group in inhibitor 2 is exposed to solvent and has weak electron density, suggesting it may also be flexible. The pyridyl ring is folded close to the P$_3$ phenyl ring of the inhibitor and occupies the S$_2$ pocket (Fig. 3). The lack of additional interactions of this pyridyl group with renin is consistent with the observation that this compound is as potent as inhibitor 1 (Fig. 1). The pyridyl group of the inhibitor bound to the renin molecule in the open conformation is involved in crystal-packing interactions, which may explain its positional difference from that of the pyridyl ring in the inhibitor bound to the renin molecule in the closed conformation (Fig. 3).

The plane of the amide group of the P$_4$ residue is perpendicular to that of the P$_3$ residue in all three inhibitors (Fig. 3). The N-methyl group is pointed toward the side chain of Tyr$^{220}$ in the S$_2$ pocket. In comparison with the structures of renin in complex with polyhydroxymonoamide inhibitors (9), which lack a P$_4$ group, the side chain of Tyr$^{220}$ rotates by about 20$^\circ$ and 60$^\circ$ across the $\chi_1$ and $\chi_2$ torsion angles to avoid steric contact with this methyl group (Fig. 4). This change in the conformation of the Tyr$^{220}$ side chain is observed in both the open and closed conformations of renin for all three inhibitors. The orthogonality of the amide planes of the P$_4$ and P$_3$ residues projects the methyl group deeper into the S$_2$ pocket as compared with the P$_4$ residue in the compound CGP 38'560 (Fig. 5) (7). A smaller change in the position of the Tyr$^{220}$ side chain is observed in the latter complex, which is in the closed conformation.

In contrast to earlier structure studies (7, 9), which showed the phenyl group at the P$_3$ position having a common binding orientation (Fig. 5), the P$_3$ phenyl groups of the three inhibitors studied here assume a variety of orientations, due mostly to changes in the $\chi_2$ torsion angle (Fig. 3). The methyl group on the benzylic carbon of the P$_3$ side chain is projected into the solvent. The side chains in these inhibitors are connected to a planar amido nitrogen atom rather than a tetrahedral carbon as in other peptidomimetic inhibitors (7, 9). Consequently, the side chain enters the S$_2$ binding pocket from a different direction as compared with the inhibitors with a tetrahedral carbon (Fig. 5). The amino acid side chains of renin forming the S$_3$...
The P₂ group of the polyhydroxymonoamide inhibitors in our earlier study (9) is the smaller cyclopropylmethyl group. A water molecule was observed at the base of the S₂ pocket in those structures. In the current structures, the larger aminothiazole ring fills the S₂ pocket more fully. The amino group displaces the water molecule observed in the earlier structures (9) and is hydrogen-bonded to the side chain hydroxyl group of Ser²²² and the main chain carbonyl group of Tyr²²⁰ (Fig. 4). The sulfur atom of the aminothiazole ring is surrounded mostly by side chains of hydrophobic residues (Ala³⁰⁰, Ile²⁹¹, Met²⁸⁹, and Leu²¹³). The higher polarizability of the sulfur atom may probably give rise to stronger van der Waals interactions with these side chains. The bulkier sulfur atom, and possibly the additional hydrogen-bonding interactions of the amino group, also cause a shift in the position of the P₂ (2-amino-4-thiazolyl)methyl residue as compared with the position of the P₂ His residue in the CGP 38 560 complex (Fig. 5).

The change in conformation of the Tyr²²⁰ side chain due to the P₄ residue of these inhibitors is coupled with a change in conformation of the His²⁸⁷ side chain, which was observed to be located close to the Tyr²²⁰ side chain and away from the S₂ pocket in the earlier structures (9). In the renin molecule with the open conformation, a small movement is observed for the His²⁸⁷ side chain to avoid steric contact with the Tyr²²⁰ side chain. The His²⁸⁷ side chain in the new position still maintains interactions with the side chain of Asp²⁴⁴. In the renin molecule with the closed conformation, due to the proximity of residues 243–245, the His²⁸⁷ residue undergoes a large conformational change (including a change in $\chi_1$ of 140°). Its side chain is located close to the S₂ pocket in the new position, where it interacts with the ring nitrogen of the thiazolyl group through a water molecule at the opening of the S₂ pocket (Fig. 4). Two other polar atoms, the main chain amido nitrogen of Tyr²²⁰ and the carbonyl oxygen of the P₄ residue, complete the tetrahedral coordination of this water molecule (Fig. 4). A change in the position of the Met²⁸⁹ side chain is also observed (Fig. 4).

In the structure of renin in complex with the inhibitor CGP 38'560 (7), the His²⁸⁷ side chain was found to be close to the S₂ pocket, interacting with a water molecule at the opening of the S₂ pocket. The hydrogen bond between this water and the His side chain of the inhibitor, however, was not observed. A water molecule at the opening of the S₂ pocket was also observed in our earlier study, where the His²⁸⁷ is away from the S₂ pocket (Fig. 4) (9).

The dihydroxyethylene transition state analog in the P₁ and P₁' positions is bound in a conformation similar to that observed in the earlier study (Figs. 3 and 4) (9). The first hydroxyl group of the diol is located between the catalytic aspartic acid residues 32 and 215. The ACHPA-γ-lactam transition state analog in inhibitor 3 occupies similar spatial positions as the dihydroxyethylene analog (Fig. 3). As predicted from modeling studies (5), the gem-dimethyl group on the lactam ring superimposes with the isopropyl group of the diol analog, mimicking the Val side chain in the natural substrate. The carbonyl oxygen atom of the lactam occupies a position similar to that of the
second hydroxyl group of the dihydroxyethylene transition state analog (Fig. 3).

These \( P_2\)-\( P_3\) butanediamide inhibitors are about 40-fold more potent than the polyhydroxymonoamide inhibitors of renin (9). The crystal structures show that the \( P_2\)-\( P_3\) butanediamide inhibitors have stronger interactions with renin at the \( P_2 \) position and additional interactions due to the \( P_4 \) residue. These may explain the increased potency of the inhibitors. The contribution of the \( P_3 \) residue is difficult to evaluate, and it is not clear whether the two different series of inhibitors have similar interactions with renin at this position.

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