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Impact of the Cu(II) ions on the chemical and biological properties of goserelin – coordination pattern, DNA degradation, oxidative reactivity and *in vitro* cytotoxicity

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ABSTRACT

Goserelin acetate (Gos) as a synthetic analog of the mammalian gonadotropin-releasing hormone (GnRH) is widely used in the treatment of sex hormone-related conditions. In this paper we present the chemical and biological aspects of its interaction with Cu(II) ions. The mode of Cu(II) binding and the thermodynamic stability of the obtained complexes were characterized by potentiometry, UV–Vis and CD spectroscopic methods. The DFT calculations were applied in order to investigate and confirm the molecular structure of the studied systems. The experimental and theoretical results clearly indicated the involvement of three nitrogens from the peptide and two oxygens from the acetate moieties in the Cu(II) coordination under physiological conditions. The investigated metallopeptide caused single- and/or double cleavage of the sugar-phosphate backbone of the plasmid DNA in the reaction accompanied by endogenous substances such as hydrogen peroxide or ascorbic acid. The degradation of the DNA molecule occurred *via* the free radical mechanism. Calculations based on measured spectra allowed determining the kinetic parameters of $^{\cdot}OH$ formation. The cytotoxic effects of Gos and its metallo-derivative were tested *in vitro* towards two cancer cell lines (A549 – human lung adenocarcinoma, CT26 – mouse colon carcinoma).

1. Introduction

Despite their beneficial functions, estrogens, androgens and progesterone also play a key role in the progression of the hormone-dependent forms of carcinoma [1,2]. Numerous studies have indicated the impact of the steroid hormones in the pathogenesis of certain types of diseases, including: prostate, breast, ovarian, endometrial and colon cancers, as well as endometrial hyperplasia [3,4]. For instance, about 70%–80% of breast cancers and approximately 70% of ovarian cancers are estrogen receptor-α and/or progesterone receptor positive. Similarly, the presence of androgen receptor is a defining feature of the prostate carcinoma [5–8]. Production of the gonadal hormones is controlled by the pulsatile secretion of GnRH (gonadotropin-releasing hormone), the primary regulator of the hypothalamus-pituitary-gonadal axis (HPG) [9,10]. The mammalian GnRH is a decapeptide with β-II-turn-type conformation. Its folded shape is provided by the bend around the flexible Gly^6 residue and is essential for binding to the pituitary receptors (GnRHRs) [11]. The GnRH episodic secretion and activation of the endogenous GnRHRs are crucial for the normal reproductive function [12]. Apart from the nervous system, the receptors are highly expressed in human reproductive (breast, endometrium, ovary and prostate) and non-reproductive tissues (heart, adrenal, bladder, colon and lung), both in physiological and pathological conditions. Therefore, a novel modulatory role of GnRH in the context of tumors growth promotion, metastasis and angiogenesis has been reported [13–15]. Treatment of the hormone-dependent tumors is connected with the desensitization of the pituitary which suppress the secretion of GnRH and sex steroids, leading to pharmacological castration [15,16].

Comprehensive studies have indicated that metal ions may affect

Abbreviations: A549, human lung adenocarcinoma cell line; cAMP, cyclic adenosine monophosphate; CT26, mouse colon carcinoma cell line; DFT, Density Functional Theory; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; FDA, fluorescein diacetate; Gos, goserelin acetate; Glp, pyroglutamic acid; GnRH, gonadotropin-releasing hormone; GnRHR, gonadotrophin-releasing hormone receptor; HAc, acetic acid; HAsc, ascorbic acid; HPG, hypothalamus-pituitary-gonadal axis; ID, propidium iodide; IP3, inositol trisphosphate; MTT, 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide; NDMA, *N*,*N*-dimethyl-4-nitrosoaniline; PBS, phosphate-buffered saline; ROS, reactive oxygen species; TRF, thyrotropin releasing hormone

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the endocrine activity of GnRH [16]. The Cu(II)-GnRH complex is a unique hormonal analog. It contains a Cu(II) ion which is bound to three nitrogen atoms (one is derived from the imidazole ring of the His residue and two from the deprotonated amide groups) [17]. The *in vitro* and *in vivo* assays have reported that in comparison with the native hormone, the metallopeptide exhibited: (a) higher resistance against enzymatic degradation, (b) greater efficiency in binding towards the rat, ship and pig pituitary receptors and (c) greater ability in the ovulation induction and in consequence it revealed higher endocrine activity [16,18–20]. Moreover, the metal-containing derivatives induced a distinct intracellular signaling pathway which refers to the cAMP production, while the non-coordinated GnRH favoured the production of IP3 [21].

Those results prompted us to undertake relevant studies on the agonist of GnRH-goserelin (brand name ZOLADEX) commonly used in the treatment of the breast and prostate cancers [22]. Comparing to the native neuropeptide, the structural sequence of goserelin acetate (Gos) was modified in two positions: the Gly^6 residue was substituted by the bulky D-Ser(tBu) and the C-terminal Gly^{10} was replaced by the aza-Gly moiety (Fig. 1). Such modifications stabilized the required β-turn of the peptide drug and increased its binding affinity towards the membrane receptors [22,23].

Since the GnRHR (gonadotrophin-releasing hormone receptor) receptor is a promising target for the hormone therapy [24] and the Cu (II)-GnRH complex exhibited a greater potential in the context of the HPG axis regulation, we decided to investigate how Cu(II) ions may affect the chemical and biological properties of goserelin. The aim of this work was to: (a) study the interaction mode of Gos towards the Cu (II) ions, (b) verify the ability of this complex to promote the ROS (reactive oxygen species) generation and the DNA cleavage, (c) determine its cytotoxic effect against two cancer cell lines.

2. Experimental section

2.1. Materials

Goserelin acetate, gonadotropin releasing hormone, the pBR322 plasmid, $CuCl_2$ (with purity 97%) and other small chemicals were purchased from Sigma-Aldrich and used without further purification. Copper(II) complexes were synthesized *in situ*. Cell cultures were obtained from Professor Luis G. Arnaut's group (Chemistry Department, University of Coimbra, Portugal). All cell culture fluids were purchased from Immuniq (Poland).

2.2. Potentiometric studies

Potentiometric titrations of Gos and its Cu(II) complexes were performed at a constant temperature of 298 K under an argon atmosphere using a Metrohm 905 Titrando pH-meter. The measurements were carried out over the pH range 2.5–10.5 using a total volume of 1.5 mL. The CO_2-free NaOH solution at a concentration of 0.1021 M was used as a titrant. Before each measurement, the combined glass-Ag/AgCl electrode (Metrohm, Biotrode) was calibrated daily by titration of 6 mM HCl with a strong base. The titration of ligand and complexes were performed in water solution of 6 mM HCl at 0.1 M KCl ionic strength. The ligand concentration was 1 mM and 2.5 mM and metal-to ligand molar ratios of 1:1.1, 1:2, 1:2.5 were used. Due to the complex precipitation, titration curves only of the molar ratio 1:2.5 were incorporated into the investigations. The exact concentrations and the purities of the ligand solutions were confirmed by the Gran method. Since Gos was synthesized as the acetate salt, the protonation of acetic acid and its profound effect on the pH of the solutions were included into the calculations. Stability constants ligand and Cu(II) complexes were calculated using the SUPERQUAD and HYPERQUAD programs. Standard deviations (σ values) quoted were given by the program itself and referred to random errors only. They were a good indication of the importance of particular species involved in the equilibria.

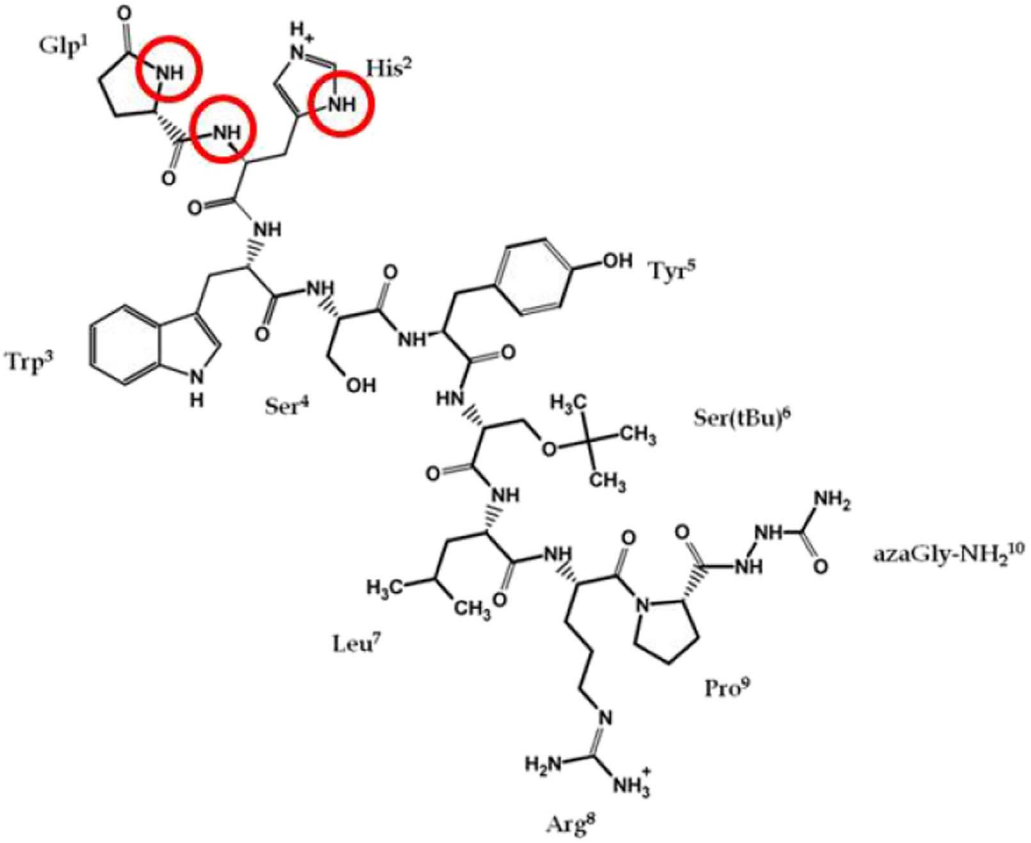

Fig. 1. The skeletal formula of goserelin in its fully protonated form. The Cu(II) binding sites are marked.