Electron Redistribution of Clavalanate on Binding to a β-Lactamase

Sang-Hyun Park and Hojing Kim

Department of Chemistry, Seoul National University, Seoul 151-742
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A class A β-lactamase from *Staphylococcus aureus* PC1 complexed with 3R,5R-clavulanate is studied. The starting geometry for the computations is the crystal structure of the β-lactamase. Docking of the clavulanate to the enzyme is done exploiting the requirements of electrostatic and shape complementarity between the enzyme and clavulanate. This structure is then hydrated by water molecules and refined by energy minimization and short molecular dynamics simulation. In the energy refined structure of this complex, the carboxyl group of the clavulanate is hydrogen bonded to Lys-234, and the carbonyl carbon atom of the clavulanate is adjacent to the Oγ of Ser-70. It is found that a crystallographic water molecule initially located at the oxyanion hole, which is formed by the two -NH group of Ser-70 and Gln-237, is replaced by the carbonyl oxygen atom of the 3R,5R-clavulanate after docking and energy refinement. The crystallographic water molecules are proved to be important in ligand binding. Glu-166 residue is found to be repulsive to the binding of clavulanate, which is in agreement with experimental observation. Arg-244 residue is found to be important to the binding of clavulanate as well as to interaction with C2 side chain of the clavulanate. The electron density redistribution of the clavulanate on binding to the β-lactamase is studied by an *ab initio* quantum-mechanical calculation. A significant redistribution of electron density of the clavulanate is induced by the enzyme, toward the enzyme, toward the transition state of the enzymatic reaction.

Introduction

β-Lactam antibiotics are used as effective, substrate-analogue-based drugs targeted against the bacterial cell-wall synthetic enzymes, peptidoglycan transpeptidases and D-alanyl-D-alanine carboxypeptidases[1]. β-Lactamases constitute a group of bacterial enzymes that destroy these clinically useful antibiotics by cleaving and hydrolyzing the sensitive four-membered β-lactam ring. Their growing incidence in the clinical environment and the need to design β-lactamase-resistant penicillins have made necessary the study of their structure and function at the molecular level. Most β-lactamases characterized to date contain a reactive serine at the catalytic site. The serine enzymes are grouped into classes A, C, and D, depending on their amino acid sequences and specificity for penicillins or cephalosporins[2]. The class A β-lactamases are serine hydrolases produced by both Gram-positive and Gram-negative bacteria. Crystal structures of the Gram-positive β-lactamases from *Staphylococcus aureus*[3,4] and from *Bacillus licheniformis*[5,6] are known at 2.0 Å resolution. Recently, a report on the 2.5 Å resolution crystal structure of the Gram-negative β-lactamase, RTEM-1 from *Escherichia coli* has been published[7]. A wealth of kinetic data is available for many of the class A β-lactamases and their site-directed mutants[8-20]. Despite all of this information there seems to be no consensus regarding the nature of the general base involved in the acylation step of the reaction[21].

The system studied here is a class A β-lactamaes from *Staphylococcus aureus* PC1 complexed with clavulanate. Clavulanate, first isolated from *Streptomyces clavuligerus,* is a naturally occurring β-lactam that inactivates β-lactamases from a variety of Gram-negative and Gram-positive bacteria *in vitro* and *in vivo*[22]. Clavulanate possesses weak, though broad-spectrum, activity as an antibiotic, but it is potent progressive inhibitor of a wide range of β-lactamases. A combination of amoxycillin and potassium clavulanate showed dramatic synergistic effects against some β-lactamase-producing bacteria and is used therapeutically[23]. The structure of clavulanate contains all the essential features of a good β-lactam substrate but with the differences: it has an oxygen atom instead of a sulfur atom in position 1, and an exocyclic double bond at aposition 2 (Figure 1).

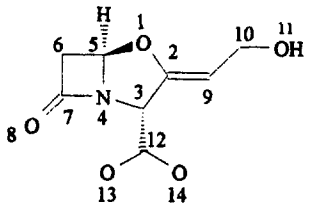

Figure 1. Structure and numbering systme of 3R,5R-clavulanate.

Studies on the mechanism of inactivation for class A β-lactamases have been carried out in many laboratories. Kinetic studies using β-lactamase from *Staphylococcus aureus* showed a 1 : 1 ratio of inhibitor-enzyme complex formation with a first-order rate constant of 2.7×10^5 $M^{-1}min^{-1}$ with respect to the concentration of sodium clavulanate[24]. After the initial formation of the inhibitor-enzyme complex, clavulanate disappears slowly and linearly with time, leading to complete regeneration of enzymatic activity.

Methods

The starting geometry for the computations is a crystal structure of *Staphylococcus aureus* PC1 β-lactamase[4] obtained from the Brookhaven Protein Data Bank (accession number 3BLM)[25]. The Ambler standard numbering scheme will be used throughout this paper[26].

The position of the catalytically important groups and

other side chains, and relatively rigid nature of the β-lactam molecules suggest a mode of binding for the ligand. Docking of clavulanate to the enzyme was done exploiting the requirements of electrostatic and shape complementarity between the enzyme and the clavulanate. For the docking of 3R,5R-clavulanate in the enzyme active site, the guess geometry was defined by a relative enzymeligand disposition such that the carbonyl carbon atom of clavulanate lies in the vicinity of the Oγ atom of Ser-70, and the carboxylate group of clavulanate is oriented twoards the Lys-234. This structure is then hydrated by 408 water molecules and refined by energy minimization and short molecular dynamics simulation. The entire structure is consisted of 6014 atoms including hydrogen atoms. Standard point charges on the amino acid residues and a dielectric constant of 1 are used throughout for the calculation of the Coulombic term. Initial minimization of the complex is carried out until the energy gradient is less than 0.1 kcal/mol·Å. In this process, each heavy atom of β-lactamase and water molecules are first fixed to its initial position, thus allowing added hydrogen atoms to adjust to a static crystallographically defined environment. After this process, minimization is repeated allowing only the clavulanate to move in order to make adequate docking. Subsequently, a constraint of 100 Å^2 kcal/mol was applied to the α-carbon atoms of β-lactamase during minimization. A short molecular dynamics simulation is then carried out to equilibrate the added solvent molecules. Finally, the entire system is minimized with no constraint until the energy gradient is less than 0.1 kcal/mol·Å. Same procedures are repeated for the other enantiomers of clavulanate.

We have used the local density functional (LDF) approach, an *ab initio* electronic structure technique[27-30]. Unlike approaches bases on Hartree-Fock theory, the LDF method treats the energy of a molecular system as a function of the total electron density rather than of the wavefunction. This leads to one of the significant advantages of LDF calculations over conventional Hartree-Fock, namely, that, wherease the computational time needed for the latter methods scales with the number of atoms or basis functions to the fourth power, LDF calculations scale only with the third power. LDF methods can, therefore, treat larger systems, which is of significance for calculations on complex biological systems. In addition, LDF theory includes some electronic correlation effects and may be more accurate than Hartree-Fock in cases where these effects are important. The LDF method has been shown to give good agreement with experimental data in studies of the electron density of atomic surfaces[31] and small molecules[32-35], and has also been applied to studies of structure, conformation, energetics and vibrational frequencies[30,36]. The calculations described here on the protein-ligand systems are carried out in three steps[37]. First, the electron density of the isolated clavulanate in its binding conformation is calculated. Then, the electron density calculation of the clavulanate is repeated in the electrostatic filed of the hydrated complex. Finally, the difference electron density (boundisolated) is calculated and examined using computer graphics[38]. In these calculations, all atoms of the clavulanate are treated quantum mechanically, employing a basis set with polarization functions, equivalent in size to a Gaussian 6-31G** basis set. To mimic the electrostatic environment formed by the hydrated β-lactamase in the calculation of the bound clavulanate, the protein and the water molecules of the hydration shell are represented as point charges at the position of each of the atoms, including all hydrogen atoms. The Coulombic potential arising from all point charges is then included in the Hamiltonian of the LDF calculation. The self-consistent LDF solution including this potential gives the electron density of the clavulanate when bound to the β-lactamase. Atomic charges for the clavulanate are calculated using a Mulliken population analysis[39].

Table 1. RMS Deviations Between the Minimized and the X-Ray Crystal Structure (Å)

	3R,5R	3S,5R	3S,5S
Cα-carbon atoms	0.5425	0.6628	0.6029
Backbone atoms	0.5856	0.7030	0.6929
All atoms	0.9567	1.7477	1.5587

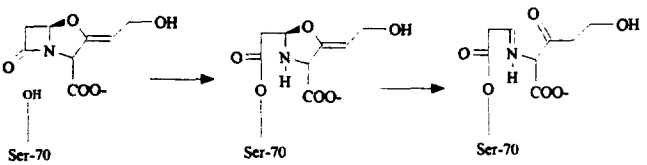

Figure 2. Proposed mechanism for the inactivation of β-lactamase by clavulanate found in the literature.

Results and Discussion

Energy minimized structures are superimposed onto the corresponding initial crystal structure using a least-square fit of the Cα carbon atoms and backbone atoms. Table 1 summarizes the results of several RMS deviation analysis for each of the complexes.

Various mechanisms have been proposed for the inhibition of β-lactamase by the clavulanate[8,40,41] (Figure 2). All share the first two steps. First, the active site serine residue reacts with the carbonyl carbon of the clavulanate to form a tetrahedral intermediate. Second, the C-N bond in the β-lactam ring is cleaved and an acyl enzyme is formed. These two steps are thought to be common for a normal substrate and the inhibitors. The divergence starts at the acyl enzyme. Active site labeling experiments on several class A β-lactamases invariably point to a serine at position 70 as the residue involved in the formation of an acyl enzyme intermediate with the carbonyl carbon of the β-lactam ring[42-44]. Ser-70 lies in one of two crevices across the enzyme surface formed at the interface of the helical domain with the β-sheet domain. Many of the amino acid residues in this area are conserved in four class β-lactamase sequences[2]. The side chain of Ser-70 lies in the floor of this crevice, and the ammonium group of Lys-73, another conserved residue, is adjacent to it. Ser-70 is situated at the amino terminus of the buried central helix a2 of the helical domain, suggesting a role for the dipole of this helix in the catalytic mechanism[45].

The mechanism of proton abstraction and donation during the hydrolysis of β-lactam compounds by the β-lactamases is still the subject of considerable discussion. Hydrogen-bonding schemes around the clavulanate in our model Mi-

Figure 3. Ribbon representation of the β-lactamase and the 3R,5 R-clavulanate.

Table 2. H···A Distances and D···H···A Bond Angles, Where A and D are the acceptor and Donor Atoms Involved in Hydrogen-Bonding, in the Complex of β-Lactamase and 3R,5R-Clavulanate

Donor atom	Acceptor atom	Distance (Å)	Angle (degree)
O11 of clav.	HOH-103H	1.69	169.66
N of Ser-70	O8 of clav.	1.98	158.03
N of Gln-237	O8 of clav.	1.92	172.06
Oγ of Ser-130	O14 of clav.	1.59	162.97
Nζ of Lys-234	O14 of clav.	1.86	160.13

chaelis complex are summarized in Table 2. In the structure of this complex, the carboxylate group of the clavulanate is hydrogen bonded to Lys-234 and Ser-130, and the carbonyl carbon atom of the clavulanate is adjacent to the Oγ of Ser-70. It is found that a crystallographic water molecule initially located at the oxyanion hole, which is formed by the two -NH group of Ser-70 and Gln-237, is replaced by the carbonyl oxygen atom of the 3R,5R-clavulanate after docking and energy refinement (Table 2 and Figure 4). It should be noted that the conformation of 3R,5R-clavulanate and the relative positions of Ser-70 and Lys-234 are sufficient conditions to locate the carbonyl oxygen of β-lactam ring at the oxyanion hole. But the carbonyl oxygen atoms of 3S,5R- and 3S,5S-clavulanate are found at a distance from the oxyanion hole (about 3-4 Å). Polarization of the β-lactam carbonyl bond prior to nucleophilic attack by Ser-70 is accomplished by this oxyanion hole, hydrogen-bonding to the backbone amides of Ser-70 and Gln-237, leading to a α-face attack on the β-lactams.

The nonbond interaction energies of 3R,5R-clavulanate with various residues in the active site of β-lactamase is given in Table 3. The result of our calculation reveals that the crystallographic water molecules are important in ligand binding. It has been proposed by Lamotte-Brasseur *et al.*[46] that the carboxylate group of β-lactams is attracted by Ser-

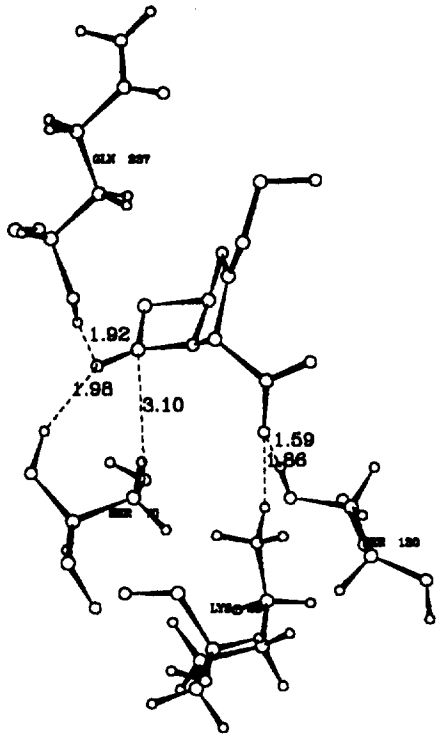

Figure 4. Atomic structure near the active site of our model Michaelis complex. A Molecule with no label is the 3R,5R-clavulanate. Hydrogens of the clavulanate are omitted for clarity. Ser-70, Lys-73, Ser-130, Lys-234, and Gln-237 residues are shown. Interatomic distances are given in Å.

Table 3. The Nonbond Interaction Energies of 3R,5R-Clavulanate with Various Residues in Active Site of β-Lactamase (in kcal/mol)

Residues	Sum	VDW	Coulombic
Lys-234	−93.48	0.25	−93.72
Arg-244	−54.48	−2.11	−52.37
Lys-73	−52.62	−0.56	−52.06
water 103	−19.25	2.59	−21.84
Ser-130	−17.53	0.31	−17.84
water-71	−15.95	−0.12	−15.83
water-111	−12.46	1.37	−13.83
water-64	−11.55	0.88	−12.44
Ser-70	−6.10	−2.00	−4.10
Gln-237	−4.92	−4.70	−0.215
Gln-166	+34.71	−0.24	+34.95
Total	−280.22	−19.02	−261.20

130, Lys-234, and Ser-235. In our model Michaelis complex, however, Ser-235 weakly repels the clavulanate (Figure 4).

Lys-234 is important for attracting and orienting the carboxylate group of β-lactams. More recently, site-directed mutagenesis was used by Fink *et al.* to substitute the conserved Lys-234 with glutamic acid[13]. That the mutant shows a 10-fold increase in K_m and a 200-fold decrease in V_{max} supports our result that a positive group at position 234 of β-strand b3 in important for attracting and orienting the C3-carboxy-

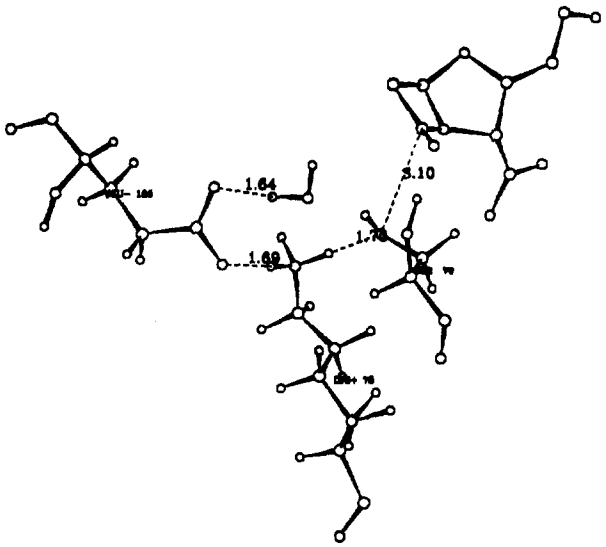

Figure 5. Atomic structure near the active site of our model Michaelis complex. A Molecule with no label is the 3R,5R-clavulanate. Hydrogens of the clavulanate are omitted for clarity. Ser-70, Lys-73, Glu-166, and a water molecule are shown. Interatomic distances are given in Å.

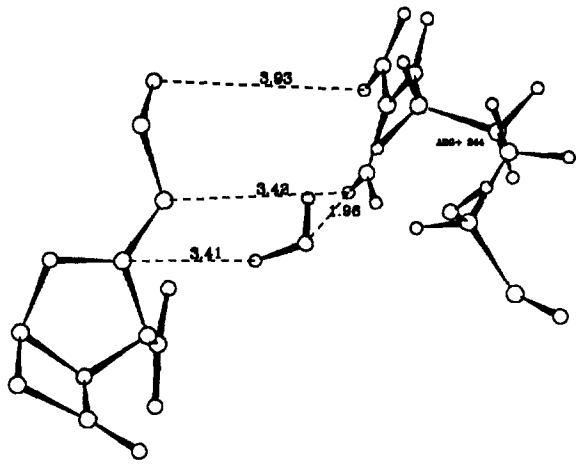

Figure 6. Atomic structure near the active site of our model Michaelis complex. A Molecule with no label is the 3R,5R-clavulanate. Hydrogens of the clavulanate are omitted for clarity. Arg-244, and a water molecules are shown. Interatomic distances are given in Å.

late group of β-lactams.

Prior to and within the a2 helix is the conserved sequence Phe-66-X-X-X-Ser-70-X-X-Lys-73, which is present in all class A and C β-lactamases and some PBP sequences[1]. The Phe-66 is quite far from the reactive Ser-70, and lies against the back face of the β-sheet. The Lys-73 in this sequence is actually adjacent to Ser-70 by virtue of being one turn up the a2 helix. The two residues are within hydrogen bonding distance.

The hydroxyl group of the reactive Ser-70 could be activated in at least two ways. First, the dipole moment of helix a2 provides a formal half positive charge[45] which may assist in lowering the *pK* of the hydroxyl proton. Second, the charged amino group of Lys-73 may repel and orient the serine's proton for direct transfer to the β-lactam during formation of the acyl intermediate[5]. In our model Michaelis complex, the amino group of Lys-73 is only 1.75 Å apart from the hydroxyl group of Ser-70. But the distance between the serine's proton and the nitrogen atom of 3R,5R-clavulanate is 3.57 Å.

The strong repulsive interaction between 3R,5R-clavulanate and Glu-166 residue is noteworthy. Site-directed mutagenesis to substitute the Glu-166 with tyrosine[47] showed that most β-lactams bind to the mutant more strongly than to the corresponding native enzyme. This experimental result supports the reliability of our model Michaelis complex. In fact, the mutant was reported to behave much like a class C β-lactamase, with high affinity and low hydrolytic activity. The inhibition and inactivation constants of clavulanate to the mutants are, however, reported to be unmodified. The author has proposed that both acylation and deacylation are affected at the same ratio, thus yielding an unmodified inactivation constant[47]. But we propose here that the substitution of Glu-166 to tyrosine residue would remove the strong repulsive interaction between 3R,5R-clavulanate and Glu-166, while lowering hydrolytic activity of the β-lactamase. In other words, Glu-166 would play a role in the β-lactamase induced hydrolysis of clavulanate, although it lowers the affinity of the β-lactamase toward clavulanate simultaneously.

Glu-166 is postulated by Madgwick *et al.* to facilitate the transfer of protons in the acylation and deacylation steps[48]. In our energy refined structure of the complex Oε of Glu-166 is hydrogen-bonded to side chain of Lys-73, and the latter is hydrogen-bonded againto Oγ of Ser-70. Glu-166 may assist in the deprotonation of Ser-70 hydroxy group in the acylation step *via* Lys-73 (Figure 5). A crystallographic water (water 81 in the coordinate deposited in the Brookhaven Protein Data Bank) is also located between the side chains of Ser-70 and Glu-166. This water has been proposed by Herzberg[4] to deacylate the acyl-enzyme complex.

The strong attractive interaction between the clavulanate and Arg-244 is very interesting. Arg-244 is hydrogen-bonded to a crystallographic water molecule (water 111 in the coodinate deposited in the Brookhaven Protein Data Bank), which is adjacent to the carboxylate oxygen of 3R,5R-clavulanate. The reactive double bond of 3R,5R-clavulanate at C2 position is 3.72 Å from the positive side chain of Arg-244, which is much closer than Lys-73 or Lys-234. The only known β-lactamase without arginine at position 244 is *Streptomyces albus G* with Asn-244, and this enzyme is less effectively inhibited by clavulanate. The number of turnovers before inactivation is 20,000 for *Streptomyces albus G* enzyme compared with 200-400 for other β-lactamases[8,49]. This result suggests that a detailed investigation of the interactions between the C2 side chain of clavulanate and Arg-244 is required to design a good β-lactamase inhibitor, although there are little experimental data on the role of Arg-244. In fact, modifications of the C2 side chain of clavulanate have been reported to give dramatic changes in inhibitory activity[50].

The spatical arrangement of protein charges that induces the redistribution of electron density is identified and found to be consistent with the proposed reaction mechanism. Net charge changes of atoms of 3R,5R-clavulanate induced by

Table 4. Influence of the Enzyme Environment on the Charge Redistribution Within 3R,5R-Clavulanate[a]

	C2	C5	C7	O8	C9	C10	O11	O13	O14
Isolated	0.314	0.039	0.241	−0.401	−0.304	−0.485	−0.681	−0.457	−0.453
Bound	0.302	0.027	0.268	−0.464	−0.294	−0.513	−0.763	−0.526	−0.560
ΔCharge[b]	−0.012	−0.012	0.027	−0.063	0.010	−0.028	−0.082	−0.069	−0.107

[a] Detemined by a Mulliken population analysis[39]. [b] The difference in atomic net charge (Δcharge = bound-isolated).

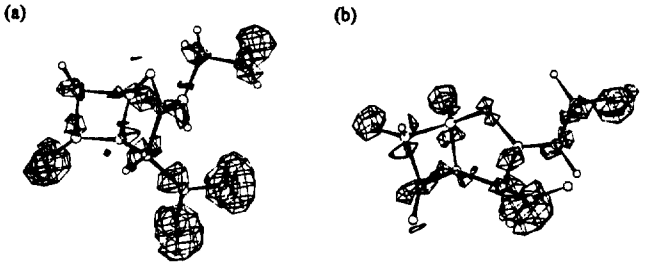

Figure 7. Migration of electrons is 3R,5R-clavulanate induced by binding to β-lactamase. The changes in electron density is shown by difference electron density contours (bound-isolated). The regions where (a) the increase of electron density is more than 0.002 e/Å^3 and (b) the decrease of electron density is more than −0.002 e/Å^3 are displayed respectively.

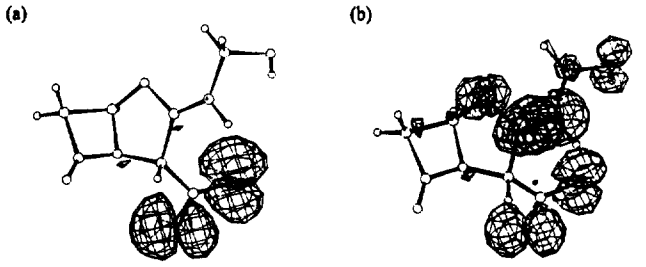

Figure 8. Changes of the HOMO of 3R,5R-clavulanate on binding to β-lactamase. Contours of HOMO's of isolated (a) and bound (b) clavulanate are shwn.

the β-lactamase are summarized in Table 4. The changes in electron density of 3R,5R-clavulanate on binding to β-lactamase is shown in Figure 7 by difference electron density contours. A significant redistribution of electron density of the clavulanate is induced by the enzyme, toward the transition state the enzymatic reaction. Polarization of the carbonyl bond (C7-O8) is calculated, which would enhance nucleophilic attack of Oγ of Ser-70. This is induced by the oxyanion hole, the hydrogen-bonding to the backbone amides of Ser-70 and Gln-237. The most significant change in atomic net charge is occurred at the carboxylate oxygen O14 due to the interaction with Lys-234 and Ser-130. Net charge change of another carboxylate oxygen O13 is less significant because this atom is not interact with Ser-130. Another significant redistribution of electron density is calculated at the C2 side chain (C10 and O11 atoms). This result stresses again the importance of C2 side chain. Contours of HOMO (highest occupied molecular orbital) of isolated and bound 3R,5R-clavulanate is shown in Figure 8. A significant change in the shape of HOMO induced by the enzyme can be seen at a glance. Recalling the Frontier Molecular Orbital theory[51], it can be interpreted such that the double bond at C2 side chain and O1 atom become reactive after the binding to enzyme. Comparing the shape of HOMO induced by the enzyme with the proposed reaction mechanism shown Figure 2, one can find that there are close relationships between them. This result implies that the spatial arrangement of the point charges of the enzyme drives the ligand to the transition state before the real enzymatic reaction.

The contours of LUMO (lowest unoccupied molecular orbital) is not shown here because there is little change on binding to the enzyme.

In the X-ray crystal structure of acyl-enzyme complex formed by clavulanate and *Staphylococcus aureus* PC1 β-lactamase[40], decarboxylation of the carboxylate group of clavulanate has been observed. The migration of of electron density from C3 to C12 atom on binding to the enzyme is calculated in our model complex (Figure 7). However, we could not assert that the result indicates the observed decarboxylation because there is rather small change in electron density in the interatomic region of C3 and C12

Conclusion

A crystallographic water molecule initially located at the oxyanion hole, which is formed by the two -NH group of Ser-70 and Gln-237, is replaced by the carbonyl oxygen atom of the 3R,5R-clavulanate after docking of the clavulanate and energy refinement. But the carbonyl oxygen atoms of 3S,5R- and 3S,5S-clavulanate are found to be at a distance from the oxyanion hole (about 3-4 Å) These results suggest that the conformation of 3R,5R-clavulanate and the relative positions of Ser-70 and Lys-234 are sufficient conditions to locate the carbonyl oxygen of β-lactam ring at the oxyanion hole. It has been proposed that the carboxylate group of β-lactams is attracted by Ser-130, Lys-234, and Ser-235. In our model Michaelis complex, however, Ser-235 weakly repels the clavulanate. The crystallographic water molecules are calculated to be important in ligand binding. Glu-166 residue is found to be repulsive to the binding of clavulanate, which is in agreement with experimental observation. Arg-244 residue is found to be important to the binding of clavulanate as well as to the interaction with C2 side chain of the clavulanate. A significant change in the shape of HOMO of clavulanate induced by the enzyme indicates that the double bond at C2 side and O1 atom become reactive after the binding to enzyme.

Understanding the mechansims of action of known inhibitor is of great importance to the design of new effective drugs. On the basis of the structure of β-lactmase molecular-mechanical and quantum-mechanical studies have allowed us to get a better understanding of the mechansim of β-lac-

tamase inactivation.

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References

1. J.-M. Frére and B. Joris, *CRC Crit. Rev. Microbiol.*, **11**, 299 (1985).
2. R. P. Ambler, *Philos. Trans. R. Soc. London Ser. B*, **289**, 321 (1980).
3. O. Hertzberg and J. Moult, *Science*, **236**, 694 (1987).
4. O. Hertzberg, *J. Mol. Biol.*, **217**, 701 (1991).
5. P. C. Mowes, J. R. Knox, O. Dideberg, P. Charlier, and J.-M. Frére, *PROTEINS*, **7**, 156 (1990).
6. J. R. Knox and P. C. Mowes, *J. Mol. Biol.*, **220**, 435 (1991).
7. C. Jelsch, F. Lenfant, J. M. Masson, and J. P. Samama, *FEBS Lett.*, **299**, 135 (1992).
8. J. R. Knowles, *Acc. Chem. Res.*, **18**, 97 (1985).
9. H. Christensen, M. T. Martin, and S. G. Waley, *Biochem. J.*, **266**, 853 (1990).
10. R. M. Gibson, H. Christensen, and S. G. Waley, *Biochem. J.*, **272**, 613 (1990).
11. H. Adachi, T. Ohta, and H. Matsuzawa, *J. Biol. Chem.*, **266**, 3186 (1991).
12. W. A. Escobar, A. K. Tan, and A. L. Fink, *Biochemistry*, **30**, 10783 (1991).
13. L. M. Ellerby, W. A. Escobar, A. L. Fink, C. Mitchinson, and J. A. Wells, *Biochemistry*, **29**, 5797 (1990).
14. G, Dalbadie-McFarland, J. J. Neitzel, and J. H. Richards, *Biochemistry*, **25**, 332 (1986).
15. M. T. Martin, and S. G. Waley, *Biochem. J.*, **254**, 923 (1988).
16. R. Virden, A. K. Tan, and A. L. Fink, *Biochemistry*, **29**, 145 (1990).
17. W. J. Healey, M. R. Labgold, and J. H. Richards, *PROTEINS*, **6**, 275 (1989).
18. F. Jacob, B. Joris, O. Dideberg, J. Dusart, J.-M. Ghuysen, and J.-M. Frére, *Protein Enginereering*, **4**, 79 (1990).
19. F. Jacob, B. Joris, S. Lepage, J. Dusart, and J.-M. Frére, *Biochem. J.*, **271**, 399 (1990).
20. S. J. Cartwright, A. K. Tan, and A. L. Fink, *Biochem. J.*, **263**, 905 (1989).
21. J.-M. Ghuysen, *Ann. Rev. Microbiol.*, **45**, 37 (1991).
22. C. Reading and M. Cole, *Antimicrob. Agents Chemother.*, **11**, 852 (1977).
23. A. G. Brown, *Drug Des. Deliv.*, **1**, 1 (1986).
24. C. Reading and P. Hepburn, *Biochem. J.*, **179**, 67 (1979).
25. F. C. Bernstein, T. F. Koetzle, G. J. B. Williams, E. F. Meyer, Jr., M. D. Brice, J. R. Rodgers, O. Kennard, T. Shimanouchi, and M. Tasumi, *J. Mol. Biol.*, **112**, 535 (1977).
26. R. P. Ambler, A. F. W. Coulson, J.-M. Frére, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Walley, *Biochem. J.*, **276**, 269 (1991).
27. P. Hohenberg and W. Kohn, *Phys. Rev. B*, **136**, 864 (1964).
28. W. Kohn and L. J. Sham, *Phys. Rev. A*, **140**, 1133 (1965).
29. B. Delley and D. E. Ellis, *J. Chem. Phys.*, **76**, 1949 (1982).
30. L. Versluis and T. Ziegler, *J. Chem. Phys.*, **88**, 322 (1988).
31. A. J. Freeman, C. L. Fu, M. Weinert, and S. Ohnishi, *Hyperfine Interact.*, **33**, 53 (1987).
32. B. Delley, *Chem. Phys.*, **110**, 329 (1986).
33. F. W. Kutzler, P. N. Swepston, Z. Berkovitch-Yellin, D. E. Ellis, and J. A. Ibers, *J. Am. Chem. Soc.*, **105**, 2996 (1983).
34. M. P. C. M. Krijn and D. Feil, *J. Chem. Phys.*, **89**, 4199 (1988).
35. M. P. C. M. Krijn, H. Graafsma, and D. Feil, *Acta Crystallogr. B*, **44**, 609 (1988).
36. H. J. F. Jansen and A. J. Freeman, *Phys. Rev. B*, **35**, 8207 (1987).
37. DMOL, Vers. 2.2, Biosym Technologies Inc., San Diego, 1992.
38. INSIGHT-II, Vers. 2.1.2., Biosym Technologies Inc., San Diego, 1992.
39. R. S. Mulliken, *J. Chem. Phys.*, **23**, 1833 (1955).
40. C. C. H. Chen and O. Herzberg, *J. Mol. Biol.*, **224**, 1103 (1992).
41. I. Rizwi, A. K. Tan, A. L. Fink, and R. Virden, *Biochem. J.*, **258**, 205 (1989).
42. V. Knott-Hunziker, S. G. Waley, B. S. Orlek, and P. G. Sammes, *FFBS Lett.*, **99**, 59 (1979).
43. S. J. Cartwright and A. F. W. Coulson, *Philos. Trans. R. Soc. London Ser. B*, **289**, 370 (1980).
44. C. Kemal and J. R. Knowles, *Biochemistry*, **20**, 3688 (1981).
45. W. G. J. Hol, *Prog. Biophys. Mol. Biol.*, **45**, 149 (1985).
46. J. Lamotte-Brasseur, G. Dive, O. Dideberg, P. Charlier, J.-M. Frére, and J. M. Ghuysen, *Biochem. J.*, **279**, 213 (1991).
47. M. Delaire, F. Lenfant, R. Labia, and J.-M. Masson, *Protein Engineering*, **7**, 805 (1991).
48. P. J. Madgwick and S. G. Waley, *Biochem. J.*, **248**, 657 (1987).
49. J.-M. Frére, C. Dormans, V. M. Lenzini, and C. Duyckaerts, *Biochem. J.*, **207**, 429 (1982).
50. A. G. Brown, D. F. Corbett, J. Goodacre, J. B. Harbridge, T. T. Howarth, R. J. Ponsford, I. Stirling, and T. J. King, *J. Chem. Soc., Perkin Trans. 1*, 635 (1984).
51. I. Fleming, *Frontier Orbitals and Organic Chemical Reactions*, A John Wiley and Sons, 1976.