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Metal Modulation of Oxytocin Structure, Function, and Receptor Interactions

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Abstract

Oxytocin is a nine-amino acid peptide hormone essential for reproduction, social bonding, and neuromodulation. Accumulating evidence demonstrates that divalent metal ions play critical roles in modulating oxytocin structure, receptor binding, and biological activity. Despite nearly five decades of research, a comprehensive molecular-level understanding of metal–oxytocin interactions and their functional consequences remains incomplete. This review synthesizes current knowledge of how Cu^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, and other metals influence oxytocin's conformational landscape and receptor interactions through distinct coordination modes. We highlight emerging applications in biosensor development and therapeutic formulation that leverage these metal-binding properties. Advancing our understanding of metal-mediated modulation of oxytocin function will enhance our knowledge of peptide hormone regulation and provide new avenues for therapeutic intervention in conditions involving dysregulated oxytocin signaling.

Keywords

bioinorganic chemistry; G protein-coupled receptor signaling; metal coordination; oxytocin; peptide hormones

1 | Introduction

Oxytocin (OT), discovered in the early 20th century by British physiologist Sir Henry Dale, has emerged as one of the most thoroughly studied peptide hormones due to its critical roles in reproduction, social bonding, and neurological function [1–3]. Dale initially identified OT's role in stimulating uterine contractions during childbirth, and in 1953, Vincent du Vigneaud and his group successfully synthesized OT, making it the first polypeptide hormone to be artificially produced [4]. The term "oxytocin" means "quick birth" in Greek, aptly reflecting its primary role in reproductive functions in stimulating labor and promoting

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Author Contributions

J.P. and M.C.H. conceptualized the work. J.P. wrote the main manuscript text, M.C.H. revised the text critically for important intellectual content and provided funding for the work, and J.P. and M.C.H. created the figures and tables.

Conflicts of Interest

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milk ejection during lactation in women [5]. Additionally, OT has significant neural impacts across all vertebrates, influencing social bonding, social memory, and mental health [2, 5].

OT is synthesized from a larger precursor protein (15–16 kDa) primarily in the hypothalamus. Following proteolytic processing, it is cleaved to produce and release OT as a nonapeptide with the sequence Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–Gly–NH_2 with a C-terminal amide (Figure 1) along with neurophysin, a carrier protein that transports OT to the posterior pituitary gland [2, 3, 5]. From there, OT is secreted into peripheral circulation, acting as both a hormone and a neuromodulator [3]. Under physiological conditions, the two Cys residues in OT can form a disulfide bond, to make its characteristic cyclic peptide structure (3D NMR solution structure of the -COOH analog shown in Figure 1A). OT undergoes several posttranslational modifications (PTM) that influence its structure and activity, including amidation of the C-terminal Gly, the formation of a disulfide bond between two Cys at the 1^{st} and 6^{th} positions, and acetylation [7–9]. These PTMs may significantly contribute to OT's functional diversity in different tissues and its roles in processes like childbirth, social bonding, and emotional regulation.

Despite the wealth of reports on the physiological and pathological significance of OT, several gaps in understanding remain regarding the molecular basis of its bioactivity and signaling mechanisms. One particularly understudied area is the role of metal ions in modulating OT structure and function. The potential influence of metals on OT activity was first reported in 1973 by Soloff and Swartz, who discovered that OT binding to rat mammary gland tissue could be strongly influenced by divalent metal ions [10]. Specifically, they demonstrated that the binding interaction is enhanced by Mn^{2+}, Co^{2+}, Mg^{2+}, and Zn^{2+}, with Co^{2+} enhancing binding similarly to Mn^{2+} [10]. Interestingly, this interaction is not affected by monovalent metal ions (Na^+, K^+, or Li^+) or Ca^{2+} [10]. Subsequently, Pearlmutter and Soloff found that Ni^{2+} also enhances OT binding to rat mammary gland tissue, whereas Fe^{2+} and Ci^{2+} showed no enhancement [11]. However, at slightly acidic pH (pH 6.5), OT binds to Cu^{2+} at least 20 times more tightly than to Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} (Table 1) [11].

Interestingly, the order of effectiveness for enhancing OT binding does not follow the Irving–Williams series for metal–ligand stability ($Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$) [12], suggesting that factors beyond simple thermodynamic stability of metal/peptide species govern these interactions. Given that mammary gland tissue has strong expression of the OT receptor (OXTR) [13], these early studies suggest potential involvement of metal ions in OT–OXTR interaction. However, many questions remained, including whether metal ions interact with the receptor, OT, or both. Additionally, the use of rat mammary gland tissue, which contains many proteins, complicated the determination of each metal ion's effect on the OXTR–OT interaction.

Recent reports underscore the biological stakes of gaining molecular understanding into the OXTR–OT interactions and the potential influence of metal ions. OT has been shown to protect dopaminergic circuits in Parkinson's models [14], attenuate β-amyloid toxicity and social memory loss in Alzheimer's paradigms [15], and mitigate social isolation-induced pathology [16]; in parallel, updated reviews are highlighting copper dyshomeostasis and

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cuproptosis in CNS disease [17], iron/metal metabolism in neurodegeneration [18, 19], and even lithium deficiency as a risk factor for AD [20].

Given the challenges of isolating OXTR and investigating the metal-mediated interaction between OXTR and OT, researchers have shifted focus to studying OT's metal binding properties through various biophysical approaches. Toward developing a comprehensive picture on the potential role of metal ions in OT signaling, this review compiles current understanding of metal-mediated OT's bioactivities with OXTR and characterization of the metal binding of both gaseous and aqueous OT in various biophysical methods. We focus primarily on interactions with biologically relevant divalent metal ions, particularly Cu^{2+} and Zn^{2+}, while also discussing interactions with Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Pd^{2+}. Most studies in this arena do not specify the redox state of OT, as they generally assume that OT remains in its oxidized form under physiological conditions. Therefore, if the redox state is not mentioned, it can be assumed that oxidized OT (oxOT) was used. We note that while metal interactions with modified OT variants, such as *N*-methylated OT analog, or posttranslationally modified forms (e.g., *N*-acetylated or glycosylated OT) warrant investigation, such reports are scarce, and are thus not discussed in this review. However, such studies with these modified OT variants offer promising future directions for revealing additional layers of regulation of OT structure and signaling.

2 | Metal-Mediated OT–Receptor Interactions: Biological Evidence

Early investigations using rat mammary gland tissue homogenates revealed that divalent metal ions modulate OT–receptor interactions [10, 11], though these complex systems made it difficult to determine whether metals interact with OT, the receptor, or both. These studies determined the effectiveness of metal ions in facilitating the OT–OXTR interactions following the order: $Co^{2+} = Mn^{2+} > Mg^{2+} > Ni^{2+}$ [10, 11]. Additionally, monovalent metal ions, Cu^{2+}, and Ca^{2+} appeared to be inactive in enhancing receptor binding (though whether these ions inhibited binding was not explored) [11]. While both Mn^{2+} and Co^{2+} enhance binding, the kinetics of their interaction differ. The kinetics of association and dissociation of OT with high Mn^{2+} level (5 mM) is independent of Mn^{2+} concentration [11]. While Mn^{2+} can affect the concentration of OXTR available for binding, it does not alter the equilibrium association constant [11]. In contrast, with Co^{2+}, the association rate increases while the dissociation rate decreases [11]. This analysis led researchers to propose a metal binding mechanism for OXTR with two metal binding sites. Metal binding to one site increases the number of available binding sites without affecting the affinity to OT, whereas metal binding to another site increases the affinity to OT [11].

However, more recent cellular studies have revealed that Cu^{2+} and Zn^{2+} can also influence OT signaling [21, 22], though potentially through mechanisms distinct from the receptor binding enhancement observed with Mn^{2+} and Co^{2+}. Building on emerging evidence for direct Cu^{2+} and Zn^{2+} binding to the OT peptide (detailed in subsequent sections), our lab recently investigated the biological role of Cu^{2+}–OT and Zn^{2+}–OT in HEK 293 T cells expressing OXTR by examining MAPK signaling activation [21]. OT exhibits different behaviors in response to Cu^{2+} and Zn^{2+} depending on its redox state. Both Cu^{2+}- and Zn^{2+}-oxOT suppress MAPK activation, whereas Zn^{2+}-reduced OT (rOT) induces more

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MAPK signaling activation [21]. This suggests that metal-bound oxOT may not strongly interact with OXTR to induce MAPK signaling, whereas Zn^{2+}-rOT somehow induces a conformational change in OXTR that activates MAPK signaling pathway [21]. This cellular work thus provides a functional bridge between the molecular binding studies and the earlier tissue-based observations, helping to reconcile the apparent discrepancies in Cu^{2+} and Zn^{2+} activity. Consistent with this context-dependent signaling model at the cellular level, Kleszczewski et al. investigated whether Cu^{2+}–OT complex formation affects OT bioactivity by altering contractile responses in pregnant human myometrium [22]. Given OT's high affinity for Cu^{2+} and the stability of Cu^{2+}–OT complexes, they hypothesized that Cu coordination could modulate OT-mediated myometrial contraction. Interestingly, Cu^{2+} alone did not significantly alter OT activity or induce measurable changes in myometrial contraction in vitro. However, this finding suggests that Cu^{2+} does not simply enhance or abolish OT–OXTR interactions at the tissue level. Instead, these results are consistent with a model in which Cu^{2+} tunes OT signaling in a context- and pathway-dependent manner, potentially by modulating ligand conformation, availability, or signaling bias rather than grossly changing receptor-binding potency. Together, the metal-and redox-dependent MAPK responses in OXTR-expressing HEK293T cells and the myometrial contractility results highlight that Cu^{2+} and Zn^{2+} can play physiologically relevant roles in modulating OT–OXTR signaling.

OT is also known to increase intracellular Ca^{2+} levels in the uterine myometrium by activating the $G_{q/11}$ signaling pathway upon OT binding to OXTR [23, 24]. This activation stimulates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol trisphosphate (IP3) and diacylglycerol (DAG). IP_3 binds to its receptor localized on the endoplasmic reticulum (ER), inducing the release of Ca^{2+} from the ER into the cytoplasm. The elevated intracellular Ca^{2+} levels affect diverse biological pathways, such as hormone secretion, uterine contractions during childbirth, and milk ejection during lactation. In a related study, Pearlmutter and Soloff showed that Ca^{2+} alone did not affect the binding of OT to the mammary gland receptor, but in the presence of 5 mM Mg^{2+}, Ca^{2+} between 0.2 and 0.6 mM decreased the binding [11]. However, higher than 1 mM Ca^{2+}, in the presence of Mg^{2+}, enhanced the OT affinity to OXTR [11]. From this dual effect, they hypothesized that Ca^{2+} affects both OT affinities and the concentration of the active OXTR [11]. This study also provided insights into Mg^{2+}'s biological role in the uterus, but the relationship between Mg^{2+}, OT, and OXTR was not fully understood.

Subsequent research using OT agonists and radioiodinated antagonists in the rat uterus showed that OT's potency is dose dependent on Mg^{2+} [25]. In the absence of Mg^{2+}, OT potency decreases by 1000-fold compared to conditions with 1 mM Mg^{2+} [25]. It was hypothesized that Mg^{2+} stabilizes the receptor's conformation and is necessary for binding agonists [25]. However, the mechanism by which Mg^{2+} mediates OXTR activation by OT remains unclear. Recent cryo-EM structural studies by Meyerowitz et al. revealed the OT–Mg^{2+}–OXTR complex structure, suggesting that Mg^{2+} plays a crucial role in OXTR activation by cyclic OT (see Section 6) [26].

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3 | Structural Characterization of Cu^{2+}–OT Complexes

To complement tissue-based studies, researchers have investigated direct Cu^{2+} interactions using purified peptide. Cu^{2+} binds effectively to cyclic peptide hormones due to its preference for nitrogen atoms, forming a stable Cu^{2+}—N bond [27]. Because of this binding preference, Cu^{2+} is particularly attractive for OT-like peptides with disulfide bonds, as they can easily accommodate the metal binding despite steric constraints [27].

The interaction was first reported by Zhu and colleagues who investigated the system by electrospray mass spectrometry (ESMS) at pH 2, pH 5, and pH 9 with Mn^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} [28]. At pH 5 and 9, OT could bind Cu^{2+}, but not at pH 2 [28]. Additionally, OT could bind Ni^{2+}, but did not bind any metal ions at acidic pH [28]. At pH 5, OT formed various metal-containing species, including $[M+OT]^{2+}$, $[M+OT\text{-}H^{+}]^{+}$, $[M+OT + ClO^{-}_{4}+H^{+}]^{2+}$, and $[M+OT + ClO^{-}_{4}]^{+}$ [28]. Later studies also investigated the interaction between OT and Cu^{2+} along with Ni^{2+}, Co^{2+}, and Zn^{2+}, using collision-induced dissociation (CID)–MS and electron capture dissociation (ECD) [29]. Cu^{2+}–OT complexes exhibited different CID cleavage products compared to other metal ions–OT complexes [29], suggesting a unique reaction of OT to Cu^{2+}, possibly involving the redox activity of OT. However, the reduction of Cu after binding to OT remains unclear.

The Bowers group conducted comprehensive studies of OT–Cu^{2+}through MS and ion mobilization combined with density functional theory (DFT) and molecular dynamics (MD) calculations [30]. At pH 3, OT could bind diverse divalent metal ions, such as Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+}, whereas at pH 11, OT can form a strong complex with Cu^{2+} only [30]. Accordingly, they proposed Cu^{2+}–OT coordination. When Cu^{2+} is ligated to the cyclic OT, there are four proton loss signals from three amide backbones of the C-terminal and the N-terminal amine [30]. From this observation, DFT and MD suggested that Cu^{2+} is bound to OT with a planar 4N coordination and the backbone nitrogen of Cys6, Leu8, and Gly9 and the free amine of Cys1 are ligated to Cu^{2+} (Figure 2A) [30].

Additional MS and theoretical studies suggested various potential Cu^{2+}–OT binding modes (Figure 2A): 1) backbone nitrogen of Tyr2, Ile3, Gln4, and Asn5; 2) backbone nitrogen of Ile3, Gln4, Asn5 and Cys6; or 3) four oxygens involving Tyr2, Ile3, Cys6, and Gly9 at acidic pH. These variances might be due to pH-dependent Cu^{2+} coordination [31, 32]. These studies provided insights into pH-dependent metal binding of the cyclic OT but had limitations to probe its Cu^{2+} binding under physiological conditions because of lower ionization efficiency and weak signal at physiological pH in MS.

Potentiometric and spectroscopic methods have also been used to elucidate the structural and optical properties of Cu^{2+}–OT complexes [33], potentially explaining the mechanism of interaction between OXTR and Cu^{2+}–OT complexes. Early spectroscopic studies explored the secondary structure of vasopressin-like peptides, OT and Ala-vasopressin, with Cu^{2+}, as observed by circular dichroism and electron paramagnetic resonance (EPR), showing the formation of remarkably stable complexes upon Cu^{2+} binding [33]. However, there was limited information about the specific coordinated residues of OT with Cu^{2+}.

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Antoine and coworkers studied the gas phase of Cu^{2+}–OT coordination experimentally and theoretically [34]. They used photofragmentation of Tyr2 combined with MS and calculated the absorption of Tyr2 using resolution of identity (RI)—the second-order approximate coupled cluster singles and doubles model (CC2), time-dependent density functional theory, and semiempirical ab initio Model 1 (SAM1) methods [34]. OT undergoes a significant conformational change upon Cu^{2+} binding with large changes in the dihedral angles of specific amino acids, Tyr2 and Asn5, based on the crystal structure of OT complexed with neurophysin [34]. They hypothesized that Tyr2 might always point away from the ring and might not be strongly affected by Cu^{2+}, showing an abundance of cleaved peptides between Tyr2 and Ile3 and subtle differences in calculated Tyr2 absorption [34]. However, these theoretical calculations are based on the result obtained from the gas phase of OT, so OT might behave differently in an aqueous state, especially in a cellular context.

For a better understanding of OT behavior in a biologically relevant environment, Alshanski et al. determined the NMR structure of Cu^{2+}-oxOT by paramagnetic relaxation enhancement NMR (PRE NMR) (Figure 2B) [35]. It showed that a free Cys1 is required for Cu^{2+} binding, and the amide backbone of Cys1, Tyr2, Ile3, and Gln4 may coordinate Cu^{2+} with a π–cation interaction between the aromatic ring of Tyr2 and Cu^{2+} [35]. Our lab also reported characterization of both oxOT and rOT upon Cu^{2+} and Zn^{2+} addition by various biophysical methods in the aqueous state for comparing Cu^{2+} –OT and Zn^{2+}–OT [21]. We investigated their secondary structural changes through CD spectroscopy at 196 nm and 225 nm, representing disordered and alpha helicity regions, respectively [21]. Cu^{2+} increased the molar ellipticity of oxOT at both regions, but Zn^{2+} does not show a similar effect, indicating that Cu^{2+} induces different structural changes compared to Zn^{2+} [21]. In contrast, rOT appears highly disordered regardless of whether metal ions are bound [21]. We also applied CD spectroscopy for a competition assay of Cu^{2+} and Zn^{2+} binding to oxOT and rOT, showing tighter affinity toward Cu^{2+} than Zn^{2+}, consistent with previously reported studies [21]. We applied electronic absorption to visualize the effect of metal ions on Tyr2 absorption. Cu^{2+} induces a blueshift from 275 to 270 nm with increased intensity, whereas other metal ions cause a redshift to 295 nm [21]. This blueshift induced by Cu^{2+} was also consistent with the spectral increase at 515 nm, which indicates a *d*–*d* transition at the Cu^{2+} center [21].

These structural insights emphasize how Cu^{2+} coordination can remodel OT's backbone and aromatic environment in ways that could influence receptor signaling and oxidative stability under physiological conditions. Further investigation of Cu^{2+}- dependent redox modulation within OT systems may help clarify whether such effects extend to neural or cellular environments where the metal ion has been implicated in other pathogenic processes [17, 36].

4 | Structural Characterization of Zn^{2+}–OT Complexes

Zn^{2+} has also been extensively studied for its interactions with OT, potentially mediating the interaction between OT and OXTR. The Zhu group found that Zn^{2+} coordinates with OT at pH 5.0, but not at basic pH using ESMS study [28]. The Bowers group later investigated the coordination of Zn^{2+}–OT under physiological pH using MS and theoretical calculations,

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including Chemistry at Harvard Macromolecular Mechanics (CHARMM) and DFT [37]. They created CHARMM structures based on ion mobility experiments and hydration energy measurements [37]. These structures predicted that Zn^{2+} forms a nearoctahedral geometry coordinated with six backbone carbonyl oxygens (6O) from Tyr2, Ile3, Gln4, Cys6, Leu8, and Gly9 (Figure 3A) [37]. Additionally, the N-terminus of Zn^{2+}–OT is free from direct Zn^{2+} binding, with the side chains of Ile3, Gln4, and Asn5 pointing away from the Zn^{2+} binding cavity [37]. This creates a more hydrophobic surface on the Zn^{2+}–OT complex.

Hydration studies showed that Zn^{2+} is hidden from the water ligands [37]. Based on these results, they hypothesized that the hydrophobic surface of the Zn^{2+}–OT complexes and the free N-terminus might facilitate docking to OXTR [37]. A few years later, the Bowers group compared the coordination of OT with Cu^{2+} and Zn^{2+} as determined by MS and theoretical studies at acidic pH [30]. They reported a different theoretical structure of Zn^{2+}–OT determined by MD and DFT at acidic pH, showing that Zn^{2+} stills form an octahedral coordination geometry, but the N-terminus can be a competitive ligand (Figure 3B) [30]. In other words, this structure showed a distorted octahedral coordination with five backbone carbonyl oxygens (one loosely bound) and the N-terminal-NH_2 group (5O-1N) [30].

Continuing the study of Zn^{2+}–OT interactions, in 2016, the Clemmer group used ion mobility spectrometry–mass spectrometry (IMS–MS) to investigate OT conformations in the absence of Zn^{2+} at pH 3 [38]. They observed that OT has two conformations due to Cys6 and Pro7 existing in both *cis*- and *trans*-configured populations (Figure 4) [38]. However, upon Zn^{2+} binding, most Zn^{2+}–OT complexes adopt the *trans*-configuration [38]. The binding affinities of Zn^{2+} for each configuration differ, with the *trans*-isomer having a K_a of 1.43 μM^{-1} and the *cis*-isomer having a K_a of 0.42 μM^{-1} [38]. They hypothesized that the active configuration of Zn^{2+}–OT is the *trans*-isomer, suggesting that the unbound *trans*-isomer binds Zn^{2+} and then interacts with OXTR [38]. Additionally, they proposed another scenario where Zn^{2+} induces isomerization from *cis* to *trans*, and this complex may then bind with OXTR [38]. However, proving the detailed mechanism of this metal-related isomerization is experimentally challenging.

The above studies focused on the predicted coordination of gasphase OT with Zn^{2+} binding. In contrast, many researchers have investigated the aqueous state of Zn^{2+}–OT to identify OT degradation products under various solvent and temperature conditions and to improve OT stability using divalent metal ions [39–42]. OT is highly unstable at elevated temperatures, which is particularly problematic in tropical countries [43]. Even at 30°C or higher, OT easily dimerizes [44]. Avanti and coworkers showed the effect of the buffers and metal ions, such as Ca^{2+}, Mg^{2+}, and Zn^{2+} on the stability of OT using LC–MS, isothermal titration calorimetry (ITC), reversed-phase high-performance liquid chromatography (RP-HPLC), and size exclusion HPLC (HP-SEC) [39, 41]. They found that OT is more stable in citrate buffer at pH 4.5 when it binds Zn^{2+} compared to Ca^{2+} and Mg^{2+}, even at 70°C [39]. However, OT is unstable in acetate buffer [39]. They hypothesized that Zn^{2+}-bound OT becomes more hydrophobic, which may prevent water molecule-induced aggregation or dimerization [41]. Similarly, OT is stable in aspartate buffer at pH 4.5, and the presence of divalent metal ions suppresses OT dimerization under the same solvent conditions, as monitored by various NMR techniques, such as nuclear Overhauser effect spectroscopy

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(NOESY), $^{1}H–^{13}C$ heteronuclear single-quantum coherence (HSQC), and $^{1}H–^{15}N$ HSQC [40]. Consistent with the effect of citrate, Zn^{2+} stabilizes OT more effectively than Ca^{2+} and Mg^{2+} in the aspartate buffer [42]. Their biophysical studies suggest that divalent metal ions in both citrate and aspartate buffers may prevent disulfide bond formation, resulting in the suppression of dimerization [41]. They also hypothesized that the complex of metal and citrate may suppress Cys-mediated intermolecular or intramolecular interactions, and Zn^{2+} particularly interacts with OT in the aspartate buffer, leading to a more hydrophobic and stable complex [41]. Although these studies provided insights into the stability of aqueous OT with metal ions, which may affect storage and formulation, connecting these findings to biological implications remains challenging due to pH differences and diverse cellular buffering systems. Recent work continues to underscore the role of Zn^{2+} as a structural modulator of peptide conformation and intermolecular association. Applying these principles specifically to oxytocin could reveal how Zn^{2+} coordination shapes its aggregation propensity, stability, and receptor engagement [18, 45].

5 | Oxytocin-Based Metal Sensors and Applications

Due to its distinct binding characteristics with Cu^{2+} and Zn^{2+}, OT has emerged as an attractive platform for developing peptide-based technologies, including sensors to detect these metal ions in various samples, such as disease biomarkers and environmental contaminants [46–50]. Yitzchaik and coworkers developed a series of OT-based surface sensors by covalently anchoring OT to either gold or glassy carbon electrodes for detecting Cu^{2+} and Zn^{2+} [46, 47]. They were able to monitor Cu^{2+} and Zn^{2+} at physiological pH, with detection limits of 500 fM and 100 fM for Cu^{2+} and Zn^{2+} ions, respectively [46]. However, these sensors lacked selectivity between the two metal ions at physiological pH. For example, in the presence of Cu^{2+}, the probes could not selectively detect Zn^{2+}. The researchers found that these sensors could bind Cu^{2+} selectively at high pH [48]. To shift the metal selectivity toward Zn^{2+} over Cu^{2+}, they modified the N-terminal amine of OT with lipoic acid, reasoning that blocking the N-terminal amine would disrupt Cu^{2+} binding due to the metal ion's expected square planar coordination involving the N-terminal amine [49]. Indeed, this *N*-acetylated OT-gold surface probe demonstrated higher Zn^{2+} selectivity than Cu^{2+} at physiological pH detection of Zn^{2+} from sub-pM to sub-μM levels [49]. Similarly, another selective Zn^{2+}–OT was developed by functionalizing the N-terminal amine of OT with a dodecyl group, resulting in high sensitivity to Zn^{2+} [50]. These studies provide insights into the distinct coordination chemistry of Cu^{2+} and Zn^{2+}, as well as strategies for tuning metal selectivity of OT toward Zn^{2+} over Cu^{2+}.

6 | Other Metal Ion–Oxytocin Complexes

6.1 | Ca^{2+} and Mg^{2+}

The interaction of OT with Ca^{2+} has been investigated because OT can cause Ca^{2+} influx in the uterine myometrium by various pathways [24]. Ananthanarayanan and coworkers reported that OT can bind two Ca^{2+} ions with K_d of 50 μM in TFE buffer, which mimics a lipid environment [51]. They hypothesized that Ca^{2+} plays a significant role in the interaction between OT and OXTR because the extracellular Ca^{2+} concentration is estimated

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in the millimolar range, and OT can increase intracellular Ca^{2+} level by activating the $G_{q/11}$ signaling pathway [51]. To understand its Ca^{2+}-dependent secondary structure, they employed CD spectroscopy [51]. Compared to apo-OT, two equivalents of Ca^{2+} induced a large conformational change in OT, presumably increasing the π–π transition of the aromatic ring of Tyr2 and altering backbone conformation [51]. To further investigate the coordination of OT with two equivalents of Ca^{2+}, they monitored tyrosine fluorescence [51]. The addition of Ca^{2+} induced a decrease in tyrosine fluorescence, indicating that the Tyr2 residue was pointing away from the ring moiety where Ca^{2+} is potentially bound [51]. These studies highlighted the presence of two Ca^{2+} binding sites, leading to investigations of two truncated forms of OT: the tripeptides Pro7–Leu8–Gly9-NH_2 and tocinoic acid, which is the ring moiety of OT [51]. As expected, the mixture of these two components upon Ca^{2+} binding did not behave similarly to intact OT, yielding different CD spectra [51]. To visualize the Ca^{2+}–OT structure, they used combined 2D NMR methodologies, such as correlation spectroscopy, total correlation spectroscopy, and NOESY, along with molecular modeling studies under the same conditions [52]. They identified two Ca^{2+} binding sites: one in the ring moiety and one in the tail segment [52]. From these collective data, they proposed a two-step Ca^{2+} binding mechanism for OT [51, 52]. Initially, the first Ca^{2+} interacts with the tail segment of OT, moving it closer to the ring moiety. Subsequently, the second Ca^{2+} binds to the ring moiety, causing the tail to detach from the ring. They suggested that this 2 Ca^{2+}–OT complex might interact with OXTR to trigger the signaling pathway [51, 52]. Although this study provided insights into the potential role of Ca^{2+} in OT structures and activities, correlating these findings to physiological conditions is challenging because the TFE buffer used for in vitro studies does not accurately reflect the membrane and extracellular matrix environment.

The effect of Mg^{2+} on the structure of OT was similarly investigated. While Ca^{2+} induces a large conformational change in OT to a more helical structure, Mg^{2+} causes a smaller and opposite structural change compared to its Ca^{2+} binding [51]. This suggests that OT behaves differently when binding to two equivalents of Mg^{2+}. The authors also observed tyrosine fluorescence of OT with Mg^{2+}, showing similar trend as Ca^{2+} binding in TFE buffer [51]. These findings led them to hypothesize that there is a structural difference between Ca^{2+}–OT and Mg^{2+}–OT complexes and the difference may result in different bioactive forms and receptor interactions [51].

Another study investigated the conformation of OT with Mg^{2+} to understand OT's stability, alongside Zn^{2+}. The stability of OT with Mg^{2+} in the aqueous solutions, such as aspartate or citrate buffer at acidic pH, was measured by NMR, ITC, and LC–MS [39, 41, 42]. Unlike Zn^{2+}, Mg^{2+} does not stabilize OT, showing relatively weak affinity [39, 41, 42], subtle heat change in ITC [41], and small chemical shifts from the backbone of Cys1, Tyr2, and Ile3 in NMR [42]. These small chemical shift changes in the presence of Mg^{2+} may result from a cation–π interaction between Mg^{2+} and the aromatic ring of Tyr2 [42]. However, the interaction between Mg^{2+} and OT is so weak that Mg^{2+} cannot protect against OT's degradation [42].

Recently, Meyerowitz et al. reported the cryo-EM structure of the OT–Mg^{2+}–OXTR complex (Figure 5) [26], suggesting that Mg^{2+} plays a role in OXTR activation by cyclic

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OT. This structure represents the first and currently only available high-resolution model of OXTR bound to a metal–OT complex. Mg^{2+} is surrounded by water molecules that form hydrogen bonds with the backbone of Pro7 of OT and Arg, Asp, and Glu residues of OXTR within a 15 Å distance from Mg^{2+}. They also showed that Mg^{2+} is not required for OT binding only but is essential for activation of OXTR [26]. This structure provides insights into the metal-mediated coordination between OT and OXTR, but the mechanism of Mg^{2+}-dependent OXTR activation by OT and the metal specificity of OT and OXTR still remains unclear.

6.2 | Mn^{2+}

Mn^{2+}–OT coordination was also investigated by NMR in DMSO, which provides a hydrophobic solvent environment, using different OT variants [53]. They found that, particularly in Thr4-OT mutant (substitution of Gln with Thr at 4^{th} position of OT), Mn^{2+} coordinates with the amide carbonyls of Asn5 and Gly9, and potentially forms a hydrogen bond between the hydroxyl group of Thr4 and a water molecule [53]. Another combined computational and ESMS study proposed a different model [28], suggesting that Mn^{2+} coordinates octahedrally with OT without a water molecule. This indicates that the carbonyl oxygen atoms from the backbone are involved in Mn^{2+} coordination, with no water molecule occupying the axial position. At pH 9, they predicted that Mn^{2+} coordinates with four nitrogen atoms from the OT backbone in a square planar and two oxygens from the amide of Gln4 and Cys6 at the axial positions, with Tyr2 pointing away [28]. Although these studies provide insights into the coordination of Mn^{2+}–OT and its potential biological role in activation of OXTR, it remains challenging to determine the direct effects of Mn^{2+} on the OT–OXTR interaction under physiological conditions, including Mn^{2+} levels, pH, and solvents.

6.3 | Co^{2+}

Co^{2+} induces the highest effect on the interaction between OT and its receptor in the rat mammary gland tissue [10, 11], and subsequent computational studies confirmed the OT peptide binding to Co^{2+} in both gas and solution phases, aligning with the experimental results [54]. Nevertheless, it remains unclear whether under physiological conditions, Co^{2+} directly coordinates with OT or its receptor. Due to the difficulty in studying Co^{2+} binding to OXTR, researchers have turned their attention to understanding the coordination chemistry of Co^{2+}–OT. To investigate this interaction, Co^{2+}–OT complexes were analyzed using ECD–MS [29], alongside Cu^{2+}–OT complexes. The most abundant cleavage products were observed at the amide bond between Tyr2 and Ile3, similar to other metal ions like Ni^{2+} or Zn^{2+} [29]. This suggests that Co^{2+} shares a similar octahedral coordination with these ions. Despite Co^{2+} appearing to enhance the OT–OXTR interaction in mammary gland tissue, the low physiological levels of ionic Co^{2+} and the relative low binding affinity of OT for Co^{2+} make it challenging to determine how Co^{2+} contributes to OXTR activation by OT.

6.4 | Ni^{2+}

Ni^{2+} has also been studied to determine the binding kinetics and the coordinated residues in comparison to Cu^{2+}–OT coordination. Pettit and coworkers demonstrated that at pH levels above 9.0, Ni^{2+} coordinates with four nitrogen atoms in a square planar manner,

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showing slow interaction kinetics spectroscopically [27]. Later ESMS studies and molecular modeling confirmed that at pH 9.0, Ni^{2+} forms stable 4N complexes, similar to Cu^{2+} [28, 54]. It was proposed that three amide nitrogen atoms are coordinated to Ni^{2+} at three corners of the square, while the amino nitrogen of Cys1 projects outside, away from the fourth corner, with Tyr2 pointing away [28]. This coordination geometry likely reflects both electrostatic effects and a high-pH-driven backbone deprotonation mechanism for Ni^{2+} binding, and therefore may not directly translate to physiological conditions. Accordingly, the direct relationship between Ni^{2+} and OT in activation of OXTR remains largely unexplored, and specific studies are needed to determine any significant biological interactions or effects.

6.5 | Pd^{2+}

Pd^{2+}–OT complexes have been investigated to understand their carcinostatic and toxic side effects as well as to explore potential new catalytic processes or reaction mechanisms related to OXTR activation [55, 56]. An early study using ESMS proposed that Pd^{2+} coordinates with OT via the N-terminal amino group and one sulfur atom from two Cys residues in acidic conditions [28]. However, it was unclear which sulfur atom was coordinated. A subsequent study using Pd(en) suggested that in the *trans* OT, Pd^{2+} binds with one sulfur atom from Cys1 and the nitrogen atom from the amino group of Cys1, while Tyr2 points slightly away from the tocin ring [57]. These findings provide insights for the development of diagnostic tools and therapeutic strategies in medicine and catalysis.

7 | Comparative Analysis, Physiological Relevance, and Future Directions

7.1 | Summary of Metal–Oxytocin Interactions

The diverse metal-binding properties of OT reveal distinct coordination modes and biological effects for different divalent metals (Table 2). Cu^{2+} exhibits the highest binding affinity (K_d = 11 μM) [11] and unique redox chemistry, forming predominantly nitrogen-coordinated complexes with significant structural rearrangements. In contrast, Zn^{2+} shows moderate affinity (K_d = 200 μM) [11] and forms oxygen-coordinated octahedral complexes that increase OT's hydrophobicity and influence *cis*-/*trans*-isomerization. Mg^{2+} and Ca^{2+} demonstrate essential roles in receptor activation despite relatively weak direct binding to OT, suggesting that their primary function involves stabilizing the OT–OXTR complex rather than forming stable OT chelates.

7.2 | Physiological Considerations

The physiological relevance of these metal–OT interactions must be considered in the context of tissue-specific metal availability and concentrations. Extracellular Mg^{2+} concentrations (≈1 mM) [58] and Ca^{2+} concentrations (≈2.5 mM) [59] are well above the binding affinities measured for OT, suggesting that these interactions are physiologically relevant. Cu^{2+} and Zn^{2+} concentrations in biological fluids are typically much lower (μM to nM range) [60, 61], but their high binding affinities, particularly for Cu^{2+}, suggest that selective interactions may still occur under specific physiological conditions.

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The tissue-specific expression of metal transporters and the local microenvironment around OXTR-expressing cells may create conditions where metal–OT interactions become functionally significant. For example, synaptic clefts and specialized cellular environments may concentrate specific metals sufficiently to influence OT signaling [62].

7.3 | Future Directions and Implications

Several critical knowledge gaps remain in understanding metal–OT interactions and their biological significance. First, the precise mechanisms by which different metals modulate OXTR activation require further investigation using reconstituted systems with purified components. Second, the physiological conditions under which these metal–OT interactions occur in vivo need clarification through tissue-specific metal concentration measurements and functional studies. The development of OT-based metal sensors represents a promising application of this fundamental research, with potential uses in environmental monitoring and clinical diagnostics. The ability to tune metal selectivity through chemical modification of OT's N-terminus opens possibilities for developing highly specific detection systems. From a therapeutic perspective, understanding metal–OT interactions may inform the development of more stable OT formulations for clinical use, particularly in regions with challenging storage conditions. Additionally, metal-mediated modulation of OT activity could provide new approaches for treating conditions involving dysregulated oxytocin signaling, such as autism spectrum disorders or social anxiety.

Recent studies have further broadened context for these efforts. OT has been shown to protect dopaminergic neurons and preserve nigrostriatal signaling in Parkinson-like models [14] and to attenuate β-amyloid-associated toxicity and cognitive decline in Alzheimer paradigms [15, 16, 63]. These findings highlight OT's emerging neuroprotective potential and point toward possible intersections with metal-regulated pathways. In parallel, new work on copper homeostasis and "cuproptosis" [17, 64], zincdriven peptide aggregation [45], and the homeostasis of iron and other brain metals [18, 19, 65] has clarified how metal imbalance contributes to neuronal dysfunction. Even nontraditional cations, such as lithium, have been implicated, with lithium deficiency recently linked to accelerated Alzheimer's onset [20]. Together, these discoveries suggest that advancing our molecular understanding of OT–metal coordination could bridge mechanistic bioinorganic chemistry with the physiology of neuroprotection.

Going forward, efforts to integrate structural and cellular perspectives will be essential. Determining how metal binding alters OT conformational ensembles under physiological redox and ionic conditions, and whether these changes bias OXTR signaling pathways, represents a critical next step. Bridging in vitro coordination chemistry with in vivo neurochemical models could reveal whether metal-bound OT states participate directly in regulating neuronal excitability, oxidative balance, or peptide stability. Ultimately, such insights may guide the rational design of OT analogs and delivery systems that exploit metal coordination for enhanced stability, receptor selectivity, and therapeutic durability.

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8 | Conclusion

OT, a peptide hormone with nine amino acids, plays a significant role in reproduction, childbirth, lactation, nervous system, and social bonding across all vertebrates. Its ability to bind metal ions, such as Mn^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+} and Co^{2+}, has been a subject of study for several decades. Early studies showed that divalent metal ions enhance OT binding to its receptor (OXTR), with Mn^{2+} and Co^{2+} being particularly effective. Recent studies have further examined the distinct roles of Zn^{2+} and Cu^{2+} in modulating OT's bioactivities, structure, and receptor interaction. Cu^{2+} tends to stabilize OT through nitrogen coordination, inducing significant structural changes and affecting receptor binding differently than Zn^{2+}. Meanwhile, Zn^{2+} alters OT's hydrophobicity and isomerization, impacting its interaction with OXTR. Mg^{2+} and Ca^{2+} play essential roles in receptor activation and signaling, despite showing relatively weak direct binding to OT.

Biophysical approaches have provided deeper insights into the metal-binding properties of OT, but challenges remain in fully understanding which metal ion interacts with OT and OXTR and the molecular mechanisms controlling these interactions, particularly in physiological environments. The development of OT-based sensors for Cu^{2+} and Zn^{2+} detection further highlights OT's potential as a platform for detecting metal ions. However, the precise coordination chemistry, stability, and bioactivity of OT–metal complexes, especially in the cellular context, require further investigation to elucidate their roles in biological processes and potential therapeutic applications.

The emerging pictures suggest that metal ions serve as important modulators of OT function, with different metals contributing distinct regulator mechanisms. Understanding these interactions may lead to new therapeutic strategies and improved clinical formulations of this crucial neuropeptide hormone.

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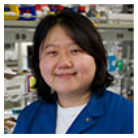
Biographies



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Data Availability Statement

No datasets were generated or analyzed during the current study.

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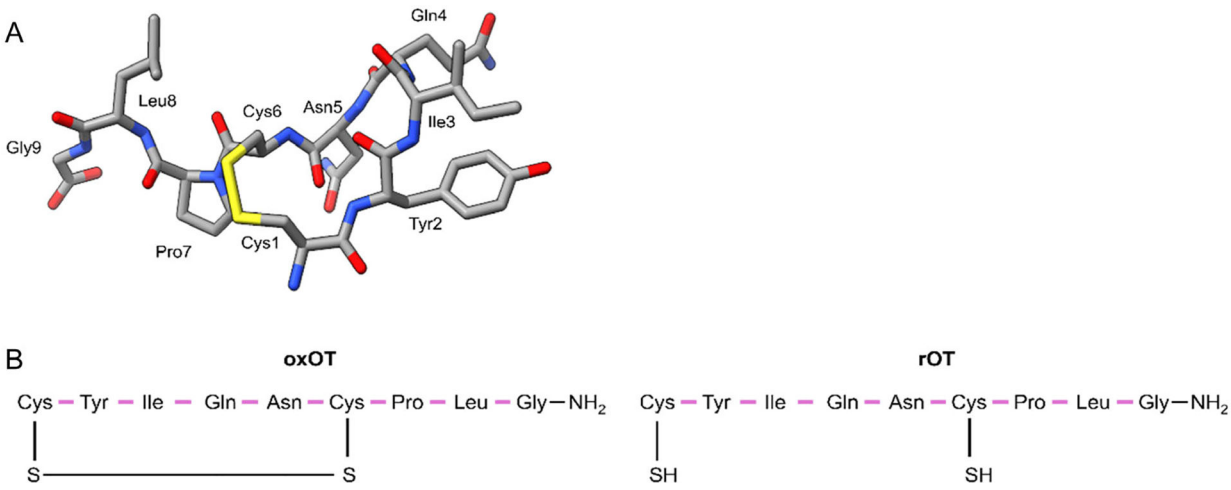

FIGURE 1 |.

Chemical structures of oxidized oxytocin (oxOT) and reduced oxytocin (rOT). (A) NMR solution structure of oxOT (PDB: 2MGO), with heteroatoms colored as follows: N (blue), S (yellow), and O (red) [6]. The deposited PDB coordinates depict a free C-terminal carboxylate [6], though native oxOT is believed to be natively produced with an amidated C-terminus. (B) Amino acid sequences of oxOT and rOT with a C-terminal amide (-NH_2). A corresponding experimental 3D structure for rOT is not currently available. Because NMR structures are typically determined in solution, rOT readily undergoes reoxidation during analysis, which complicates structural determination of a stable rOT structure under standard conditions.

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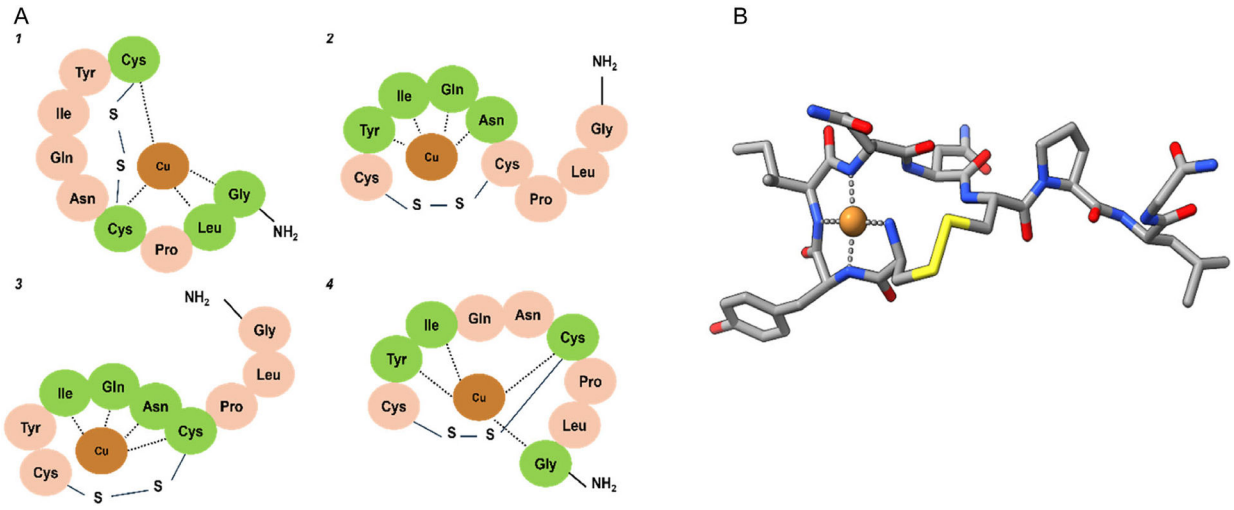

FIGURE 2 |.

Cu^{2+} binds oxOT with different coordination modes. (A) Different predicted Cu^{2+}-oxOT binding modes determined by CID-MS. Residues involved in Cu coordination are highlighted in green, and the specific coordinating atoms are described in the text. Dashed lines indicate coordination to Cu. (1: pH 10, 2: pH 8–9, 3: pH 7, 4: pH 3) (B) NMR structure of Cu^{2+}-bound oxOT. (PDB: 7OTD). The coordination modes shown here represent oxOT. Experimental characterization of Cu coordination to rOT is still needed and may reveal coordination geometries distinct from those proposed for oxOT.

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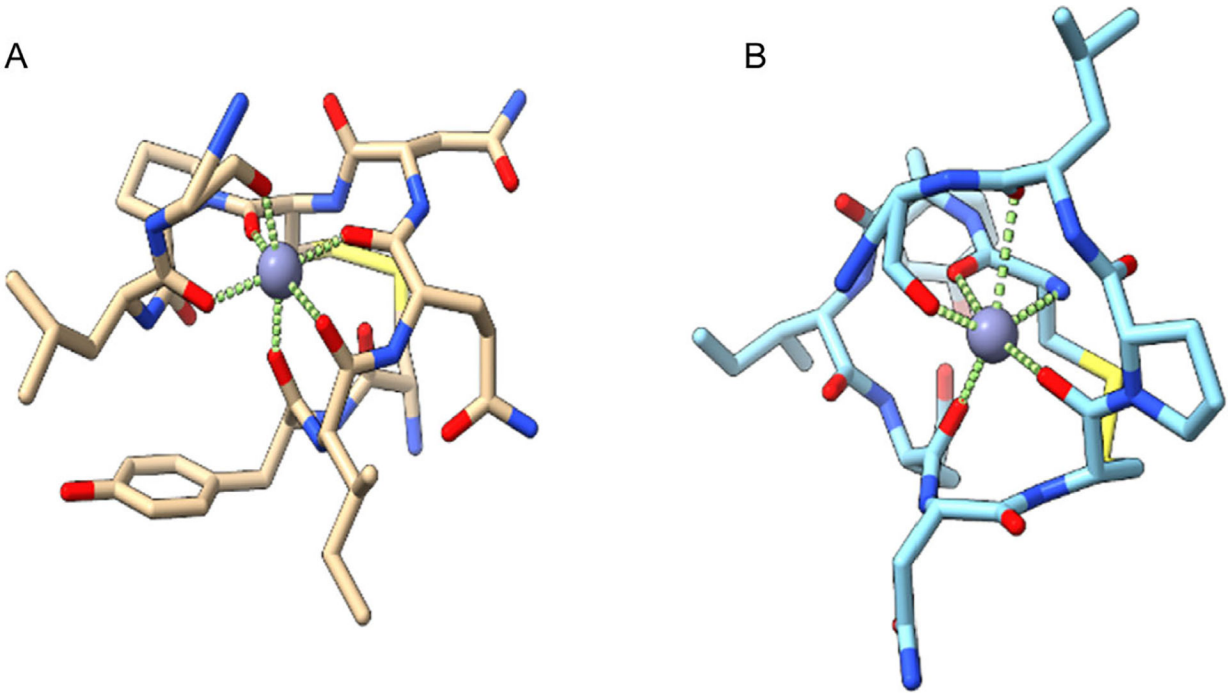

FIGURE 3 |.

Predicted Zn^{2+}-oxOT coordination determined by MS and DFT at acidic pH. (A) 6O coordination. (B) 5O-1N coordination. The greendashed line represents a loosely bound C═O or N–H to Zn^{2+}. MS = Mass spectrometry; DFT = density functional theory.

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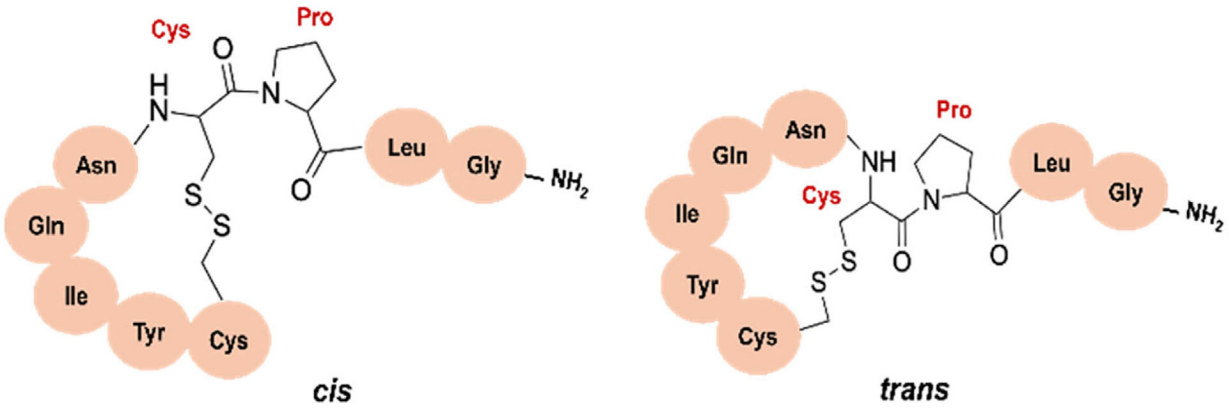

FIGURE 4 |.
Configurations of the *cis*- and *trans*-isomers of OT at the Cys6—Pro7 bond.

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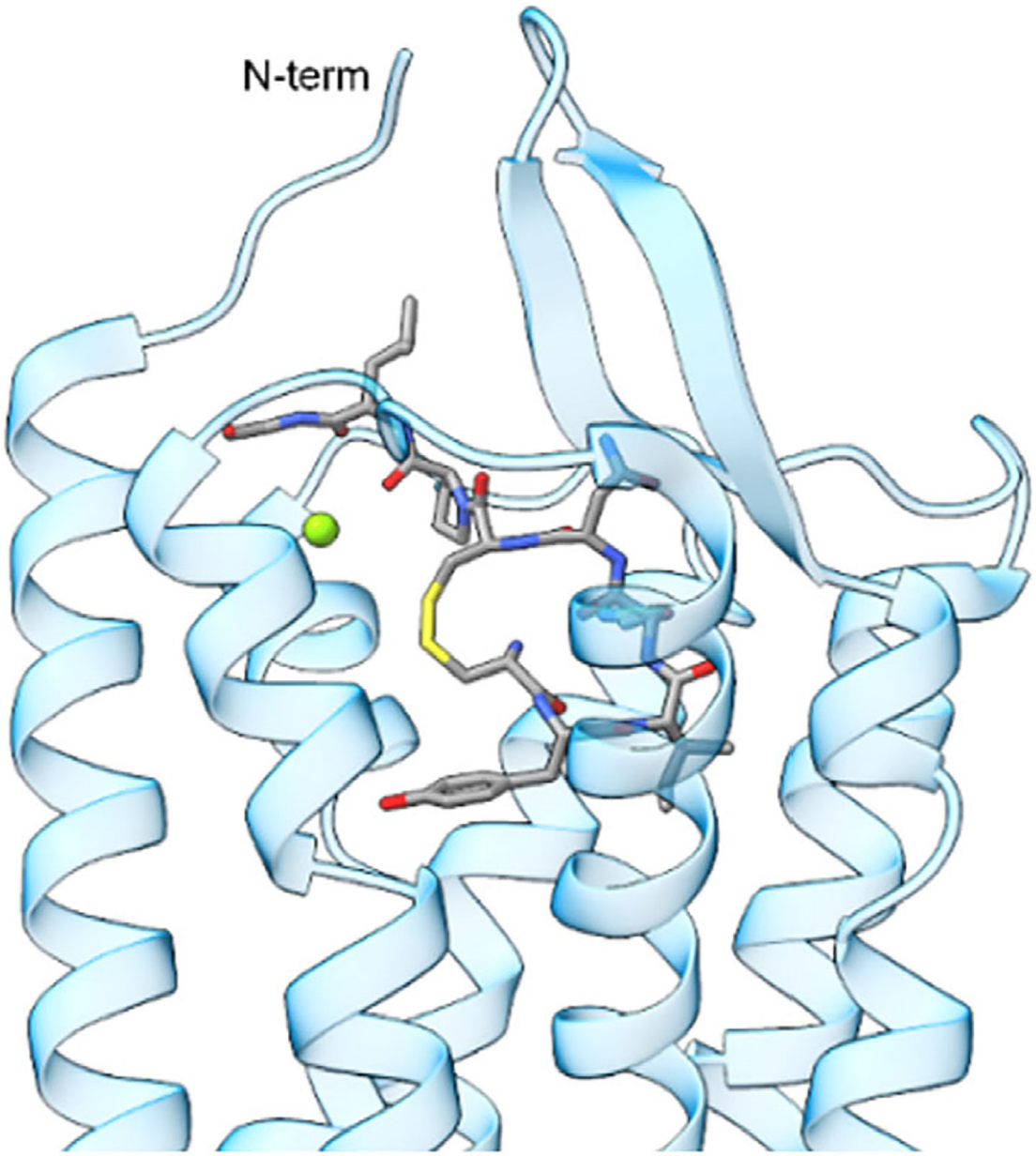

FIGURE 5 |.
Cryo-EM structure showing the interaction between Mg^{2+} (green), oxOT (dark gray), and OXTR (blue). (PDB: 7RYC).

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TABLE 1 |

Determination of oxOT's affinities to divalent metal ions at pH 6.5 determined by Cu^{2+} competition assay using a solid-state Cu^{2+} electrode [11].

Metal ions, M^{2+}	K_d, μM
Cu	11
Zn	200
Co	250
Mg	330
Ca	500
Ni	500

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TABLE 2 |

Comparative summary of msetal–OT interactions.

Metal ion	Binding affinity, K_d, μM [11]	Primary coordination mode	Structural effects	Key methods	Biological function	Cell/tissue models
Cu^{2+}	11	4N square planar	Significant conformational change, Tyr^2 interactions	MS (ESMS/ECD/CID/IMS-MS), DFT/MD, NMR, EPR, CD, UV-Vis	MAPK suppression (oxOT)	Rat mammary tissue, human uterine tissue, HEK293T-OXTR
Zn^{2+}	200	6O octahedral	Increased hydrophobicity, *cis-/trans*-isomerization	MS (ESMS/IMS-MS/LC-MS), ITC, RP-HPLC/HP-SEC, NMR, DFT/MD/CHARMM	MAPK activation (rOT)	Rat mammary tissue, HEK293T-OXTR
Co^{2+}	250	6O octahedral	Similar to other divalent metals	ECD-MS, DFT	Enhanced receptor binding	Rat mammary tissue
Mg^{2+}	330	Weak cation-π	Minimal direct structural change	NMR, CD, Tyr^2 fluorescence, ITC, LC-MS, cryo-EM	Essential for OXTR activation	Rat mammary tissue, rat uterus
Ca^{2+}	500	2-site binding	Tail-ring conformational changes	NMR, CD, Tyr^2 Fluorescence	Dual effects on receptor binding	Rat mammary tissue
Ni^{2+}	500	4N square planar	Similar to Cu^{2+} at high pH	ESMS, potentiometry	Limited biological data	Rat mammary tissue

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