THEORETICAL STUDIES ON $\beta$-LACTAM ANTIBIOTICS

III. Conformational Flexibility of the Five Membered Ring in Clavulanic Acid and Penicillin and the Binding Specificity of $\beta$-Lactamases

N. V. JOSHI, R. VIRUDACHALAM AND V. S. R. RAO
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

ABSTRACT

The possible conformations of clavulanic acid and penicillin were analysed by semiempirical potential energy calculations. The oxazolidine ring of clavulanic acid favours only one type of puckered conformation. On the other hand, the thiazolidine ring of penicillin favours two types of puckered conformations similar to those observed in ampicillin and penicillin G or V in the solid state. The present study predicts that both these conformations can coexist in solution in agreement with the NMR results. Comparison of the possible conformations of penicillin with that of clavulanic acid indicates that the C$_3$ puckered conformation of the thiazolidine ring (as in penicillins G or V in the solid state) is important for the binding of penicillin with $\beta$-lactamases.

INTRODUCTION

PENICILLIN is one of the widely used antibiotics to combat many bacterial diseases. However, in recent years its use has been limited by the emergence of resistant bacteria. These strains achieve their resistance mainly by producing $\beta$-lactamases which inactivate the antibiotic by hydrolysing the lactam peptide bond before the antibiotic reaches the target enzyme (transpeptidase).

Recently we have studied the binding specificity of transpeptidase by theoretical methods and have shown that for optimal binding with the enzyme, the aminoacyl and the carboxyl groups should assume specific conformations$^{1-3}$. For the binding specificity of $\beta$-lactamases, such an information is not available. Recent observation that clavulanic acid is a potent inhibitor of various $\beta$-lactamases indicates that to bind with $\beta$-lactamases the aminoacyl group of the antibiotic is not as essential as the lactam ring and the carboxyl group. Since the carboxyl group of penicillin can assume different orientations due to different modes of puckering of the thiazolidine ring it is not known which of these forms is essential for binding with $\beta$-lactamases. In order to understand this, a detailed analysis of the conformational flexibility of the five membered ring in clavulanic acid and penicillin was made by semiempirical potential energy calculations, and, the results are presented in this paper. These studies are important for the design of a suitable $\beta$-lactam compound active against resistant bacteria.

METHODS

Nomenclature

The numbering of the atoms and the dihedral angles in clavulanic acid and penicillin are shown in Fig. 1. R$_1$ and R$_2$ (Fig 1) were treated as methyl groups for calculational simplicity. The torsional angles $\phi_1$, $\phi_2$ and $\phi_3$ (in clavulanic acid) specifies the relative orientation of the planes (C$_5$, O$_1$, C$_3$, (C$_2$, C$_5$, N$_2$) and (N$_4$, C$_5$, C$_9$, C$_6$, C$_3$) respectively with respect to the reference plane (C$_5$, N$_2$, C$_9$). Hence in this notation $\phi_1$ and $\phi_2$ define the conformation of the five membered ring and $\phi_3$ defines its orientation with respect to the lactam ring. In the initial conformation ($\phi_1$, $\phi_2$, $\phi_3 = 0$) all the ring atoms (O$_1$, C$_5$, C$_9$, N$_2$, C$_3$, C$_2$ and C$_7$) are in one plane as in Fig. 1a. Clockwise rotation about the virtual or real bonds (indicated by arrows) looking
along C₂-C₃, C₁-N₄ and N₄-C₄ is considered as positive for the dihedral angles a₁, a₂ and a₃. The definition of the torsional angles (q², ψ²) which define the orientation of the carboxyl group is according to the IUPAC-IUB nomenclature. Similar definition holds good for penicillin also. The superscript 'c' in (q², ψ²) refers to clavulanic acid. Similarly 'A' in (q², ψ²) refers to the antibiotic penicillin.

**Energy Calculations**

The total conformational energy (V_{tot}) of a molecule can be expressed as

\[ V_{tot} = V_{nb} + V_{es} + V_\theta + V_{tor} \]

where

- \( V_{nb} \) is the non-bonded interaction energy,
- \( V_{es} \) is the electrostatic interaction energy,
- \( V_\theta \) is the energy due to bond angle strain and
- \( V_{tor} \) is the torsional potential energy.

The form of the functions and the parameters used to calculate \( V_{nb} \), \( V_{es} \), \( V_\theta \) and \( V_{tor} \) are described elsewhere.

Since our main aim is to study the flexibility of the five membered ring, the lactam ring was fixed in a planar conformation⁴ and the energy of the molecule was calculated as a function of \((a₁, a₂)\). These dihedral angles were varied in the range \(-60^\circ\) to \(+60^\circ\) at \(10^\circ\) intervals and at each grid point, the energy was minimized with respect to \((a₁, \beta, \beta₂)\) in clavulanic acid and \((a₁, \psi₂, \psi₂, \beta₂, \beta₂)\) in penicillin. In both these compounds \(\psi₂\) or \(\psi₂\) was fixed at \(30^\circ\) which is the preferred value in simple peptides⁵,⁶. Iso-energy contours of these molecules on the \((a₁, a₂)\) plane are shown in Figs. 2 and 3. The projection diagrams

**Fig. 2.** Iso-energy contours for clavulanic acid in kcal. mole⁻¹. ● — Global minimum; □ — solid state conformation.

**Fig. 3.** Iso-energy contours for penicillin in kcal. mole⁻¹. In region I the thiazolidine ring is in the C₂ puckered conformation and in region II it is in the C₃ puckered conformation. ● — Global minimum. Solid state conformations of the thiazolidine ring in ampicillin trhydrate (■); ampicillin anhydride (□); penicillin V (▲) and penicillin G (△).

The form of the functions and the parameters used to calculate \( V_{nb} \), \( V_{es} \), \( V_\theta \) and \( V_{tor} \) are described elsewhere.

**Table I**

Calculated and crystal structure values of \( a₂ \) and \( \psi₂ \) or \( \psi₂^{A} \)

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Relative orientation of the bicyclic ring system ((180^\circ-a₂))</th>
<th>Orientation of the carboxyl group ((q₂^0) or (q₂^{A}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clavulanic acid</td>
<td>Calculated⁷ Experimental⁸</td>
<td>Calculated⁸ Experimental⁸</td>
</tr>
<tr>
<td>Penicillin (C₂ puckered)</td>
<td>122° 120°</td>
<td>148° 161°</td>
</tr>
<tr>
<td>Penicillin (C₃ puckered)</td>
<td>143° 147°</td>
<td>111° 113°</td>
</tr>
</tbody>
</table>

**a — Corresponding to the minimum energy conformations.**

**b — Data taken from References 4, 9 and 12.**

**RESULTS AND DISCUSSION**

Figure 2 shows a single minimum for the oxazolidine ring of clavulanic acid indicating that the ring is highly rigid and favours only one type of conformation. The energy minimum occurs at \((a₁, a₂) = (10^\circ, -10^\circ)\).
In the solid state the ring assumes a conformation around (10°, -20°) and is very close to the energy minimum. However, the solid state conformation has about 2 kcal. mole⁻¹ higher energy than the energy minimum which may be compensated by lattice energy in the crystal. The relative orientations of the two rings (α₁) and the orientation of the carboxyl group (φ₂) as predicted from theory are in agreement with the crystal structure results (Table 1).

Figure 3 shows two minima for the thiazolidine ring of penicillin. The global minimum occurs at (α₁, α₂) = (40°, 10°) in region I and the local minimum at (−10°, −25°) in region II. The solid state conformations of the thiazolidine ring as observed in ampicillins⁴,⁻¹⁰ fall in region I and that of penicillin G or V⁺ in region II. When the ring assumes a conformation in region I the C₈ atom is puckered and in region II the C₃ atom is puckered. The barrier between the two minima is small (4–5 kcal. mole⁻¹) and the ring can easily flip over from one conformation to the other. The global minimum in region I has about 0.5 kcal. mole⁻¹ lower energy than the minimum in region II. Therefore, conformations in region I will predominate at equilibrium. In other words, the population of the C₂ puckered conformation will be higher than that of the C₃ puckered conformation in solution. This agrees with the recent NMR studies¹³.

Depending upon the mode of puckering the relative orientations of the lactam and the thiazolidine rings (α₃) and the orientation of the carboxyl group (φ₂) vary. The values of (α₃, φ₂) at the minimum energy conformations are very close to the experimental values (Table 1). The influence of the thiazolidine ring puckering on the preferred orientation of the aminoacyl group is not significant and the latter favours a conformation around φ₁ ≈ 170°. Interestingly, this is the conformation required for optimal binding with transpeptidase³,⁵.

It is interesting to note that clavulanic acid which exists in only one type of conformation is a potent inhibitor of β-lactamases. This suggests that this molecule has the right conformation to interact with...
OBSERVATION OF HIGHER SEISMICITY ASSOCIATED WITH LOWER PREMONSOON RAINFALL IN NORTH-EAST INDIA

SURYYA K. SARMAH

Physics Department, Gauhati University, Gauhati 781 014

ABSTRACT

A comparison of the temporal variation of the premonsoon rainfall with that of seismicity for about fifty-five years in North-Eastern Indian region shows that both premonsoon rainfall and seismicity in the same year are inversely related to each other. However, heavy premonsoon rain seems to occur a year or so before every earthquake of magnitude seven or greater. Influence of premonsoon rainfall on earthquake occurrence in this region is discussed.

INTRODUCTION

According to plate tectonic theory, the N.E. Indian region is marked by underthrusting of the Indian plate to the north and Burmese plate to the east and south-east. The region has the highest seismicity compared to the rest of the country. The region is also characterised by high rainfall especially during monsoon (June to September). Since more than 50% of the earthquakes of this region having magnitudes greater than seven (M > 7) occurred during the first half of the monsoon season, it is suspected that premonsoon rainfall may have some influence on the occurrence of earthquake in this region. If some relation between the two phenomena can be found, it will be a very convenient method for prediction of earthquakes in the North-East India by observing only the variation of premonsoon rainfall.