Computer Graphics / Molecular Mechanics Studies of $\beta$-Lactam Antibiotics. Geometry Comparison with X-Ray Crystal Structures

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Geometries for a number of representative $\beta$-lactam antibiotics (penams, cephems and monobactams) have been calculated by computer graphics/molecular mechanics energy minimization procedures using both MM2 and AMBER force fields. The calculated geometries have been found in reasonable agreement with the geometries reported in the X-ray crystal structures, especially in terms of the pyramidal character of the amide nitrogen in the $\beta$-lactam ring and the Cohen distance. Based on these calculations, it is suggested that the nitrogen atom in the monobactams may also have pyramidal geometries in the biologically active conformations.

Introduction

Computer-assisted molecular design (CAMD) is recently becoming an important new tool in the molecular research areas such as organic synthesis, enzyme catalysis, drug-receptor interactions and protein engineering.1-10 The CAMD technique commonly involves the interactive manipulation of three-dimensional molecular structure information by means of a sophisticated computer graphics system; the requisite 3-D structural information is typically obtained from X-ray crystallographic data and from theoretical computations. In the case of "small molecules" (excluding proteins and other macromolecules) X-ray crystallographic methods provide highly precise unambiguous information of molecular structure and conformation in the solid state. Subject to certain limitations, e.g. disorder in the crystal lattice, occluded solvent, polymorphism, unusual intermolecular interactions or crystal packing forces, the X-ray experiment provides a time-averaged model of the low energy conformations of the given molecule within a given crystal lattice environment. More recently, 2D-NMR techniques such as NOE measurements are becoming an increasingly powerful tool for exploring molecular conformations in solution.

Theoretical calculation methods including ab initio, semiempirical quantum mechanics and molecular mechanics (energy minimization, grid searching of conformational space, Monte Carlo searches and molecular dynamics simulations) have been employed to generate and study 3-D molecular conformations. Ab initio and semiempirical quantum mechanics calculations have profitably been applied to a wide range of chemical problems but they have not yet been shown practical for studying molecular interactions involved with, for example, enzyme catalysis and ligand-receptor interactions.11 Molecular mechanics calculations have been very useful in studying organic molecules in non-polar solvents and in the gas phase. They use simple analytical functions to represent bond stretching, bending, and torsional and non-bonded (dispersion, attraction exchange repulsion and electrostatic interaction) energies of molecules.12 Since evaluation of these analytical functions is computationally rapid and efficient the molecular mechanics methods can be applied to the study of complex molecular systems and interactions.

In connection with our research program of applying the CAMD techniques to the design of physiologically important molecules,12 we desired to evaluate the reliability and utility of the computer graphics/molecular mechanics method in the molecular design of several types of antibiotics. Thus, we have generated a number of energy minimized molecular conformations of the $\beta$-lactam antibiotics by using molecular mechanics calculations, compared them with the corresponding solid state X-ray crystallographic conformation and herein report the results.

Results and Discussions

Among the representative $\beta$-lactam antibiotics whose X-ray crystal structures are available either through the published literature or accessible Cambridge Structures Database(CSD), we have selected three penams(1-3), two cephems(4,5), three oxacarbenams(6-8), and two monobactams(9,10) as test examples. The structures were interac-

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1 DCG, Inc. 1326 Carol Road, Meadowbrook, PA 19046, U.S.A.
System,\textsuperscript{12} In the geometry refinements based on the energy
minimization procedures, we employed both the MM-2\textsuperscript{14} and
AMBER\textsuperscript{15} force field parameters. It has generally been
acknowledged that the MM-2 force field is an all-atom field
which is useful for small molecules, whereas the AMBER is
a unite-atom field which is useful for peptide and nucleic acid
modeling.\textsuperscript{3,4}

The molecular parameters obtained from the reported
X-ray crystal structures and the molecular mechanics calcu-
lations are listed in Table 1 through 4 for penams,
cepems, oxacephalosporins, and monobactams, respectively.
The bond distances of the calculated geometries generally
agree with those of the solid state geometries within several
percentage point deviation in all the structures studied,
although some exceptions are noted where the deviation is as
large as 10 percent in the cephaloridine(4) structure. No ap-
parent and predictable advantages of one force field over the
other has been observed.\textsuperscript{16} The bond angles in the calculated
structures differ from those in the X-ray structures substan-
tially more than the usual standard deviations observed in
the X-ray crystallography. Geometry calculations on the
zwitter ionic form of 6-aminopenicillanic acid(1) and the non-
ionized form of ampicillin(3) have also been carried out. No
significant differences are observed between the gross
molecular geometries of the non-ionized and zwitter ionic
structures, although the molecular parameters in the im-
mediate vicinity of the ionization sites have been somewhat
altered.

It has generally been understood that the gas phase
molecular structures agree reasonably well with those found
in crystal, although some significant deviations are also
observed in certain cases. For example, biphenyl has a twist-
ed conformation in the gas phase, but is planar in the crystal.
Furthermore, molecular mechanics calculations of the iso-
lated molecule gave a conformation with a central bond length
and torsional angle close to the gas phase values.\textsuperscript{11} The
deviations of molecular structures in crystal from the results
of quantum mechanical or molecular mechanics calculations
are most often explained in terms of crystal packing effects
and the influence of the crystal forces on the geometry.\textsuperscript{17}

Despite the extensive research work in both biology and
medicinal chemistry of \(\beta\)-lactam antibiotics, the detailed
understanding of the 3-D molecular geometry requirements
for the biological activities is still limited. The origin of the
biological mode of action of these antibiotics is believed to be
related to their ability to irreversibly acylate the active site of
the transpeptidase enzyme involved in the biosynthesis of the
peptidoglycan moiety of the bacterial cell wall. It has been
suggested by Strominger that the transpeptidase should rec-
ognize the D-al daughters of the pentapeptide frag-
ments of the nascent cell wall in order to carry out the
necessary cross linking and that the antibiotic behaves as a
structural analog of the endogenous substrate, thus aborting
the cross linking.\textsuperscript{18,19}

The currently recognized minimum structures of \(\beta\)-lactam
antibiotics required to show the biological activity are the
chemical reactivity of the \(\beta\)-lactam amide bond and a suitable
distance geometry between the oxygen atom of the \(\beta\)-lactam
amide functionality and the carbon atom of the carboxy
group (Cohen Distance).\textsuperscript{20} The chemical reactivity is related
to the pyramidality of the amide nitrogen atom, thus hinder-
ing the amide resonance in the \(\beta\)-lactam ring. The required
pyramidalization of the amide bond is usually created by either the strain of the ring fusion as in penem or by electron delocalization through enamine resonance outside the lactam ring as in cephalosporins. In the biologically active compounds the Cohen distances range 3.0-3.9 Å. Presumably, this distance has something to do with the 3-D stereochemical features necessary for the recognition of the antibiotics by the enzyme.

The molecular parameters considered to be important for biological activities are listed in Table 5 for compounds 1-10. It is instructive to note that in the Cohen distance comparisons, the X-ray crystal structures agree far better with the results obtained from the MM-2 than the AMBER calculations. On the other hand, in comparing the sums of the bond angles around the amide nitrogen (a measure of pyramidality), the MM-2 calculations tend to overestimate the pyramidality while the AMBER tends to underestimate it. The average values between these two numbers agree reasonably well with the experimental values found in the crystal structures. It is very intriguing that the AMBER calculations show significant pyramidalities for the amide

<table>
<thead>
<tr>
<th>A. Interatomic distances (Å)</th>
<th>X-ray</th>
<th>MM-2</th>
<th>Amber</th>
<th>X-ray</th>
<th>MM-2</th>
<th>Amber</th>
<th>X-ray</th>
<th>MM-2</th>
<th>Amber</th>
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<td>93.8</td>
<td>92.1</td>
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<td>89.8</td>
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<td>91.7</td>
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<td>86</td>
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<td>86.0</td>
<td>89.8</td>
<td>92</td>
<td>85.9</td>
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<td>86.4</td>
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<tr>
<td>(S)-C(11)-C(12)</td>
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<td>120.2</td>
<td>120.1</td>
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<td>117.9</td>
<td>120.2</td>
<td>118.4</td>
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<td>(S)-C(16)-C(7)</td>
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<td>93.1</td>
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Table 1. Molecular Parameters for Penem Derivatives

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<tr>
<th>6-APA (1)</th>
<th>Pen V (2)</th>
<th>Ampicillin (3)</th>
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<tr>
<td>X-ray</td>
<td>MM-2</td>
<td>Amber</td>
</tr>
<tr>
<td>X-ray</td>
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Molecular Mechanics Studies of Antibiotics
nitrogen in the monobactams 9 and 10. The antibacterial activities observed in monobactams such as sulafazectin have been considered to be rather puzzling from the standpoint of established structure-activity relationships. The β-lactam nitrogens in all monobactams whose structures are investigated by X-ray diffraction is planar, although the distance between the oxygen of the amide group and the sulfur atom (3.355 Å) is reportedly compatible with the Cohen distance.²¹

It appears possible that monobactams may exist in pyramidal conformation in gas and solid phases, although they show planar geometry in crystals, perhaps due to the crystal packing and other effects. This possibility may be examined by molecular mechanics calculations with included crystal packing effects. ²²²³ We further suggest that since the monobactam ring is considerably more susceptible to conformational distortions than the bicyclic systems such as penams and cephalosporins, a substantial degree of pyramidalization may also be induced upon its interaction with the enzyme active site. In other words, the biologically active conformation of the monobactams inside the enzyme cavity might possess a substantial degree of pyramidalization, thus showing an enhanced chemical reactivity towards the enzymatic nucleophiles.

### Experimental Methods

The molecular modeling system at POSTECH consists of Evans & Sutherland PS 3600 graphics station linked to VAX 8600 running the MacroModel Molecular Modeling Software (version 2.0 update)¹⁹. The MacroModel implementation of MM-2 differs from the standard version¹⁴ in several ways. The major distinction is in electrostatics—whereas the standard version uses dipole/dipole interactions, MacroModel uses partial atomic charges which correspond to the MM-2 dipoles. The field is also set for a distance-dependent dielectric to mimic polarization effects. MacroModel also uses an improper torsion calculation in place of the MM-2 out-of-plane bending. Also incorporated is a preliminary set of parameters for Lennard-Jones hydrogen bonding as used in AMBER. The AMBER field¹⁵ used in the MacroModel is set up for distance-dependent dielectric electrostatics which functions as a crude approximation of polarization effects.

The energy minimizations were carried out to the preset convergence criterion (RMS energy gradient 0.05 KJoule/A) initially by the Steepest Descent(SD) method followed by block diagonal Newton-Raphson(BDR) method.¹⁴ For the the given structure, molecular hydrogens were added in the Organic Input Mode before the MM-2 calculations. Since the AMBER method requires hydrogens only at the heteroatoms, other types of hydrogens were deleted from the MM-2 minimized structures before the AMBER

### Table 2. Molecular Parameters for Cephalosporins

<table>
<thead>
<tr>
<th></th>
<th>Cephaloridine (4)</th>
<th>Cephalosporin C₁₅ (5)</th>
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<tr>
<td></td>
<td>X-ray MM-2 Amber</td>
<td>X-ray MM-2 Amber</td>
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<td>S(C₁-2)</td>
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<td>C₁(C₂)</td>
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<td>1.486 1.502 1.498</td>
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<tr>
<td>C₁(C₃)</td>
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<td>1.329 1.396 1.375</td>
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<td>C₁(C₄)</td>
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<td>1.476 1.499 1.481</td>
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<tr>
<td>C₁(C₅)</td>
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<td>C₁(C₆)</td>
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<td>1.459 1.498 1.423</td>
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<tr>
<td>C₁(C₇)</td>
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<tr>
<td>N(C₂(C₃))</td>
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<td>C₁(C₆)</td>
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<td>1.535 1.587 1.554</td>
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<td>C₁(C₇)</td>
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<td>1.527 1.533 1.505</td>
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<td>C₁(C₉₋₁₁)</td>
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<td>C₁(C₁₀₋₁₁)</td>
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<tr>
<td>CO₃⁻</td>
<td>1.528 1.517 1.529</td>
<td>1.489 1.520 1.522</td>
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### Table 3. Molecular Parameters for Oxa/Carbaspenems

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<tr>
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<th>X-ray MM-2 Amber</th>
<th>X-ray MM-2 Amber</th>
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<tr>
<td>X₁(C₁₋₂)</td>
<td>1.401 1.374 1.372</td>
<td>1.485 1.511 1.501</td>
<td>1.325 1.343 1.330</td>
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<tr>
<td>X₁(C₁₋₅)</td>
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<tr>
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<tr>
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<td>1.372 1.377 1.463</td>
<td>1.482 1.452 1.452</td>
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<tr>
<td>C₃₋₅(C₄₋₅)</td>
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<td>1.437 1.332 1.484</td>
<td>1.481 1.514 1.517</td>
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<tr>
<td>C₃₋₅(C₆₋₅)</td>
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<td>1.467 1.484 1.481</td>
<td>1.514 1.517 1.512</td>
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</table>
The image contains a page with tables and text that appears to be from a scientific paper. The tables and text are related to molecular mechanics studies of antibiotics. The tables include data on bond distances and angles, molecular parameters for monobactams, and structural characteristics of β-lactams. The text and tables are in English, and the page number is 189.
calculations. The total force field potential energy values are listed in Table 6. The X-ray crystal molecular parameters were obtained from literature for compound 1-5, and from the CSD sources for compounds 1-5, and from the CSD sources for compounds 6-10.

Acknowledgement. We wish to thank Professors W. C. Still(Columbia University) for loaning the MacroModel Molecular Modeling Program, and W.-C. Shin(Seoul National University) for providing some of the X-ray crystal coordinates. The financial supports from Korea Science and Engineering Foundation and POSTECH are gratefully acknowledged.

References

16. The overall geometry fit of the β-lactam nucleus (as rigid body) in the calculated and the X-ray structures have been evaluated by the computer graphical superposition method. The geometry superpositions were slightly better between the X-ray and the corresponding MM2 structures (RMS = 0.031-0.074 Å) than the superpositions between the X-ray and the corresponding AMBER structures (RMS = 0.064-0.249 Å).