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Inorganica Chimica Acta 358 (2005) 1247–1252

Inorganica Chimica Acta

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Note

Effective heterogeneous hydrolysis of phosphodiester by pyridine-containing metallopolymers

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Received 16 March 2004; accepted 17 October 2004
Available online 19 November 2004

Abstract

The copper (II) complex of a simple pyridine- and amide-containing copolymer serves as an effective catalyst for heterogeneous hydrolysis of the prototypical phosphodiester substrate bis(*p*-nitrophenyl)phosphate at pH 8.0 and 25 °C. The catalysis has a first-order rate constant of $k_{cat} = 8.3 \times 10^{-6}\ s^{-1}$, corresponding to a catalytic proficiency of 75-thousand folds relative to the uncatalyzed hydrolysis with a rate constant of $k_0 = 1.1 \times 10^{-10}\ s^{-1}$ in aqueous buffer solution at pH 8.0. This observation suggests that polymers can be designed to include various functional groups feasible for effective metal-centered catalysis of phosphodiester hydrolysis.

Keywords: Phosphoester; Hydrolysis; Metal; Cu; Metallopolymer; Polymer

1. Introduction

A number of functional and reactive polymers have recently been developed to show broad applications in catalysis, such as hydrolytic and oxidative catalysis [1]. The latter includes oxidative cleavage of nucleic acids [2], and the former is involved in many important biological, industrial, and environmental processes. For example, the reversible hydrolysis of phosphoester bonds and phosphorylation are the key steps in biological signaling, which trigger many normal and disease-related metabolic pathways [3]. Hydrolysis also plays a critical role in degradation of nucleic acids, phosphoester-containing pesticides, and nerve agents, including the G-agents (Sarin and Soman) and the VX-agent [4]. During hydrolysis, the nucleophilicity of a water molecule is greatly enhanced by a general base or a metal center and becomes substantially more acidic compared to free water molecules [5,6], which renders hydrolysis possible around neutral pH. The substrate can be significantly polarized by the metal center and becomes more susceptible to nucleophilic attack [5]. A number of metal complexes, including those of Co^{3+}, Cu^{2+}, Zn^{2+}, and lanthanides (III), have been synthesized in recent years as models for different families of metallohydrolases, such as peptidases, nucleases, and phosphoesterases [6]. The investigation of these model complexes has provided further insight into the mechanism of metal-centered hydrolysis.

Heterogeneous catalysis has been well recognized to be a convenient process in industry and laboratory owing to easy separation of the products, recovery of the catalysts, and incorporation into a continuous flow process. However, it has not been widely applied to hydrolytic processes. Some recent reports about effective hydrolysis by metal centers incorporated onto silica- and organopolymer-based matrices suggest potential applications of heterogeneous hydrolysis [7]. In this

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doi:10.1016/j.ica.2004.10.024

communication, we demonstrate that an easily prepared metallopolymer system can perform proficient heterogeneous hydrolysis toward phosphodiester bond, which can thus serve as a functional model system to provide further insight into metal-centered hydrolysis of phosphoesters.

2. Experimental

The reagents 2- and 4-vinylpyridine were obtained from Across Organics (Fair Lawn, NJ), acrylamide from BioRad (Richmond, CA), and the buffers HEPES, CAPS and MOPS, sodium acetate, Chelex resin, and bis(*p*-nitrophenyl) phosphate (BNPP) from Sigma-Aldrich (St Louis, MO). The buffer solutions were treated with Chelex resin to remove any trace amount of metal ions. Copolymers of 4-vinylpyridine (4Vp) and acrylamide (Ac) were prepared according to published procedures [8] by mixing different ratios of freshly distilled 4VP and recrystallized Ac and the initiator 2,2′-azoisobutyronitrile (AIBN) in DMF to produce copolymers of different average repeating units (RU) of $4Vp_x/Ac_y$. The stoichiometry of the RU of the copolymer was found to vary with the 4Vp/Ac ratio and the amount of AIBN in the reaction mixture. The copolymers were precipitated in ethyl acetate, then filtered and dried in a vacuum oven at 40 °C. Copolymers of 2-vinylpyridine (2Vp) and Ac were prepared with the same method as the 4Vp–Ac copolymers. The formation of the polymers was verified with a Bruker ADX250 NMR spectrometer. A total recycle time of ~10 s was used for spectrum acquisition to ensure complete relaxation of the ^{1}H NMR signals.

The hydrolytic activity of the metallopolymer complex was determined by measuring the initial rate of the hydrolysis of BNPP in 1:1 methanol/HEPES buffer of 25 mM at pH 8.0 and 25 °C, wherein the increase of the absorption at 405 nm ($\epsilon = 17\,500$ M^{-1} cm^{-1} due to the hydrolytic product *p*-nitrophenolate) with time was obtained on a Varian Cary 3E spectrophotometer. A plot of the formation of the product with respect to time gives the rate.

Upon binding with Cu^{2+}, the resulting complex precipitates out as blue solid, which is the active catalyst toward phosphodiester hydrolysis. The optimum metal-to-RU stoichiometry for the catalysis was determined by titrating 1.0-mM polymer solution with Cu^{2+} and checking the resulting heterogeneous solution for activity toward hydrolysis of 1.0-mM BNPP, a prototypical phosphodiester substrate, at pH 8.0 and 25 °C.

The stoichiometry of metal complexes can also be conveniently determined by means of the Job plot [9], wherein the optical density is monitored under different mole fractions of the metal (X_M) or the ligand ($X_L = 1 - X_M$) with a constant total concentration of the metal and the ligand ([M] + [L]). The mole fractions that exhibit the maximum absorption afford the stoichiometry of the complex. Thus, a maximum at $X_M = X_L = 0.5$ reflects the formation of a complex of $\mathrm{X_L}$ and a maximum at $X_M = 0.33$ ($M_L = 0.67$) indicates the formation of a complex of ML_2.

3. Results and discussion

Vinyl pyridine and acrylamide were chosen for the preparation of copolymers that are expected to bind transition metals through the pyridine moiety and form H-bonding using the amide, thus may loosely mimic the active-site environment of various metallo-hydrolytic enzymes [5]. The formation of the polymers can be verified with ^{1}H NMR spectra, which exhibit very broad signals of up to ~500 Hz for the polymers and the disappearance of the sharp monomer signals. The broadness of the signals is the consequence of the very slow rotational correlation time of the "polymer molecules" and the irregular monomer sequence in the polymers.

Depending on the Vp/Ac ratio and the amount of the initiator in the preparation, copolymers with different amounts of Vp and Ac can be obtained. The use of a molar ratio of 4Vp:Ac = 1:3 in the presence of 1% molar amount of AIBN produced a copolymer with a stoichiometry of $4Vp_3Ac_1$ for the RU based on the integration of the ^{1}H NMR signals of the pyridine ring and the amide NH_2 protons (Fig. 1). The latter signals disappear upon addition of a few drops of D_2O into the polymer in DMSO. The chemical shifts of the solvent exchangeable NH_2 signals in the copolymer are consistent with those of Ac at 6.4 and 6.9 ppm. The integration gives a 4Vp:Ac ratio of 3:1, with $4Vp_3Ac_1$ as the average RU for this copolymer. The stoichiometry of $4Vp_3$-Ac_1 gives an apparent RU formula mass of 386.5 Da, which was used for the calculation of the mole fractions. The

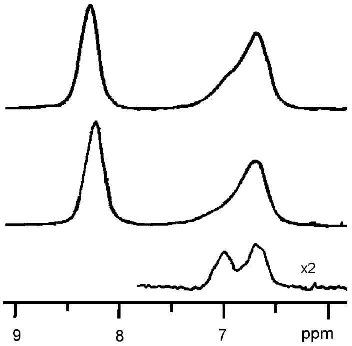

Fig. 1. The ^{1}H NMR signals of the pyridine ring and the amide NH_2 protons of the copolymer in DMSO (top trace) and in DMSO upon addition of a few drops of D_2O, where the NH_2 signals are wiped out (middle trace). The change can be clearly seen in the difference spectrum which reveals the NH_2 signals (bottom trace).