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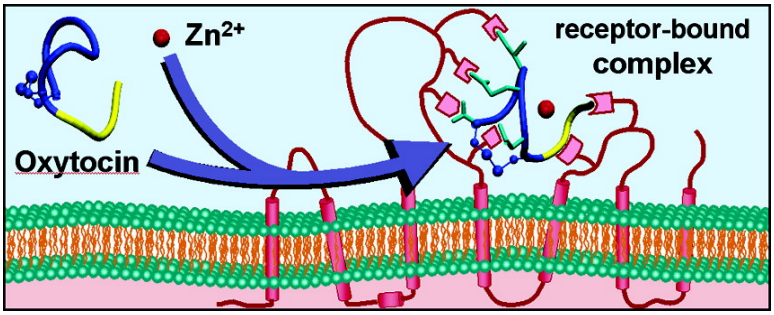

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Oxytocin-Receptor Binding: Why Divalent Metals Are Essential

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Oxytocin was the first peptide hormone to have its sequence determined and the first to be chemically synthesized in its biologically active form.[1] OT is well characterized for its role in the reproductive cycle, where it stimulates smooth muscle contractions in the uterus during labor and the mammary glands during lactation.[2] OT is further implicated to play a pivotal role in the complex process of "affiliation" in mammals (maternal behavior, infant separation distress, and mate formation).[3] The peptide consists of nine amino acids (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly) and an amidated C-terminus. The secondary structure is characterized by a disulfide bridge that links the first and sixth residues, which results in the formation of a 20-atom ring.[1]

The presence of doubly charged cations, such as Zn^{2+} or Cu^{2+}, has been found to be essential for the specific binding of OT to its receptor.[4] There is uncertainty, however, whether the metal ions primarily interact with the receptor, OT, or both. While receptor−metal ion interactions are very difficult to study experimentally, there have been a number of studies of the binding of divalent metal ions with OT using potentiometric and spectroscopic probes.[5] While providing useful information, there was little evidence of the metal ion binding site or of conformation change in OT upon metal binding. These are crucial points as will be described shortly.

Here we present results from experiment and theory that directly assess the effects of Zn^{2+} on OT conformation. The two mass spectrometry-based experimental methods employed to probe the molecule structure include cross section[6] and hydration energy[7] measurements. Associated with these are extensive molecular mechanics calculations using the CHARMM force field[8−10] and density functional theory calculations (DFT) using the TURBOMOLE family of programs.[11,12]

Figure 1a shows the nanoelectrospray (nano-ESI) mass spectrum obtained for a 50 μM OT solution[13] using an instrument previously described.[14] The dominant peaks in the spectrum are due to the singly and doubly protonated oxytocin. In solution at physiological pH oxytocin should be singly protonated since it has only one basic site (N-terminus) and the C-terminus is amidated. Hence, in the absence of doubly charged metal ions the conformation of OT will be that of the $[OT+H]^+$ ion. A typical calculated structure using CHARMM in explicit water solution is given in Figure 2a. Of importance is the fact that the protonated N-terminus is significantly solvated by the peptide itself, making it unavailable for interaction with the receptor. Also of importance is the fact that the side chains of Ile3, Gln4, and Asn5 all point in different directions and do not form a cohesive quasi-planar surface. Both of these factors suggest $[OT+H]^+$ will not bind effectively to the receptor as will be discussed shortly.

The CHARMM procedure[9,10] for generating structures can be tested experimentally. Ion mobility measurements yield a cross section for the solvent-free $[OT+H]^+$ of 230 ± 3 Å^2 in excellent agreement with the 228 Å^2 obtained for the solvent-free $[OT+H]^+$ family of structures generated by the CHARMM procedure.[9] A typical structure is shown in Figure 2b. A second probe of the structure of $[OT+H]^+$ can be obtained by measuring hydration energies using equilibrium methods. These are given in Table 1 for the addition of the first three water ligands. It is clear by comparing these hydration energies with the model systems listed in Table 1 that the N-terminal NH_3^+ group in $[OT+H]^+$ is fully solvated by the peptide, fully consistent with the CHARMM generated family of lowest energy structures.

Figure 1b shows the mass spectrum after 200 μM $ZnCl_2$ is added. The protonated species are completely suppressed, and only $[OT+Zn]^{2+}$ is observed. Hence Zn^{2+} ions very effectively coordinate with OT. The Zn^{2+} affinity of OT was reported[4] to be 5000 M^{-1}.

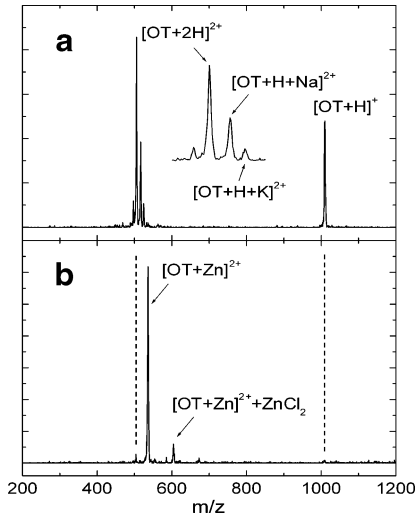

Figure 1. Nanoelectrospray mass spectra of (a) a 50 μM oxytocin solution and (b) the same solution with 200 μM $ZnCl_2$ added.

Table 1. Hydration Enthalpies ($-\Delta H_n°$, kcal/mol) for Singly Protonated Oxytocin $[OT+H]^+$, the Doubly Charged Zinc−OT Complex $[OT+Zn]^{2+}$, and Some Comparison Systems[a]

	number of water ligands			
peptide	1	2	3	reference
$[OT+H]^+$	7.4	8.3	7.4	this work
$[OT+Zn]^{2+}$	9.6	8.6	−	this work
exposed NH_3^+ (alkylamine)[b]	14.8	12.1	9.6	7
buried NH_3^+ (peptide)[c]	≤7	−	−	7
bare Zn^{2+}	96	87	61	16
$(peptide)^{2+}$ [d]	10.3	8.9	9.6	7

[a] Errors ±0.3 kcal/mol. [b] Data for *n*-decylamine. Essentially the same results were obtained for the peptide AA. [c] Data for $(Ac\text{-}A_xK)H^+$, $x = 4$ to 8. [d] Data for neurotensin (ELYENKPRRPYIL).

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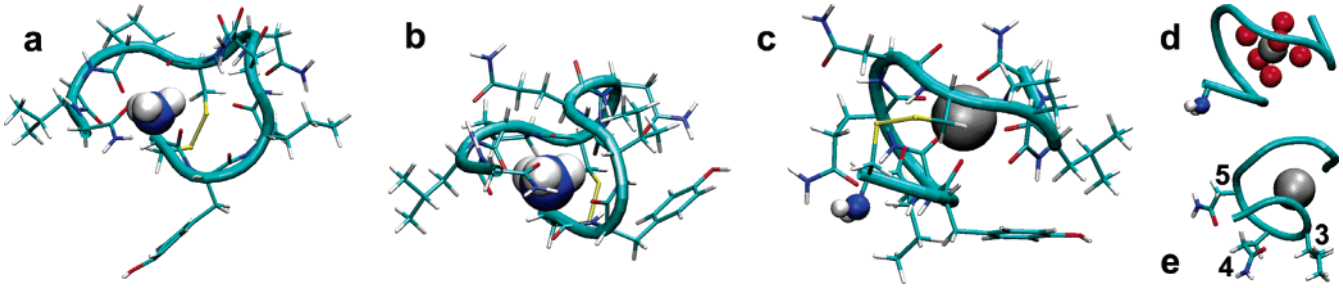

Figure 2. Typical examples from minimum energy families of structures generated by CHARMM. The N-terminal NH_3^+ group is enlarged for emphasis. (a) Structure of $[OT+H]^+$ calculated in explicit water solvation. (b) Structure of $[OT+H]^+$ calculated in a solvent-free environment. (c) Calculated structure of $[OT+Zn]^{2+}$. (d) Same $[OT+Zn]^{2+}$ structure showing the octahedral orientation of the six carbonyl backbone oxygens surrounding the Zn^{2+} ion. (e) Same $[OT+Zn]^{2+}$ structure emphasizing side chain orientation of Ile3, Gln4, and Asn5.

The $[OT+Zn]^{2+}$ complex can be characterized by the same methods used to characterize $[OT+H]^+$. The experimentally measured cross section is 232 ± 3 Å^2, and the theoretical cross section from the lowest energy CHARMM/DFT structural family[9,12] is also 232 Å^2 (Figure 2c). This structure has the Zn^{2+} ion forming a near-perfect octahedral complex (Figure 2d) with six of the backbone carbonyl oxygens (associated with Tyr2, Ile3, Gln4, Cys6, Leu8, and Gly9). This near octahedral cage has zinc−oxygen distances of 204−215 pm, with an average of 211 pm. These distances compare favorably with the sum of the zinc and oxygen ionic radii, 214 pm.[15] The coordination of Zn^{2+} with OT lines up the side chains of Ile3, Gln4, and Asn5 to form a cohesive, near-planar surface amenable for coordination with the receptor (Figure 2e). Furthermore, it frees the N-terminus from its coordination with the peptide and makes it available for bonding with the receptor.

Hydration studies are fully consistent with a peptide completely shielding the Zn^{2+} metal center from the water ligands. The energies for addition of the first two waters (9.6 and 8.6 kcal/mol) are expected for coordination to a doubly charged peptide (Table 1). A free Zn^{2+} ion binds water much more strongly (96 kcal/mol for the first, 29 kcal/mol for the fifth water).[16]

The question is, do the dramatic conformational changes induced by Zn^{2+} coordination to OT account for the great enhancement that divalent metal ions have on OT-receptor binding? The OT receptor has been sequenced and modeled and is a typical G-protein with seven transmembrane α-helices, three extracellular loops, and three intracellular loops.[17] It appears that the cyclic portion of OT binds to one extracellular loop and the linear to another.[18] Hormone positions 3, 4, and 5 are especially important with a hydrophobic interaction involving Ile3 and interactions of Gln4 and Asn5 with a receptor Tyr and a Gln, respectively.[19] Our model of $[OT+Zn]^{2+}$ facilitates these interactions by directing the side chains at positions 3−5 to point in similar directions approximately perpendicular to the backbone. This conformation is robust and held in place by the strong octahedral binding of the six backbone carbonyl oxygens to the Zn^{2+}. In $[OT+H]^+$, on the other hand, the side chains point in random directions and are not readily available for interaction with the receptor. Further, the surface of $[OT+Zn]^{2+}$ is generally hydrophobic since the carbonyl oxygens are directed toward the core so they can interact with the Zn^{2+}, a general condition that aids in desolvation of OT and promotes receptor binding.

A previous docking calculation study of OT and its receptor suggested an important salt bridge was formed between the protonated N-terminus of OT and a conserved acidic residue on the receptor.[20] In $[OT+H]^+$ such an interaction would be prevented by the self-solvation of the protonated N-terminus by the OT peptide itself (Figure 2a,b). However, in $[OT+Zn]^{2+}$ the N-terminus is freed and ideally situated for this interaction (Figure 2c).[21]

In summary, a combination of experimental and theoretical evidence indicates oxytocin undergoes substantial conformational change when coordinating with Zn^{2+}. These changes directly facilitate binding of specific residues in OT with specific residues in its receptor. Metal ions might also play a role in the receptor conformation, although that does not appear to be necessary given the results presented here.

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Supporting Information Available: Details of computational results: range of structures and cross section distributions. This material is available free of charge via the Internet at http://pubs.acs.org.

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