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MODEL OF SERINE PROTEASES CHARGE RELAY SYSTEM – PCILO STUDY ☆

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The PCILO (Perturbative Configuration Interaction Using Localized Orbitals) method has been used to determine the electronic structure of the active center of serine proteases. The results show that the carboxyl group of the aspartic acid residue is the ultimate proton acceptor of the catalytic triad (Asp, His, Ser)$^-$. In the absence of a substrate the negative charge of the active centre is delocalized, causing polarization of the Ser O^γ–H bond and an increase of the nucleophilicity of the O^γ atom. The proton of the O^γ–H bond of the Ser residue is, however, only partially transferred to the $N^{\epsilon 2}$ atom of imidazole His. The hydration of the model charge relay system is also investigated.

1. Introduction

The hydrogen bond chain connecting aspartic acid, histidine and serine residues is commonly found in the active sites of serine proteases – the enzymes which catalyze hydrolysis of proteins, low molecular amide and ester substrates [1]. Throughout the article we will use the chymotrypsin numbering scheme to identify the amino-acid sequence. According to this, the active center is composed of the following residues: Asp102-carboxyl group, His57-imidazole group and Ser195-hydroxyl group.

Considerable amount of experimental data show [2] that the acylenzyme is formed as intermediate during the catalytic process. In the acylenzyme a carbonyl carbon of the substrate scissile bond is covalently bonded to the O^γ atom of Ser195. Usually the hydroxyl group of serine is not very reactive and Blow et al. [3a,b] suggested the "charge relay system" (CRS) to explain the enhancement of O^γ Ser195 nucleophilicity. According to this mechanism, at neutral pH (maximum catalytic activity) the negative charge of the buried carboxyl group of Asp102 is transferred through H-bonds to the O^γ atom of Ser195, forming a very reactive alkoxide anion (fig. 1).

IR studies [4] of H-bonds between aspartic acid and imidazole and between alcoholate (EtO^-) and imidazole support Blow's idea and show that H-bonds of the charge relay system are easily polarizable H-bonds. The results of that study also show that the degree of proton transfer is dependent on the polarity of the environment. Theoretical studies (CNDO/2 and PRDDO methods) predict that in the substrate free state the boundary structure with negative charge located on the carboxyl of Asp102 (fig. 1–structure I) is the most stable structure [6,7]. It has been also found that the carboxyl group of Asp102 plays a key role in the enhancement of O^γ Ser195 nucleophilicity [5b]. Amidon [5a] concluded, on the basis of CNDO/2 results, that solvation stabilizes the structure with negative charge located on the O^γ atom of Ser195 (fig. 1–structure II).

According to the IR studies, however, solvation stabilizes the structure with the proton bonded to O^γ Ser195 [4]. Also, high resolution NMR spectra [8] of chymotrypsin in substrate free state (in which Ser195 and one side of the imidazole ring of His57 are solvated) show that the O^γ–H bond proton of Ser195 is only partially transferred to histidine.

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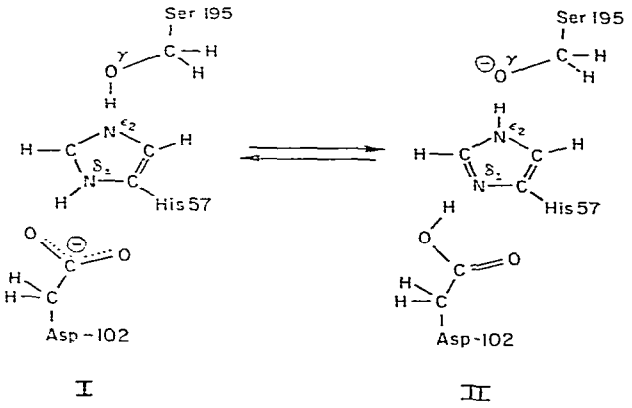

Fig. 1. The charge-relay system of serine proteases active centre-pH 7.

Recent studies of intermolecular hydrogen bonds have shown [9] that PCILO gives results which are in very good agreement with experimental data and ab initio SCF calculations. In the present work we have used PCILO [10,11], modified for geometry optimization [12], to study the H-bonds and hydration of the charge relay system model.

2. The charge relay system model

In our calculations the active site residues—charge relay system (Asp102, His57 and Ser195) were modeled by acetic acid, imidazole and methanol. The internal geometry of imidazole used in this study was determined by X-ray diffraction [13]. Standard bond lengths and bond angles were used for acetic acid and methanol internal geometries [14].

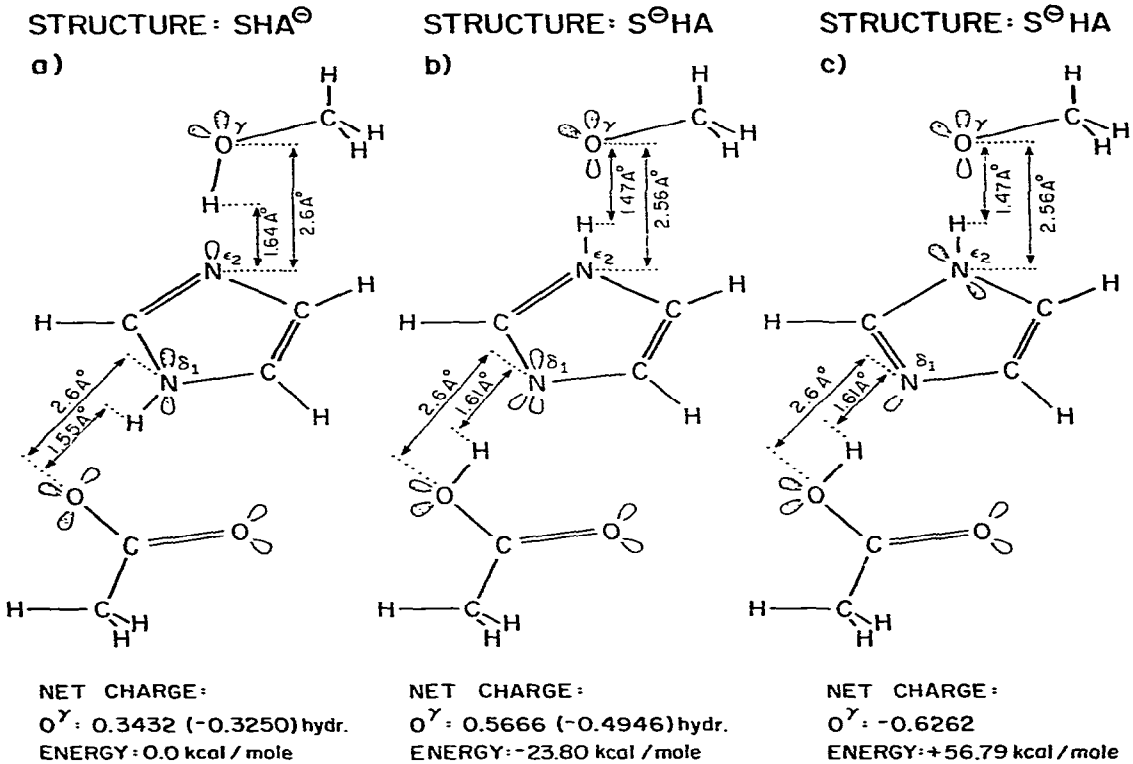

Fig. 2. Canonical structures of model charge relay system optimized by PCILO method.

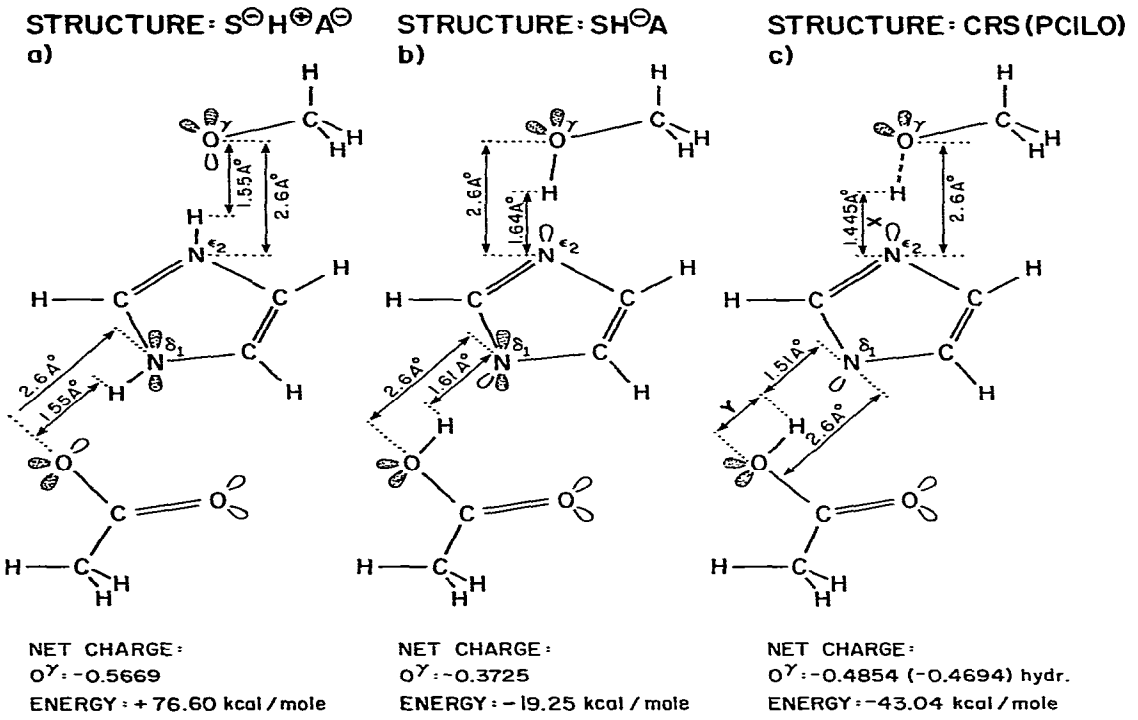

Fig. 3. Canonical structures of model charge relay system optimized by PCILO method.

The geometry of the boundary structure of the charge relay system with negative charge located on the carboxyl of Asp102 (SHA^-) was then optimized by PCILO method. The optimized geometry corresponds to the structure with the imidazole ring in plane with the principal bonds of aspartic acid and methanol. The distances between the electronegative atoms of the H-bond are 2.6 Å for both bonds (fig. 2a). The calculated distances are shorter than those observed in the chymotrypsin crystal, which ranges from 2.8–3.0 Å [3c].

The total energy of each canonical structure calculated in this work will be referred to the total energy of the structure $SHA^{\ominus}$ which will be taken as the reference zero energy.

For the canonical form $SHA^{\ominus}$ we have calculated also the structure with the bifurcated H-bond between imidazole and the carboxyl group. This structure is about 2.2 kcal/mole less stable than the structure with the linear H-bond. The bifurcated H-bond can be stabilized by the entropy factor due to the thermal fluctuations of the H atom between the carboxyl oxygens [7]. At the physiological temperature, however, the entropy contribution is less than 2.2 kcal/mole. For this reason we have limited our investigation of all other boundary structures to the types containing only linear H-bonds.

The boundary structure with negative charge located on the O^{γ} atom of Ser195 ($S^{\ominus}HA$) and with Π lone pair on the $N^{\delta 1}$ atom of imidazole is about 23.8 kcal/mole more stable than structure ($SHA^{\ominus}$) – fig. 2b. However, the same boundary structure ($S^{\ominus}HA$) but with the Π lone pair located on the $N^{\epsilon 2}$ atom of imidazole is about 56. 8 kcal/mole less stable than $SHA^{\ominus}$ (see fig. 2c).

Proton transfer from the O^{γ}–H bond of Ser195 to the $N^{\epsilon 2}$ atom of imidazole, without concomitant proton transfer from $N^{\delta 1}$ of imidazole to the carboxyl group of Asp102 (starting from structure $SHA^{\ominus}$) leads to the structure $S^{\ominus}H^{\oplus}A^{\ominus}$ (fig. 3a). This struc-

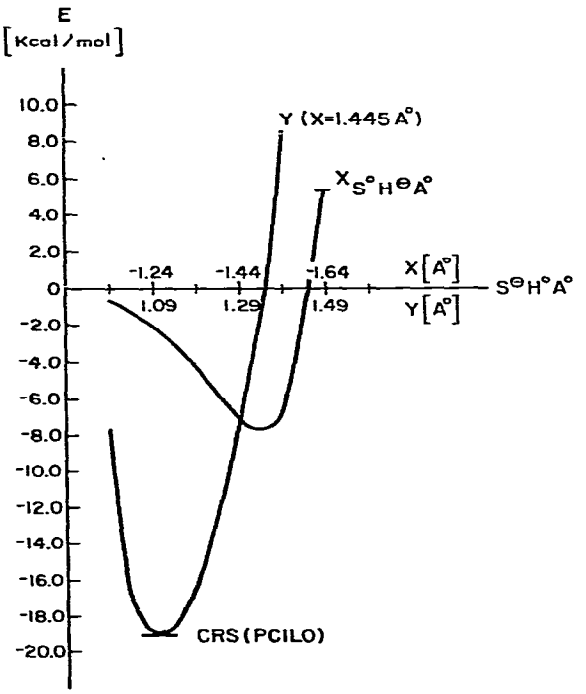

Fig. 4. PCILO potential curves for proton transfer from $O^{\gamma}H$ bond of methanol to $N^{\epsilon 2}$ of imidazole (X) and from $N^{\delta 1}$ imidazole to carboxyl group of acetic acid (Y – position of first proton is fixed at X = 1.445 Å). Starting structure $SH^{\ominus}A$.

ture is about 76.6 kcal/mole less stable than $SHA^{\ominus}$. On the other hand, proton transfer from $N^{\delta 1}$ of imidazole to the carboxyl group without concomitant proton transfer from the O^{γ}–H of bond Ser195 to the $N^{\epsilon 2}$ imidazole produces the structure $SH^{\ominus}A$ (fig. 3b), which is about 19.2 kcal/mole more stable than $SHA^{\ominus}$. The proton transfer from His57 to Asp102 can then be considered as a first step in the formation of alkoxide anion on Ser195.

When we examine the proton transfer from O^{γ}–H bond of Ser195 to imidazole (starting from the structure $SH^{\ominus}A$, fig. 4 – curve X), we find an intermediate structure with partial proton transfer which is about 31 kcal/mole more stable than the structure $SHA^{\ominus}$ (i.e., about 7 kcal/mole than $SH^{\ominus}A$). Optimization of the proton position in the H-bond between carboxyl and imidazole (fig. 4 – curve Y) for this intermediate

Table 1
PCILO total energy decomposition for some canonical structures of charge relay system *

Energy contribution	Structure		
	$S^{\ominus}HA$ a)	$S^{\ominus}HA$ b)	CRS (PCILO)
Zero order en. ΔE_0	+82.78	+34.29	+47.27
Polarization en.	0.0	0.0	0.0
Delocalization en.	−110.92	−5.82	−78.88
Intra bond corr. en.	14.54	−6.98	−12.62
Inter bond corr. en.	+5.87	−8.95	−9.55
Second order en. ΔE_2	−100.505	−21.75	−101.06
$\Delta E_0 + \Delta E_2$	−17.52	112.74	−53.79
Deloc. deloc. int.	−1.38	+29.55	+0.04
Intra-inter corr.	−4.34	+8.67	+6.89
Inter-inter corr.	−1.48	+6.13	+3.78
Third order en. ΔE_3	−6.29	+44.05	+10.75
$\Delta E_0 + \Delta E_2 + \Delta E_3$	−23.81	+56.79	−43.04

* The reference zero energy is energy of the structure $SHA^{\ominus}$.
a) Lone pair localized on $N^{\epsilon 2}$ atom of imidazole.
b) Lone pair localized on $N^{\delta 1}$ atom of imidazole.

structure leads to the most stable PCILO structure. In this structure, which will be referred to as CRS-(PCILO) [optimized structure of charge relay system by PCILO method], the proton from $N^{\delta 1}$ of imidazole is nearly completely transferred to the carboxyl group, while the proton in the H-bond between the imidazole $N^{\epsilon 2}$ atom and O^{γ} of Ser195 is in the intermediate position. The CRS(PCILO) structure (fig. 3c) is about 43 kcal/mole more stable than $SHA^{\ominus}$.

The energy partitioning of the PCILO total energy is given in table 1 for the two $S^{\ominus}HA$ structures and CRS(PCILO) structure. The results are discussed in section 4.

3. Hydration of the charge relay system

The charge relay system as a part of the complex structure of enzymes is only partly accessible to solvation [3,15]. Asp102 is buried in the interior of the enzyme and protected from solvation by other amino-acid residues. The OH bond of Ser195, which is on the enzyme surface and one side of the imidazole ring of His57 is, however, accessible to water molecules. From this point of view, it would be difficult if not

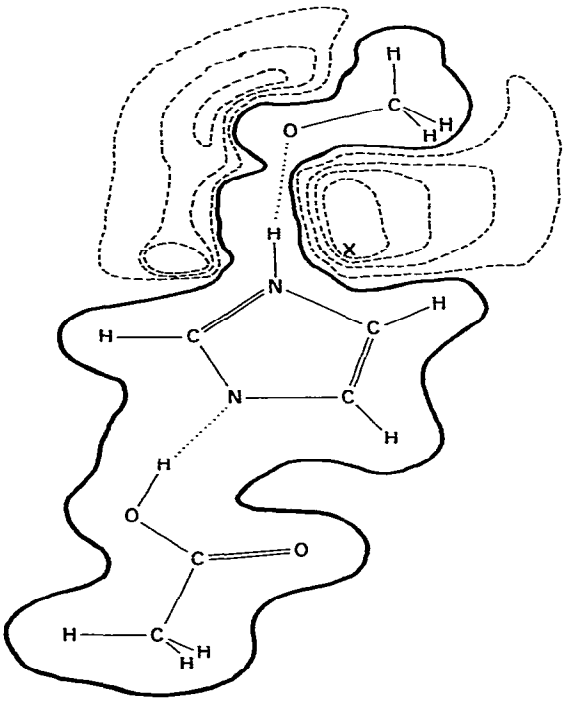

Fig. 5. Electrostatic potential map of the structure $S^{\ominus}HA$.

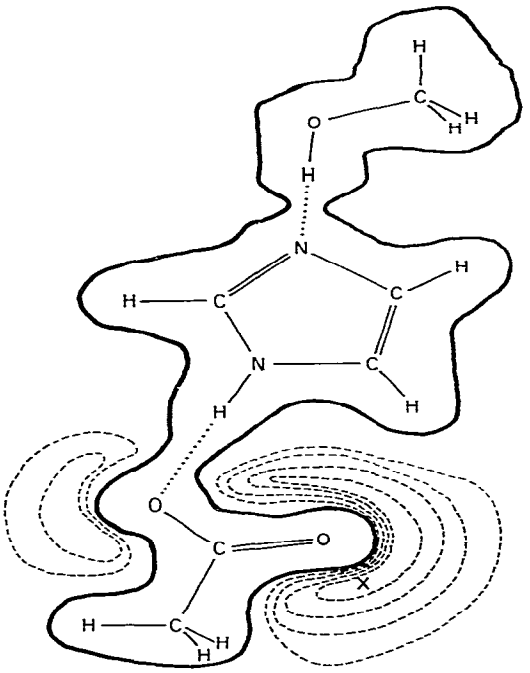

Fig. 6. Electrostatic potential map of the structure $SHA^{\ominus}$.

impossible to apply the continuum model [16] to the calculation of the solvation energy because of the complicated shape of the cavity. What is obviously needed is a formulation which can take account of solvent effect on a local basis and which will also include contributions from molecular (i.e. translational, rotational, torsional, etc.) motion. Unfortunately, such a formalism has, as yet, not been advanced. Lacking a more complete formulation, we have decided to treat the interaction between the water molecules and the canonical structures at selected sites by the "supermolecule" method [17]. This approach assumes rigid molecular structures and, while important entropic contributions may be missing, the results should nevertheless give an accurate assessment of the energetics involved. We shall refer to the interaction energy between "first layer" of the (generally tightly bound) water molecules and solute as hydration energy reserving the broader term solvation energy to interaction which includes molecular motion.

For the model studied we can assume that the most probable places for hydration are the OH bond Ser195 and imidazole His57 in the direction of the lone pairs of O^{γ} and $N^{\delta 1}$ respectively. In an effort to determine other possible hydration regions we have calculated the electrostatic interaction between the net atomic charge of the hydrogen atom (as it is in the H_2O molecule, q_H(PCILO) = 0.113) and the PCILO charge distribution of the charge relay system:

$$E_p = \sum_A \left(\frac{0.113\, Z_A}{R_{AH_p}} - P_{AA} V_{AH_p} \right).$$

E_p is the interaction energy at point "p" between the net atomic charge of H(0.113) and the charge distribution of a particular system. Z_A is the "core" charge of atom A, R_{AH_p} the distance, P_{AA} the gross atomic population of atom A and V_{AH_p} the electrostatic interaction between valence electron (s orbital approximation) on atom A and nuclear charge of the hydrogen atom:

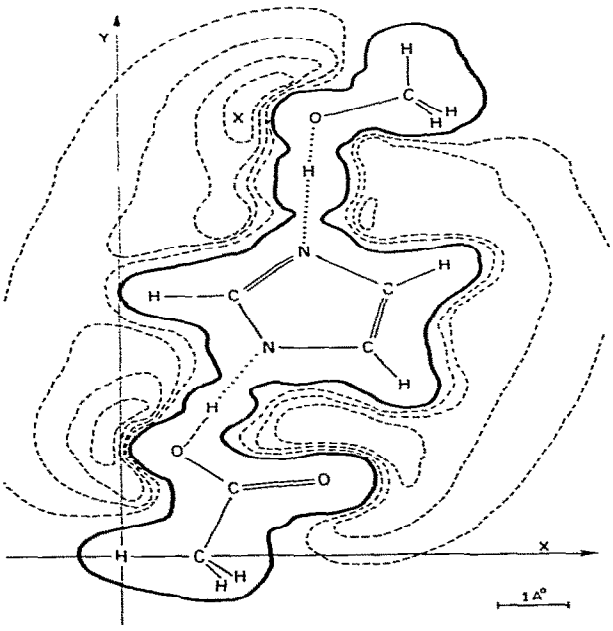

Fig. 7. Electrostatic potential map of the structure CRS (PCILO).

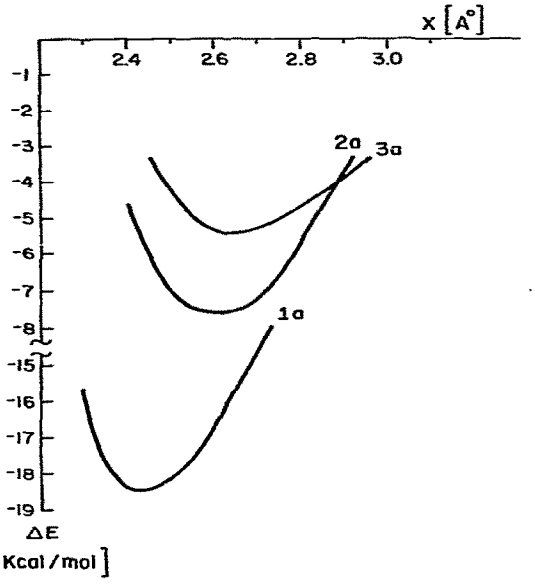

Fig. 8. Potential curves of interaction between water molecule and O^{γ} methanol lone pair of canonical structures of charge relay system. 1a, S^{-} HA; 2a, CRS(PCILO); 3a, $SHA^{\oplus}$.

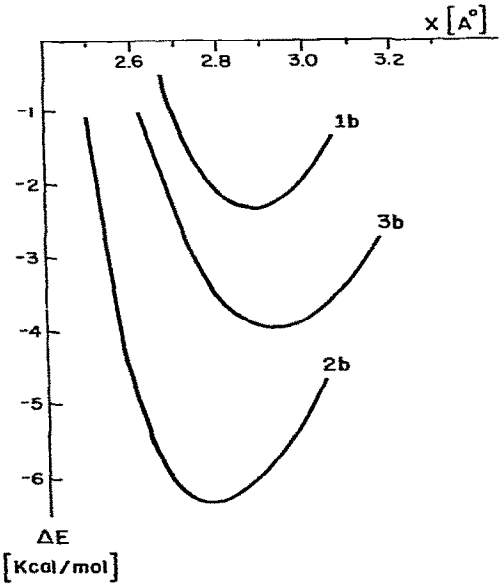

Fig. 9. Potential curves of interaction between water molecule and $N^{\delta 1}$ imidazole lone pair of canonical structures of charge relay system. 1b, $S^{\ominus}HA$; 2b, CRS(PCILO); 3b, $SHA^{\oplus}$.

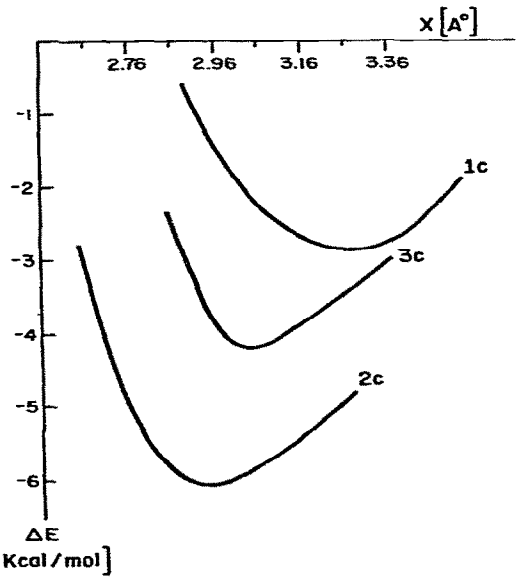

Fig. 10. Potential curves of interaction between water molecule and $N^{\epsilon 2}$ imidazole (XY plane) of canonical structures of charge relay system. 1c, $S^{\ominus}HA$; 2c, CRS(PCILO); 3c, $SHA^{\oplus}$.

Table 2
PCILO hydration energy of some canonical structures of charge relay system *

Structure	Hydration energy (kcal/mole)				Total energy after hydration (kcal/mole)
	O^{γ} lone pairs	$N^{\delta 1}$ lone pair	$N^{\epsilon 2}$	Total	
$S^{\ominus}HA$	2 × (−18.44)	−2.31	2 × (−2.82)	−44.83	−68.63
CRS (PCILO)	2 × (−7.61)	−6.29	2 × (−6.02)	−33.55	−76.59
$SHA^{\ominus}$	2 × (−5.39)	−3.43	2 × (−4.18)	−22.57	−22.57

* The reference zero energy is energy of nonsolvated structure $SHA^{\ominus}$.

$$V_{AH_p} = 0.113 \int s_A^2 (R_{AHp})^{-1} \, dv .$$

For these calculations we have used a slightly modified program of Giessner-Prettre [18].

Figs. 5–7 display the electrostatic energy maps of the canonical structures $S^{\ominus}HA$, CRS(PCILO) and $SHA^{\ominus}$. The heavy line around the molecular system represents zero electrostatic energy X denotes the position of maximum electrostatic interaction (minimum energy) and the dotted lines are isoenergy curves for energies $E_i = E_X - C_i$ (C_i = 2, 5, 8 and 11 kcal/mole, respectively). As is obvious from figs. 5 and 6, two nearly equivalent hydration sites exist in the XY plane on both sides of the $N^{\epsilon 2}$ atom of imidazole. The hydration sites of the lone pairs are out of the XY plane.

The interaction energy of a water molecule with a particular atom of the model charge relay system (lone pairs of O^{γ} Ser and $N^{\delta 1}$ imidazole and $N^{\epsilon 2}$ in XY plane) was then calculated by the "supermolecule" approach. In all cases water is a proton donor. Figs. 8–10 show plots of the calculated interaction energies versus the distance between the water oxygen atom and a particular electronegative atom of the canonical structure. Table 2 lists the total hydration energies calculated on the assumption that the oxygen of Ser195 and $N^{\epsilon 2}$ of imidazole are hydrated by two water molecules. Tables 3–5 show the partitioning of PCILO hydration energy.

As we can see from table 2, the hydration energy of the structure $S^{\ominus}HA$ is about 11 kcal/mole larger than for the structure CRS(PCILO). In spite of this difference, the hydrated structure CRS(PCILO) is still about 8 kcal/mole more stable than structure $S^{\ominus}HA$.

Table 3
Decomposition of PCILO hydration energy – structure CRS (PCILO)

Interaction energy contribution	O^{γ} lone pair hydration	$N^{\delta 1}$ lone pair hydration	$N^{\epsilon 2}$ (XY plane) hydration
Zero order en. ΔE_0	+7.26	+6.22	+1.79
polarization en.	0.0	0.0	0.0
Delocalization en.	−20.59	−15.39	−25.48
Intra-bond corr. en.	+0.36	−0.61	+0.15
Inter-bond corr. en.	−0.66	−1.08	−0.88
Second order en. ΔE_2	−20.89	−16.54	−26.35
$\Delta E_0 + \Delta E_2$	−13.63	−10.32	−24.56
Deloc.-deloc. int.	+5.59	+3.51	+17.41
Intra-inter corr.	+0.18	+0.44	+0.44
Inter-inter corr.	+0.20	+0.41	+0.46
Third order en. ΔE_3	+6.02	+4.29	+18.27
$\Delta E_0 + \Delta E_2 + \Delta E_3$	−7.61	−6.02	−6.29

Table 4
Decomposition of PCILO hydration energy – structure $S^{\ominus}HA$

Interaction energy contribution	O^{γ} lone pair hydration	$N^{\delta 1}$ lone pair hydration	$N^{\epsilon 2}$ (XY plane) hydration
Zero order en. ΔE_0	+14.34	+5.65	+2.27
Polarization en.	0.0	0.0	0.0
Delocalization en.	−52.94	−11.47	−16.46
Intra-bond corr. en.	+0.47	−0.11	−0.04
Inter-bond corr. en.	−0.54	−0.54	−0.81
Second order en. ΔE_2	−53.01	−12.12	−17.81
$\Delta E_0 + \Delta E_2$	−38.66	−6.48	−15.54
Deloc.-deloc. int.	+20.05	+3.36	+12.50
Intra-inter corr.	+0.09	+0.19	+0.38
Inter-inter corr.	+0.02	+0.12	+0.37
Third order en. ΔE_3	+20.22	+3.66	+13.23
$\Delta E_0 + \Delta E_2 + \Delta E_3$	−18.44	−2.82	−2.31

Table 5
Decomposition of PCILO hydration energy – structure $SHA^{\ominus}$

Interaction energy contribution	O^{γ} lone pair hydration	$N^{\delta 1}$ lone pair hydration	$N^{\epsilon 2}$ (XY plane) hydration
Zero order en. ΔE_0	+8.51	+5.23	+1.92
Polarization en.	0.0	0.0	0.0
Delocalization en.	−19.80	−11.77	−15.79
Intra-bond corr. en.	+0.25	+0.01	+0.10
Inter-bond corr. en.	−0.60	−0.79	−0.62
Second order en. ΔE_2	−20.16	−12.55	−16.32
$\Delta E_0 + \Delta E_2$	−11.65	−7.32	−14.40
Deloc.-deloc. int.	+5.90	+2.63	+9.84
Intra-inter corr.	+0.17	+0.28	+0.27
Inter-inter corr.	+0.16	+0.26	+0.37
Third order en. ΔE_3	+6.26	+3.14	+10.46
$\Delta E_0 + \Delta E_2 + \Delta E_3$	−5.39	−4.18	−3.93

4. Discussion

The results of the present study show that in the charge relay system are present extremely strong hydrogen bonds with the character of charge-transfer complexes. From the decomposition of the PCILO total energy (table 1) follows that the main stabilization contribution comes from the second order delocalization term. The importance of this term in the PCILO scheme is clearly established by comparison of the results for the structure $S^{\ominus}HA$ with two different canonical forms of imidazole (fig. 2 – $S^{\ominus}HA$ b,c). For the structure with the Π lone pair located on the $N^{\delta 1}$ atom the second order delocalization contribution is about 105 kcal/mole larger than for the structure with the Π lone pair located on the $N^{\epsilon 2}$ atom. This difference arises mainly from the difference in energy associated with electron excitation from the Π lone pair on the nitrogen atoms to the antibonding Π orbital of the carboxyl group. Note that the distance between the Π lone pair of nitrogen and the carboxyl group is about twice as large for structure 2c as it is for 2b (fig. 2). We can see from table 1, structure $S^{\ominus}HA$, that the dispersion energy (inter-bond correlation) is positive. This does not mean that the dispersion interaction is repulsive – rather it is the value relative to the dispersion energy of structure $SHA^{\ominus}$. The dispersion energy of $S^{\ominus}HA$ is actually attractive and contributes about 25% to the total attraction energy (25% intrabond correlation and 50% delocalization) in the second order. The polarization energy is zero because the free parameters of PCILO are determined by minimization of the polarization energy.

The main contribution to the repulsive energy of the investigated structures of the charge relay system relative to the $SHA^{\ominus}$ energy comes from the zero order energy, ΔE_0. It is not possible to judge from the numerical value of ΔE_0 alone which terms (in perturbation sense) are responsible for the destabilization. It is clear, however, that exchange repulsion term is not included, because of ZDO approximation. The third order contributions are in general repulsive, as is evident from the definition [12a,b].

In regard to the hydration energy, the situation is roughly the same. In all cases (tables 2, 3) the main attractive contribution comes from the second order delocalization energy. The main repulsive contributions come from ΔE_0 and from third order delocalization–delocalization interactions.

The PCILO method uses CNDO/2 parametrization of integrals and thus underestimates the short range repulsion. This is probably the reason for the shorter equilibrium distances calculated by PCILO in comparison with experiment. At the same time PCILO overestimates the attractive delocalization energy. These errors are, however, well balanced by parametrization, at least for "equilibrium" distances. A systematic study of the application of PCILO to hydrogen-bonded complexes [9] shows, that PCILO gives stabilization energies in good agreement with ab initio SCF calculations.

It must be kept in mind that the present results correspond to the optimized geometry of CRS. For longer H-bond distances and distorted H-bonds we may expect formation of double well potentials (increase of energy barriers) for proton transfer processes. This effect is well documented by nonempirical ab initio [19–20] as well as semiempirical methods [5a,7,21] for H-bond formation between a pair of electroneutral molecules and between positively charged proton donor and neutral proton acceptor. The above mentioned effect was not observed, however, for H-bond formation between the acetic acid anion and neutral imidazole molecule studied by the PCILO method [22]. It means that for the H-bond distances of 2.8 and 3.0 Å (in CRS), the relative order of stability of the boundary structures can be expected to be the same.

Koeppe et al. [23] have published the results of their IR studies of the trypsin active center. For neu-

tral pH they recorded frequencies for the Asp102 carboxylic group at 1680 and 1600 cm^{-1}. In general, when the proton is present at the carboxylic group, the C=O stretching vibration is observed at about 1715 cm^{-1}, whereas the antisymmetric and symmetric stretching vibrations of the $-CO_2^-$ group are respectively at 1575 and 1400 cm^{-1} [4]. The recorded frequencies for the trypsin active center [23] then suggest that the carboxylic group of Asp102 is strongly H-bonded to the imidazole ring of His57. It is obvious, from the IR results as well as from the ^1H, ^{13}C and ^{15}N NMR studies [8,24–26] of serine proteases, that the precise position of the protons in CRS cannot be determined experimentally.

According to our results, the structure of the charge relay system of serine proteases in substrate free state is as follows: the proton from $N^{\delta 1}$ imidazole His57 is nearly completely transferred to the carboxyl group of Asp102, while the proton from the O^{γ}–H Ser195 bond is only partially transferred to the $N^{\epsilon 2}$ atom of imidazole His57. Nevertheless, the nucleophilicity of O^{γ} Ser195 is high enough for nucleophilic attack on the carbonyl carbon of the substrate bond. The proton transfer from the O^{γ}–H Ser195 bond to $N^{\epsilon 2}$ atom of imidazole His57 is completed during the formation of a tetrahedral intermediate between Ser195 and substrate. This structure does neither contradict nor confirm the interpretation of the experimental IR and NMR measurements [23,8,24–26].

The high catalytic efficiency of serine proteases is, in our opinion, not caused by some "electronic trick" in the active center, but rather results from the presence of long-lived excited vibrational states in the enzyme molecule created after ES complex formation. The hypothesis for such a mechanism has been recently suggested [27].

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