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Structural studies of Cu(II) binding to the novel peptidyl derivative of quinoxaline: *N*-(3-(2,3-di(pyridin-2-yl)quinoxalin-6-yl)alanyl)glycine

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ABSTRACT

The coordination properties of the novel conjugate towards copper ions were investigated. The performed studies exhibited the unusual binding properties of the ligand molecule having two potential strong coordination sites, namely dipeptidic chain and pyridyl nitrogens. On the basis of potentiometric and spectroscopic studies the binding at the low pH values to the aromatic entity is suggested, while the rise of pH (including physiological one) yielded the dimeric head-to-tail complex formation. This stable species possesses three nitrogen donors involved in Cu(II) chelation: N(pirydyl), NH_2 and N^-(amide).

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1. Introduction

Quinoxaline containing molecules are often considered as the DNA interactive compounds. They are also claimed to be efficient ligands for the transition metal ions. Particularly 2,3-di(pyridin-2-yl)quinoxaline (DPQ) and dipyrido[3,2-a:2,3-c]phenazine (DPPZ) complexed with selected metal ions are of current interest in view of their binding to DNA. They often exhibit the "chemical nuclease" activity and may efficiently nick DNA causing oxidative cleavage [1] or hydrolytic one as it has been found in the case of copper(II) complex with DPPZ [2].

The importance of this issue is confirmed by the investigations on the series of ruthenium and platinum complexes with respect to their DNA binding abilities [3,4]. Other studies on complexes of pyridyl derivatives of quinoxaline with palladium [5] manganese [6], copper [7] or platinum [8] present these compounds as highly effective metal chelators that may show significant chemotherapeutic activity.

Conjugation of this type of heterocyclic compounds to peptides results in modulation of the chemical nucleases properties by changing the affinity and specificity of interactions with DNA, and enabling its cellular recognition and subsequent transport. The good example of such strategy is the synthesis of peptides coupled to 4-(butyric acid)-4′-methyl-2,2′-bipyridine connected to the peptide N-terminus. This allowed formation of tertiary complexes with phenanthroline and DPPZ [9]. Literature data show many examples of targeting achieved by adding ligand moiety to the biologically active molecule, directed to certain types of binding sites. This approach is often used in cancer therapy significantly diminishing cytotoxic effect on healthy organs.

Recently we have reported that the dipeptides containing various analogues of 3-(quinoxalin-6-yl)alanine, including 2,3-bis(2-pyridyl)quinoxaline, dipyrido[3,2-a:2,3-c]phenazine, and 2,3-bis(2-phenyl)quinoxaline interact with Cu(II) and Fe(II) ions. We have also evidenced that these compounds, including the Cu(II)-DPQa-Gly cleave plasmid DNA with the oxidative mechanism leading to the formation of single and double strand damage products [10]. For the synthesis of the studied quinoxaline-peptide derivatives we developed an efficient solid-phase method [11].

In this paper we report the coordination pattern of *N*-(3-(2,3-di(pyridin-2-yl)quinoxalin-6-yl)alanyl)glycine (DPQa-Gly) (Fig. 1).

2. Materials and methods

2.1. Materials

Method for the synthesis of peptide-heterocycle conjugates was developed, based on the condensation of diaminophenylalanine

Abbreviations: DPQa-Gly, *N*-(3-(2,3-di(pyridin-2-yl)quinoxalin-6-yl)alanyl)glycine; DPQ, 2,3-di(pyridin-2-yl)quinoxaline; DPPZ, dipyrido[3,2-a:2,3-c]phenazine.

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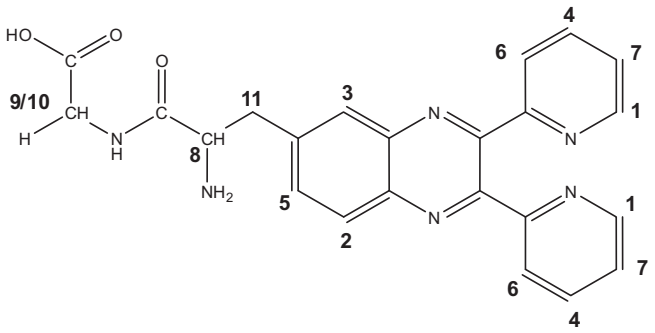

Fig. 1. Molecular structure of the studied ligand (protons are labeled in order of appearance in the NMR spectrum).

with dicarbonyl compounds [11]. The formation of the studied complexes occurs spontaneously following mixing of the water solution containing metal salt with the ligand. As a metal ion source $Cu(NO_3)_2 \times 3H_2O$ (Merck) was used. KNO_3, NaOH, HNO_3 and ethylene glycol were Merck products. D_2O, ammonium acetate, acetonitrile and other simple chemicals were purchased from Sigma–Aldrich.

2.2. Potentiometric measurements

Potentiometric titrations of the ligand and its complexes with Cu(II) in aqueous solution in the presence of 0.1 mol dm^{-3} KNO_3 were performed at 298 K under argon atmosphere using pH-metric titrations (Molspin automatic titrator, Molspin Ltd., Newcastle-upon-Tyne, UK). The CO_2-free NaOH solution at a concentration of 0.10156 M was used as a titrant. The samples were titrated in the pH region 2.0–10.5 using a total volume of 1.5 cm^3. Changes in pH were monitored with a combined glass–Ag/AgCl electrode (ThermoRussel CMAW 711, Russell pH Ltd., Fife, UK) calibrated daily in concentration using HNO_3 titration [12]. Ligand concentration was 1×10^{-3} mol dm^{-3}, and metal-to-ligand molar ratios of 1:1 and 1:2 were used. These data were analyzed using the SUPERQUAD program [13]. Standard deviations were computed by SUPERQUAD and refer to random errors only. They are, however, a good indication of the importance of the particular species involved in the equilibria.

2.3. EPR

The solutions for EPR measurements with ethylene glycol (water:glycol, 2:1, v/v) were prepared to ensure homogeneity of frozen samples. Solutions were of similar concentrations and metal-to-ligand molar ratio to those used in potentiometric measurements. The spectra were recorded at 120 K (liquid nitrogen) on a Bruker ESP 300E spectrometer (Karlsruhe, Germany) at the X-band frequency (9.3 GHz). The EPR parameters were calculated for the spectra obtained at the maximum concentration of the particular species for which well-resolved components were observed.

2.4. UV–Vis

Absorption spectra were recorded on a Cary 50 Bio spectrophotometer (Varian Inc., Palo Alto, CA) in the 800–220 nm range, using 1 cm cuvette. Solutions were of similar concentrations and metal-to-ligand molar ratio to those used in potentiometric studies. The values of ε parameter were calculated at the maximum concentration of the particular species obtained from potentiometric curves.

2.5. Circular dichroism

CD spectra were recorded at 298 K on Jasco J-715 spectropolarimeter (JASCO, Japan Spectroscopic Co., Hiroshima, Japan) over the range of 800–220 nm, using 1 cm and 0.1 cm cuvettes. The concentrations and metal-to-ligand molar ratio were similar to those used in potentiometric studies. Spectra are expressed in terms of $\Delta\varepsilon = \varepsilon_1 - \varepsilon_r$, where ε_1 and ε_r molar absorption coefficients for left and right circularly polarized light, respectively. The values of $\Delta\varepsilon$ were calculated at the maximum concentration of the particular species obtained from potentiometric data.

2.6. NMR studies

^1H and ^{13}C NMR spectra were recorded on Bruker Avance 600 MHz instruments at controlled temperatures (±0.1 K) in D_2O using 1D and 2D {HMQC (Heteronuclear Multiple Quantum Correlation), COSY (Correlation Spectroscopy), HMBC (Heteronuclear Multiple Bond Correlation), T1-pseudo 2D} experiments. The pH was adjusted to the desired values by use either DCl or NaOD. The required concentration of copper ions was achieved by using a stock solution of copper nitrate in D_2O. Each sample was it was carefully deoxygenated repeated freezing–thawing cycles. The ligand concentration was 3.75 mM and used metal to ligand ratio 1:350. Chemical shifts were referenced to internal 3-trimethylsilyl[$^2H_4$]propanesulfonate (TSP-d**4**).

Paramagnetic contributions to proton longitudinal relaxation rates R_{1p} (=$1/T_{1p}$) in case of fast exchange of the ligand between the free (f) and the metal-bound (M) forms can be measured by:

$$\frac{1}{T_{1p}} = \frac{1}{T_{1obs}} - \frac{1}{T_{1f}} \cong \frac{p_M}{\tau_M + T_{1M}} \quad (1)$$

where p_M is the fraction of bound ligand and τ_M is the inverse of the off-rate constant. In case of large excess of the ligand, p_M can be approximated to the molar fraction (or its double if the complex is considered 2:1) as all the copper can be safely assumed to be bound even in case of small affinity. If p_M and τ_M are known, relaxation times of protons in the bound ligand T_{1M}, can be easily evaluated from Eq. (1) and interpreted by using the Solomon equation [14,15]:

$$\frac{1}{T_{1M}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\mu_B^2 g_e^2 \gamma_H^2 S(S+1)}{r^6}\left[\frac{3\tau_c}{1+\omega_H^2\tau_c^2} + \frac{7\tau_c}{1+\omega_e^2\tau_c^2}\right]$$
$$\frac{1}{\tau_c} = \frac{1}{\tau_R} + \frac{1}{\tau_e} + \frac{1}{\tau_M} \quad (2)$$

where μ_o is the permeability of vacuum, S is the electron spin quantum number, μ_B is the electron Bohr magneton, g_e is the free-electron g factor, γ_H is the proton magnetogyric ratio, ω_H and ω_e are the proton and electron Larmor frequencies, τ_R is the rotational correlation time of the metal complex, and τ_e is the electron spin relaxation time (usually ranging between 1 and 5 ns in copper complexes). The contact contribution to T_{1M} was neglected, as it is usual for Cu(II) complexes with relatively small ligands where large values are generally found for the transverse electronic relaxation time.

Paramagnetic contributions T_{1M} can be converted into distance restraints by Eq. (2) provided a value for the correlation time τ_c is independently known. In estimating the effective correlation time, τ_M^{-1} and τ_e^{-1} can both be neglected due the relatively low values generally encountered in Cu(II) complexes [16]. The rotational correlation time of the metal complex τ_R was instead calculated by the Stokes–Einstein equation as proton-Cu(II) distances in (2) are not largely affected by the accuracy of this value (due to the sixth power of r in Eq. (2)):

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