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Mechanism of action of aspartic proteinases: Application of transition-state analogue theory

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Summary

Applying the semiempirical MO methods AM1 and PM3 as well as the density functional theory to the model of the catalytic site composed of ca. 160–190 atoms, we have carried out studies aimed at the explanation of three aspects of the mechanism of action of aspartic proteinases: the site of dissociation within the catalytic diad COOH/COO^- (i) in the free enzyme and (ii) in the Michaelis complex, and (iii) the energy changes associated with the catalytic paths. We have found that the state of dissociation within the catalytic diad is ligand-sensitive. In the free enzyme and in the intermediate complexes, Asp^{33} prefers to be dissociated with the outer oxygen of Asp^{213} protonated, while in the Michaelis and product complexes the opposite holds true. This is in agreement with recent mechanistic hypotheses and with some experimental results by FTIR and NMR. The energy diagram for the catalysis indicates that electronic effects are responsible most of all for the relative reduction of energy of the intermediates and possibly transition states on the catalytic reaction path. The shape of the diagram qualitatively agrees with the transition-state analogue theory for the enzymatic reactions.

Introduction

Aspartic proteinases are one of the four major groups of proteolytic enzymes. Since 1985 they have evoked immense interest, when it became evident that an aspartic proteinase is the only proteolytic enzyme involved in the replication and mutation of the retroviruses HIV-1 and HIV-2 [1,2]. Since then, the development of efficient inhibitors of HIV proteinases has become one of the major targets of combating AIDS [3]. The amino acid sequence and molecular architecture of the catalytic center are unique and very conservative within otherwise quite divergent aspartic proteinases. This is reflected in the fact that the respective atoms forming this center and its nearest environs (ca. 100 atoms) project almost perfectly on each other (the largest rms is equal to 0.53 Å) when overlapped together from 10 selected solid-state structures [4,5] of various aspartic proteinases. The catalytic center with its nearest neighborhood is given in Fig. 1.

Studies aimed at the kinetics and mechanism of action of aspartic proteinases have been carried out since their discovery [6–9]. It is known that two aspartic acid side-chain carboxyls (catalytic diad), with a structural water molecule captured between them, are essential for the catalytic activity of these enzymes. In a typical state, one of the carboxyls is dissociated so that the catalytic center as a whole acts as a monoanion [10]. In 1985, James and Sielecki [10], by a comparison of several structures of penicillopepsin, both free and complexed with its inhibitors, first proposed a reasonable mechanism of action of aspartic proteinases. In 1992, the original mechanism was slightly modified, independently by three groups, on the basis of resolution of the structures of microbial aspartic proteinases, complexed with transition-state mimics [11–13]. The latter are inhibitors that, while in the catalytic site of aspartic proteinases, are capable of sustaining a gem-diol produced, for example, in situ by addition of the structural water molecule to a ketone. The gem-diol can in turn mimic a gem-diolamine, believed to be an intermediate in the proteolysis [11–13], Fig. 2.

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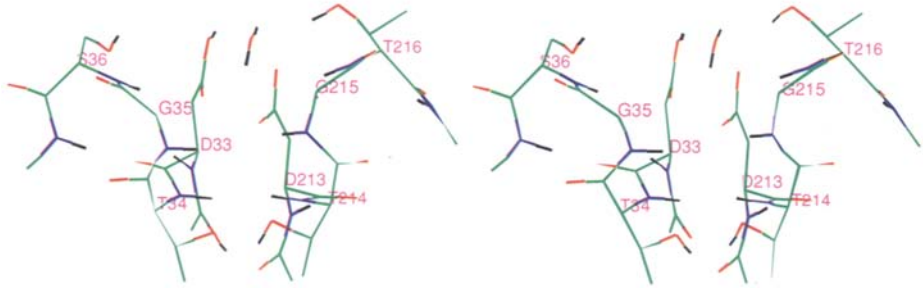

Fig. 1. Stereoview of the catalytic center of penicillopepsin [23], Brookhaven Protein DataBank file 3APP (red: oxygen; green: carbon; blue: nitrogen; black: hydrogen). Hydrogens bonded to carbon atoms are not shown.

(1) In the first step, the structural water nucleophilically attacks the carbonyl carbon of the substrate's peptide bond. This is accompanied by a synchronous rearrangement of the Asp^{33} (notation for penicillopepsin [11]) carboxylic proton to the substrate's carbonyl oxygen and of the attacking water proton to the outer Asp^{213} carboxylate oxygen.

(2) The intermediate gem-diolamine thus formed inverts at the nitrogen so that the free-electron pair gets exposed to a subsequent protonation either from the environs or from Asp^{213} being now protonated, see above.

(3) The last step consists of this protonation, accompanied by a synchronous rearrangement of one of the gem-diolamine OH protons to the Asp^{33} carboxylate and

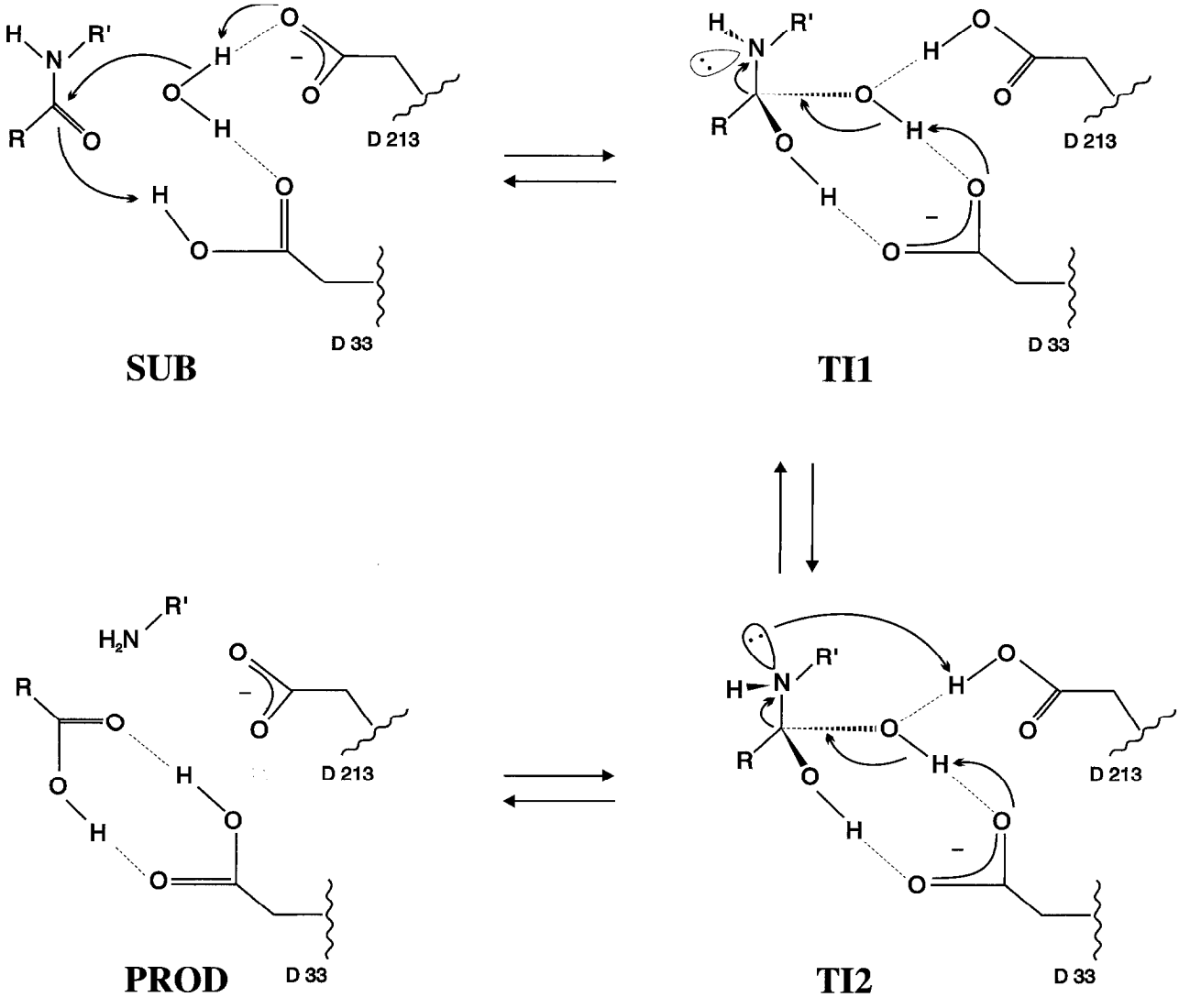

Fig. 2. Proposed mechanism of action of aspartic proteinases [11–13].

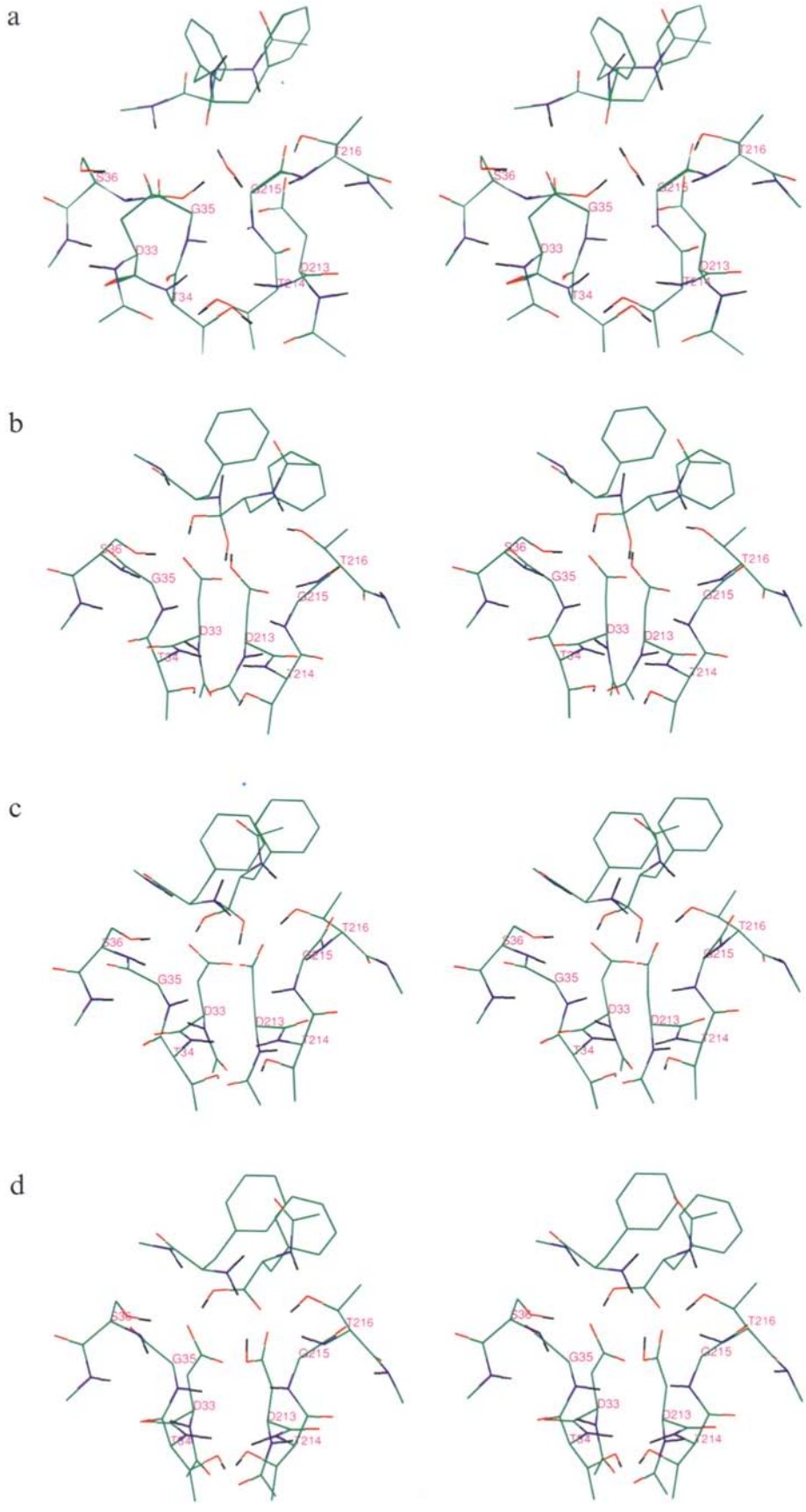

Fig. 3. Stereoviews of the AM1 optimized structures corresponding to the ground states on the catalytic path of the proteolysis by aspartic proteinases: (a) SUB; (b) TI1; (c) TI2; and (d) PROD.

TABLE 1
COMPARISON OF THE RELATIVE ENERGIES (AM1 AND PM3) OF FOUR STATES OF PROTONATION WITHIN THE CATALYTIC DIAD COOH/COO$^-$, USING THE MODEL OF ASPARTIC PROTEINASES COMPATIBLE WITH FIG. 1, WITH NO SUBSTRATE BOUND

Protonation site	MMX	AM1	PM3
Asp^{33} outer oxygen		7.9	6.9
	(0.41)	(0.31)	(0.36)
Asp^{33} inner oxygen		8.0	7.5
	(0.44)	(0.34)	(0.39)
Asp^{213} outer oxygen		0.0[a]	0.0[b]
	(0.53)	(0.46)	(0.51)
Asp^{213} inner oxygen		0.3	1.0
	(0.39)	(0.36)	(0.42)

The rms values relative to the crystal structure of rhisopuspepsin [31] are given in parentheses. The energies (heats of formation) are in kcal/mol; rms deviation values are in Å. The energy values by MMX have no significance since the results from molecular mechanics are not comparable among different valence topologies.
[a] Heat of formation: –925.4 kcal/mol.
[b] Heat of formation: –898.7 kcal/mol.

fission of the C-N bond in the intermediate. Thus, the product acid and amine are formed and the original configuration within the catalytic center is restored, in which Asp^{33} is protonated and Asp^{213} is dissociated.

The above mechanism is based on the assumptions that (i) the standard state of the catalytic center is a monoanion; and (ii) it is the outer oxygen of Asp^{33} that is protonated, see above.

A 1991 kinetic study, using ^{18}O NMR on the ^{18}O-labeled substrates [14,15], has confirmed the multistep character of this mechanism and has simultaneously suggested that the last step, i.e. the division of the gem-diolamine (see above), is the rate-controlling one. This supports the mechanism as depicted in Fig. 2 [10–13], which is now believed to be true.

The purpose of this work is to study the processes occurring on the catalytic centers of aspartic proteinases, using methods of theoretical chemistry. The current investigation has concentrated on two aspects: (i) the state of protonation within the catalytic diad; and (ii) energetic relationships between (among) the ground states on the catalytic path, Fig. 2.

Methods

All semiempirical MO calculations were carried out with the program package MOPAC, v. 6.01 [16], using the AM1 [17] and/or PM3 [18] methods. All computing was done with the PRECISE option turned on and geometry was optimized using the BFSG procedure. The density functional theory (DFT) calculations were done using the program DMOL, v. 2.30 [19], implementing the JMW [20] density functional in the minimal basis (MIN), and in the local approximation of the electron density (LDA, local density approximation). Because of the size of the systems (the models used were composed of ca. 160–190 atoms), in the DFT calculations only the single point energy evaluations (with no optimization of geometry) were possible; they were applied to the geometries initially refined by the semiempirical AM1 MO method. All computations were done on a CRAY EL-98 (eight processors) at the Interdisciplinary Center for Mathematical and Computer Modelling at the University of Warsaw. A typical optimization of geometry by a semiempirical MO method took 10–15 h while a typical DFT point calculation took 32–35 h of CPU time. All structure modeling and editing was done using the PCMODEL program [21]. Initial refinements of geometry were done using an MMX force field [22] implemented within PCMODEL.

Results and Discussion

Using the solid-state structure of penicillopepsin [23] and molecular mechanics, a model of the catalytic center, typical of aspartic proteinases and comprising some 160 atoms, was elaborated. It consisted of two peptide sequences, corresponding to Ac-Asp^{33}-Thr^{34}-Gly^{35}-Ser^{36}-NHMe and Ac-Asp^{213}-Thr^{214}-Gly^{215}-Thr^{216}-NHMe, and the structural water (see above) cut out of the PDB structure for penicillopepsin [23] (see Fig. 1). Having hydrogens added, the energy was minimized by the MMX force field, after which the resultant structure did not drift away from the starting one to any significant extent (rms lower than 0.6 Å). Thus, the mimic was stable relative to the reference structure [23] and maintained all geometric features typical of a true catalytic center.

Site of protonation in the free catalytic center of aspartic proteinases

Using the program PCMODEL, four structures comprising the catalytic center were prepared, representing four possible sites of protonation within the Asp^{33}/Asp^{213} COO^-/COOH catalytic diad. Subsequently, the structures were minimized, using the MMX force field initially and the AM1 and PM3 methods finally. The results are given in Table 1.

TABLE 2
RELATIVE ENERGIES (AM1 AND PM3) FOR FOUR STATES OF PROTONATION WITHIN THE CATALYTIC DIAD COOH/COO$^-$, USING THE MODEL OF ASPARTIC PROTEINASES, SEE FIG. 3a

Protonation site	AM1 (kcal/mol)	PM3 (kcal/mol)
Asp^{33} outer oxygen	0.00[a]	0.00[b]
Asp^{33} inner oxygen	16.2	12.4
Asp^{213} outer oxygen	3.3	2.6
Asp^{213} inner oxygen	14.4	8.7

[a] Heat of formation: –5192.4 kcal/mol.
[b] Heat of formation: –4834.5 kcal/mol.

TABLE 3
RELATIVE ENERGY VALUES (AM1, PM3 AND DFT (MIN/JMW)) RELATING SUB, TI1, TI2 AND PROD, SEE TEXT

Method	SUB	TI1	TI2	PROD
AM1	73.5	0.00[a]	19.3	33.9
	(0.44)	(0.32)	(0.30)	(0.47)
PM3	57.2	0.00[b]	25.0	24.9[c]
	(0.40)	(0.28)	(0.22)	(0.22)
DFT (MIN/JMW)[d]	106.4	0.00	36.4	53.7

The rms values relative to the crystal structure of rhisopuspepsin [31] are given in parentheses. The energies are in kcal/mol; rms deviation values are in Å.
[a] Heat of formation: −5192.4 kcal/mol.
[b] Heat of formation: −4834.5 kcal/mol.
[c] PROD converts into TI2 under minimization.
[d] Total energy: −4431.824207 au.

The data in the table prove the stability of the model facing various theoretical methods. The stability is reflected in the nominal drifts of the atoms during the minimizations. It is seen that the outer oxygen of Asp^{213} demonstrates the highest proton affinity, in agreement with the recent results of Iliadis et al. [24] by FTIR. On the other hand, the proposed mechanism (see Fig. 2) assumes the Asp^{33} outer oxygen to be protonated initially and finally, and Asp^{213} to be protonated at the intermediate step. The discrepancy may suggest that the substrate/inhibitor/intermediate binding may induce changes in the maximum proton affinity between the outer Asp^{213} and the outer Asp^{33} oxygens [25].

Site of protonation in the substrate-binding catalytic center of aspartic proteinases

The structures mimicking the complex of the catalytic center with a minimal substrate (Ac-FF-NHMe) (SUB), the tetrahedral intermediates (TI1 and TI2), and the product (PROD) were adopted from our former work [26], in accordance with Fig. 2. Using the PCMODEL program, four variants of the SUB (see Fig. 3a) complex were generated, representative of the four possible states of protonation within the catalytic diad. The structures were subsequently optimized using the MMX force field initially and the AM1 and PM3 semiempirical MO methods finally. The results are given in Table 2. They confirm that the outer oxygens in both Asp^{213} and Asp^{33} prefer to be protonated over the inner ones.

Simultaneously, the data in Tables 1 and 2 provide support for the hypothesis that the state of protonation within the catalytic diad may vary depending on whether the enzyme is free or is binding a ligand (substrate/inhibitor/intermediate) in its catalytic pocket [11–13,25].

Catalytic mechanism and transition-state analogue theory [27,28]

Figure 3 presents the optimized (AM1) structures for the successive steps of the proteolysis. Table 3 includes the energy values for SUB, TI1, TI2 and PROD while Fig. 4 presents the same relationships in a diagrammatical form.

The profile in Fig. 4 appears to be a surprise, in view of the expectation that for a 'classical' reaction the intermediates should be of higher energy than the reactants and products. The apparent paradox can be rationalized in terms of the transition-state analogue theory, which supports the conclusion that the shape of the profile in Fig. 4, with TI1 and TI2 of lower energy than both SUB and PROD, provides for a due manifestation of the catalytic mechanism of an enzyme [27,28].

Still in the 1940s Pauling [29] postulated that one of the characteristics of an enzymatic reaction is a perfect fit of the catalytic pocket to the transition states and/or the intermediates. This would be accompanied by a contribution to the binding energy, which would substantially compensate for the barrier to activation. In a true enzyme and not a mimic like this, the steric constraints imposed on the catalytic center by the rest of the enzyme's 'body', while reducing the barrier, prevent the energies of the intermediates from falling below the level typical of SUB and/or PROD. On the contrary, in the mimic used, which lacks the surrounding body and is thus amenable to expansion, the depression in energy observed for TI1 and TI2 (50–70 kcal/mol for semiempirical MO methods, see Fig. 4) may, in our opinion, provide for a measure of a

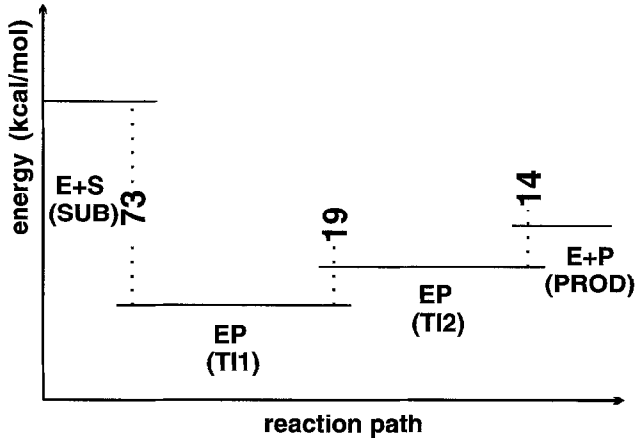

Fig. 4. Energy (AM1) diagram relating SUB, TI1, TI2 and PROD; see text.

pure electronic effect associated with a perfect fit of TI1 and TI2 into the catalytic pocket. Indeed, the calculations with partially constrained positions, by imposing force constants on specific atoms in the tetrapeptides Ac-Asp^{33}-Thr^{34}-Gly^{35}-Ser^{36}-NHMe and Ac-Asp^{213}-Thr^{214}-Gly^{215}-Thr^{215}-NHMe of the mimic, indicate that while the profile remains similar to that in Fig. 4, the energetic collapse from SUB to TI1 and TI2 has been notably alleviated (not shown). Similar results for aspartic proteinases have been recently obtained by Goldblum [30] on a model consisting of as many as ca. 280 atoms by the semiempirical MNDO/H MO method.

Conclusions

Using the formerly developed model of the catalytic center of aspartic proteinases [26], it was found that: (i) In a free enzyme the outer oxygen of Asp^{213} prefers to be protonated and that of Asp^{33} prefers to be dissociated within the catalytic COOH/COO$^-$ diad, in agreement with the finding of Iliadis et al. [24]. (ii) In the Michaelis (SUB) and in the PROD complexes the reverse is true, while in the intermediate IT1 and IT2 complexes the Asp^{213} prefers to be protonated again, in agreement with the proposed mechanism [11–13]. Thus, the proton affinities within the diad seem to be ligand-sensitive. (iii) The energy diagram for the catalysis qualitatively agrees with the transition-state analogue theory for the enzymatic reactions.

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