Polyhedron 55 (2013) 216–224

Contents lists available at SciVerse ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

Sialorphin and its analog as ligands for copper(II) ions

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ARTICLE INFO

Article history:
Received 21 May 2012
Accepted 26 October 2012
Available online 20 March 2013

Keywords:
Sialorphin
Peptides
Copper(II) ion
Metal ions complexes
Synthesis
Potentiometry
Stability constants
UV–Vis
CD
NMR
Theoretical calculations

ABSTRACT

In this study the sialorphin (Gln-His-Asn-Pro-Arg) and its analog (Glp-His-Asn-Pro-Arg) were analyzed in terms of metal binding ability. Both peptides were synthesized using the solid-phase method. The application of number analytical methods: potentiometry, spectroscopy (UV–Vis, CD, NMR) and mass spectrometry allowed for a detailed characterization of the coordination abilities of presented peptides. The analysis of the obtained results has shown that both peptides are able to form a series of complexes. However due to the presence of free N-terminal amino group the sialorphin is more effective in metal ion binding. Nevertheless, in basic conditions both peptides involve the amide nitrogen belonging to the side chain of Asn3 moiety and form 4N complex with square planar structure. This unusual ability has been confirmed by the results obtained from the NMR studies.

1. Introduction

Sialorphin (Sia) is a hormonal messenger involved in an intracellular communication. This signaling mediator, which was recently identified by the pharmaco-chemical genomic approach, is synthesized in the submandibular gland and prostate of rats [1]. Sialorphin is secreted in response to environmental stress and for this reason it can help in mediating the homeostatic response of rats to stressful situations. Moreover, this peptide plays an important role in many physiological processes, e.g. in the control of social behavior and pain perception. However, the most studied function of sialorphin is its role in sexual behavior [2–4]. Characteristic feature of sialorphin is the Gln-His-Asn-Pro-Arg sequence (Fig. 1a).

The data available in the literature show that under physiological conditions the N-terminal Gln can be transformed into the cyclic structure (Fig. 1b) called pyroglutamine (pGlu, Glp) [5,6]. This residue (Glp) occurs in hormones, e.g. thyrotropin-releasing hormone (TRH, Glp-His-Pro-NH_2) and luliberin (LHRH, luteinizing hormone releasing hormone, Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2). Thyrotropin-releasing hormone is a tripeptide that can improve functional recovery after neurologic dysfunction. Furthermore, TRH is one of the factors responsible for regulation of body temperature and stimulation of hepatic blood flow. LHRH is the decapeptide amide which is the main factor mediating in the neuroregulation of the secretion of gonadotropins. On the other hand, it is well known that the metal ions can be important regulators of activity of many peptides [7–9]. For example, the studies on LHRH showed that the biological properties are strongly influenced by the presence of metal ions, i.e. it was proved that Cu(II) ions most effectively affect biological functions of peptides. Less effective are metal ions such as Ni(II) and Zn(II) [7,10,11]. In vivo studies showed that the presence of metal–LHRH complexes may influence considerably the ovulation process. Moreover, Kochman et al. demonstrated that the complexation process may also affect the release of LH and FSH [11]. As it has been pointed out by the Ogawa et al. synaptic membranes that contain TRH receptors lose their function in the presence of certain metal ions. For example, they found that Ni(II) ions can reduce the binding capacity of

Abbreviations: TRH, thyrotropin-releasing hormone; LHRH, luliberin; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

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http://dx.doi.org/10.1016/j.poly.2012.10.055

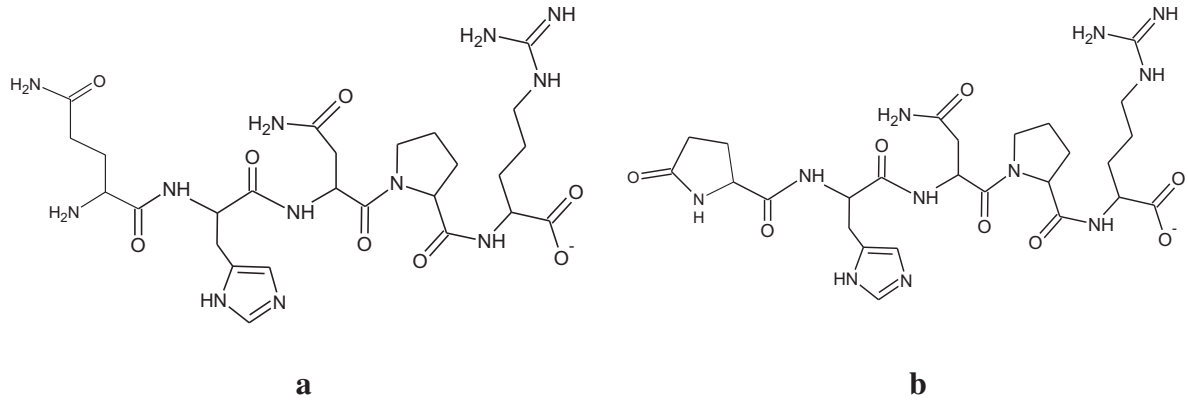

Fig. 1. Chemical structure of studied ligands: (a) sialorphin (Gln-His-Asn-Pro-Arg) – Sia and (b) [pQ[1]]sialorphin (Glp-His-Asn-Pro-Arg) – qSia.

TRH-receptor while Cu(II) and Zn(II) increased the binding properties of mentioned receptor [12].

The aim of the present study is synthesis and characterization of coordination abilities of the sialorphin (Sia) and its analog pGlu-His-Asn-Pro-Arg (qSia) towards Cu(II) ion (Fig. 1). In order to shed some light on the properties of the metal–peptide complexes we analyze them using potentiometric and spectroscopic techniques. Experimental data are supported with the results of quantum-chemical calculations.

2. Material and methods

2.1. Synthesis

Sialorphin (Sia) was synthesized manually by the solid-phase method on a 2-chlorotrityl chloride resin (loading 1.3 mmol/g, 1% DVB, 100–200 mesh, Tianjin Nankai Hecheng Science & Technology Co., Ltd., China) using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry [13,14].

N-α-Fmoc-protected amino acids were purchased from Iris Biotech GmbH (Germany); 1-hydroxybenzotriazole (HOBt), piperidine, trifluoroacetic acid (TFA) and chloranil were provided by Fluka (Switzerland); *N*,*N*-diisopropylethylamine (DIEA), triisopropylsilane, *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and phenol were purchased from Sigma–Aldrich (Poland); acetonitrile (ACN), *N*,*N*-dimethylformamide (DMF), diethyl ether were provided by Polish Chemicals (Poland).

The *N*-α-Fmoc-amino acids were protected on the side chain as follows: 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl (Pbf) for arginine and trityl (Trt) for glutamine, asparaginine and histidine. Attachment of the first amino acid to the resin was performed according to the method proposed by Barlos et al. with a loading of 0.5 mmol per gram [15].

Deprotection of the Fmoc group was carried out over 5 and 15 min with 25% piperidine in DMF. All the amino acids were coupled with a 3-fold molar excess of the protected amino acid (Fmoc-AA) dissolved in DMF using TBTU with addition of HOBt in the presence of DIEA for 2 h (Fmoc-AA:TBTU:HOBt:DIEA, 1:1:1:2). The completeness of each coupling step was monitored by the chloranil test. The peptide was cleaved from the resin after synthesis with a trifluoroacetic acid/triisopropylsilane/water (95:2.5:2.5 v/v/v) mixture for 2 h. The cleaved peptide was precipitated with diethyl ether and lyophilized. The crude peptide thus obtained was purified by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) on a Kromasil C8 column (8 × 250 mm, 5 μm particle size) with several linear gradients of acetonitrile (ACN) in 0.1% TFA. The eluates were fractionated and analyzed by the analytical RP-HPLC. The purity of the peptide was checked on an analytical Beckman chromatograph with a Kromasil C8 column (4.6 × 250 mm, 5 μm particle size) using several linear gradients of ACN in 0.1% TFA. Fractions containing the pure peptide (>98%) were pooled and lyophilized. The obtained Sia was analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF).

The analog pGlu-His-Asn-Pro-Arg (qSia) was obtained from sialorphin. The cyclization reaction of the N-terminal Gln residue was conducted in 30% acetic acid at room temperature. The progress of the reaction was monitored by RP-HPLC. After cyclization, acetic acid was evaporated. The residue was dissolved in water and lyophilized. The obtained peptide qSia, was purified by *RP-HPLC* as described. The pure qSia was analyzed by MALDI-TOF MS. Physicochemical characteristics of the synthesized peptides are presented in Table 1.

2.2. Potentiometric measurement

Potentiometric measurements were carried out using Molspin pH-meter system with a Mettler Toledo InLab 422 semimicro combined electrode at 25.0 °C calibrated in hydrogen ion concentration using HNO_3 [16]. The water ionization constant was pK = 13.77. The ligand solutions were prepared with 1×10^{-3} HNO_3 and an ionic strength 0.1 M KNO_3, the concentration of ligand were 1×10^{-3}M. Samples of the complexes were prepared by adding 0.05 M $Cu(NO_3)_2$, were ligand to metal ratio was 1.5:1. The titrations of ligands and complexes were started at 3.0 pH and finished at 11.5 pH. The sample volumes were 1.5 mL. Alkali (0.1 M NaOH) was added dropwise by using a 0.25 ml micrometer syringe. Stability constants and stoichiometry of the complexes were calculated from titration curves using SUPERQUAD program [17].

2.3. UV–Vis and CD measurement

Visible spectra of complexes were collected on Varian Carry 50 Bio spectrophotometer. The spectra were recorded with a cuvette with an optical path 10 mm at 25.0 °C. The CD spectra in the visible

Table 1
Physicochemical characteristics of the peptides.

Symbol	Peptide	HPLC R_t(min)[a]	$[M+H]^+$ found (calculated)
Sia	Sialorphin	9.4	651.2 (651.3)
qSia	[pQ[1]]sialorphin	10.2	634.3 (634.3)

[a] Linear gradient from 3 to 45% of [B] for 20 min, flow rate of 1 ml/min.The following solvent system was used: 0.1% aqueous TFA, 0.1% TFA in acetonitrile with Kromasil C8 column (4.6 × 250 mm, 5 μm particle size).