Coordination Chemistry Reviews 255 (2011) 1824–1833

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

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

Review

Tetrazole peptides as copper(II) ion chelators

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Contents

ARTICLE INFO

Article history:
Received 25 November 2010
Accepted 27 February 2011
Available online 26 March 2011

Keywords:
Tetrazole peptides
Enkephalins
Casomorphins
Copper complexes

ABSTRACT

The 1,5-disubstituted tetrazole ring a mimetic of the *cis*-amide bond is an unique element modifying the ability of peptides to chelate copper(II) ions. The position of the tetrazole ring system in the peptide backbone plays a critical role in the stabilization of the metallopeptide molecule. The insertion of a tetrazole between amide groups leads to enhancing the stability of the complex and to obtaining a very effective peptide chelating agent. These findings can provide important information for modeling biologically relevant peptide–metal binding sites. Some aspects of biological activity of tetrazole modified exogenous opioid peptides in the presence of copper(II) ions are also presented in this review.

1. Introduction

Research into complex systems containing metallopeptides is particularly useful for the rational design of coiled-coil protein structures [1–6]. Metallopeptide systems are unique and can effect the biological activity of peptides. Studies on the influence of peptide sequences and peptide bond modification on the structure and thermodynamic stability of metallopeptides are very important to understanding both the biological role of peptides and the influence of metal ions on protein conformation. Moreover, data obtained on the thermodynamic stability of metallopeptides can lead to the recognition of some peptides as ligands competitive to other ligands present in human body fluids.

This review centers on endogenous and exogenous opioid peptides. These peptides play a significant role in the regulation of physiological processes not only in the brain, but also in almost all tissues. They can act as hormones, neuromodulators and neurotransmitters [7–12]. Their proper functioning depends on many factors, and in particular on the presence of copper ions [13]. Copper is a trace element in the human body but plays a fundamental role in the biochemical processes occurring in the nervous system [14]. Therefore, the interactions between copper(II) ions and opioid peptides or their analogues were the focus of the reported work. The aim of reviewed studies was to investigate the coordinating effects of a new class of peptide chelators, tetrazole opioid peptide analogues [15–22], on copper(II) ions. The 1,5-disubstituted tetrazole ring is a *cis* amide bond surrogate [23–26]. Enkephalins and β-casomorphin-7 were subjected to tetrazole modification. These peptides exhibit the same receptor activity as morphine, and hence they could be useful as potential natural analgesics [27,28]. The tetrazole ring affects both the conformation of the pep-

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doi:10.1016/j.ccr.2011.02.023

tides and their interactions with opiate receptors [29]. Therefore, the influence of the *cis* -amide bond surrogate in metallopeptides was investigated from the chemical and biological points of view. The studied compounds were tetraalanine **(1)**, [Leu^5]enkephalin ([Leu^5]EK) (**2–5**), and β-casomorphin-7-amide (**6–8)** analogues:

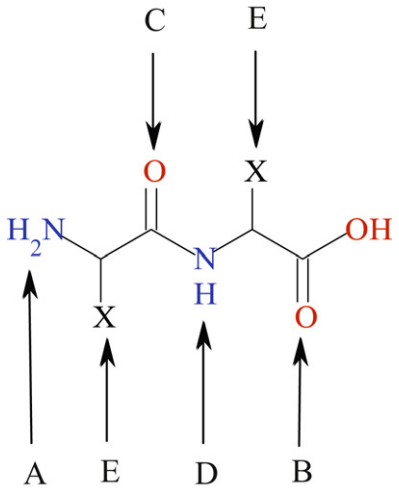
1. Ala-AlaΨ(CN_4)Ala-Ala
2. Tyr-GlyΨ(CN_4)Gly-Phe-Leu
3. Tyr-Gly-GlyΨ(CN_4)Phe-Leu
4. Tyr-D-Ala-GlyΨ(CN_4)Phe-Leu
5. Tyr-D-Ala-Gly-Phe-LeuΨ(CN_4)CH_3
6. Tyr-ProΨ(CN_4)Phe-Pro-Gly-Pro-Ile-NH_2
7. Tyr-Pro-PheΨ(CN_4)Ala-Gly-Pro-Ile-NH_2
8. Tyr-Pro-Phe-ProΨ(CN_4) Gly-Pro-Ile-NH_2

The examination of coordination equilibria in aqueous solution containing copper ions and peptide ligands was performed by using potentiometric measurements combined with spectroscopic techniques: electronic absorption (UV–vis), circular dichroism (CD) and electron paramagnetic resonance (EPR). Potentiometry enables the detection of different metallopeptide species formed in the solution as well as the determination of their thermodynamic stability and species distribution relative to solution pH. Spectroscopic data can be used to identify the type and number of peptide donor atoms involved in metal ion coordination. Thanks to the above methods, it has been possible to define the metal binding ability of tetrazole analogues of peptides. The most interesting problems to solve were: (i) how does the tetrazole ring, as a *cis*-amide bond surrogate, affect the formation and thermodynamic stability of complex species at different pH ranges? and (ii) what is the competitive copper binding efficiency of tetrazole peptides as compared to other opioid oligopeptides? In order to obtain the most accurate pattern of tetrazole functioning in metallopeptides, the authors investigated the chelating abilities of systems containing endogenous enkephalin Tyr-Gly-Gly-Phe-Leu [19], exogenous α-caseins Arg-Tyr-Leu-Gly-Tyr-Leu (casein 90–95) [15] and Arg-Tyr-Leu-Gly-Tyr-Leu-Glu (casein 90–96) [16], and β-casomorphins: bovine Tyr-Pro-Phe-Pro-Gly-Pro-Ile and human Tyr-Pro-Phe-Val-Glu-Pro-Ile [17,18].

2. Formation of metallopeptides. Peptides as chelating agents for metal ions

Peptides are effective and specific ligands for metal ions [30–33]. In the peptide molecule there are several potential coordinating sites (Scheme 1).

The most important donor center is the N-terminal amine nitrogen atom (A). The amine group is usually primary, although it can be secondary, as in the case of proline. The oxygen donor atoms occur in all peptides, the C-terminal carboxylate group (B) being the most effective. Carbonyl oxygen donors of the peptide bond (C) can also participate in metal ion coordination. Copper(II) and platinum metals promote the hydrogen ionization of the peptide bond (D) and form very stable N^-–metal bonding. Amino acid side chains can also contain different donor centers (E). The most important ones include histidine imidazole [34–40], cysteine sulfhydryl, aspartic carboxylate [41] and tyrosine phenolate [42]. The hydroxylate groups of serine or threonine, the amide carbonyl group of asparagine or the lysine amine side chain are seldom involved in metal coordination. The stability of the metallopeptide molecule depends not only on the interaction of the donor atoms directly involved in metal binding but also on the hydrophobic action of non-coordinating amino acid side chains [33]. Aliphatic or aromatic chains form a spatial shield preventing the hydrolysis of the N^-–metal bond and increasing the structural strength of the complex molecule [43]. Peptide conformation has a substantial impact on coordinating equilibria in the metal–peptide system both in thermodynamic and structural terms. Oligoglycine and oligoalanine are examples of simple peptides without side chains. In these systems, Cu(II) coordination starts from the N-terminal nitrogen atom, which is regarded as the "anchoring" site for the metal [30–33,44]. The adjacent carbonyl oxygen is the second donor atom to close the chelating ring. The deprotonation of consecutive amide nitrogen atoms in the presence of metal ions in a medium with increasing pH leads to the formation of successive N^-–Cu(II) bonds and eventually to the production of the $CuH_{-3}L$ complex. In this metallopeptide species four nitrogen atoms are bonded to the Cu(II) ion in three five-membered chelating rings (Fig. 1).



Scheme 1. Potential donor atoms in peptide molecule.

The involvement of amide nitrogen atoms in copper(II) coordination was also confirmed in the solid phase of complexes the di-, tri-, tetra- and pentaglycine [45–49]. The formation of the chelating ring by consecutive nitrogen donor atoms is a driving force for the coordination process leading to a decrease in the p*K* value of the first amide group by up to 10 log units as compared with the free peptide ligand [30,31,44]. In the case of Cu(II) peptide complexes, the deprotonation of the adjacent amide groups are usually well separated from each other. This indicates that the binding process occurs in a non-cooperative manner.

3. Tetrazole peptide chelators

3.1. Copper(II) complexes with the tetrazole analogue of tetraalanine

The tetrazole ring incorporated into the peptide backbone chain mimics the *cis*-amide bond conformation, i.e. the α-carbons of the amino acid residues connected by the tetrazole are placed at the same side of the C–N bond (Scheme 2).

Such a modification dramatically changes the ability of tetraalanine to coordinate to Cu(II) ions [22]. The incorporation of the 1,5-disubstituted tetrazole ring results in the absence of the consecutive amide nitrogen in copper bonding, just as in proline-containing peptides. In Pro peptides, the second proline nitrogen atom in the peptide backbone is unable to undergo ionization and participate in the formation of the $N^- \rightarrow$ Cu(II) bond. Therefore, it acts as a "break-point" in metal coordination [50–53]. The characteristic feature of the tetrazole moiety distinguishing it from the Pro residue is the presence of N(4) nitrogen atom as a potential donor center for a metal ion (Scheme 2). Potentiometric and spectroscopic results [22] revealed that tetrazole incorporation into the Ala^2-Ala^3