Polyhedron 28 (2009) 2329–2334

Contents lists available at ScienceDirect

Polyhedron

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

Interaction of Cu-dipeptide complexes with Calf Thymus DNA and antiproliferative activity of [Cu(ala-phe)] in osteosarcoma-derived cells

Gianella Facchin [a], Eduardo Kremer [a], Daniel A. Barrio [b], Susana B. Etcheverry [b], Antonio J. Costa-Filho [c], María H. Torre [a,*]

[a] Química Inorgánica, DEC, Facultad de Química, Gral. Flores 2124, CC1157 Montevideo, Uruguay
[b] Bioquímica Patológica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115 (1900) La Plata, Argentina
[c] Grupo de Biofísica Sérgio Mascarenhas, Instituto de Física de São Carlos, Universidade de São Paulo, C.P. 369, 13560 São Carlos (SP), Brazil

ARTICLE INFO

Article history:
Received 18 September 2008
Accepted 22 April 2009
Available online 3 May 2009

Keywords:
Cu(II)complexes
DNA-interaction
EPR
CD
Osteosarcoma

ABSTRACT

In this work the study of Calf Thymus DNA interaction with several Cu(L-dipeptide) complexes was reported. The binding stoichiometry (Cu(mmol)/DNAmol base) was determined and in an attempt to clarify the binding mode, EPR and CD experiments were performed. All the studied complexes interacted with DNA, in a more selective way than $[Cu(H_2O)_6]^{2+}$, being the [Cu(ala-phe)] the complex with the highest interaction. The EPR experiments suggested that the monomeric species formed in solution were coordinated through a nitrogen atom of the DNA bases (inner-sphere binding) and the CD studies showed structural changes upon the DNA–complex interaction. Besides, the ratio Cu(mmol)/DNAmol base obtained by the binding stoichiometry experiments was close to that found by EPR and CD determinations. The effect on cell proliferation determined by the crystal violet bioassay on UMR106 rat osteosarcoma-derived cells showed that the [Cu(ala-phe)] complex exerted an antiproliferative action against this tumor line.

1. Introduction

The interaction of metal complexes with nucleic acids and their constituents has been a subject of interest in bioinorganic chemistry, especially since the discovery of cisplatin and their analogues. These studies have been useful for the development and the comprehension of the activity of new chemotherapeutic agents designed for the treatment of numerous diseases [1–3]. Many of these chemotherapeutic agents act by inhibition of the synthesis of the deoxyribonucleic acid (DNA), a natural target due to its predominant role in cellular replication. One way to achieve this inhibition is by direct DNA binding in either a noncovalent interaction by intercalation, groove-face binding or external electrostatic binding or a covalent interaction due to an outer-sphere or inner-sphere binding and (or consecutive) strand breakage [4,5].

In particular, in the search for new drugs the proposal that complexes based on essential metals may be less toxic than those with non essential ones led to the study of copper based drugs, some of them having an important cytotoxic effect [6–8]. Consequently, active copper compounds and their interaction with DNA have attracted great interest [5,9–11]. These studies have mostly used methods that focus on changes in the DNA structure as electronic absorption titration, fluorescence spectroscopy, viscosity measurements and circular dichroism, among others. For instance, chemotherapeutic copper complexes with polypyridil ligands showed an important interaction with DNA as intercalators as determined by UV–Vis and circular dichroism (CD) spectroscopies and viscosity [3]. Moreover, Sarkar's group studied the DNA interaction of Cu(II) complexes with piroxicam and meloxicam (anti-inflammatory drugs) which exhibited anticancer activity by UV–Vis and circular dichroism (CD) spectroscopies. They showed that their complexes bind strongly to DNA-backbone possibly with intercalation [12].

On the other hand, there is much less work using methods based on properties related to the metal ion. In this area relevant studies were performed by Chikira's group that provided information on the binding of copper complexes with amino acids or peptides to DNA fibers by electronic paramagnetic resonance (EPR) [13–20].

As a part of our research on metal-based drugs we have studied several copper complexes with oligopeptides as potential antitumoral agents encouraged by the antecedents in the literature [21,22]. In particular, we have synthesized and structurally characterized in solid state and in water solution a series of copper complexes with dipeptides [23–30] and analysed their magnetic interactions [31,32].

Due to these previously reported antecedents and with the aim of obtaining structural information about the interaction of DNA with copper-dipeptide complexes we explored changes in the environment of the copper ion and in the DNA upon coordination.

* Corresponding author. Tel.: +598 2 9249739; fax: +598 2 9241806.
E-mail address: mtorre@fq.edu.uy (M.H. Torre).

0277-5387/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.poly.2009.04.029

In particular, the study of the DNA interaction with several Cu(L-dipeptide) complexes previously characterized by our research group was performed. Several dipeptides commercially available were selected in an effort to include ligands with inverted sequences of aminoacids and to cover a series containing aminoacids with different sizes. The aim of this selection was to perform a screening that provides structural information useful for biggest systems. Firstly, the direct quantification of the amount of copper bound to the DNA (binding stoichiometry) was measured. Secondly, EPR experiments were made with the complex that presented the best binding stoichiometry and with two complexes that presented medium interaction, in order to test whether there was any change in the Cu(II) coordination sphere when binding to the DNA. CD technique was used to explore the distortion introduced in the DNA structure upon binding with complexes or the distortion in the Cu(dipeptide) complexes.

Besides, to evaluate potential pharmacological activity of the complexes, a cell proliferation assay on UMR106 rat osteosarcoma-derived cells was performed.

2. Materials and methods

2.1. Synthesis of the complexes and analytical characterization

The copper (II) complexes with L-dipetides (SIGMA) ala-ala, ala-val, ala-ile, ala-leu, ala-thr, ala-phe, ala-tyr, phe-ala, phe-leu, phe-phe, val-phe, val-gly were obtained and characterized as described previously [26,27,29,30].

Table 1 summarizes the stoichiometry and analytical data for the Cu(II) complexes. Elemental analysis was performed with a CARLO ERBA EA 1108 equipment.

2.2. Spectroscopic measurements

Electronic absorption spectra of aqueous solution samples were recorded on a Milton Roy Spectronic 3000 spectrophotometer, using 1-cm path length quartz cells.

The copper concentration was measured by atomic absorption spectroscopy using a Perkin–Elmer 5000 instrument, with a Photron lamp for copper analysis, at 325 nm with air/acetylene flame.

X-band (9.5 GHz) EPR measurements were carried out on frozen aqueous solutions using a Varian E109 spectrometer and cavity with 100 kHz field modulation. The measurements were performed at N_2 liquid temperature. The *g*-values were obtained from spectral simulations using the QPOW program [33].

The CD spectra were recorded using a Jasco 720 instrument in a 1-cm path length quartz cell, in the 200–300 nm range.

2.3. Determination of the stoichiometry of the interaction Cu(II)dipeptide–DNA

The technique used in order to determine the stoichiometry of the interaction of the complexes with DNA was adapted from Mahnken et al. [34]. An aqueous solution of Calf Thymus DNA (SIGMA) (c.a. 1.7 mg/mL, 1 mL) was allowed to react with an aqueous solution of Cu-dipeptide (c.a. 1 mM, 1 mL) for 24 h at 37 °C.

The DNA was precipitated afterwards by addition of absolute ethanol (4 mL) and an aqueous solution of NaCl (2 M, 0.2 mL), and it was centrifuged (30 min at 50 rpm). The supernatant was discarded. The precipitate was dissolved in 2 mL of H_2O. This process was repeated three times. DNA and copper concentrations were monitored in the final solution. DNA concentration was determined at 260 nm using $\varepsilon_M = 6000\ M^{-1}\ cm^{-1}$. Copper concentration was measured by atomic absorption spectroscopy as described in Section 2.1. The solubility of the complexes was checked in the conditions of the precipitation of the DNA. The interaction was expressed as the ratio: Cu(mmol)/DNA(mol base).

2.4. Cu(II)dipeptide–DNA binding: characterization by EPR measurements

The EPR spectra of an aqueous solution of Calf Thymus DNA (SIGMA, 2.45 mg/mL) in presence of increasing amounts of copper complexes were recorded. The dilution scheme for each Cu(II) complex covered a 10–500 μM range, while the concentration of the DNA solution varied from 500 to 700 μM in DNA bases (pH 7). After each addition of copper complex the mixture was allowed to mix for 15 min at room temperature and then an aliquot was frozen at N_2 liquid temperature in order to register the EPR spectrum.

2.5. Cu(II)dipeptide–DNA binding: characterization by CD measurements

The CD spectra of an aqueous solution of Calf Thymus DNA (SIGMA) in presence of increasing amounts of copper complexes were recorded. The dilution scheme for each Cu(II) complex covered a 0.5–5 μM range, while the concentration of the DNA solution (DNA concentration was determined at 260 nm using $\varepsilon_M = 6000$) remained approximately constant at 50 μM in DNA bases (pH 7). As the data were obtained in excess of DNA, the total molar concentration remained nearly constant in all the spectra of each complex. This enabled us to perform a Job analysis of the data at 218 nm in order to estimate a binding constant for DNA–[Cu-peptide] [35].

Table 1
Stoichiometry, code and analytical data for the Cu(II) complexes.

Complex code	Complex stoichiometry	%C		%N		%H	
		Calc.	Exp.	Calc.	Exp.	Calc.	Exp.
1	$[Cu(ala\text{-}ala)]\cdot 2H_2O$	27.93	27.57	10.86	10.41	5.40	4.89
2	[Cu(ala-val)]	38.44	38.40	11.21	11.15	5.60	5.66
3	[Cu(ala-ile)]	40.94	40.85	10.61	10.55	6.06	6.18
4	$[Cu_3(ala\text{-}leu)_3(H_2O)_3(CO_3)]\cdot PF_6\cdot H_2O$	31.40	32.23	7.85	8.17	5.54	6.09
5	$[Cu(ala\text{-}thr)]\cdot {}^1/_2H_2O$	32.22	32.30	10.74	10.66	4.98	5.05
6	$[Cu(ala\text{-}phe)]\cdot {}^1/_2H_2O$	48.35	48.15	9.40	9.30	4.70	4.81
7	$[Cu(ala\text{-}tyr)]\cdot H_2O$	43.40	43.33	8.44	8.54	4.82	4.98
8	$[Cu(phe\text{-}ala)]\cdot {}^1/_2H_2O$	46.93	47.12	9.13	9.19	4.89	4.23
9	[Cu(phe-leu)]	53.01	53.21	8.24	8.52	5.93	6.26
10	[Cu(phe-phe)]	57.82	57.42	7.49	7.57	4.85	4.76
11	[Cu(val-phe)]	51.60	51.96	8.60	8.71	5.57	6.01
12	[Cu(val-gly)]	35.67	35.92	11.88	11.96	5.13	5.58