Structural Examination of the Peptide-Zinc interaction in the Divalent Oxytocin Complex

Alexandra Seuthe, Dengfeng Liu, Oli Th. Ehrler, Xiaohua Zhang, Thomas Wyttenbach and Michael T. Bowers
Oxytocin (OT) Structure

- Synthesized in posterior pituitary and released into circulation.
- Receptor is G-protein found in smooth muscle cells.
- Conformation of OT ligand dramatically affects binding to receptor.

Virtually all vertebrate species have an OT-like hormone:
- Disulfide bridge b/t residues 1 and 6
- Cyclic portion, 3 residue amidated tail
- Synthesized in posterior pituitary and released into circulation.
- Receptor is G-protein found in smooth muscle cells.

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH₂)

\[ \text{S} - \text{S} \]
Oxytocin (OT) Function

- OT is found in equivalent concentration in both sexes.
- OT has been linked to several physiological activities:
  1) Uterine contractions during birth
  2) Lactation

Also responsible for “Affiliation” in mammals.

Establishment of complex social and bonding behaviors related to reproduction and the care of offspring.

*i.e.* maternal behavior, infant separation distress, mate formation

- Linked to autism.
Metal-OT Complexes

Why Zinc?

Zinc Fingers: Protein motif that binds DNA
Transcription Factors necessary for DNA replication.

OT and Divalent Metal Cations

• Essential elements and other metals have been found to form complexes with OT.
  \[ \text{i.e.: Cu}^{2+}, \text{Zn}^{2+}, \text{Co}^{2+}, \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Ni}^{2+} \]

• The presence of divalent cation is essential for specific binding of OT to receptor.

~ 30 residues
OT = 9 residues
Research Objectives

Characterize the OT-Zinc complex.

**Observation:**

Divalent metal cations required for OT-Receptor binding.

OT has high affinity for divalent metal.

Lock and key model: Receptor binding is conformation-dependent.

**Question:**

Does Zinc bind to receptor or ligand?

What are the binding properties of the OT-metal complex?

Does Zinc cause a conformational change which will enhance binding?

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH₂)
Experimental methods

- Mass spectrometry
- Cross section (collisions with helium)
- Hydration (equilibrium with water vapor)

Theoretical methods

- Molecular mechanics
- Density functional theory

Cross section

Protons/Metal ions
Molecule size/shape
Molecule surface

Cross section

Molecule structure
Instrumentation

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector
Instrumentation
Instrumentation

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector

To Pump

Drift Cell

Quad Analyzer

Ion Funnel

Ion Optics

Detector
Instrumentation
Instrumentation
Instrumentation

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector
Hydration under equilibrium conditions

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector

M⁺ in 1–2 torr H₂O Drift cell  M⁺•(H₂O)ₙ out
Cross section measurements

ESI Ion Source → Ion Funnel → Drift Cell → MS → Detector

1–5 torr He Drift cell

E → in → out
Cross section measurements

Slow Component: Large Cross Section
Fast Component: Small Cross Section
Experimental Results

Mass Spectrum Oxytocin

![Mass Spectrum of Oxytocin](image)

- [O T+H]
- [O T+H+K] 2+
- [O T+H+Na] 2+
- [O T+2H] 2+
- [O T + H] +1

m/z

200 400 600 800 1000 1200

+2

+1
Mass Spectrum of Oxytocin
Mass Spectrum
Oxytocin

with ZnCl$_2$
Mass Spectrum
Oxytocin

with ZnCl$_2$
How does zinc interact with OT?
Theory Results: OT-H⁺
Bare OT

Theory: MM - CHARMM force field
DFT - SVP basis set; BP86 functional

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH₂)

228 Å² (calc)
230 Å² (exp)
Theory Results: OT-Zn\(^{2+}\) Complex

- **Theory:** MM - CHARMM force field
  - DFT - SVP basis set; BP86 functional

- **Zn–O distance:** 204-215 pm
- **Zn\(^{2+}\) + O ionic radius:** 214 pm

- **Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH\(_2\))**
- **S – S**

- **Octahedral coordination sphere**

- **236 Å\(^2\) (calc)**
- **236 Å\(^2\) (exp)**
### Isotocin (IT)

<table>
<thead>
<tr>
<th>OT</th>
<th>Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S – S</td>
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</table>

<table>
<thead>
<tr>
<th>IT</th>
<th>Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly(NH₂)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>S – S</td>
</tr>
</tbody>
</table>

**Osteichthyes – Bony Fish**
Isotocin (IT)

OT: Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH$_2$)
    S – S

IT: Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly(NH$_2$)
    S – S

Osteichthyes – Bony Fish

- donating backbone oxygen

- donating backbone oxygen
## Comparison With Experimental Results

1.) Cross Section

<table>
<thead>
<tr>
<th></th>
<th>$\sigma$ [Å²]</th>
<th>Exp.</th>
<th>MD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(OT+H)^+$</td>
<td>230</td>
<td></td>
<td>228</td>
</tr>
<tr>
<td>$(OT+Zn)^{2+}$</td>
<td>236</td>
<td></td>
<td>236</td>
</tr>
<tr>
<td>$(IT+Zn)^{2+}$</td>
<td>222</td>
<td></td>
<td>225</td>
</tr>
</tbody>
</table>

2.) Hydration
Hydration of \((\text{Oxytocin} + \text{Zn})^{2+}\)

\[(\text{OT} + \text{Zn} + n \, \text{H}_2\text{O})^{2+}\]

0.5 torr H\(_2\)O
300K
# Hydration data

(OT+H)<sup>+</sup>  

<table>
<thead>
<tr>
<th>$n \text{(H}_2\text{O})$</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H_{\text{hydr}}$ [kcal/mol]</td>
<td>-7.4</td>
<td>-8.3</td>
<td>-7.4</td>
</tr>
<tr>
<td>$\Delta S_{\text{hydr}}$ [cal/mol·K]</td>
<td>-14.9</td>
<td>-18.6</td>
<td>-15.8</td>
</tr>
</tbody>
</table>

(OT+Zn)<sup>2+</sup>  

<table>
<thead>
<tr>
<th>$n \text{(H}_2\text{O})$</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H_{\text{hydr}}$ [kcal/mol]</td>
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<td>-8.6</td>
</tr>
<tr>
<td>$\Delta S_{\text{hydr}}$ [cal/mol·K]</td>
<td>-18.9</td>
<td>-15.9</td>
</tr>
</tbody>
</table>
Water binding energies

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding Energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn(^{2+}) \cdots \text{H}_2\text{O}</td>
<td>96</td>
</tr>
<tr>
<td>Peptide \cdots \text{H}_2\text{O}</td>
<td>10</td>
</tr>
<tr>
<td>(OT+H(^{+})) \cdots \text{H}_2\text{O}</td>
<td>7</td>
</tr>
</tbody>
</table>

Consistent with OT-water interaction rather than zinc-water interaction

[2] Lui et al. JACS 2003, 125, 8458
Water binding energies

\[
\begin{align*}
\text{Zn}^{2+} & \cdots \text{H}_2\text{O} & 96 \text{ kcal/mol} \quad [1] \\
\text{Peptide} & \cdots \text{H}_2\text{O} & 10 \text{ kcal/mol} \quad [2] \\
(\text{OT}+\text{H})^+ & \cdots \text{H}_2\text{O} & 7 \text{ kcal/mol} \\
(\text{OT}+\text{Zn})^{2+} & \cdots \text{H}_2\text{O} & 10 \text{ kcal/mol} \\
\end{align*}
\]

Consistent with OT-water interaction rather than zinc-water interaction

[2] Lui et al. JACS 2003, 125, 8458

Hydration and Cross Section Measurements confirm that Zinc is buried in the structure.
Oxytocin (OT) Receptor Interaction

• OT receptor sequence is known, but ligand binding is not.

• The cyclic portion of OT binds to extracellular loop. The linear portion binds to another extracellular loop.

• Isoleucine in third position has been found to be crucial to receptor binding.
Oxytocin (OT) Receptor Interaction

Why do divalent metals increase OT binding?

OT
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH₂)

\[
\text{Residue 3-5 are found to be crucial for cyclic hormone-
receptor interaction.}
\]

\[
\text{Hydrophobic residue Ile/Phe-3 forms hydrophobic pocket
interaction}
\]

\[
\text{Conserved Gln-4 and Asn-5 interact with conserved residues
in the receptor.}
\]
Side Chain Conformation

- Bare OT
- OT- Zinc Complex
Interaction of the cyclic portion is stabilized by salt bridge interaction between NH3+ and Glutamic Acid in receptor.

Zinc displaces N-terminus from the interior of peptide.
Compact vs. Extended

- Bare OT
- OT-Zinc Complex
Conclusions

Experiments confirm that Zinc is buried in peptide structure.

- A dramatic conformational change occurs as OT binds to zinc.
  Similar change is seen in IT.
- Conformational change appears more favorable for receptor binding.
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