Chemical speciation and molecular modelling studies on interaction of cephalothin with metal ions

R K Sharma*, Deepi Goel, Sachin Mittal & Shallu Sindhwani
Department of Chemistry, University of Delhi, Delhi 110 007, India
Received 5 June 2000; revised 15 March 2001

Chemical speciation and molecular modelling studies have been carried out for accessing the interaction of metal ions with cephalothin and distribution of metal cephalothin species in biological fluids. Chemical speciation has been carried out using the program BEST and SPE and the method of Bjerrum and Calvin, as modified by Irving and Rosso. It is found that Zn(II) binds to cephalothin through the nitrogen of dihydrothiazine ring and carboxylate group at C-4.

Among the semisynthetic derivatives of cephalosporins, cephalothin (Structure I) is the most widely used. It occurs as a white to off-white crystalline powder that is practically odourless. It is freely soluble in water and insoluble in most organic solvents. Cephalothin forms chelate with metal ions and this property of cephalothin is responsible for their antibacterial action.

\[ \text{O} \quad \text{C} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{S} \quad \text{H} \quad \text{S} \quad \text{H} \quad \text{CH}_2 \text{OAc} \]

(I)

In the present work, chemical speciation and molecular modelling studies have been carried out for accessing the interaction of metal ions with cephalothin and distribution of metal cephalothin species in biological fluids. Molecular modelling studies were applied for obtaining the interaction sites of cephalothin with zinc.

Experimental

Cephalothin was procured as sodium salt from M/s Sigma Chemical Co. USA. Its purity was checked by TLC and melting point. All other chemicals viz. perchloric acid, sodium perchlorate, metal sulphates/potassium sulphate used were of analytical grade. The pH-metric method, as described by Bjerrum and Calvin and modified by Irving and Rosso, is used in the present study for the determination of $pK_a$ values of the ligands and metal stability constants. The pH-metric titrations have been carried out with the digital pH-meter (pH-5651) with combined glass electrode. The pH-meter was calibrated before performing the titrations. To ensure the constant ionic strength during the titration, an inert electrolyte sodium perchlorate, NaClO₄ (Fluka) was added in requisite amounts. A standard solution of tetramethylammonium hydroxide (TMAH) (E. Merck) in water has been used as the titrant. Its solution was standardized by standard solution of oxalic acid. Perchloric acid (HClO₄) was standardized with standard base (TMAH) solution. Metal ion solutions were prepared from analytical grade salts of the corresponding metal sulphates and were standardized by conventional methods. The titrations were performed in a covered glass jacket titration cell under nitrogen stream presaturated with water. All measurements were made at a constant temperature (25 ± 0.5°C) maintained constant by using Julabo VC type thermostat. Solution concentration of ligand in the presence and absence of metal ions were in the order of 0.005 M. The description of the sets performed is as follows:

- Set (i): 3.0 ml HClO₄ (0.05 M) + 1.0 ml NaClO₄ (2 M) + 0.20 ml K₂SO₄ (0.01 M) + 15.8 ml H₂O; (ii) 3.0 ml HClO₄ (0.05 M) + 1.0 ml NaClO₄ (2 M) + 0.20 ml K₂SO₄ (0.01 M) + 4.0 ml ligand (0.005 M) + 11.8 ml H₂O; (iii) 3.0 ml HClO₄ (0.05 M) + 1.0 ml NaClO₄ (2 M) + 0.20 ml MSO₄ (metal sulphates) (0.01 M) + 4.0 ml ligand (0.005 M) + 11.8 ml H₂O.

A Pentium computer was used for computing the results and investigating the interaction sites of ligands. Stepwise protonation constants within the range of potentiometric titration (up to pH-12.0) were calculated by fitting the pH data with the help of PKAS.
Table 1 - Stability constants of bivalent metal complexes with CET in water

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Bjerrum's half integral method</th>
<th>Weighted least squares method</th>
<th>$S_{\text{min}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\log K_1$</td>
<td>$\log K_2$</td>
<td>$\log \beta_2$</td>
</tr>
<tr>
<td>H$^+$</td>
<td>5.0</td>
<td>9.04</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>7.55</td>
<td>7.05</td>
<td>14.60</td>
</tr>
<tr>
<td>Ni</td>
<td>7.20</td>
<td>5.65</td>
<td>12.85</td>
</tr>
<tr>
<td>Zn</td>
<td>7.05</td>
<td>5.40</td>
<td>12.45</td>
</tr>
<tr>
<td>Cd</td>
<td>6.75</td>
<td>4.60</td>
<td>11.35</td>
</tr>
<tr>
<td>Co</td>
<td>6.20</td>
<td>4.15</td>
<td>10.35</td>
</tr>
<tr>
<td>Mn</td>
<td>5.40</td>
<td>3.60</td>
<td>9.00</td>
</tr>
<tr>
<td>Mg</td>
<td>4.95</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Calculations

Molecular mechanics calculations are done with HyperChem Suite release 5.1 professional version. Chemical speciation has been carried out using BEST and SPE. The basic algorithm in BEST can be stated in terms of equation,

$$T_i = \sum_{j=1}^{NS} e_{ij} \beta_j \prod_{k=1}^{i} \left[ C_k \right]^{e_{ij}}$$

which is a statement of the mass balance of the i-th component in terms of the j-th species summed over all species present NS. Each species concentration consists of a product of the overall stability constant and individual component concentration ($C_k$) raised to the power of the stoichiometric coefficient e$_{ij}$. The standard deviation in pH units is obtained using of equation,

$$\sigma_{fi} = \left( \frac{U}{N} \right)^{1/2}$$

where $N = \Sigma W$

$$U = \Sigma (\text{pH obsd.} - \text{pH calcd.})^2$$

Results and discussion

The order for the magnitude of stability constants (Table 1) of divalent metal complexes of cephalothin (CET) is as follows:

Mg(II) < Mn(II) < Co(II) < Cd(II) < Zn(II) < Ni(II) < Cu(II)

According to investigations of Irving and William, the order of complex forming abilities in the series of similar metal ions is as follows:

Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II)

The order can be accounted for by the decrease in metal ionic radius from Mn to Zn and by the crystal field stabilization energy increasing from Fe to Cu.

The $d$-orbitals of Zn(II) are completely filled. So the stabilization energy is released in its complex formation. This is why the order changes after copper.

Chemical speciation diagram as a function of pH are depicted in a representative Fig. 1. Only the ML and ML$_2$ species exist in the pH range studied. Persual of species distribution diagram reveals that 76% ML species is formed at pH 5.8 and 99% ML$_2$ species is formed at pH range 8.7 in case of zinc-cephalothin complex.

From the formation constant and chemical speciation studies, it is concluded that cephalothin forms strong complex with zinc and the antibacterial
action of cephalothin is due to the strong interaction of cephalothin with zinc containing enzymes e.g. carboxypeptidase and transpeptidase etc. resulting in their inactivation.

Chelating sites on the basis of molecular modelling

These calculations were done with HyperChem Suite release 5.1 Professional Version, an interactive software that allows for rapid structure building, geometry optimization and molecular display. Cephalothin has two ionizable hydrogen ions i.e. it has two $pK_a$ values corresponding to -NH of amide group (N-9) and carboxylate group at C-4. The two possible sites of cephalothin, that are available for coordination are nitrogen of dihydrothiazine ring, carboxylate group at C-4 and nitrogen of amide group (N-9), carbonyl oxygen of $\beta$-lactum ring. In our experiment, molecular mechanics has been used to determine the minimization energy and thus the ideal site for metal binding. HyperChem has the ability to handle transition metals and transition states. Energy minimization was repeated several times to find the global minimum. The minimization energy value is found to be lower for Zn(II) binding through nitrogen of dihydrothiazine ring and carboxylate group at C-4 (Fig. 2) as compared to Zn(II) binding through nitrogen of amide group (N-9) and carbonyl oxygen of $\beta$-lactum ring, thus indicating former to be more stable.

Acknowledgement

One of us (RKS) thanks the UGC, New Delhi, India for the award of Research Scientist and SM thanks CSIR, New Delhi, India for financial assistance.

References

1. Nagrady, Medicinal chemistry. a biochemical approach (OUP, NY) 1998.