Aerobic Reactions of Antitumor Active $Rh_2(CH_3COO)_4$ with Glutathione, Cysteine and Its Derivatives

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The notable antitumor activity of the dirhodium(II) carboxylates has attracted considerable attention since the 1970s. Dirhodium(II) complexes can bind to DNA forming inter-strand cross-links, resulting in disruption of the transcription process and ultimately cell death. However, before reaching its main target these compounds can also interact with amino acid residues in proteins and enzymes. It has been suggested that the $Rh_2(CH_3COO)_4$ complex can decompose *in vivo* probably due to its interaction with proteins / enzymes that have thiol groups from cysteine residues at or near their active sites [1]. Few studies have been carried out on the complex formation between $Rh_2(CH_3COO)_4$ and thiol-containing ligands [2], including cysteine [3], but no structural information of the reaction products with biomolecules has been available. Here, we present the results of our investigations about the reactions between $Rh_2(CH_3COO)_4$ and glutathione (H_3A), the most abundant thiol containing peptide in cells, as well as cysteine (H_2Cys) and its derivatives penicillamine (H_2Pen; 3,3'-dimethylcysteine) and *N*-acetylcysteine (H_2NAC) under aerobic conditions in aqueous solution at the physiological pH.

The reactions were monitored using electrospray ionization mass spectrometry (ESI-MS) and UV-vis. spectroscopy. The reaction products were purified using size exclusion chromatography, and characterized by ^{13}C NMR, sulfur K-edge XANES, and Rh K-edge EXAFS spectroscopic techniques.

The ESI-mass spectra of the products were dominated by mass peaks corresponding with $[Rh^{III}_2L_4]^{2-}$ (L = Pen, Cys, NAC) and $[Rh^{III}_2(HA)_4]^{2-}$ ions. Presence of hydrogen peroxide in solution mixtures confirmed that oxidation of Rh(II) to Rh(III) occurs via reduction of O_2. Rh K-edge EXAFS spectroscopy revealed that reactions of $Rh_2(CH_3COO)_4$ with glutathione and *N*-acetylcysteine, with only thiol group as their binding site, produce oligomeric species of dimeric units, in which tri-thiolate bridges are formed between the two Rh(III) ions (Rh···Rh 3.11 ± 0.02 Å). In the reaction products with penicillamine and cysteine, which are able to form (*S*,*N*)-chelates, the Rh(III) ions are bridged by only two thiolate groups, resulting in Rh···Rh distances > 3.5 Å [4, 5].

The results of this study shed a light on the fate of antitumor active dirhodium(II) tetracarboxylates in body, and their interactions with thiol-containing enzymes and proteins, such as metallothioneins.

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