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Stereoelectronic Effects in Serine Proteases: Ab Initio Molecular Orbital Calculations

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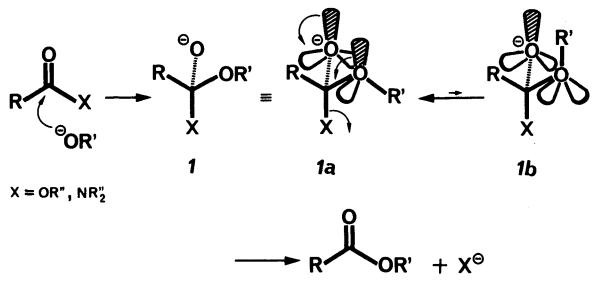
Ab initio molecular orbital calculations have been performed on the reaction profile for the addition/elimination reaction between ammonia and formic acid, proceeding via a tetrahedral intermediate: $NH_3+HCO_2H\rightarrow H_2NCH(OH)_2\rightarrow NH_2CHO+H_2O$. For the addition of ammonia two transition state conformations, of which one possesses a lone pair, on the hydroxyl oxygen of formic acid, antiperiplanar to the attacking nitrogen (T1 conformer) and the other possesses a hydrogen atom, of the OH group, antiperiplanar to the nucleophile ammonia (T1′ conformer), have been identified as characteristic transition state structures. Calculated transition state energies for the first addition step of the reaction revealed that a lone pair on the oxygen of the OH group, which is antiperiplanar to the attacking nitrogen (T1 conformer), stabilized the transition state by 7.4 kcal mol^{-1} compared to the T1′ conformer. Similarly, for the second water elimination step of the reaction, two conformers (T2 and T2′) have been identified as transition state structures, of which the T2 conformer possesses a lone pair on the nitrogen, antiperiplanar (app) to the leaving water and the T2′ conformer possesses a hydrogen atom, of the NH_2 group, app to the leaving water molecule. Again, calculated transition state energies for the T2 and T2′ structures revealed that a lone pair on the nitrogen of the NH_2 group, which is app to the scissile C-O bond (T2 conformer), stabilized the transition state by 3.1 kcal mol^{-1}, thus supporting the hypothesis of stereoelectronic control for this reaction. The significance of these stereoelectronic effects for the mechanism of action of serine proteases is discussed and a novel mechanism is proposed.

Stereoelectronic control, or the role of orbital orientation in organic and enzymatic reactions, has been of considerable interest over the last decade.[1-31] Deslongchamps and cowokers[2] in studying tetracovalent carbon species have demonstrated selective cleavage of bonds which are *trans*-antiperiplanar (app) to lone pair on directly bonded oxygen and/or nitrogen atoms. There has been a growing recognition that conformationally-dependent orbital interactions can play a significant role in the structure and reactivity of biomolecules.

Thus, as shown in the reaction below, addition of alkoxide to the acyl carbon, will yield the tetrahedral intermediate **1**. In the stereoelectronic theory, breakdown of this intermediate with cleavage of the C-X bond is facilitated by two non-bonded electron pairs oriented app (shaded) to the C-X bond in conformation **1a**. In conformation **1b** only one non-bonded electron lone pair is oriented app to the scissile C-X bond. In the stereoelectronic theory, cleavage of the C-X bond of the tetrahedral intermediate **1** is presumed to be controlled by the number of *app* lone pairs and thus should proceed through conformation **1a** rather than **1b** (two app paris in **1a** vs. one app pair in **1b**).



Research groups of Lehn,[5-9] Pople,[10] Gorenstein,[11-17] and others have provided theoretical justification for these stereoelectronic effects in tetracovalent carbon species and phosphates and pentacovalent phosphoranes. We have recently suggested that this stereoelectronic effect has a major impact on the relative energies of transition states, much more so than on ground states or intermediates.[14,15] It is thus important to use calculated transition state energies as a guide in the evaluation of the stereoelectronic effect. Although earlier calculations[1-13] supported the existence of the operation of stereoelectronic effects in tetracovalent species, these comparisons were based only on stable molecular structures that, again, would tend to minimize the stereoelectronic effect. Following the detailed quantum mechanical studies by Oie et al.,[32,33] for the addition-elimination reaction profile between ammonia and formic acid proceeding via a tetrahedral intermediate, we have recently initiated

$$NH_3+HCO_2H\rightarrow H_2NCH(OH)_2\rightarrow NH_2CHO+H_2O \quad (1)$$

an examination of the stereoelectronic effect in the well-characterized transition states.[16] Separate transition states were observed for the first addition step and the second elimination step, with a metastable tetrahedral intermediate.[16,32,33] Related molecular orbital calculations on acyl addition reactions have characterized only a single transition state, failing to find any stabilization of a metastable tetrahedral intermediate.[32,34] Although we could identify for the first

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addition step two transition state structures (T1 and T1′) which were important for the stereoelectronic effect comparison, only one conformer (T2) could be characterized for the second elimination step as a real transition state structure, thus making our work half complete.[16] We report here a complete characterization of all four of the transition state conformers (T1, T1′, T2, and T2′) which are ideal for testing the stereoelectronic theory.

Methods of Calculation

In our original study,[16] the SCF LCAO-MO ab initio molecular orbital calculations utilized the GAUSSIAN 70 series of programs with a STO-3G minimal basis set.[35] Initial structures for the addition-elimination transition states of reaction 1, as shown in Fig. 1, were partially taken from the optimized structures of Oie et al.[32] The geometries for different conformations about the C-N and C-O-H bonds for both transition states were then optimized by sequentially varying all bond lengths, bond angles, and torsional angles until the total energy has been effectively minimized when it was clear that only very small (<0.02 kcal mol^{-1}) decreases in energy were possible by further calculation. For optimization of parameter χ, the following Eq. 2 was used, by calculating three energies (E_1—E_3) around the previously increment of dχ, which assumes a parabolic harmonic vibration function:

$$\chi_{OPT} = \chi_0 - \frac{(E_2 - E_3)d\chi}{2(E_2 + E_3 - 2E_1)} \quad (2)$$

where $E_1 = a\chi_0^2 + b\chi_0 + c$

$E_2 = a(\chi_0 + d\chi)^2 + b(\chi_0 + d\chi) + c$

$E_3 = a(\chi_0 - d\chi)^2 + b(\chi_0 - d\chi) + c$

Since $a=(E_2+E_3-2E_1)/2d\chi^2$, the force constant is negative when the numerator of coefficient a is negative. In those structures (T1, T1′, and T2) identified as transition states (a saddle point with only one negative force constant), the reported energy was maximized with respect to this single geometric parameter defining the reaction coordinate, and all other geometric parameters were relaxed to their minimum energy values.[16] Although this procedure is feasible for calculating stable intermediates or ground state structures, it may not be the best choice for calculations of transition state structures unless one geometric parameter can clearly be identified as the reaction coordinate. In general, however, the reaction coordinate consists of the translating bond coupled with other vibrations. A newer optimization routine of the GAUSSIAN 80 program[36] can now automatically search for the transition state. The initially optimized geometries[16] obtained from the GAUSSIAN 70 program were utilized in the full transition state optimization with the GAUSSIAN 80 program. All four transition state structures (T1, T1′, T2, T2′; see Fig. 1) are now optimized and the final geometries are given in Table 1.

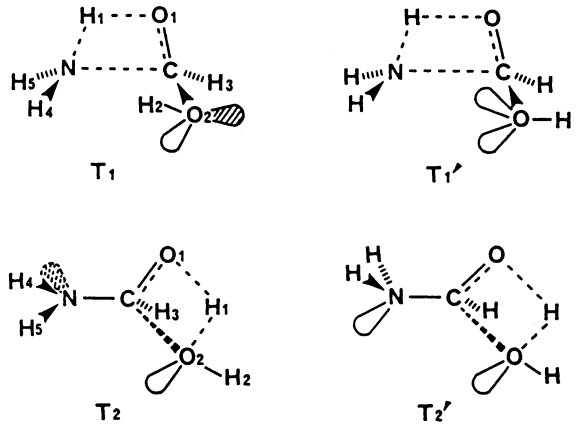


Fig. 1. Structures for different conformations of tetrahedral transition states in the acyl addition-elimination reaction 1.

Table 1. STO-3G Optimized Structures of Transition States and Intermediate Molecule[a]

Parameter[b]	T1	T1′	T2	T2′
C-O_1	1.315 (1.315)	1.308	1.312 (1.312)	1.304
C-O_2	1.402 (1.402)	1.438	1.720 (1.720)	1.678
<O_1CO_2	119.5	121.3	88.09 (88.1)	89.88
C-N	1.756 (1.756)	1.706	1.441 (1.441)	1.486
<NCO_1	89.93 (89.9)	92.25	120.9 (120.6)	122.5
<NCO_2	107.9 (107.9)	101.2	108.6	102.9
H_1-O_1	1.404 (1.404)	1.461		
H_1-O_2			1.078 (1.078)	1.061
<H_1O_1C	79.80 (79.8)	78.37		
<H_1O_2C			72.25 (72.3)	75.73
τ H_1O_1CN	−0.41 (−0.4)	0.81		
τ $H_1O_2CO_1$			−0.70 (−0.5)	−1.11
H_2-O_2	0.990	0.990	0.990	0.991
<H_2O_2C	103.4	103.2	113.9	115.3
τ H_2O_2CN	−88.97	180.0[c]	133.2	129.1
H_3-C	1.112	1.119	1.112	1.112
<H_3CN	101.9	104.0	109.3	108.5
τ $H_3CO_1H_1$	−125.0	−125.1		
<H_3CO_1			122.8	122.7
$H_{4(5)}$-N	1.034	1.034	1.027	1.035
<H_4NC	120.9	117.9	111.3	105.5
τ H_4NCO_1	−110.6	−112.6		
τ H_4NCO_2			63.26	180.0[c]
<H_5NC	119.7	120.3	112.5	105.9
τ H_5NCO_1	111.0	110.9		
<H_5NH_4			109.5	103.5

a) Bond length in Å, bond angles (<XYZ) and torsional angles (τWXYZ) in degrees; see Fig. 1 for definition of atoms W, X, Y, Z. Optimized (at STO-3G level) parameters in parentheses from Ref. 32. b) See Fig. 1 for definition of structures. c) Assumed.

Results

Shown in Fig. 1 are the four optimized transition state structures of which T1 and T1′ correspond to the first addition step of the reaction and T2 and T2′ to the second elimination step. T1 possesses a lone pair on oxygen (shaded), which is antiperiplanar (app) or trans to the attacking nitrogen (nucleophile). Similarly, T2 possesses a lone pair on nitrogen (shaded), which is trans-app to the leaving water molecule. On the other hand, both T1′ and T2′ do not have an app lone pair with respect to the nucleophile (ammonia) or the leaving group (water). Instead they possess a hydrogen atom which is app to the translating bond.

Shown in Fig. 2 are the reaction profiles for formation of the amide bond in the reaction of ammonia and formic acid. The profiles differ with respect to the conformations about the C-N and C-OH bonds. The relative T1 and T2 transition state energies (Table 2) and geometries (Table 1) calculated in the present study agree well with the earlier calculations.[32] The lowest reaction pathway (solid line in Fig. 2) proceeds via T1 and T2 transition states, with T1 being 1.9 kcal mol^{-1} more stable than T2. It is important to note that both T1 and T2 possess a lone pair (shaded), which is app to the translating bond, in agreement with the stereoelectronic theory.[14,15] For the first addition transition state (formation of the C-N bond), the conformation T1 with an oxygen lone pair on $O_{(2)}H$ app to the C-N bond is 7.4 kcal mol^{-1} lower in energy than T1′, which has no lone pair orbital on $O_{(2)}$ app to the C-N bond. We have previously reported[16] that the energy difference between T1 and T1′ is 3.9 kcal mol^{-1}, which is much smaller than the present 7.4 kcal mol^{-1}, indicating the limitation of the stepwise optimization procedure for the characterization of transition state structures. Similarly, for the second elimination transition state, the conformation T2 with a nitrogen lone pair app to the scissile C-$O_{(2)}$ is 3.1 kcal mol^{-1} lower in energy than T2′, which has no lone pair on nitrogen app to the breaking C-$O_{(2)}$ bond. In our previous study,[16] we failed to locate the T2′ transition state when we assumed that we could approximate the 3N-6

Table 2. Ab Initio Energies and Population Analysis for Various Conformations of Transition States T1 and T2

	Conformation			
	T1	T1′	T2	T2′
Rel. energy[a] / kcal mol^{-1}	0.0[a]	7.41	1.87	4.96
bond				
C-O_1	0.6433	0.6595	0.6577	0.6730
C-O_2	0.5400	0.4901	0.1860	0.2131
C-N	0.2286	0.2637	0.6748	0.6048
H_1-O_1	0.1772	0.1533	0.1784	0.1524

a) Total energy of T1 was −241.59257 hartrees.

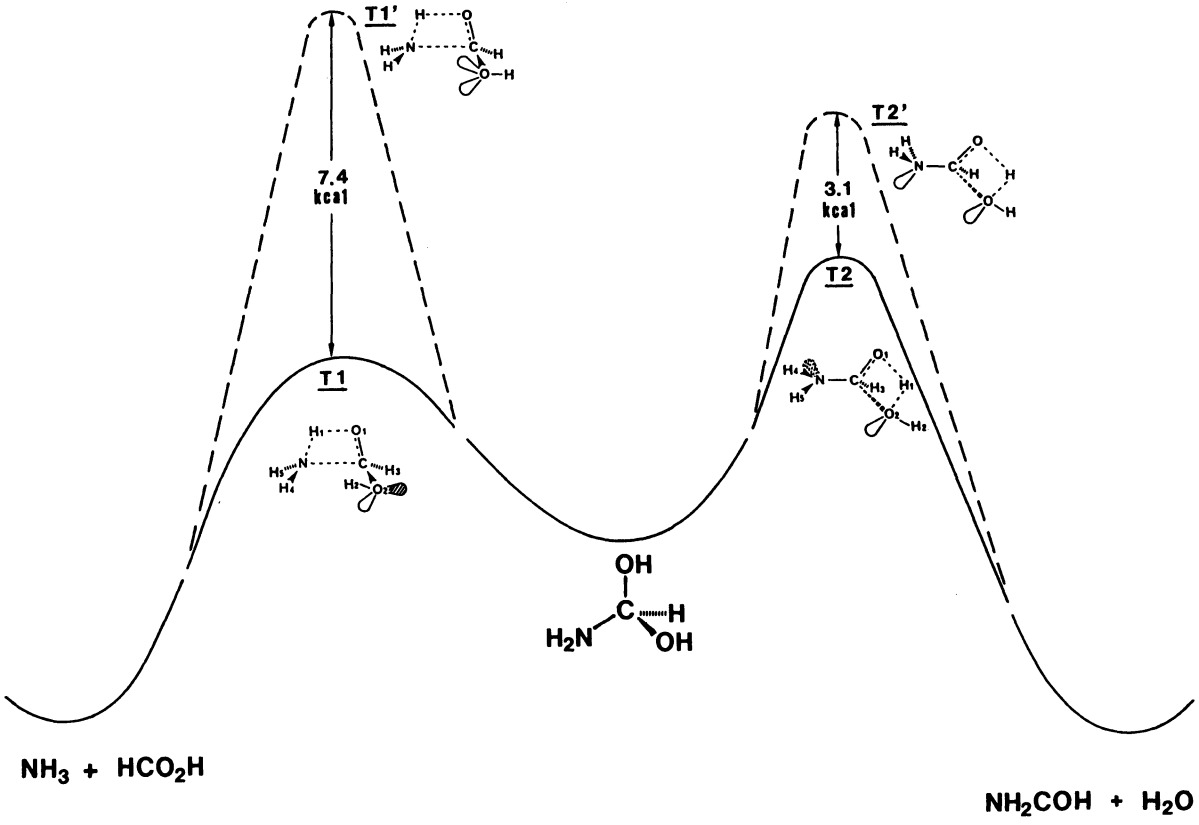


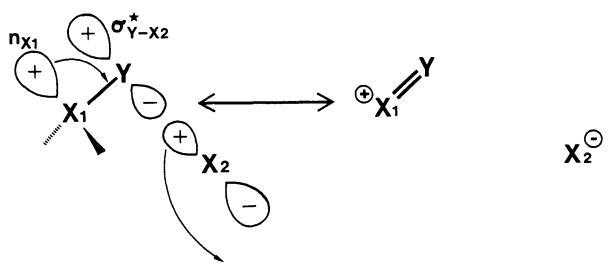
Fig. 2. Reaction profiles for acyl addition-elimination reaction 1 are shown assuming various conformations about the C-N and C-$O_{(2)}$ bonds. All conformations are optimized as transition states.

eigenvectors and eigenvalues of the quadratic force constant matrix by treating them as classical vibrational modes (bond stretches, bends, etc.) and force constants. For a col point or transition state, only one of the eigenvalues (force constant) can be negative, and this corresponds to the imaginary vibration frequency defined as the reaction coordinate. In reality, however, it is an oversimplification to assign a single classical vibrational mode to the reaction coordinate eigenvector, although it has the advantage of giving us better chemical intuition for the nature of chemical reactivity. Apparently, the geometric parameters of T2′ were strongly coupled and thus could not be treated in a classical fashion.[16]

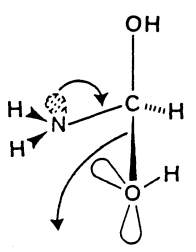
Oie et al. have established that the relative stabilities of the various species were generally unaffected by using more extensive basis sets and inclusion of correlation effects.[32] Because we are only interested in relative energies of transition states differing only in conformation (not atomic bonding), any errors resulting from neglect of correlation and polarization in our study should essentially cancel.[32] The STO-3G optimized geometries, population analyses, and energies for various transition state conformations are shown in Tables 1 and 2.

The stereoelectronic effect is believed to arise from the interaction of an app lone pair (n_{X_1}) on X_1 (oxygen or nitrogen) with the σ^* antibonding Y-X_2 orbital (Scheme 1).[1-28]

In terms of a simple resonance picture for the stereoelectronic effect, stabilization of the structure is thought to derive from an anomeric type, double bond-no bond resonance contribution. The bond opposite to the app lone pair has some no bond character and the bond containing an app lone pair has some double bond character. The overlap population analysis[37] of Table 2 confirms this general picture. As expected, the overlap population of a bond that is app to a lone pair is smaller than the overlap populaton of a bond that is not app to a lone pair (assuming we are comparing otherwise identical structures). Thus the C-N overlap population is 0.264e in the T1′ conformation, whereas it is 0.229e in the T1 conformation (Table 2). The presence of an app lone pair to the scissile C-N bond in T1 conformation weakens this bond (reduces the overlap population). As expected from the double bond-no bond resonance description of the stereoelectronic effect, the C-$O_{(2)}$ overlap population in T1 (0.540e) is larger than in T1′ (0.490). The app lone pair on $O_{(2)}$ to the C-N bond in T1 imparts some double bond character to the C-$O_{(2)}$ bond. Similar overlap population stereoelectronic effects are observed for the T2 and T2′ conformation. T2, with an app lone pair on nitrogen to the scissile C-$O_{(2)}$ bond, has a lower C-$O_{(2)}$ bond overlap population (0.186e) than T2′ (0.213e), and also has a higher C-N bond population (0.675e) than T2′ (0.605e), in accordance with the double bond-no bond resonance structure of Scheme 1.



Scheme 1.



Scheme 2.

Discussion

We have earlier established that the kinetic stereoelectronic effect will largely be manifested in energy differences at the transition state.[14,15,17] On the basis of one-electron molecular orbital theory,[38] the two-electron stabilization from the interaction of a doubly occupied MO(n) with a vacant nondegenerate MO(σ^*) may be approximated as being inversely proportional to the energy separation of the two MO's and directly proportional to the square of their overlap.

$$SE(\mathrm{n},\sigma^*) \propto \frac{S^2_{n\sigma^*}}{\Delta E_{n\sigma^*}} \qquad (3)$$

In the above example, $S_{n\sigma^*}$ is the overlap integral between the app lone-pair orbital(n) on oxygen or nitrogen and the vacant antibonding orbital(σ^*) of the translating bond, and $\Delta E_{n\sigma^*}$ is the energy difference between these orbitals. The app lone-pair stereoelectronic effect presumably derives from this orbital interaction since maximal overlap between n and σ^* is found for the app orientation. Of course, if the stereoelectronic effect does not have its major impact in the transition state, little activation energy differences and no kinetic acceleration will result from these interactions.

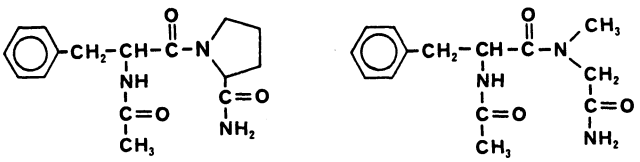
By microscopic reversibility, it should be easier to break or make the bond that possesses no bond character (Scheme 1), and indeed as shown in Fig. 2 and Table 2, this has been confirmed in these calculations of transition state structures. For the addition transition state, the conformation T1 with an oxygen lone pair that is app to the translating C-N bond is 7.4 kcal mol^{-1} lower in energy than T1′, which has no lone pair app to the C-N bond. A similar stereoelectronic effect operates on the second water elimination step:

T2 with a nitrogen lone pair app to the scissile $C-O_{(2)}$ bond is 3.1 kcal mol^{-1} lower in energy than T2′, which has no lone pair app to the $C-O_{(2)}$ bond. Note in our discussion we ignore the lone pair orbitals on $O_{(1)}$, which also are app to the C-N or $C-O_{(2)}$ bonds and hence stereoelectronically facilitate C-N or $C-O_{(2)}$ bond translation. Considering also the counterbalancing stereoelectronic effect,[16] the most favored tetrahedral carbon transition state is one that possesses a lone-pair app to the adjacent scissile bond while at the same time does not have a lone pair on the cleaving atom, which is app to a polar adjacent bond (bond which possesses double bond character; see Scheme 2).

This stereoelectronically most favored structure, derived from the comparisons of transition state conformations, supports Lehn and Wipff's similar conclusions[5-9] based upon overlap population and energy differences in stable tetrahedral intermediates.

Reversing the addition-elimination reaction of Eq. 1 (going from right to left in Fig. 2), one can consider this reverse reaction to be a model study of serine proteases, the water molecule representing a serine hydroxyl group and ultimately hydrolyzing a peptide bond. Although enzymic catalysis is believed to derive from the enzyme's special capacity to stabilize (and hence lower the energy of) the transition state of the reaction,[39-41] it is important to recognize that for most enzymatic reactions, and certainly including peptide bond hydrolysis, there is probably no one unique transition state. Thus, as Albery and Knowles[42] have argued, in the "evolutionary perfection" of an enzyme catalyst in facilitating a multistep reaction, eventually the enzyme must find a mechanism that lowers the barrier to each elementary step. Chymotrypsin and most likely all other serine proteases proceed via an acyl enzyme intermediate in which formation of the acyl enzyme intermediate and hydrolysis of the intermediate in turn proceed via tetrahedral intermediates.[43] Concentrating only on the reaction path between Michaelis comple and acyl enzyme, the enzyme must catalyze formation and breakdown of the tetrahedral intermediate in a minimum two-step reaction. If chymotrypsin is "evolutionarily perfect," then each of these steps is partially rate limiting. The enzyme must thus be sufficiently conformationally flexible at its active site to stabilize both transition states in the formation (T2) and breakdown (T1) of the tetrahedral intermediate. If stereoelectronic effects operate for one transition state, they will likely operate for both. Since the optimal stereoelectronic conformation for formation of the tetrahedral intermediate (T2 conformation) must be different from the optimal stereoelectronic conformation for breakdown of the intermediate (T1 conformation), passage from one transition state to the other will require single-bond rotation about C-O and C-N in the tetrahedral intermediate. Although recent papers have addressed the importance of stereoelectronic effects in the mechanism of action of the serine proteases, such as α-chymotrypsin,[29-31] no mention has been made to the important C-O bond rotation, which is required by an additional counterbalancing stereoelectronic effect.[16] We argue that a most important function of the enzyme is to provide for these conformational changes in the tetrahedral intermediate complex.

Experimental support for the stereoelectronic effects in the mechanism of action of α-chymotrypsin is provided by the elegant work of Bizzozero and coworkers.[29-31] Although peptide bonds involving the carbonyl group of amino acid residues with large hydrophobic side chains are known to be easily hydrolyzed by α-chymotrypsin, if the amide nitrogen is contributed by a proline residue, the peptide bond is resistant to chymotryptic cleavage. This finding has led Bizzozero and Zweifel to examine the reactivity of dipeptides, possessing an alkyl substituent on the leaving group nitrogen, such as *N*-acetyl-L-phenylalanyl-L-prolinamide and *N*-acetyl-L-phenylalanyl-sarcosinamide (Scheme 3). Both dipeptides were found to be unreactive but proved to be good competitive inhibitors, indicating that they form enzyme-dipeptide complexes and their unreactivity must be due to some factors arising during the transformation leading to or from the acyl-enzyme intermediate.



Scheme 3.

Shown in Fig. 3 is our modified description of the catalytic events in the acylation reaction as presented by Dugas and Penny.[31] This modified description is equivalent to Figs. 5 and 6 in our previous article[16] and also to Fig. 4.7 of Deslongchamps' proposal,[31] and this Fig. 3 contains the important C-O bond rotation, in addition to the nitrogen inversion. For the nucleophilic attack by the hydroxyl group of Ser-195, the most stereoelectronically favored transition state is E-T2, which possesses a nitrogen lone pair app to the developing SerO-C_α bond while at the same time does not have a lone pair on the nucleophile serine-oxygen, which is app to a polar N-C_α bond (see also Scheme 2). The important point to be considered here is that the app lone-pair orbital on nitrogen points toward the solvent (backside of the page) and the N-H′ bond toward the inside of the enzyme active site. The distance between the nitrogen and the imidazole ring of His-57 in the E-T2 conformation is only 3.5 Å (based upon model building derived from analogous X-ray crystal structures), and is just sufficient to accommodate a hydrogen atom. When the N-H′ hydrogen is

replaced by an alkyl group, as in the case of a proline residue, this substituent would come too close to the imidazole ring of His-57. Hence it can be argued that a dipeptide containing an alkyl substituent on nitrogen is inactive toward α-chymotrypsin hydrolysis because the steric hindrance prevents formation of the tetrahedral intermediate in the stereoelectronically required E-T2 conformation: In Fig. 3, H′ on nitrogen must be replaced by an alkyl group. The E-T2 conformation is not possible because the distance between the nitrogen and the imidazole ring is only 3.5 Å. Then when H′ is replaced by an alkyl group, the lone pair orbital on the nitrogen can never be app to the nucleophile (Ser-OH), corresponding to the T2′ conformation in Fig. 2-this prevents the nucleophilic attack by Ser-OH.

Note in Fig. 2 that the energy difference between T2 and T2′ is 3.1 kcal mol^{-1}. However, this is the minimum estimate of the corresponding energy difference between E-T2 and E-T2′ (H′ in Fig. 3 is replaced by an alkyl group and rotated to be app to Ser-OH as in the T2′ conformation) since the energy different between T2 and T2′ would have been greater if methyl groups (any alkyl groups) had been used in place of hydrogen on the amide nitrogen. This originates from the expectation that SE (n,σ^*)$>SE(\sigma_{O\text{-}H},\sigma^*)$; SE(n,σ^*)$>SE$ ($\sigma_{C\text{-}H},\sigma^*$)$>SE$ ($\sigma_{C\text{-}C},\sigma^*$); and SE (n,σ^*)$>SE$ ($\sigma_{N\text{-}C}$, σ^*)$>SE$ ($\sigma_{N\text{-}H}$,n*)[44] in Eq. 3. This implies that electron donation from the $\sigma_{N\text{-}H}$ bond into the σ^* bond is better than the $\sigma_{N\text{-}C}$ bond, resulting in a stabilization of the T2′ conformation compared to the corresponding alkyl substituted counterpart, such as E-T2′ with the replacement of H′ by an alkyl group.

If the E-T2 conformation is stereoelectronically best for lowering the energy of the transition state for SerO-C_α bond formation, as discussed above, it must be the worst for N-C_α bond cleavage. For the cleavage of the N-C_α bond, stereoelectronically the lone pair on the serine oxygen must be app to the translating (scissile) N-C_α bond and no lone pair on the N should be app to the adjacent SerO-C_α bond (E-T1 conformation). This E-T1 conformation is achieved by N-inversion[30] accompanied by torsion about the Ser-195 bonds. The equilibrium between the two intermediates, E-T2 and E-T1 in Fig. 3, is likely to be largely on the side of the E-T1 conformer since E-T2 is destabilized by the unfavorable dipole-dipole repulsion between the N-H group of His-57 and the N-H′ group of the nitrogen. In E-T1 this unfavorable interaction is replaced by a favorable hydrogen bond interaction between the N-H group of His-57 and the lone pair orbital of the nitrogen. It is interesting to note[30] that due to this particular equilibrium position the tetrahedral intermediate is now locked into a conformation (E-T1) where reversion to the enzyme-substrate complex is no longer feasible, since the SerO-C_α bond no longer has an app lone pair on N. Recall that an app lone pair on N is responsible for stabilizing the transition state for C-O cleavage by 3.1

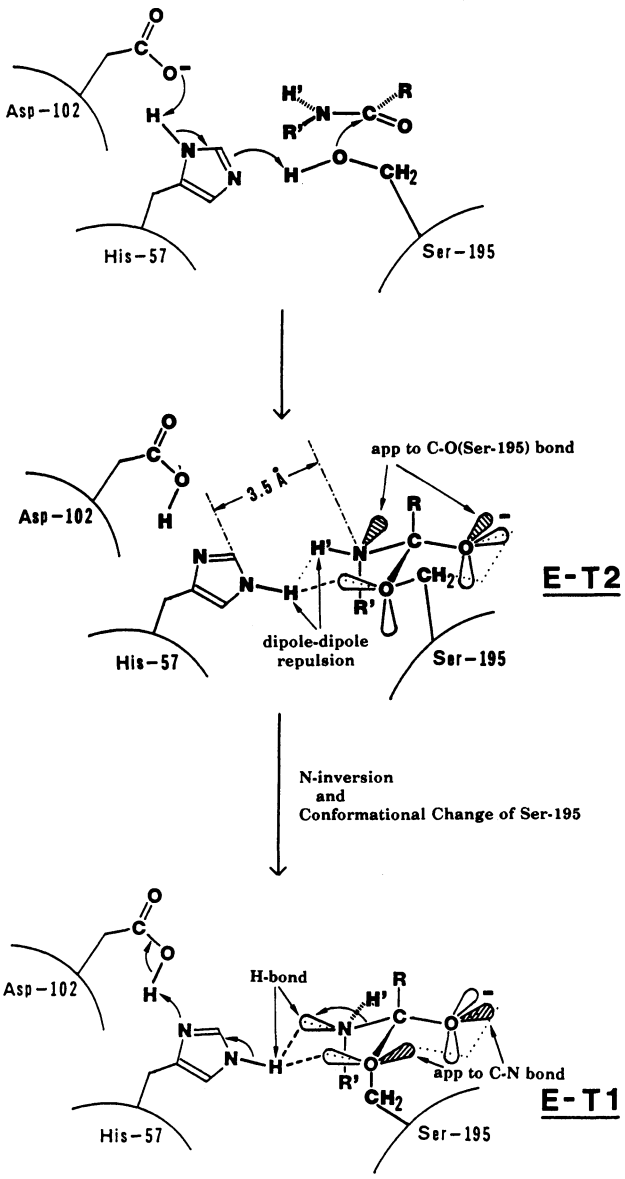


Fig. 3. Schematic drawing of steps in acylation reaction of α-chymotrypsin partially taken from Dugas and Penny.[31] Antiperiplanar lone pairs to the scissile bond are shaded.

kcal mol^{-1}. This N-inversion step implies that the leaving nitrogen acts as a switch controlling which of the two critical bonds of the tetrahedral intermediate, the C-O bond or the C-N bond, is broken.[30] In the switch position of E-T2, where the lone pair on N points toward the solvent (backside), C-O bond formation is allowed by the stereoelectronic principle, whereas C-N bond cleavage is prevented by lack of an app lone pair on serine oxygen and also by lack of N-protonation by the imidazole. In the other switch position of E-T1, where the lone pair on N points toward the inside of the enzyme active site, C-O bond cleavage is forbidden by the stereoelectronic principle, whereas C-N bond cleavage is facilitated by the app lone pair on the serine oxygen as well as by the N-protonation.

Since the tetrahedral intermediate is more stable in E-T1 than in E-T2 (even without considering the dipole-dipole interaction, the T1 transition state is 1.9 kcal mol^{-1} more stable than the T2 transition state; see Table 2), the breakdown of the amide bond (forward direction) is favored over that in the backward direction. Note also the additional important conformational change of Ser-195, going from E-T2 to E-T1, to gain 7.4 kcal mol^{-1} stabilization (Fig. 2) for C-N bond cleavage, which has been missed in the previously recognized literature (actually, the counterbalancing stereoelectronic effect[16] in E-T2 has been ignored in the literature).

The important mobility of the imidazole, to swing between a position allowing formation of a hydrogen bond with the serine oxygen, and one allowing formation of a hydrogen bond with the leaving nitrogen, has been discussed.[16,30] Detailed experimental support for the movement of important atoms has also been given in our earlier publication.[16] Together with the mechanistically required nitrogen inversion, conformational change of the Ser-195, and the observed flexibility of the imidazole group, it would appear as though the serine protease is designed to provide the stereoelectronically optimal transition states for both the acyl addition and elimination steps. These stereoelectronic effects should now be included in a more detailed quantum mechanical calculation of the reaction profile for the serine proteases.

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References

1) A. J. Kirby, "The Anomeric Effect and Related Stereoelectronic Effect at Oxygen," Springer-Verlag, Berlin (1983).
2) P. Deslongchamps, "Stereoelectronic Effects in Organic Chemistry," Pergamon Press, Oxford (1983).
3) D. R. Storm and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **94**, 5815 (1972).
4) W. L. Mock, *Bioorg. Chem.*, **4**, 270 (1975).
5) J. L. Lehn and G. Wipff, *J. Chem. Soc., Chem. Commun.*, **1975**, 800.
6) J. M. Lehn and G. Wipff, *J. Am. Chem. Soc.*, **96**, 4048 (1974).
7) J. M. Lehn and G. Wipff, *J. Am. Chem. Soc.*, **98**, 7498 (1976).
8) J. M. Lehn and G. Wipff, *Helv. Chim. Acta*, **61**, 1274 (1978).
9) J. M. Lehn and G. Wipff, *J. Am. Chem. Soc.*, **102**, 1347 (1980).
10) L. Radom, W. L. Hehre, and J. A. Pople, *J. Am. Chem. Soc.*, **94**, 2371 (1972).
11) D. G. Gorenstein, J. B. Findlay, B. A. Luxon, and D. Kar, *J. Am. Chem. Soc.*, **99**, 3473 (1977).
12) D. G. Gorenstein, B. A. Luxon, J. B. Findlay, and R. Momii, *J. Am. Chem. Soc.*, **99**, 4170 (1977).
13) D. G. Gorenstein, B. A. Luxon, and J. B. Findlay, *J. Am. Chem. Soc.*, **99**, 8048 (1977).
14) D. G. Gorenstein, B. A. Luxon, and J. B. Findlay *J. Am. Chem. Soc.*, **101**, 5869 (1979).
15) D. G. Gorenstein, B. A. Luxon, and E. M. Goldfield, *J. Am. Chem. Soc.*, **102**, 1757 (1980).
16) D. G. Gorenstein and K. Taira, *Biophys. J.*, **46**, 749 (1984).
17) K. Taira and D. G. Gorenstein, *J. Am. Chem. Soc.*, **106**, 7825 (1984).
18) D. G. Gorenstein and R. Rowell, *J. Am. Chem. Soc.*, **101**, 4925 (1979).
19) D. G. Gorenstein, R. Rowell, and J. B. Findlay, *J. Am. Chem. Soc.*, **102**, 5077 (1980).
20) D. G. Gorenstein, R. Rowell, and K. Taira, "Phosphorus Chemistry," p. 69, ACS Symposium No. 171, Washington, D. C. (1981).
21) R. Rowell and D. G. Gorenstein, *J. Am. Chem. Soc.*, **103**, 5894 (1981).
22) D. G. Gorenstein and K. Taira, *J. Am. Chem. Soc.*, **104**, 6130 (1982).
23) K. Taira, T. Fanni, and D. G. Gorenstein, *J. Am. Chem. Soc.*, **106**, 1521 (1984).
24) K. Taira and D. G. Gorenstein, *Tetrahedron*, **40**, 3215 (1984).
25) K. Taira, T. Fanni, and D. G. Gorenstein, *J. Org. Chem.*, **49**, 4531 (1984); D. G. Gorenstein, A. Chang, and J.-C. Yang, *Tetrahedron*, **43**, 469 (1987); J.-C. Yang and D. G. Gorenstein, *Tetrahedron*, **43**, 479 (1987).
26) K. Taira, W. L. Mock, and D. G. Gorenstein, *J. Am. Chem. Soc.*, **106**, 7831 (1984).
27) J.-C. Yang and D. G. Gorenstein, *Tetrahedron Lett.*, **25**, 4627 (1984).
28) K. Taira, K. Lai, and D. G. Gorenstein, *Tetrahedron*, **42**, 229 (1986).
29) S. A. Bizzozero and B. O. Zweifel, *FEBS Lett.*, **59**, 105 (1975).
30) S. A. Bizzozero and H. Dutler, *Bioorg. Chem.*, **10**, 46 (1981).
31) H. Dugas and C. Penney, "Bioorganic Chemistry. A Chemical Approach to Enzyme Action," Springer-Verlag, New York (1981).
32) T. Oie, G. H. Loew, S. K. Burt, J. S. Binkley, and R. D. MacElroy, *J. Am. Chem. Soc.*, **104**, 6169 (1982)
33) T. Oie, G. H. Loew, S. K. Burt, and R. D. MacElroy, *J. Am. Chem. Soc.*, **105**, 2221 (1983).
34) I. H. Williams, D. Spangler, D. A. Femec, G. M. Maggiora, and R. L. Schowen, *J. Am. Chem. Soc.*, **102**, 6621 (1980); H. Yamataka, S. Nagase, T. Ando, and T. Hanafusa, *ibid.*, **108**, 601 (1986).
35) W. J. Hehre, W. A. Lathan, R. Ditchfield, M. D. Newton, and H. A. Pople, Quantum Chemistry Exchange Program, Indiana University, No. 236, W. J. Hehre, R. F. Stewart, and J. A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969).
36) GAUSSIAN 80: Quantum Chemistry Program Exchange University of Indiana.
37) R. S. Mulliken, *J. Chem. Phys.*, **36**, 3428 (1962).
38) W. T. Borden, "Modern Molecular Orbital Theory for

Organic Chemists," Prentice-Hall, Englewood Cliffs, N. J. (1975).
39) L. Pauling, *Chem. Eng. News*, **24**, 1375 (1946).
40) R. Wolfenden, *Acc. Chem. Res.*, **5**, 10 (1972).
41) G. E. Lienhard, *Ann. Rep. Med. Chem. Res.*, **7**, 249 (1972).
42) W. J. Albery and J. R. Knowles, *Biochemistry*, **15**, (1976).
43) A. Fersht, "Enzyme Structure and Mechanism, 2nd ed," Freeman, NY (1985).
44) A. S. Cleplak, *J. Am. Chem. Soc.*, **103**, 4540 (1981).