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Copper(II) complexes of methylated glycine derivatives: Effect of methyl substituent on their DNA binding and nucleolytic property

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ABSTRACT

A set of copper(II) complexes of glycine and methylated glycine derivatives, $Cu(aa)_2$, consisting of C-dimethylglycine, L-alanine, *N*-dimethylglycine and sarcosine, was investigated for their DNA binding and nucleolytic properties by means of EPR and visible spectroscopy, and electrophoresis. They bind weakly to DNA with apparent binding constants in the range $1.8–2.9 \times 10^3\ M^{-1}$ with very similar orientation. No DNA cleavage is observed in the absence of exogenous agents. Copper(II) complexes of *N*-methylated derivatives bind to DNA more stereo-specifically and less strongly, and their oxidative DNA cleavage is less efficient than those of the corresponding C-methylated derivatives in the presence of hydrogen peroxide (H_2O_2) alone, or sodium ascorbate (NaHA) alone or tandem H_2O_2–NaHA. The oxidative DNA cleavage mechanism in the three systems involves a common copper(I) species. Neocuproine can inhibit DNA cleavage by these complexes.

1. Introduction

Although, copper is essential to animals, it can also induce toxicity. This toxicity is due to those reactions of the redox-active copper ions which generate reactive oxygen species (ROS) via a Fenton or Haber–Weiss mechanism [1]. Copper(II), Cu^{2+}, has been implicated in many diseases such as Wilson's disease, Menkes disease, Alzheimer's disease, Parkinson's disease, prion disease, other neurodegenerative diseases and cataract [2]. ROS can be produced by Cu^{2+} in the presence of hydrogen peroxide or ascorbate anion or both hydrogen peroxide and ascorbate [1–5]. The ROS thus produced can cause damage to protein, DNA or cellular membrane. Recently, it was reported that even the antioxidant metalloenzyme, copper–zinc superoxide dismutase (Cu–Zn-SOD) could cleave DNA in the presence of hydrogen peroxide or mercaptoethanol [6].

Copper complexes with amino acids are both important biologically and interesting. Many low-molecular-weight copper complexes with amino acids and amino acid derivatives act as anti-inflammatory, antiulcer, anticonvulsant, anticancer, and/or radiation protection agents [7]. They can assume a variety of coordination geometries, from irregular square planar, distorted planar, flattened tetrahedral, and distorted square–pyramidal to distorted octahedral, as observed in their experimental crystal structures [7].

However, copper(II) ion chelated with amino acid can harm DNA by either oxidative or hydrolytic cleavage, depending on the amino acid involved [8–10]. A recent study has investigated the effects of pH, concentration of both amino acid and hydrogen peroxide, and the type of amino acids (glycine, lysine, L-alanine) on the kinetics of ·OH radicals formation from the reaction of copper(II)-amino acid mixtures with hydrogen peroxide [11]. Similar reaction is also involved in the oxidative cleavage of DNA by copper(II) complexes of *N*,*N*′-dimethylglycine and other ligands [8]. A widely accepted view of the mechanism of DNA cleavage by copper(II) complexes details the binding of the complexes to the DNA and their subsequent reaction with hydrogen peroxide to generate the ·OH radicals on site to cleave the DNA. Of relevance are the studies of the binding of copper(II) complexes of some amino acids with DNA fibers using EPR spectroscopy and the results have shown that the nature and orientation of binding of the complexes were influenced by the amino acids used (glycine, serine, threonine, lysine and arginine) [12,13]. This paper reports the effect of the number and position of methyl substituent(s) on (i) the relative quantity of hydroxyl radicals produced by the reaction of the copper(II) chelated methyl-derivatives of glycine (general structure of ligands is depicted in Scheme 1), $Cu(aa)_2$, with hydrogen peroxide, and (ii) the oxidative cleavage of DNA by these complexes in the

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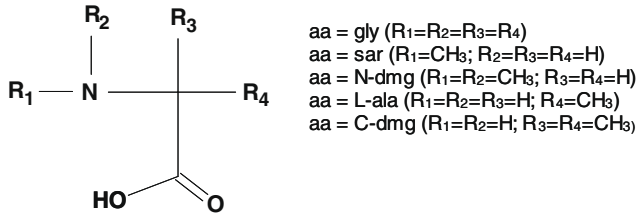

Structure of amino acid, aa

Scheme 1.

presence of either hydrogen peroxide or sodium ascorbate or both these exogenous reagents. In addition, the interaction of these complexes with DNA was investigated by EPR and ethidium bromide (EB) quenching assay studies.

2. Experimental

2.1. Reagents and materials

Most of the reagents were of analytical grade and were used as supplied. The pBR 322, gene ruler 1 Kb DNA ladder, ethidium bromide, loading buffer, Tris and agarose were bought from BioSyn Tech (Fermentas). Salmon testes DNA was purchased from Sigma Chemical Co. (USA). All solutions for DNA experiments were prepared with ultra-pure water from a Elga PURELAB ULTRA Bioscience water purification system with UV light accessory. The borate buffer was prepared from 0.1 M boric acid aqueous solution in which the pH was adjusted with NaOH aqueous solution till pH 8.5. The borate buffer pH 8.5 contains borate at 0.1 M. All stock solutions of H_2O_2 in water and metal complexes in borate buffer were freshly prepared daily.

2.2. Physical measurements

The C, H and N microanalysis was carried out with a Perkin Elmer 2400 CHN analyser. Infrared spectra of all compounds were recorded as KBr pellets using Perkin–Elmer FT-IR spectrometer in the frequency range 4000–400 cm^{-1}. UV–Vis spectroscopic measurement was carried out on a Perkin Elmer Lambda 40. Fluorescence measurements were performed using a Perkin–Elmer LS55 photoluminescence spectrometer. The EPR data of the copper complexes were obtained with a JEOL RE-2X spectrometer.

2.3. Synthesis of $Cu(aa)_2$ complexes

The aqua*bis*(*N*,*N*-dimethylglycinato)copper(II), Cu(N-dmg)$_2$ and *cis*-aqua*bis*(glycinato)copper(II), *cis*-Cu(gly)$_2$, complexes were synthesized by previously reported procedures [8,14]. The FTIR of Cu(N-dmg)$_2$ and *cis*-Cu(gly)$_2$ were identical with those of aqua*bis*(*N*,*N*-dimethylglycinato)copper(II) [8] and *cis*-aqua-*bis*(glycinato)copper(II) [14], respectively. The *bis*(C-dimethylglycinato)copper(II), Cu(C-dmg)$_2$, *bis*(L-alaninato)copper(II), Cu(L-ala)$_2$, and *bis*(sarcosinato)copper(II), Cu(sar)$_2$ were prepared by reacting freshly prepared copper(II) hydroxide with the respective amino acid as in the previously reported procedure [8].

2.3.1. Elemental analysis for $Cu(aa)_2$ complexes

Cu(C-dmg)$_2$: *Anal.* Calc for Cu($C_8H_{16}N_2O_4$): C, 35.88; H, 6.02; N, 10.46. Found: C, 35.91; H, 5.84; N, 10.36%. For Cu(sar)$_2$: *Anal.* Calc. for Cu($C_6H_{12}N_2O_4$)(H_2O)$_2$: C, 26.13; H, 5.85; N, 10.16. Found: C, 26.32; H, 5.66; N, 10.15%. For Cu(L-ala)$_2$: *Anal.* Calc. for Cu($C_6H_{12}N_2O_4$): C, 30.06; H, 5.05; N, 11.69. Found: C, 30.11; H, 4.88; N, 11.61%. For Cu(N-dmg)$_2$: *Anal.* Calc. for Cu($C_8H_{16}N_2O_4$)(H_2O)] · 2H_2O: C, 29.86; H, 6.89; N, 8.70. Found: C, 29.95; H, 6.85; N, 8.53%.

2.4. PNDA assay of ·OH radicals from $Cu(aa)_2 + H_2O_2$

The reactions of Cu(aa)$_2$ and copper(II) salt in borate buffer pH 8.5 with H_2O_2 were carried out merely to investigate the relative amount of hydroxyl radicals produced. The reactions were monitored at 5-min intervals over duration of 15 min. A PNDA assay was used for this purpose and the procedure has been previously described [9]. The production of ·OH radicals from the reactions of the copper nitrate and the Cu(aa)$_2$ complexes (100 μM) in borate buffer pH 8.5 with 20 μM H_2O_2 was assayed by the use of *p*-nitrodimethylaniline (PNDA) indicator (56 μM) according to a previous procedure [9]. The % bleaching of the PNDA after time *t* (minutes) was calculated by using the formula $(A_o - A_t)/A_t \times 100$, where A_o and A_t are the absorbance of the PNDA in the reaction mixture at initial time and at time *t*. Initial absorbance reading was measured within 30 s.

2.5. Preparation of DNA pellets and fibers

The DNA from salmon testes was dissolved in 20 mM NaCl to make a solution of approximately 1 mM DNA base pair concentration. Subsequently, the complex solution was added drop wise to the DNA, and stirred for approximately 12 h at 4 °C. The resulting solutions were adjusted to a pH of 8.4 with sodium hydroxide and hydrochloric acid throughout the experiments. The DNA-pellet was obtained by ultracentrifugation of the mixed solution at 60 000 rpm for 7 h. The DNA fibers were prepared from the pellet as previously reported [9].

2.6. EPR spectra

X-band (9.2 GHz) EPR spectra at ambient temperature and −150 °C were measured on a JEOL RE-2X spectrometer with 100 kHz field modulation of 0.5 mT. The magnetic field was calibrated with an NMR field meter EFM-2000 (ECHO Electronics LTD). The microwave frequency was measured on Anritsu MF2412A frequency counter. The EPR spectra were measured at different angles of Φ between the fiber axis and the static magnetic field.

2.7. DNA cleavage experiments

The reaction mixtures were prepared as follows: 1 μl of 10 μM H_2O_2 or 1 μl of 2 μM ascorbic acid were added to the mixture of 0.5 μl of supercoiled plasmid DNA pBR322 (0.25 μg/μl) and 2 μl of 500 μM copper(II) complexes or salt followed by dilution with borate buffer pH 8.5 to a total volume of 20 μl. The reactions were performed after incubating the reaction mixture at 37 °C for 2 h in the presence or/and absence of complexes or copper salt. 3 μl of 6× loading buffer was added to 20 μl of the reaction mixtures and electrophoresis was performed at 80 V for 90 min in Tris-acetate-EDTA (TAE) buffer, pH 8.1, using 1.25% agarose gel. After electrophoresis, the agarose gel was stained with ethidium bromide solution (0.5 μg/ml).

Agarose gel electrophoresis experiments were carried out on supercoiled plasmid DNA pBR322 (4.4 Kb) using a horizontal gel system. For the cleavage studies, each 20 μL sample consisted of the complex dissolved in buffer, DNA, and the required volume of additional buffer. All samples were incubated in the dark in a water-bath at a temperature of 37 °C for 2 h. For the oxidative or reductive cleavage studies, incubation of the samples was similarly carried out. The DNA cleavage profile was analyzed using 1.25%