Journal of Inorganic Biochemistry 173 (2017) 105–112

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

A comparison study on RNase A oligomerization induced by cisplatin, carboplatin and oxaliplatin

Delia Picone[a,*], Federica Donnarumma[a], Giarita Ferraro[a], Giovanni Gotte[b], Andrea Fagagnini[b], Giovanna Butera[b], Massimo Donadelli[b], Antonello Merlino[a,c,*]

[a] Department of Chemical Sciences, University of Naples Federico II, via Cintia, I-80126 Naples, Italy
[b] Department of Neuroscience, Biomedicine and Movement, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy
[c] Institute of Biostructures and Bioimages, CNR, Via Mezzocannone 16, I-80134 Naples, Italy

ARTICLE INFO

Keywords:
Platinated ribonucleases
Ribonuclease oligomers
Protein aggregation
Protein-metal interactions
Oxaliplatin
Carboplatin

ABSTRACT

Cisplatin (CDDP) can form interprotein cross-links, leading to the formation of platinated oligomers. A dimer, a trimer and higher oligomers of bovine pancreatic ribonuclease (RNase A) obtained upon reaction with CDDP in 1:10 protein to metal ratio at 37 °C have been previously characterized. Here, we verify the ability of carboplatin and oxaliplatin to induce RNase A oligomerization under the same experimental conditions. The amount of formed RNase A oligomers was compared with that obtained in the reaction of the protein with CDDP. Among the three anticancer agents, CDDP is the most reactive and the most effective in inhibiting the ribonucleolytic activity of the protein. Oxaliplatin is the least potent oligomerization agent. Biophysical characterizations of structure and stability of platinated dimers formed in the presence of carboplatin and oxaliplatin suggest that they have a similar thermal stability and are more prone to dissociation than the corresponding dimer obtained with CDDP. Oligomers obtained in the presence of carboplatin are the most active. X-ray structures of the monomeric adducts that RNase A forms with the three drugs provide a rational basis to explain the different effects of the three anticancer agents on enzymatic activity and protein aggregation. Although platinated oligomers of RNase A formed upon reaction with CDDP, carboplatin and oxaliplatin retain a residual ribonuclease activity, they do not show cytotoxic action, suggesting that protein aggregation processes induced by Pt-based drugs can represent a collateral drawback, which affects the functional state of protein targets and reduces the efficacy of Pt-based drug treatment.

1. Introduction

Cisplatin (*cis*-$PtCl_2(NH_3)_2$, *cis*-diamminedichloroplatinum(II), CDDP) is the most used Pt-based drug in cancer therapeutics. Carboplatin (*cis*-diammine(cyclobutane-1,1-dicarboxylate-O,O′)platinum(II)) and oxaliplatin (ethane-dioate(1*R*,2*R*)-1,2-cyclohexanediamineplatinum(II)) (Fig. 1) are two CDDP analogs that are used as an alternative to cisplatin in the treatment of tumors that are resistant or poorly responsive to CDDP [1–3]. The mechanism of action of these Pt-based drugs depends on the formation of stable adducts with DNA [4–6] that interfere with replication and transcription and lead the cell to the death by apoptosis [7–9].

The formation of DNA adducts with CDDP, carboplatin and oxaliplatin has been extensively studied and the X-ray structures of these adducts have been solved [7,8,10]. Although DNA is the main biological target of CDDP and of its second generation Pt-based drugs [11], the interaction of these molecules with proteins has drawn increasing attention in the last few years, given the role that it plays in defining the toxicity profiles of the drugs [12,13]. It has been shown that CDDP and carboplatin mainly bind the side chains of methionines [14], histidines [15,16] and cysteines [17]. Oxaliplatin efficiently binds also the side chain of aspartic/glutammic acids [18]. CDDP can bind proteins forming monodentate or bidentate adducts [12,14,19–21], i.e. interacting with one residue side chain or even with two residue side chains at the same time. Binding of CDDP to proteins can induce small structural variations in their overall native structure [12,15,22,23] or large conformational changes [24].

In the presence of high concentrations of CDDP, proteins can even modify their oligomerization state [25–27]. The CDDP-induced oligomer formation is sometimes associated with rapid loss of function [28]. The formation of platinated protein oligomers might play potential roles in the cellular toxicity associated with the use of the drug [29]. It has been shown that dimerization can also occur in the presence of oxaliplatin [30,31]. The dimerization process is accompanied with the

* Corresponding authors at: Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cintia, I-80126, Napoli, Italy.
E-mail addresses: delia.picone@unina.it (D. Picone), antonello.merlino@unina.it (A. Merlino).

http://dx.doi.org/10.1016/j.jinorgbio.2017.05.005
Received 17 March 2017; Received in revised form 27 April 2017; Accepted 7 May 2017
Available online 09 May 2017
0162-0134/

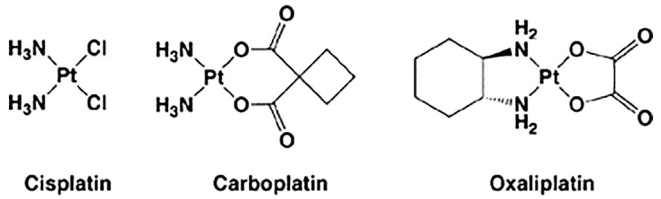

Fig. 1. Structure of cisplatin (CDDP), carboplatin and oxaliplatin.

loss of Pt ligands and partial protein unfolding [20,31].

Recently, we have characterized the products of the reaction between CDDP and bovine pancreatic ribonuclease (RNase A) [20], which is a suitable model for protein aggregation [32]. We found that, in the presence of a 1:10 protein to CDDP ratio, RNase A is able to form a dimer (Pt-D_{CDDP}), a trimer (Pt-T_{CDDP}) and higher oligomers (Pt-O_{CDDP}) [20], which are distinct from those formed by the protein through three dimensional domain swapping and well characterized [32].

Although a number of articles demonstrating the ability of CDDP to induce the formation of protein oligomers (at least at high metallodrug to protein ratio) have been published [12,25–27], no systematic studies have been performed on protein aggregation process driven by the presence of carboplatin and oxaliplatin.

This study focusses on the formation of RNase A oligomers obtained in the presence of these two drugs. The results of our work reveal that 24 h incubation at 37 °C of RNase A in the presence of an excess of carboplatin and oxaliplatin (1:10 protein to metallodrug ratio) induces the formation of platinated monomers (Pt-M_{carbo} and Pt-M_{oxa}), dimers (Pt-D_{carbo} and Pt-D_{oxa}), trimers (Pt-T_{carbo} and Pt-T_{oxa}) and a small amount of higher oligomers. Denaturing and non-denaturing polyacrylamide gel electrophoresis and size-exclusion chromatography were used to characterize the formation of the oligomers. Circular dichroism and UV–Vis spectroscopy were used to investigate their structural stability and catalytic activity. The results were compared with those obtained for CDDP under the same experimental conditions and discussed on the basis of the X-ray structures of Pt-$M_{carbo,}$ here obtained, of Pt-M_{CDDP} [20] and of the X-ray structures of adducts formed when crystals of RNase A were soaked in solutions containing cisplatin [14], carboplatin or oxaliplatin [22].

2. Materials and methods

2.1. Production and purification of platinated oligomers

CDDP, carboplatin, oxaliplatin, and yeast RNA were purchased from Sigma-Aldrich and used as received. RNase A (Type XII-A) was also purchased from Sigma Aldrich and additionally purified from deamidated species by cation-exchange chromatography onto a Source 15 S HR 16/10 column attached to an ÄKTA FPLC (fast protein liquid chromatography) system. Platinated RNase A oligomers were obtained incubating the protein (5 mg × mL^{-1}) with the two drugs (carboplatin or oxaliplatin), separately, in 0.20 M sodium phosphate buffer, pH 6.7 at 37 °C, in a 1:10 protein:metallodrug molar ratio, over a time period of 24 h. Aliquots from each reaction mixture were collected and analyzed by polyacrylamide gel electrophoresis under denaturing (SDS-PAGE) and non-denaturing (native) conditions. Platinated monomers, dimers and oligomers of RNase A obtained upon incubation of the protein with carboplatin and oxaliplatin were purified as previously described by gel-filtration chromatography either onto a Sephadex G75 (1.5 × 72 cm) or onto a Superdex 75 HR 10/300 column attached to an ÄKTA FLPC system (GE-Healthcare). Both columns were equilibrated with 0.20 M sodium phosphate at pH 6.7; samples were eluted at a flow rate of 0.15 mL × min^{-1} and 0.10 mL × min^{-1}, respectively. The fractions corresponding to the different peaks were pooled and stored at 4 °C, unless otherwise stated.

Sample homogeneity upon gel-filtration was verified on 12% polyacrylamide non-denaturing gel-electrophoresis at 4 °C, as previously described [20,33]. Concentration of all platinated species was spectrophotometrically measured at 280 nm, using the extinction coefficient of RNase A ($\varepsilon^{1\%}$ at 280 nm = 0.695).

2.2. Circular dichroism spectroscopy

The conformational and structural stability of purified Pt-M_{carbo}, Pt-M_{oxa}, Pt-D_{carbo}, Pt-D_{oxa}, Pt-T_{carbo} and Pt-T_{oxa} was analyzed by collecting far-UV circular dichroism (CD) spectra using a Jasco J-810 spectropolarimeter (JASCO Corp, Milan, Italy) and protein concentration = 0.10 mg × mL^{-1}. Operating conditions are the same used to collect CD spectra of platinated oligomers of RNase A obtained upon incubation in the presence of CDDP [20]. Briefly, each spectrum was obtained in 0.20 M sodium phosphate at pH 6.7 averaging three scans and converting the signal to mean residue ellipticity in units of deg. × cm^2 × $dmol^{-1}$. Spectra were collected using 0.1 cm path length quartz cells in the far-UV region from 190/195 to 250 nm. Other experimental settings were: scan speed = 50 nm × min^{-1}, band width = 2.0 nm, resolution = 1.0 nm, sensitivity = 50 mdeg, and response = 4 s. Thermal unfolding profiles were obtained by monitoring the CD signal at 222 nm as a function of temperature, in the range 20–90 °C, with heating rates of 1.0 °C × min^{-1}.

2.3. Enzymatic activity

The ribonucleolytic activity of the purified RNase A platinated species obtained in the presence of carboplatin and oxaliplatin was spectrophotometrically measured at 300 nm by using yeast RNA as substrate and following the Kunitz method [34]. Assays were performed at 25 °C in 0.050 M sodium acetate pH 5.0, using 0.5 mg × mL^{-1} of RNA and enzyme concentration of 0.5 μg × mL^{-1}. The activity of the platinated proteins was compared to that obtained under the same experimental conditions for RNase A and Pt-M_{CDDP} [20].

2.4. Dissociation experiments

The stability of Pt-D_{carbo}, Pt-D_{oxa}, Pt-T_{carbo} and Pt-T_{oxa} was evaluated by incubating the purified proteins at 4 °C and at 37 °C, and analyzing the quantity of monomer formed under these experimental conditions for time intervals settled between 1 and 35 days by non-denaturing gel electrophoresis.

2.5. Crystallization and X-ray diffraction data collection

Crystals of purified Pt-M_{carbo}, obtained upon RNase A incubation in 1:10 protein to metallodrug ratio, were grown according to a previously reported procedure [20]. Briefly, the adduct was crystallized at 4 °C in order to avoid the formation of higher oligomers in the crystallization mixture, using the hanging drop vapor diffusion method, protein concentration 15 mg × mL^{-1} and a reservoir solution containing 30% ammonium sulfate, 0.10 M sodium acetate at pH 5.5 and 3.0 M sodium chloride. Prior to data collection, crystals were fished with nylon loops and flash-cooled at 100 K using nitrogen gas produced by an Oxford Cryosystem without cryoprotectants, as done for many other adducts formed between RNase A and metallodrugs (see for example [35]).

Crystallographic data were collected at 100 K by using a Rigaku Micromax 007 HF generator (CuKα = 1.5418 Å) and a Saturn944 CCD detector at the CNR Institute of Biostructures and Bioimages, Naples, Italy. Data were processed and scaled using HKL2000 [36]. Crystals diffract X-rays at 2.07 Å resolution. Crystal parameters and data collection statistics are reported in Table S1.