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Note

Reactivity of [Ru^{III}(hedtra)(H_2O)] with thio-amino acids and protease inhibition

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Dedicated to Prof. Rex E. Shepherd

Abstract

Reaction of [Ru^{III}(hedtra)(H_2O)] (hedtra = *N*-hydroxyethylethylenediaminetriacetate) with thio-amino acids, L (L = cysteine, *N*-acetylcysteine, glutathione and penicilamine), was studied kinetically. Kinetic studies were performed at different concentrations of reactants, pH and temperature. Based on the kinetic results, it is suggested that the formation of S-bound substituted product takes place in a rapid ligand dependent rate determining step. Kinetic data and activation parameters are accounted for operation of an associative mechanism and discussed in reference to the data reported earlier for $edta^{4-}$ (ethylenediaminetetraacetate) complex of ruthenium(III). Results of cysteine protease inhibition studies revealed that inhibition activities of Ru–pac complexes are enzyme specific.

Keywords: Kinetics; Substitution reaction; Associative mechanism; [Ru(hedtra)(H_2O)]; Thio-amino acids; Cysteine protease inhibition

1. Introduction

The chemistry of polyaminopolycarboxylato (pac) complexes of ruthenium is intriguing in many ways [1]. The '*pac*' ligand is fairly alike in its donor character to many metallo-enzymes, which utilize carboxylate or amine donors of amino acids to bind to the metal center. A major impetus toward development of the chemistry of Ru–pac complexes had been bestowed by Professor Rex E. Shepherd of Pittsburgh University. Some emissary papers contributed from Shepherd's group exploring the potential of Ru–pac complexes in biological [2–7] and catalysis processes [8–10] are referred herein. Since there were some overlaps between the work in Shepherd's group and mine, we had been in collaboration with Shepherd till he had been with all of us. Our collaborative work has resulted in some significant publications on kinetic and mechanistic studies of Ru–pac complexes [11,12] and development of new ruthenium based oxo-transfer catalysts [13,14].

The present work stems from our continued interest [15–18] in kinetic and mechanistic studies of the interaction of Ru–pac complexes with bio-molecules with the objective of acquiring mechanistic knowledge that could be of use in rational design of a new family of metallo-pharmaceuticals. Very recently, we have discovered [19] cysteine protease inhibition activity of Ru–edta complex ($edta^{4-}$ = ethylenediaminetetraacetate). Cysteine proteases are reportedly known to have pathological role [20–22] in many health related problems. For selective inhibition of cysteine proteases, the inhibitor should

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rationally consist of an active site that can react selectively with cysteine residue of the enzyme to produce an inert covalent enzyme–inhibitor complex, and a fragment that recognizes the enzyme specifically. The reported ability of Ru^{III}–edta complex toward inhibition of cysteine protease activity [19] of both papain and bromelain enzymes was attributed to the high affinity of the $[Ru^{III}(edta)(H_2O)]^-$ complex towards binding the –SH group in the cysteine residue of the enzymes through a rapid aqua-substitution reaction and thus inhibiting the protease activity of the enzyme by forming a stable Ru(edta)–enzyme complex. It was also hypothesized that the pendant carboxylate group in $[Ru^{III}(edta)(H_2O)]^-$ complex might have some role in recognition of both the enzymes chosen for the reported investigation [19]. In order to gain more insight into the inhibition action pertaining to the kinetic behavior of the metal complexes towards binding thiol group (–SH) and possible role of pendant group of chelating pac ligand in recognition of the enzyme, we have selected [Ru(hedtra)(H_2O] complex ($hedtra^{3-}$ = *N*-hydroxyethylethylenediaminetriacetate) in the present study, which contains the $–CH_2CH_2OH$ pendant arm and the complex is considerably less labile towards aquo-substitution [23] than the corresponding $[Ru^{III}(edta)(H_2O)]^-$ complex [24]. We report herein the results of the kinetic studies of the interaction of $[Ru^{III}(hedtra)(H_2O)]$ with thio-amino acid ligands, L (L = cysteine, *N*-acetylcysteine, glutathione and penicillamine), which has not been reported so far. We also report the results of protease inhibition studies of both $[Ru^{III}(hedtra)(H_2O)]$ (**1**) and $[Ru^{III}(edta)(H_2O)]^-$ (**2**) complexes using three cysteine protease enzymes bromelain, papain and ficin, and azoalbumin, as substrate.

2. Experimental

2.1. Materials

$K[Ru^{III}(hedtra)Cl]\cdot 2H_2O$ was prepared by a published procedure [23]. Elemental *Anal.*: Calc. for $C_{10}H_{15}N_2ClO_7KRu$: C, 26.6; H, 3.4; N, 6.2. Found: C, 26.5; H, 3.2; N, 6.1%. UV–Vis data in H_2O (λ_{max}, nm (e_{max}, $M^{-1}\,cm^{-1}$): 285 (1250 ± 12). IR (cm^{-1}): 1637 (ν^{-}_{COO}). All other chemicals used were of AR grade and doubly distilled H_2O was used for preparing all solutions.

2.2. Instrumentation

A Perkin–Elmer 240C elemental analyzer was used to collect micro-analytical data (C, H, N). The electronic absorption spectra were measured with a GBC Cintra 10 spectrophotometer coupled with a JULABO water circulating bath. Infrared spectra were recorded on a Perkin–Elmer (Model 783) spectrometer using KBr pellets.

A SF-61SX2 (HI-TECH) stopped-flow spectrophotometer coupled with an online data analyzer (KinetAsyst3) was employed for the kinetic measurements. The solution temperature during kinetic experiments was maintained to within ±0.1 °C using a circulating water bath (JEIO TECH RW-1025G). The substitution reaction was followed at 510 nm where appreciable spectral changes between the reactant and product exist. Rate constant data were measured under pseudo-first order conditions of excess (10–50 fold) substituting ligands. The pH measurements were carried out with a Elico L1 127 pH meter. Acetic acid–acetate, phosphate and borate buffers were used to adjust the pH of the experimental solutions, whereas Na_2SO_4 was used to control the ionic strength of the reacting medium. Experimentally observed rate constant data (k_{obs}) were represented as an average of several kinetic runs (at least five to six) and reproducible within ±4%.

2.3. Protease inhibition studies

Enzyme inhibition studies were performed by following the procedure reported earlier [19]. Three cysteine protease enzymes bromelain, papain and ficin, are used in the present studies. The mixture of buffer (Sodium phosphate buffer (0.2 M) containing 0.001 M EDTA and 0.05 M β-mercaptol), substrate (1% azoalbumin) and enzyme (with/without inhibitor) was incubated at 37 °C for 1 h. The pH of the reaction mixture was 7.5. The reaction was terminated by adding 0.5 ml of 5% TCA and the system was kept at room temperature for 1/2 h and then subjected to centrifugation (4000 rpm) for 15 min. The filtrate (0.5 ml) was added to 0.5 ml of 0.5 N NaOH and absorbance of the resulting solution was measured at 440 nm.

3. Results and discussion

3.1. Kinetic studies

$K[Ru^{III}(hedtra)Cl]$ (**1**) was prepared by a published procedure [23] and characterized by elemental analysis and spectral data (only significant bands) given at the end of the synthetic procedure of complex – **1**. The characterization data were in good agreement with the literature data [23]. The $K[Ru^{III}(hedtra)Cl]$ complex rapidly converts into $[Ru^{III}(hedtra)(H_2O)]$ complex when dissolved in water [23]. The pK_a values of $[Ru^{III}(hedtra)(H_2O)]$ complex and substituting thio-amino acid ligands are summarized in Table 1.

Addition of thio-amino acid ligands to an aqueous solution of complex – **1** resulted in color change from pale yellow to red. The intense band appeared in the