Theoretical Proposal of a Catalytic Mechanism for the HIV-1 Protease Involving an Enzyme-Bound Tetrahedral Intermediate

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A critical step in the replication cycle of the HIV-1 virus is the processing of the two polyproteins $Pr55^{gag}$ and $Pr160^{gag-pol}$ which contain all the structural and catalytic proteins required for the formation of the viral particles.[1] The release of the proteins involves an enzyme, the HIV-1 protease (HIV-1 PR), which catalyzes the hydrolysis of some specific peptide bonds.[1,2] It has been demonstrated that the inhibition of the HIV-1 PR leads to noninfectious virions,[3] and due to these results, the HIV-1 PR has become a major target for the development of anti-HIV-1 agents.[4] The therapeutical interest of the HIV-1 PR has spurred a number of mechanistic[5] and structural studies[6−9] directed to the elucidation of the catalytic mechanism of this enzyme.

Here we present the results obtained in a theoretical study of the catalytic mechanism of the HIV-1 PR, using high-level (MP2/6-31+G*) ab initio quantum-mechanical methods, which supports the previously proposed mechanism involving the formation of a noncovalent enzyme-bound intermediate in the course of the catalytic cycle.[5] The relevance of our theoretical results to the mode of action of several HIV-1 PR inhibitors is also discussed.

According to the structural information derived from the X-ray studies,[6−9] the HIV-1 PR is a C_2 symmetric homodimeric protein, each of the monomers being formed by 99 residues. The active center is formed by two aspartic acid residues, one contributed by each subunit and in opposite states of protonation,[5c] and the water molecule involved in the hydrolysis of the peptidic bond seems to be hydrogen-bonded to the aspartyl residues.[7] Wlodawer and co-workers have proposed that the cleavage of the peptidic bond is a concerted reaction, in which no intermediate is formed.[9] This mechanism has been studied theoretically by Lee and co-workers,[10] at the HF/6-31G* level of theory. On the other hand, Meek and co-workers have presented evidence of the formation of a *noncovalent* enzyme-bound amide hydrate intermediate.[5]

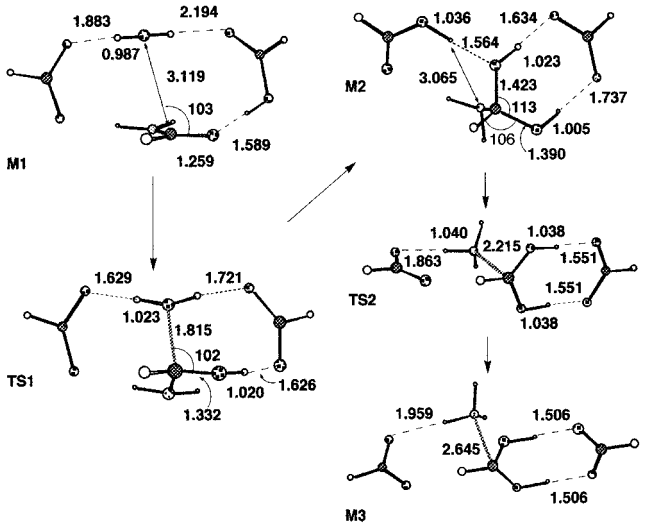

Figure 1. Relevant geometrical parameters for the fully optimized structures (MP2/6-31+G* level) of the proposed catalytic HIV-PR mechanism.

To gain mechanistic insight, high-level ab initio calculations were carried out at the MP2/6-31+G* level of theory on a model system composed of formamide, as peptidic substrate, water, and a formate/formic acid pair as a model of the catalytic residues. As a means of comparison with the results of Lee and co-workers,[10] HF/6-31G* calculations were also done. The calculations were carried out with GAUSSIAN 94,[11] and all the stationary points located were fully optimized and characterized by frequency calculations.

The starting point in the mechanism is the formation of an enzyme−substrate complex in which both the substrate and the water molecule are hydrogen-bonded to the aspartyl residues. The MP2/6-31+G*-optimized geometry of this model complex, **M1**, is shown in Figure 1. It can be seen that the water molecule is bonded to the two aspartyl residues, forming a strong hydrogen bond with the negatively charged oxygen of the carboxylate group. A very interesting feature of this structure is the strong hydrogen bond between the oxygen of the carbonyl group of the formamide and the hydrogen of the carboxylic group, with an O- - -H distance of 1.589 Å. This result may be viewed as support for the proposal by Meek and co-workers[5c] of the formation of a low-barrier hydrogen bond, which recently has been the subject of discussion in relation to enzymatic catalysis.[12] Following this mimimum, a first transition structure (**TS1**, see Figure 1) was found, in which the distance between the oxygen of the water and the carbon of

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Table 1. Relative Energies (kcal/mol) for the Stationary Points Located[a] on the Catalytic Mechanism of the HIV-1 PR

	HF/6-31G*	MP2/6-31+G*		HF/6-31G*	MP2/6-31+G*
M1	0.0	0.0	**TS2**	12.3	21.0
TS1	10.1	12.9	**M3**	9.0	20.1
M2	−0.8	9.1			

[a] All the stationary points were fully optimized at the corresponding level of theory.

the carbonyl group is reduced to 1.815 Å. In this transition structure, the hydrogen of the formic acid is almost transferred from the catalytic formic acid residue to the oxygen of the substrate, as can be deduced from the increase of the H- - -O (formic acid) distance and the decrease of the distance between this hydrogen and the oxygen of the carbonyl group. One of the hydrogens of the water is also accepted by the catalytic formate, which acts as a general base. **TS1** leads to the formation of a second minimum, **M2** (see Figure 1), in which the bond between the oxygen of the lytic water and the carbon of the carbonyl group is fully formed and one of the hydrogens of the water is transferred to the formate group. This structure is a tetrahedral intermediate on the reaction pathway that corresponds to the enzyme-bound amide hydrate found by Meek and co-workers in the molecular dynamics modeling.[5a] The distance between the nitrogen of the substrate and the hydrogen of the formate, which will be involved in the next step of the reaction, is 3.065 Å (see **M2** in Figure 1), which is very close to the 3.1 Å previously found.[5a]

The cleavage of the nitrogen−carbon bond, which leads to the formation of the reaction products (ammonia and formic acid) involves a transition structure **TS2** (see Figure 1), in which the bond between the nitrogen and the carbon atom of the carbonyl is almost fully broken, with an N−C distance of 2.215 Å, and the proton from the formic acid is almost transferred to the nitrogen atom of the substrate.[13] The single imaginary frequency of **TS2** is dominated by the N−C breaking motion. On the other hand, the hydrogens belonging to the hydroxy groups on the amide hydrate intermediate **M2** are now symmetrically shared between the two oxygen atoms of the substrate and the two oxygens of the catalytic carboxylic group. This result supports the proposal of the chemical equivalence of the two O atoms in the reaction.[5b]

According to the MP2/6-31+G* calculations (Table 1), **TS2** is less stable than **TS1** by 8 kcal/mol and the elimination step is predicted to be the rate-determining step of the catalytic mechanism. It is interesting that this result is in good agreement with the experimental evidence obtained by Meek and co-workers, indicating that the rate-determining step of the mechanism involves the collapse of the amide hydrate intermediate.[5a,b]

The final point located is a minimum (**M3**, see Figure 1) in which the N−C bond is fully broken (with an N−C distance of 2.645) and which corresponds to the reaction products complexed with the catalytic residues. In the actual enzyme, this step of the catalytic mechanism should be coupled with a conformational change in the active center involving the mobile aspartate residues[14] and allowing for the release of the products and restoring the initial situation of the catalytic process.

The analysis of the calculations presented here supports the idea that a general acid−base mechanism[15] can be invoked to explain the hydrolysis of the peptidic bond. In this mechanism, the carboxylate group of the active site acts as a base deprotonating the hydrolytic water molecule, which attacks the carbonyl group of the substrate. The proton of the other carboxylic group acts as an acid catalyst, polarizing the carbonyl bond. On the other hand, this theoretically proposed mechanism involves an addition reaction pathway, quite similar to the one proposed for explaining the hydrolysis of the amides.[16] This stepwise mechanism is an alternative to the concerted mechanism proposed by Wlodawer and co-workers[9] and was recently studied at the HF/6-31G* level of theory.[10] The HF/6-31G* calculations of Lee and co-workers predict an activation barrier of 22 kcal/mol for the concerted reaction pathway,[10] which is substantially higher than the HF/6-31G* activation barrier we computed for the stepwise mechanism (see Table 1). This result suggests that the stepwise reaction pathway involving an enzyme-bound intermediate is clearly favored, in good agreement with the experimental findings by Meek and co-workers.[5]

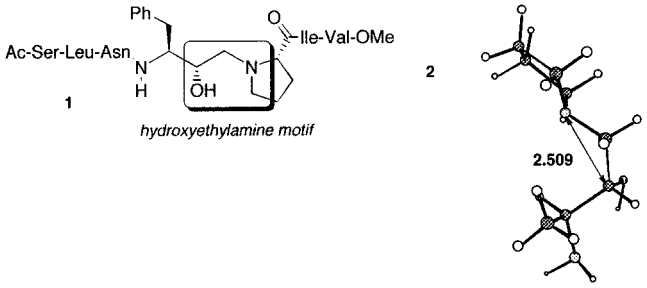

Figure 2. Structure of the HIV-1 PR inhibitor synthesized by Rich and co-workers (**1**)[17] and the HF/6-31G* optimized structure of the model inhibitor (**2**), showing the N−C distance.

The study of the catalytic mechanism of the HIV-1 PR may help to understand the mode of action of its inhibitors and, eventually, to design new ones. In this regard, it has been proposed by Meek and co-workers that some potent inhibitors of HIV-1 PR, such as the modified peptide **1** (see Figure 2) synthesized by Rich and co-workers,[17] are compounds that are able to mimic the transition state of the rate-determining step of the catalytic mechanism.[5b] To test this hypothesis we optimized, at the HF/6-31G* level, the structure of a model of the inhibitor **1** containing the hydroxyethylamine motif.[18] The optimized structure of the model inhibitor **2** is shown in Figure 2. It can be seen that the distance between the pyramidal nitrogen of the proline moiety and the tetrahedral carbon with the attached hydroxy group is 2.509 Å, which compares reasonably well with the N−C distance (2.215 Å) found in **TS2**, thus making the hydroxyethylamine motif a good mimic of the rate-determining transition state of the catalytic cycle, in good agreement with the proposal of Meek and co-workers. Interestingly, preliminary calculations show a close structural relationship between the hydroxyethylamine motif and α-aminophosphorus derivatives.[19]

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(13) In their model, Meek and co-workers[5a] proposed another step in the mechanism which involves the proton transfer from the formate group to the nitrogen of the substrate before the C−N bond scission. Actually, due to the particular characteristics of our model and without excluding its existence, we were not able to detect a similar step compatible with the actual system. We also considered that, for the purpose of locating the rate-determining step and its geometrical characteristics (**TS2**), it was not relevant.

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(19) We are currently developing the synthesis of α-aminophosphazene- and α-aminophosphine oxide-containing peptidomimetics as potential HIV-1 PR inhibitors.