

Insight into hydrolytic reaction of *N*-acetylated L-histidylglycine dipeptide with novel mechlorethamine platinum(II) complex. NMR and DFT study of the hydrolytic reaction

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Received 7th April 2011, Accepted 23rd June 2011

DOI: 10.1039/c1dt10593k

The reaction of K_2PtCl_4 with the alkylating agent mechlorethamine hydrochloride, at a molar ratio of 1 : 2, results in the formation of 2-chloro-*N*-(2-chloroethyl)-*N*-methylethylammonium-tetrachloridoplatinate(II) complex. The hydrolytic activity of the novel Pt(II) complex was tested in the reaction with *N*-acetylated L-histidylglycine dipeptide at a molar ratio 1 : 1. It was shown that the hydrolytic reaction, performed at 60 °C in acidic medium, leads to the regioselective cleavage of the amide bond involving the carboxylic group of histidine. Density functional theory was used to explore the structures of the proposed participants in the hydrolytic reaction.

Introduction

Alkylating agents are highly reactive compounds. Many of them are synthetic drugs. These compounds react with many electron-rich atoms to form covalent bonds. Nucleophilic groups, such as amino, phosphate, carboxyl, sulfhydryl or imidazolyl moieties in proteins, as well as nucleic acids, can be alkylated. The chemotherapeutic role of alkylating agents derives from their ability to interfere with genetic (DNA) material, giving mono-adducts and cross-links. Mechlorethamine ($CH_3N(C_2H_4Cl)_2$, code-name HN2) is one of the first non-hormonal chemotherapy drugs known as nitrogen mustard.¹ It is a bifunctional agent with two 2-chloroethyl moieties which react covalently with the adjacent guanine residues in each strand of DNA, building a bridge between the DNA strands. The preferred site of alkylation on DNA is the N7 position of the imidazolyl moiety of guanine.

On the other hand, cisplatin ($Pt(NH_3)_2Cl_2$), the most widely used anticancer drug at present, and its analogues (for example carboplatin) are classified as non-classical alkylating agents, or alkylating-like agents, regarding their principle function of binding to DNA. They are used for the treatment of testicular, ovarian, head, neck and lung cancer.^{2–4} However, their clinical usefulness has been limited by side effects, such as nephrotoxicity, neurotoxicity and ototoxicity, and by the emergence of cancer cells resistant to cisplatin.^{5–7} Therefore, other similar platinum complexes play an important role in the development of anticancer drugs. For all these complexes and their analogues binding occurs to the N7 position of the imidazole ring of the purine bases of DNA. Furthermore, it has been found that some Pt(II) and palladium(II) complexes can behave as artificial metallopeptidases

and promising reagents for the cleavage of unreactive amide bonds of peptides and proteins.^{8–13} It is known that the peptide bond is extremely unreactive and that the half-life for its hydrolysis in neutral solution is several hundred years at room temperature and pH 4–8.^{14,15} Hydrolytic cleavage of proteins, however, plays functional and regulatory roles in physiological processes, such as control of the cell cycle, transcription, antigen processing, and apoptosis. Selective proteolysis can be achieved with enzymes and synthetic reagents. Several proteolytic enzymes are used for cleavage, but application of enzymes is limited by their special requirements for temperature and pH. Since uncatalyzed hydrolysis of peptides is extremely slow, relatively fast methods of artificial cleavage are needed. Transition-metal complexes of Pd and Pt are promising agents for the hydrolytic cleavage of peptides and proteins.^{16–24} However, considering the clinical usefulness of Pt(II) complexes and the great affinity of Pt(II) complex ions for the nitrogen of heterocycles (for example the imidazole ring of histidine), it is surprising that such interactions have not been extensively investigated for peptides comprising of sulfur-containing amino acids (for example methionine). Namely, studies on the coordination behavior of Pt(II) complexes to histidine-containing peptides are scarcely reported and, also, the mechanism of the hydrolytic reaction of peptides in the presence of Pt(II) promoters has not been completely elucidated. For the clarification of this mechanism, it was shown to be necessary to investigate these regioselective cleavage reactions with structurally different Pt(II) complexes.

In this paper we report the synthesis, spectral characterization and hydrolytic properties of the novel 2-chloro-*N*-(2-chloroethyl)-*N*-methylethylammonium-tetrachloridoplatinate(II) complex ($[H_2N_2]^+[PtCl_4]^-$). Since the new complex contains HN2 as a classical alkylating agent, and Pt(II) ion, as a non-classical alkylating agent, it was interesting to study its reaction with imidazole-containing dipeptide. Moreover, in order to mimic

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