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Spectroscopic characterization of genotoxic chromium(V) peptide complexes: Oxidation of Chromium(III) triglycine, tetraglycine and pentaglycine complexes☆

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ABSTRACT

Evidence is growing that metabolites of Cr(III) dietary supplements are partially oxidized to carcinogenic Cr(VI) and Cr(V) in vivo. Hence, we examined oxidations of Cr(III) peptide (triglycine, tetraglycine and pentaglycine) complexes to Cr(VI) and Cr(V) by PbO_2 at 37 °C and physiological pH values between 3.85 and 7.4. The products were characterized by EPR and UV/Vis spectroscopies and electrospray mass spectrometry. At pH 3.85, the monomeric Cr(V) complexes produced were relatively unstable and degraded over min to hr under the acidic conditions. The triglycine and tetraglycine Cr(V) complexes had five-line ^{14}N-superhyperfine-coupled EPR signals; g_{iso}, (A_N) values 1.9824 (2.44×10^{-4} cm^{-1}) and 1.9825 (2.43×10^{-4} cm^{-1}), respectively. The pentaglycine Cr(V) complex had a seven-line ^{14}N-superhyperfine-coupled EPR signal: g_{iso} = 1.9844; A_N = 2.27×10^{-4} cm^{-1}. In phosphate buffer (pH 7.4 and 5.85), several Cr(V) intermediates were produced, but Cr(VI) was the end product. For the triglycine, tetraglycine and pentaglycine Cr(V) complexes, the g_{iso} (A_N, 10^{-4} cm^{-1}) values were 1.9831 (2.17), 1.9843 (2.27) and 1.9844 (2.30), respectively. A second EPR signal with unresolved superhyperfine structure was observed at g_{iso} ~ 1.966. At 1 min, the tetraglycine and pentaglycine Cr(V) complexes, had another signal at g_{iso} ~ 1.978, which decayed relative to the other signals with time. This chemistry has relevance to: (i) certain types of DNA damage produced by Cr carcinogens; (ii) the intracellular oxidation of Cr(III) to Cr(VI); and (iii) redox recycling of Cr(III) metabolites formed from both the intracellular reduction of carcinogenic Cr(VI) and from Cr(III) supplements.

1. Introduction

There is a large body of evidence that reactive Cr(V) complexes play an important role in the mechanism of Cr(VI)-induced carcinogenesis [1–29], and growing evidence that Cr(III) complexes are partially oxidized intracellularly [30] and extracellularly [14,19,20,31–34] to Cr(V) and Cr(VI). As a result, interest has been mounting in the characterization of Cr(V) complexes with biologically relevant ligands and conditions. Chromium(V) complexes are also intermediates in the Cr(VI) oxidation reactions of both inorganic [35–37] and organic [5,6,13–15, 17,18,38–42] substrates. In addition, there is evidence that they are the active species in Cr(III)-catalyzed oxidations of organic substrates in the presence of a terminal oxidant [43–53]. Chromium(III) peptide complexes are also implicated in the long-term toxic effects caused by the erosion of surgical implants [14]. Such erosion leads to the slow release of Cr(III) into the bloodstream at comparable or lower levels [14] than would be present in from chromium(III) supplementation. As such, there is considerable interest in the synthesis and structures of Cr(V) complexes with peptide ligands.

A number of Cr(V) complexes have been reported that have deprotonated amide ligands and one such complex with a macrocyclic tetraamido ligand has been characterized by X-ray crystallography [54]. This and related complexes were very stable, even in water. The MeOH reduction of Cr(VI) in the presence of the peptides: trialanine, tetraalanine, pentaalanine, triglycine, tetraglycine, and pentaglycine, and related ligands stabilize the Cr(V) oxidation state in solution. In these complexes the Cr(V) is coordinated to the amine N (for the tripeptides), and deprotonated amide *N* and carboxylate *O* atoms [44,55].

The imine/amide species, Cr(III)–bpb complexes (bpb = *N*,*N*′-bis(2-pyridine-carboxamido)-1,2-benzene), have also been used as catalysts

Abbreviations: alaalaala, trialaninato(3-); bpb, *N*,*N*′-bis(2-pyridinecarboxamido)-12-benzene; cyt P450, cytochrome P450; ehba, 2-ethyl-2-hydroxybutanoato(2-); glyglyglyH$_3$, triglycine; glyglygly, triglycinato(3-); glyglyglyglyH$_4$, tetraglycine; glyglyglygly, tetraglycinato(4-); glyglyglyglyglyH$_5$, pentaglycine; glyglyglyglygly, pentaglycine(5-); mac, 3,6,9,12,14-pentaoxo-2,2,5,5,7,7,10,10-octamethyl-13.13-diethyl-1,4,8,11-tetraazacyclotetradecane),; mampa, 5,6-(4.5-dichlorobenzo)-3,8,11, 13-tetraoxo-2,2,9,9-tetramethyl-12.12-diethyl-1,4,7,10-tetraazacyclotridecane; phen, (1.10-phenanthroline); ROS, reactive oxygen species; salen, (*N*,*N*′-ethylenebis (salicylideneiminato).

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in the oxidation of alkenes by iodosylbenzene, where oxido–Cr(V) species were proposed as the reactive intermediates. Attempts to isolate these intermediates were unsuccessful [56], although the Cr(V) species have recently been characterized by EPR spectroscopy [52]. These species are models for peptides containing His, where binding could be via both the peptide backbone and an *N*-imidazole donor.

With respect to Cr-induced cancers, Cr(V)-induced cleavage of DNA occurs with the relatively stable Cr(V) complex, sodium bis[2-ethyl-2-hydroxybutanoato(2-)]oxido-chromate(V), $[CrO(ehba)_2]^-$, which has been structurally characterized by X-ray crystallography [57] and X-ray absorption spectroscopy [58]. This complex causes stand breaks in supercoiled DNA [11,16,21] and a range of other DNA damage [22]. It is also genotoxic toward bacterial and mammalian cells in vitro [8]. Macrocyclic Cr(V) complexes of peptide-model ligands, such as mampa and mac (mampa = 5,6-(4,5-dichlorobenzo)-3,8,11,13-tetraoxo-2,2,9,9-tetramethyl-12,12-diethyl-1,4,7,10-tetraazacyclotridecane, mac = 3,6,9,12,14-pentaoxo-2,2,5,5,7,7,10,10-octamethyl-13,13-diethyl-1,4,8,11-tetraazacyclotetradecane), are also genotoxic toward both bacterial and mammalian cells in vitro, but they do not cleave DNA to any significant extent [8,9]. In addition, long-lived Cr(V)-sugar, glycoprotein and glutathione complexes are observed in bacterial [59] and mammalian cells [30,39,40] and mucus obtained from the respiratory tract [60] when treated with carcinogenic Cr(VI), even though the majority of Cr(VI) is rapidly reduced intracellularly to Cr(III) [61–63]. This led to the redox recycling mechanism of Cr-genotoxicity that was postulated to occur as a result of continuous generation of reactive and genotoxic Cr(VI), Cr(V) and Cr(IV) species [19,20,32,33,63–65], as well as reactive oxygen species from the reactions of these oxidation states with a range of biological reductants [66]. These species and model complexes have been well-characterized by EPR spectroscopy, electrospray mass spectrometry, X-ray absorption spectroscopy and, in some cases, X-ray crystallography [2,39,40,60,67–69]. The Cr(V) complexes are also able to undergo extensive pH-dependent ligand-exchange reactions amongst various biologically relevant ligands [6,31,40,70,71]. Hence, the most stable Cr(V) species are likely to differ in different organelles; for instance, lysosomes have pH values of ~4.5–5.5 [72–74]. In light of these studies, Cr complexes with non-sulfur containing peptides are candidates for active intermediates in Cr(VI)-induced carcinogenesis. Studies have also shown that such Cr(III) compounds may be genotoxic themselves either as the active carcinogenic agents [75,76], or by intracellular oxidation to Cr(V) [19,20,32,33,63].

The potential importance of Cr complexes of peptides in Cr-induced cancers was indicated by the observation that Cr(VI) interacts with DNA in the presence of a microsomal enzyme fraction to produce DNA-protein crosslinks [28]. Subsequently, it was shown that such crosslinks are markers for human exposure to carcinogenic Cr(VI) [77] and that in cultured cells, some of the crosslinks involve non-sulfur-containing amino acid residues [78]. Since Cr(III) complexes can also cause the same crosslinks [78], the question arises as to which oxidation state is involved. Hence, it was of interest to examine whether Cr(V) peptide complexes could be generated intracellularly from Cr(III), since Cr(V) tetraamide complexes damage DNA and are mutagenic in bacterial and mammalian cells [8,9]. The current study demonstrates for the first time that Cr(V) peptide complexes and Cr(VI) species can be generated from the oxidation of Cr(III) peptide compounds under physiologically relevant conditions of temperature and pH values.

In order to characterize the biochemistry of relevance to these potential extracellular and intracellular redox reactions, careful consideration of reaction conditions was required. Partial oxidation of Cr(III) peptide adducts of Cr dietary supplements to Cr(VI) by H_2O_2 in blood serum [33,34], and partial intracellular oxidation of Cr(III) supplement metabolites to Cr(V) and Cr(VI) in adipocytes [30] have been observed. This prompted us to choose suitable Cr(III) peptides oxidants that could cover the entire biologically pH range of relevance to the extracellular and intracellular environments in humans. This is important because the approximately micron size punctate structures that contain high concentrations of a mixture of Cr(III), Cr(V) and Cr(VI) in Cr(III)-treated adipocytes [30] could be: (i) mitochondria (which contain cytochrome P450 and generate a range of ROS [79], pH ~ 8 [80]); (ii) lysosomes (containing a range of oxidases; pH 4.5–5.5) [72–74] or peroxisomes (high flux of H_2O_2 used to oxidatively breakdown intracellular waste at pH values controlled by cytosolic pH value to be around 6.9–7.1 [81]). In addition, Cr(III) binding to transferrin (Tf) is proposed to be physiologically relevant [82]. This binding can occur at either the Fe binding sites of apo-Tf, which inhibits Cr uptake, or on the protein surface of the Fe(III) bound protein, Fe_2Tf, which enhances Cr uptake by cells [83]. Under physiological relevant loading of Fe to Tf in the bloodstream, Cr-binding to apo-Tf inhibits Cr uptake and is likely to be a protective mechanism against Cr toxicity [83]. However, binding to the outer surface of Fe_2Tf and other serum proteins may also be relevant to the current research and in terms of Cr(III) peptides that can carry Cr(III) into a range of intracellular environments. PbO_2 and IO_4^- were chosen as suitable oxidants that could operate over the pH range relevant to these extracellular and intracellular environments to enable a study of the pH dependence of the chemistry of Cr(V) peptide complexes and Cr(VI) obtained from oxidation of Cr(III) peptide complexes, as described herein.

While H_2O_2 acts as a good oxidant for Cr(III) complexes at pH ≥ 7.4 under biologically relevant conditions found in blood plasma [33,34], Cr(V) species undergo disproportionation under these conditions, leading to Cr(VI). This is an issue with EPR spectroscopic characterization of the Cr(V) intermediates for two reasons: (i) oxidation by H_2O_2 is somewhat slower than the chosen oxidants, which makes proper characterization of the low concentrations of Cr(V) intermediates by EPR spectroscopy difficult, i.e., the rate of Cr(V) disproportionation becomes comparable to the rate of Cr(III) oxidation; and (i) H_2O_2 tends to form mixed-ligand Cr(V) peroxido species [84,85] and the chromate(VI) product also forms Cr(VI) and Cr(V) peroxido complexes [37], all of which readily oxidize organic ligands, such as peptides. Additionally, PbO_2 and HIO_4, serve as models of biological oxido-transfer agents, such as Fe(IV) = O in cytochrome P450 (Cyt P450) or $Mo^{VI}(O)_2$ in oxotransferases, such as xanthine oxidase [32]. The latter enzyme has also been shown to result in partial oxidation of metabolites of Cr(III) supplements in blood serum [33,34] and is an important intracellular oxidation catalyst and generator of H_2O_2 and other ROS [86].

2. Experimental section

2.1. Reagents

The peptide ligands triglycine ($glyglyglyH_3$), tetraglycine ($glyglyglyglyH_4$), and pentaglycine ($glyglyglyglyglyH_5$) (all Sigma, AR grade) were used without further purification. Chromium(III) chloride hexahydrate (Merck, 98%), NaOH (Merck, 98%), KCl (Merck, 99.5%), $BaBr_2 \cdot 2H_2O$ (Sigma, AR grade), $CuCl_2 \cdot H_2O$ (Merck, 99%), PbO_2 (Merck, AR grade), periodic acid (Hopkins & Williams, 95%) and CH_3OH (Biolab Scientific, AR grade) were used as received. Chromatography columns were Lipophilic Sephadex LH-20 (Sigma) in methanol for gel filtration and DEAE A-25 Sephadex (Sigma) in water for anion exchange. Buffer solutions were prepared from the following: 100 mM acetate buffer (pH 3.85) was prepared from 100 mM solutions of sodium acetate (Merck, 99.5%) and acetic acid (BDH, 99.7%); 100 mM phosphate buffer (pH 7.40) was prepared from 100 mM solutions of Na_2HPO_4 (Merck, AR grade) and $NaH_2PO_4 \cdot H_2O$ (Merck, 99%). The pH values of the buffers were adjusted after mixing appropriate volumes of the above pairs of solutions by use of a calomel refillable pH electrode (Activon, AEP321) connected to a pH meter (Activon, model 210).

2.2. Physical measurements

Electronic absorption (UV/Vis) spectra were obtained using a Hewlett-Packard 8452A diode array spectrophotometer over the

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