Interaction of zinc(II) and copper(II) terpyridine complexes with biomolecules

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Abstract: Transition metal ions exhibit a unique role in diverse biological activities of proteins by acting as cofactors. In particular, zinc and copper ions modulate enzymes activities as well as many catalytic and oxidative/reductive processes. The kinetics and mechanism of the substitution reactions of dichloro [ZnCl2(terpy)] and [CuCl2(terpy)] (terpy = 2,2′:6′,2″-terpyridine) with biologically relevant ligands have been studied as a function of nucleophile concentrations at pH 7.38, under pseudo-first-order condition by UV-Vis spectrophotometric techniques. The interactions of Cu(II) and Zn(II) complexes with tripeptide glutathione (GSH) were investigated under pseudo-first-order conditions with respect to the complex concentration. For the substitution process of Zn(II) complex with glutathione (GSH), pre-equilibrium and chelate formation have been noted. The [CuCl2(terpy)] is more reactive than [ZnCl2(terpy)] complex and the second-order rate constants for the first step follow the order of reactivity: GSH > DL-Asp > L-Met > 5′-GMP ~ 5′-IMP for Cu(II) complex, while for Zn(II) the order of reactivity is: DL-Asp > L-Met > GSH ~ 5′-GMP > 5′-IMP. The results are discussed in terms of mechanisms of interactions between metalloproteins and biomolecules.

Keywords: Zinc(II); Copper(II); Biomolecules
Introduction

✓ Transition metal compounds play crucial roles as cofactors in metalloproteins [1]. Two essential metal ions, namely zinc and copper ions, modulate enzymes activities, catalytic and regulatory functions, oxidative-reductive processes, etc [1].
✓ Zinc(II) acts as an essential structural element in zinc-fingers, hydrolases, peptidases, anhydrases, and it is involved in gene regulation, etc [1].
✓ As a catalytic cofactor, Cu(II) is required in metalloproteins and influences biological oxidation-reduction reactions and electron transfers thanks to the couple Cu(II)/Cu(I) [1].

Zinc proteins are involved in control of nucleic acid replication, transcription and repair. They are implicated in many diseases and health complications so that they are recognized as medicinal targets [2].

The anticancer drug cisplatin, \textit{cis-}[\text{PtCl}_2(\text{NH}_3)_2], \textit{cis-DDP}, releases Zn(II) from the zinc coordination domain of polymerase-\(\alpha\) isolated from prostate cells (PA3) and inhibits the replication process [3]. The regulation of zinc-finger transcription factors has been shown by treatment of gene expression profiles of cells with cisplatin [4,5].

Cu(II) as active centre is present in Cu/Zn-superoxide dismutase (SOD1) located in cytoplasm and mitochondria. It exhibits an antioxidant defence function; it is known for its ability to detoxify free radicals [6].

Copper controls cancer development. It serves as a limiting factor for multiple aspects of tumour progression, growth, angiogenesis and metastasis [6].

Many studies are focused on the design of appropriated cofactors (e.g. Cu(II)-terpyridine complex) for G-quadruplex DNA metalloenzymes showing enantioselective catalytic effects [7,8].

Our aim of work is to investigate the mechanism of interaction between zinc(II) and copper(II) model complexes and biomolecules in proteins environment. The kinetics studies under physiological conditions were performed to provide more information for understanding structure-reactivity correlation between model cofactors pentacoordinated $[\text{ZnCl}_2(\text{terpy})]$ and $[\text{CuCl}_2(\text{terpy})]$ complexes and biological relevant nucleophiles.
Results and discussion

The substitution reactions include two steps both depending on the biomolecules concentration.
Results and discussion

- The so-obtained pseudo-first order rate constants, $k_{obsd_1}$ and $k_{obsd_2}$, calculated from the kinetic traces (absorbance/time traces) were plotted versus the concentrations of the entering nucleophiles.

- A linear dependence on the biomolecule concentration was observed for the reactions with DNA constituent (5'-IMP and 5'-GMP) and amino-acids (L-Met and DL-Asp).

Pseudo-first order rate constants as a function of nucleophile concentration for the first and second substitution reactions with DNA constituent 5'-IMP and 5'-GMP at pH 7.38.
# Results and discussion

## [ZnCl₂(terpy)]

<table>
<thead>
<tr>
<th>Biomolecule</th>
<th>$10^2 k_1$ (M⁻¹s⁻¹)</th>
<th>$10^2 k_2$ (M⁻¹s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’-IMP</td>
<td>15.4 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>5’-GMP</td>
<td>67 ± 9</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>L-Met</td>
<td>224 ± 31</td>
<td>73 ± 19</td>
</tr>
<tr>
<td>DL-Asp</td>
<td>7530 ± 449</td>
<td>685 ± 80</td>
</tr>
</tbody>
</table>

## [CuCl₂(terpy)]

<table>
<thead>
<tr>
<th>Biomolecule</th>
<th>$10^2 k_1$ (M⁻¹s⁻¹)</th>
<th>$10^2 k_1$ [Cl⁻] (M⁻¹s⁻¹)</th>
<th>$10^2 k_2$ (M⁻¹s⁻¹)</th>
<th>$10^2 k_2$ [Cl⁻] (M⁻¹s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’-IMP</td>
<td>1517 ± 90</td>
<td>3.2 ± 0.2</td>
<td>1139 ± 141</td>
<td>-</td>
</tr>
<tr>
<td>5’-GMP</td>
<td>1543 ± 261</td>
<td>-</td>
<td>134 ± 11</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>L-Met</td>
<td>2062 ± 202</td>
<td>-</td>
<td>359 ± 40</td>
<td>-</td>
</tr>
<tr>
<td>DL-Asp</td>
<td>8389 ± 1122</td>
<td>8.7 ± 0.4</td>
<td>4832 ± 393</td>
<td>3.5 ± 0.1</td>
</tr>
</tbody>
</table>

Tables 1 and 2
Second-order rate constants of the [ZnCl₂(terpy)] and [CuCl₂(terpy)] complexes with biomolecules: 5’-IMP, 5’-GMP, L-Met and DL-Asp at pH 7.38.
Results and discussion

Proposed mechanism of the substitution reactions:

\[
\begin{align*}
\text{M}^2+ & \text{Cl}^- + \text{Nu} \rightleftharpoons k_1 \rightarrow \text{M}^2+ & \text{Nu} \rightleftharpoons k_2 \\
M &= \text{Zn, Cu} \\
\text{Nu} &= 5'-\text{IMP, 5'-GMP, L-Met, DL-Asp}
\end{align*}
\]

✓ Coordination of DNA constituent to Cu(II) is occurring through phosphate group while coordination to Zn(II) complexes takes via N7 atoms for the first reaction [9].
✓ The coordination of L-Met and DL-Asp takes place via O-carboxylate donor atoms, formation of chelate O-N-amine has not been observed [10].

Results and discussion

- For the substitution reactions between [ZnCl$_2$(terpy)] and glutathione, first-order linear dependence, $k_{obsd1}$ on the complex concentration was observed at low concentration. At higher concentration, saturation kinetics was obtained.
- Fast pre-equilibrium formation of an intermediate pseudo-octahedral complex was observed, followed by rearrangement to the final complex whereas one chloride ion is substituted by GSH.
- For the reactions between [CuCl$_2$(terpy)] and glutathione, linear dependence on the complex concentration was observed for both reaction steps.

Time traces obtained for the reaction of 0.02 mM GSH and 10 and 30-fold excess of the concentration of [ZnCl$_2$(terpy)] complexes at pH 7.38 (the arrows point to the rise and fall in absorbance).
Conclusions

✓ Higher reactivity of [CuCl$_2$(terpy)] then [ZnCl$_2$(terpy)] toward biologically relevant nucleophiles was obtained.

✓ The substitution reactions includes two reactions steps both mostly depend on biomolecules concentration.

✓ The second-order rate constants for the first reaction step follow the order of reactivity: GSH > DL-Asp > L-Met > 5’-GMP ~ 5’-IMP for the [CuCl$_2$(terpy)] complex, while for [ZnCl$_2$(terpy)] the order of reactivity is: DL-Asp > L-Met > GSH ~ 5’-GMP > 5’-IMP.

✓ The π-acceptor properties of the tridentate N-donor chelate (terpy) predominantly control the overall reaction pattern.

✓ The different mechanism of interactions of the pentacoordinate complexes with 5’-GMP, 5’-IMP and GSH have been obtained.
Acknowledgments

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