 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

Further structural analysis of GnRH complexes with metal ions

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Submitted: March 13, 2005 *Accepted: April 3, 2005*

Key words: **GnRH; Metal complexes; Maldi-Tof mass spectrometry**

Neuroendocrinol Lett 2005; **26**(3):35–252 **PMID:** 15990730 NEL260305A14 © Neuroendocrinology Letters **www.nel.edu**

Abstract

MALDI-TOF mass spectrometry, ^{1}H NMR spectrometry, the continuous variation method and molecular modeling by MM3 calculation confirmed our earlier studies showing that gonadotropin-releasing hormone (GnRH) forms complex with copper(II) ion with the binding ratio 1:1. The copper(II) complex formed at physiological pH has a square planar configuration and GnRH complexes with nickel(II) and cobalt(II) ions are less stable than that of copper(II).

1. Introduction

The gonadotropin-releasing hormone (GnRH) is known to play an important role in the mediation of the neuroendocrine control of reproductive processes.

GnRH is released from nerve terminals in hypothalamus into the portal circulation in a pulsatile way and binds at the surface of gonadotropic cells in the anterior pituitary with the specific receptor. Binding of GnRH to its receptor induces the series of intracellular events leading to the biosynthesis and release of gonadotropins LH and FSH.

Copper and other metals like zinc, cobalt, manganese and molybdenum maintain living organisms through catalytic and structural roles they play in proteins and other biomolecules. Observations that ovulation in rabbits can be induced by systemic administration of copper salts [1, 2] suggested the possibility that blood-born copper could be involved in the regulation of reproduction in mammals. In the series of experiments Barnea and coworkers [3–7] demonstrated that copper complexed with amino acids or short peptides containing histidine, promoted

GnRH release both from isolated pituitary granules and tissue explants.

The previous work [8], on the binding of GnRH-metal complexes to rat pituitary receptor has shown that the Cu(II)-GnRH complex was more competitive to the GnRH receptor when compared to native GnRH, although the Ni(II) and Zn(II)-GnRH complexes were slightly less effective than the metal-free peptide. The metal complexation to GnRH has also a distinct effect on the ovulation activity of the hormone [9, 10]. Our recent work [11] has shown the high order of organization of the metal-free peptide in DMSO solution with two structured "domains" whose relative orientation is modulated by the mobility of the central glycine. The theoretical calculations performed for the Ni(II)-GnRH complex confirmed earlier studies that the metal ion could co-ordinate with four nitrogen atoms inducing a well defined arrangements of aromatic side-chains and a rigid backbone structure.

These results inspired us to further study the structure of the metal-GnRH complexes by MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time of Flight) mass spectrometry, ^{1}H NMR spectrometry, the continuous variation method [12] and the molecular modeling using the MM3 molecular mechanics calculation [13]. The earlier studies in aqueous solution with use of potentiometric and spectroscopic methods have shown that metal ions bind pyro-glutamyl-histydyl unit forming very stable equimolar complexes [14,15], especially in the case of Cu(II) and Ni(II) ions [16].

2. Materials and methods

2.1. Materials

GnRH was purchased from Peptide Institute, Inc (Osaka, Japan), and metal perchlorates, $Cu(ClO_4)$, $Ni(ClO_4)_2$ and $Co(ClO_4)_2$, were obtained Kanto Chemicals (Tokyo, Japan). All other reagents were of the highest grade commercially available.

2.2. Preparation of GnRH-metal complexes

GnRH (40 mg) was dissolved in distilled water (2 ml) and a little bit excess of the metal perchlorate salt dissolved in 15 ml of 99.5% ethanol was added. After the reaction mixture was stirred under reflux for about one hour and then cooled, diethyl ether was added to it to obtain the precipitated solid metal complex. The precipitate was filtered off, washed twice with cold ethanol and diethyl ether, and finally dried over anhydrous silica gel for a week.

2.3. MALDI-TOFMS measurement

Prepared metal (copper(II), nickel(II), cobalt(II)) complexes of GnRH were identified on a MALDI-TOF mass spectrometer (KRATOS KOMPACT MALDI) using CHCA (α-cynao-4-hydroxycinnamic acid) as matrix.

2.4. ^{1}HNMR measurements

Proton NMR spectra of GnRH-metal complexes were measured in D_2O on a JEOL JNM-A 500 spectrometer.

2.5. Continuous variation method

The binding ratio of copper(II) ion and GnRH was determined by the continuous variation method. The concentration of the original solutions of GnRH copper(II) ion were both 1.5×10^{-3} mol dm^{-3}. These two solutions were mixed at a given ratio, and the absorbance at 580 nm was measured. Absorbance of the complex (ΔA) was calculated according to the following equation,

$$\Delta A = A_{obs} - A_{ligand} - A_{metal}$$

where A_{obs} is observed of the mixture solution and A_{ligand} and A_{metal} are respectively, the absorbance of the GnRH solution and the metal ion solution with the concentration of 1.5×10^{-3} mol dm^{-3}. The binding ratio can be determined from the plot of ΔA against $X = V_{metal}/(V_{metal} + V_{ligand})$, in which V_{metal} and V_{ligand} are the added volumes of the individual solutions. The plot of Δa against X gives the maximum, whose X value corresponds to the binding ratio.

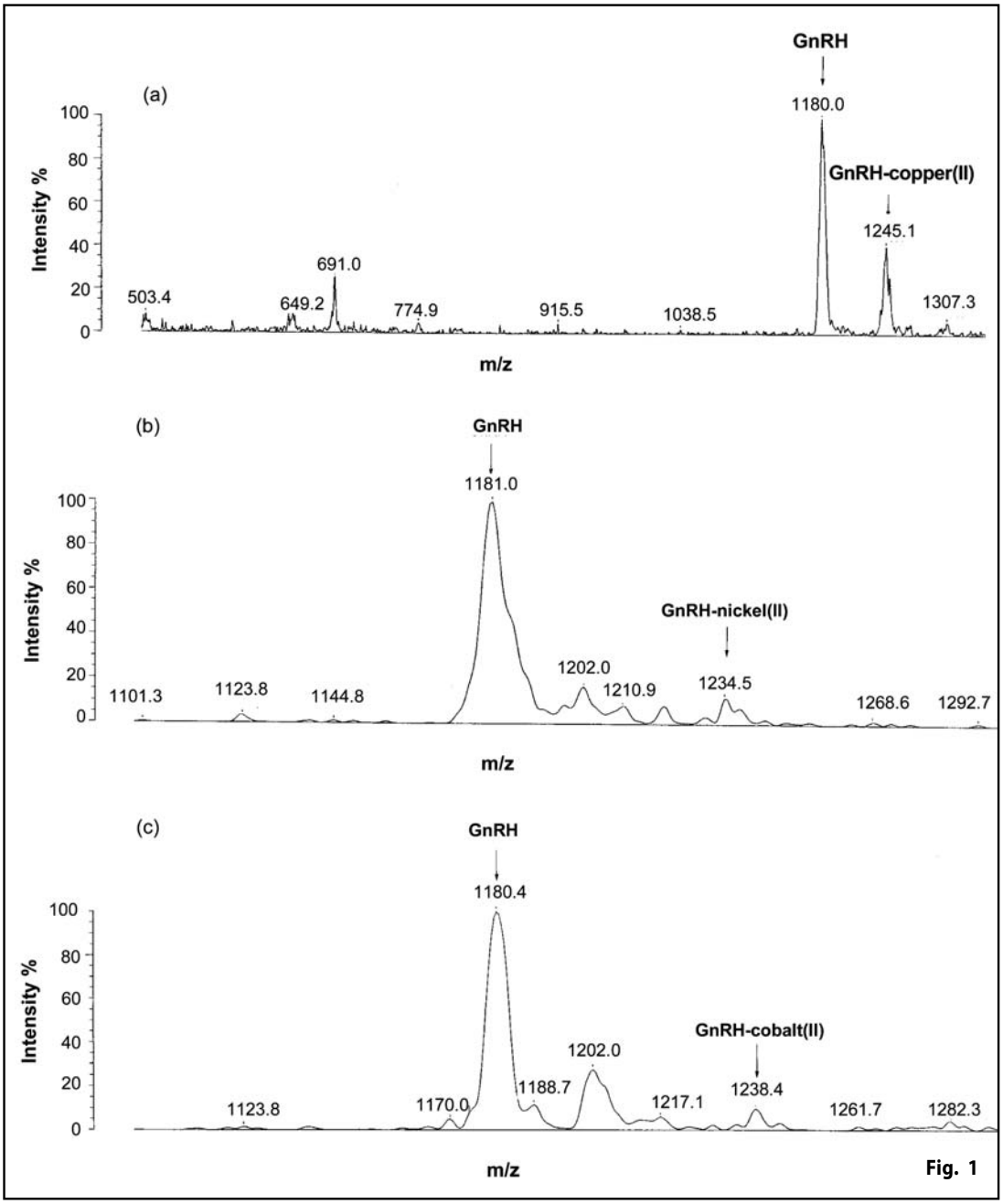
2.6. Molecular modeling

The structure of the GnRH-copper(II) complex was estimated by the molecular mechanics calculation (MM3) using the CAChe system (ver. 4.1; Oxford Molecular Group). Since the imidazole group of histidine residue is the most likely binding site to copper(II) ion [14–16], a shorter peptide composed of the N-terminal three amino acid residues (Glp-His-Trp-$NHCH_3$) was used in the MM3 calculation.

3. Results and discussion

The formation of the metal complexes was confirmed by the measurement of MALDI-TOFMS spectra (Fig. 1). It seems very likely that the 1:1 Cu(II)-GnRH complex (m/z=1245) is the most stable among the three metal complexes judging from the relative peak intensities. The stable formation of the 1:1 Cu(II) - GnRH complex was also confirmed by a FAB mass spectrum (m/z = 1244). Meta-stable signals corresponding to $[(GnRH)_n–Cu]^{m+}$(n, m#1) were, however, not observed, which suggests that the binding ratio of GnRH and copper(II) is 1:1 as it was suggested earlier [14–16].

The binding between GnRH and the metal ions was also followed by ^{1}H NMR spectroscopy. The proton signals of GnRH were significantly broadened with increasing Cu(II) ion until the ratio of GnRH and copper(II) reaches 1:1 (Fig. 2), while the signal broadening does not proceed so much when the ratio is 1:2. This may also indicate that the binding ratio of



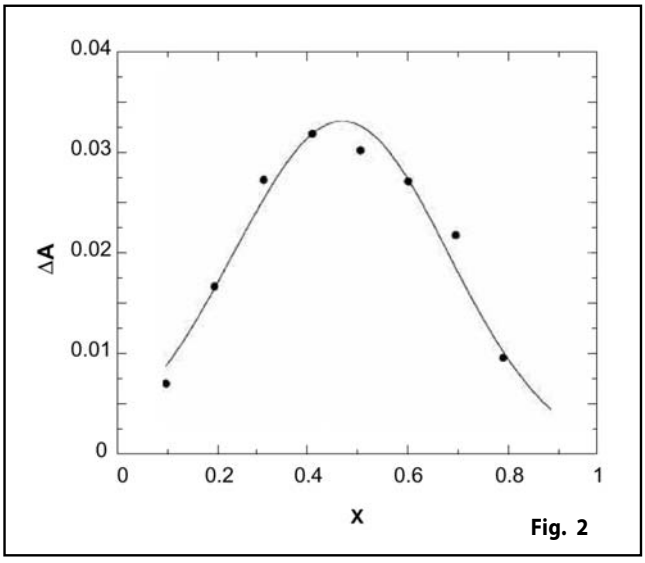


Figure 1: MALDI-TOF mass spectra of (a) GnRH-copper(II), (b)GnRH-nickel(II), and (c) GnRH-cobalt(II) complexes.

Figure 2: Continuous variation plot for the absorbance (λ=580 nm) of GnRH-copper(II) complex. The Sum of the initial concentration is 1.5×10^{-3}mol dm^{-3}.

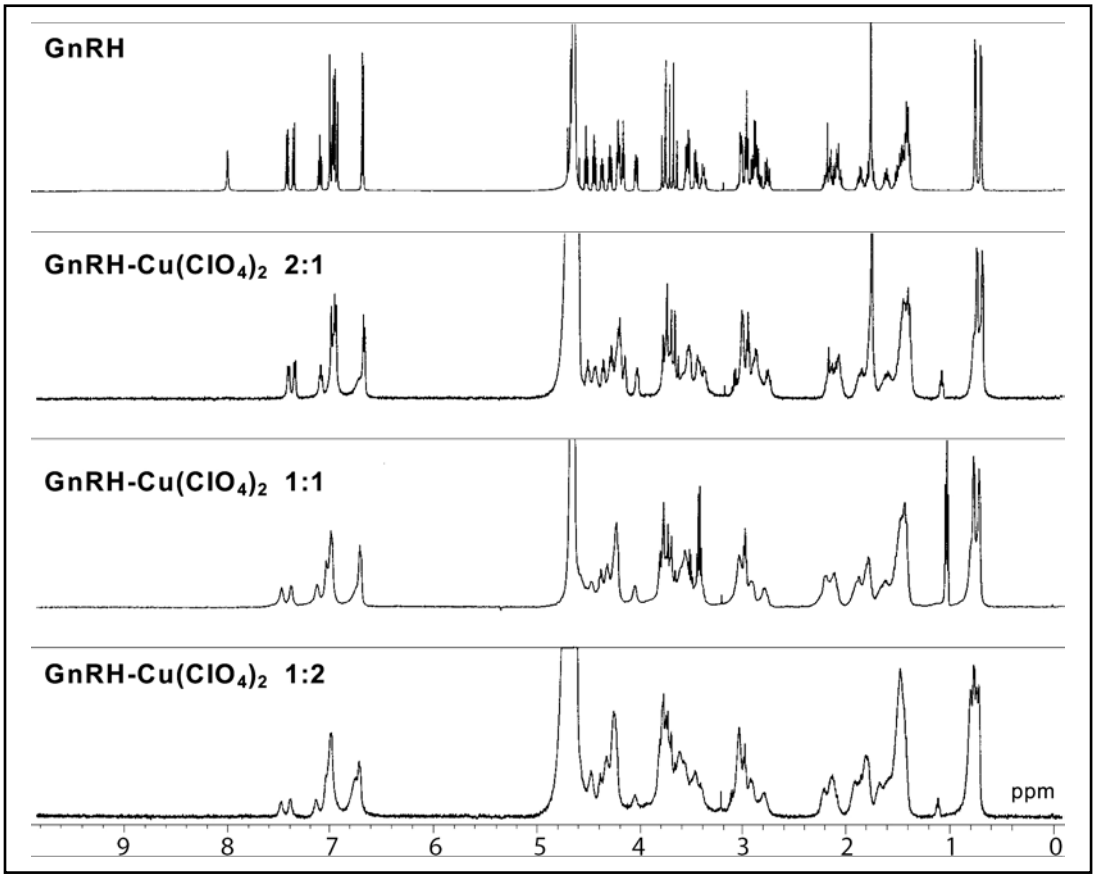


Figure 3: Effect of $Cu(ClO_4)_2$ on ^{1}HNMR spectrum of GnRH.

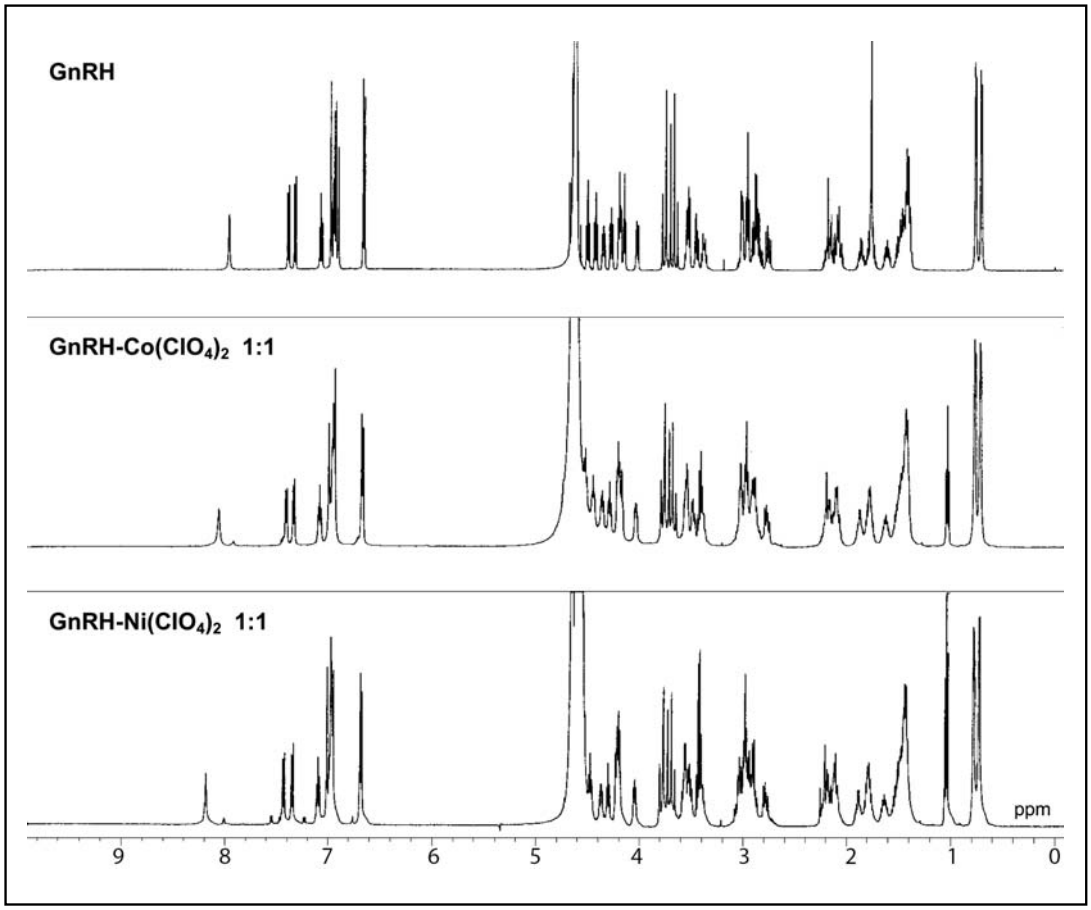


Figure 4: Effect of $Ni(ClO_4)_2$ and $Co(ClO_4)_2$ on ^{1}H NMR spectrum of GnRH.

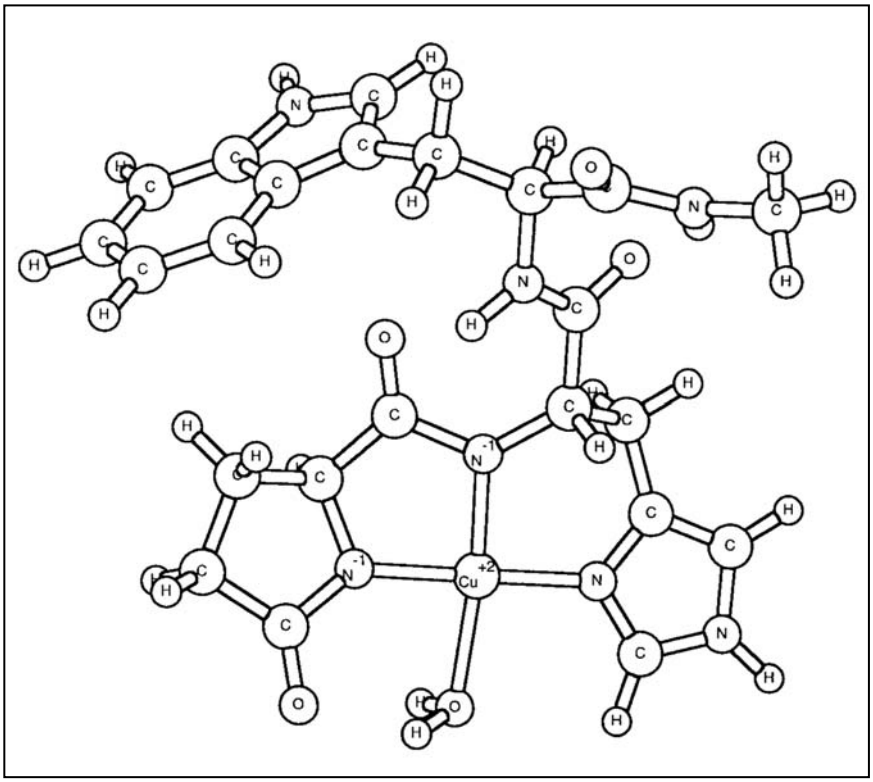


Figure 5: A proposed structure of the complex of Glp-His-Trp-$NHCH_3$ and Cu(II) obtained by MM3 calculation.

metal to GnRH is 1:1, which agrees with the results of MALDI-TOFMAS measurement.

To determine the binding ratio of GnRH and copper(II) ion, the continuous variation method was applied to the metal-peptide system. As shown in Figs. 3 and 4, there is a maximum at around X = 0.5, which obviously indicates that the binding ratio is 1:1. This result is also well compatible with those of the MALDI-TOF MS and ^{1}H NMR measurements. As the Ni(II) and Co(II) complexes do not have distinct absorption bands in the visible region, the GnRH-metal binding ratios were not determined by the this method.

Based on the fact that imidazole of His residue is the main binding site for Cu(II) ion the structure of the complex between Glp-His-Trp-$NHCH_3$ and metal ion was calculated with the MM3 calculation, though it was not fully optimized (Fig. 5). The three possible coordination sites are the imidazole nitrogen (N at position 1), the deprotonated amide nitrogen (N^-) of the histidyl residue, and the deprotonated amide nitrogen (N^-) of the pyroglutamic acid ring. The fourth coordinating site might be the oxygen (O) of a water molecule. It is well known that for Cu(II) complexes a square planar configuration is favored [14–16]. In fact, the complex illustrated in Fig.5 has a reasonable square planar configuration.

It has been suggested that the order of stability in the GnRH-metal complexes is Cu(II)>>Ni(II), Co(II). A biological assay demonstrates, on the other hand, that the Cu(II)-GnRH complex competes more efficiently in the binding to the GnRH receptor than native GnRH, while the Ni(II) and Co(II) complexes exhibit lower affinity than that of native GnRH. Nickel(II) complex formed within the physiological pH generally have a similar square planar as copper(II) complexes [14,16]. The difference in their biological activity could derive from different kinetics and complex stability (speciation) as it was revealed among others by the MALDI-TOF MS data.

These results confirm the earlier suggestions that GnRH complexes with metals could change activity in binding to the specific receptors due to modifications of GnRH molecule resulting in changes of a charge and spatial arrangements of the receptor and membrane in the neighborhood of receptor.

It has been shown that complexes of GnRH with Cu(II), Ni(II) and Co(II) modify also the intracellular signaling pathway by favoring the cAMP production, while metal-free GnRH induces only the IP3 production (unpublished results).

Further research on the properties of these complexes are rationale and can bring the new look on the mechanism of GnRH action.

Acknowledgements

These studies were performed in the frame of Japanese-Polish Governments scientific agreement No R-57 (Structural Analysis of Animal Hormones by Tofmass).

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