Study of cyclization of chelating compounds using electrospray ionization mass spectrometry

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(Received September 14, 1999)

Electrospray ionization mass spectrometry (ESI-MS) was used for the study of cyclization of organic chelating compounds (chelators). Four chelating compounds were studied: Symmetrical ethylenediaminetetraacetic acid (s-EDTA), Unsymmetrical ethylenediaminetetraacetic acid (u-EDDA), N(2-hydroxyethyl) ethylenediaminetriacetic acid (HEDTA) and N(2-hydroxyethyl)iminodiacetic acid (HEDDA). The chelators were cyclized with reagents and heating. The open and cyclized form of the chelators were semi-quantified by both positive and negative ion modes ESI-MS. The kinetics of chelator cyclization was studied as a function of reaction temperature and the pH of the matrix. The cyclization of s-EDDA was found to be a pseudo-first order reaction in s-EDDA and overall second order. The cyclizations of HEDDA and HEDTA are reversible reactions. Higher temperature and lower pH favors cyclization.

Introduction

Organic chelating compounds (chelators) such as N-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA) and ethylenediaminetetraacetic acid (EDTA) have been identified as components in high level waste at the Hanford site in southeastern Washington State. They were previously found to cyclize and form six-membered rings (intramolecular lactone and lactam, respectively) in the derivatization reaction with boron trifluoride/methanol (BF3/CH3OH). The intramolecular cyclization was also observed in the thermospray LC/MS analysis of HEDTA and EDTA, and in the thermogravimetric analysis of HEDTA. Despite the finding of the cyclization phenomena, the conditions under which the cyclization occurs and the kinetics of the cyclization were not well understood. The structures for the open and cyclized form of HEDTA are shown below:

Chelators such as HEDTA and ethylenediaminetetraacetic acid (EDTA) have been extensively employed in a wide variety of industries. Reduction of metal ion activity constitutes their most common use. The practical value of these chelators lies in their ability to form stable, water-soluble complexes with a wide range of metal ions. Diverse applications include: (1) food preservatives, (2) water-softerening agents, (3) removing poisonous elements (e.g., lead and plutonium) from the human body, (4) industrial rust and scale removal, (5) paper processing, (6) drug delivery, (7) fertilizer, and (8) processing of nuclear materials.

The in-plant chemical degradation, and post-disposal chemical and biological degradation of the chelators have generated many chelator fragments, e.g., ED3A and ethylenediamine diacetic acid (EDDA), which may have equivalent or more complexing capability than the starting chelators. Chelators and chelator degradation products are of interest from an environmental viewpoint, primarily due to their ability to form stable and water-soluble complexes with most heavy metals and radionuclides, thereby enhancing the mobility of toxic heavy metals and radionuclides through the soil column. Furthermore, some of the chelators are suspected carcinogens. As a result, characterization of chelators and chelator fragments is essential for proper handling of the industrial effluent and wastes that contain chelators. This investigation was centered on studies of cyclization of chelators. The overall objective was to try to gain a better understanding of chelator cyclization and chelator chemistry. Of particular interest was to probe the effects of reaction temperature and acidity of the matrixes on the intramolecular cyclization of the chelators. Understanding the conditions of chelator cyclization and chelator chemistry will assist in the development of methods for chelator determination and the improvement of current technologies for chelator applications.

Chelators and chelator degradation products represent a class of amino carboxylic acids containing alkyl groups bonded to one or two nitrogen that are very polar and nonvolatile. Though studies of chelator cyclization have not been reported in literature, several analytical methods have been developed for the analyses of chelators. Analytical methods for chelator determination generally involve derivatization and chromatography, which often requires extra sample handling, and may give low or variable recoveries. Liquid chromatography (LC) has been used to separate mixtures of HEDTA and EDTA metal complexes.
Themospray LC/MS has been evaluated as a potential technique for analyzing chelators and chelator fragments. However, intramolecular cyclization occurred in the analysis. Alternatively, matrix assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF-MS) was used for the direct analysis of chelators without any derivatization. Mass spectra of EDTA, HEDTA, EDDA, and NTA were obtained in such an approach. These species were analyzed alone and as mixtures in both positive and negative ion modes. It was also found that the presence of high concentrations of sodium significantly suppressed MALDI signals. A quantitative method for determination of EDTA in human plasma and urine using capillary electrophoresis (CE) has been developed. CE and CE/MS have also been used to separate and to analyze Co(II)-EDTA and Co(III)-EDTA complexes.

Electrospray ionization mass spectrometry (ESI-MS), another promising analytical method, has also been utilized to examine chelators in the negative ion mode. It is an effective method for analyzing nonvolatile chelators in solution by mass spectrometry without prior derivatization.

ESI-MS is a relatively new technique for the analysis of complex, polar, and labile molecules. Since it was introduced in the 1980s by Yamashita and Fenn, ESI-MS has been widely utilized in biochemical analyses for its ability of producing and weighing intact ions of very large biomolecules. Recently, there have been increasing applications of ESI-MS in the analysis of smaller species. One of the major advantages of ESI-MS over the other methods is that there is no need for unusual matrices such as glycerol used in fast atom bombardment (FAB) and MALDI. The solvents used in ESI-MS are the solvents (e.g., methanol, isopropanol, and water) that are usually used in wet chemistry.

Positive and negative ion mode ESI-MS methods were developed to identify the open form of the chelators (free acids) as well as their corresponding cyclized forms (lactams or lactones). Two experimental efforts were made to understand the chelator cyclization and the reaction kinetics. The first effort focused on obtaining the reaction conditions under which chelators can be cyclized. The second effort was to study the reaction kinetics as a function of the reaction temperature and the pH of the solutions.

Experimental

Chemicals

Four chelating compounds were studied as model compounds in this investigation. Reagent grade s-EDDA, HEDTA, and ammonium hydroxide (NH₄OH) were obtained from Aldrich Chemical (Milwaukee, WI, USA). u-EDDA and HEIDA were synthesized by Dani Meisels of Argonne National Laboratory (Argonne, IL, USA). Glacial acetic acid (CH₃COOH) was obtained from J. T. Baker (Phillipsburg, NJ, USA). Hydrochloric acid (HCl) was from Mallinckrodt (Paris, Kentucky, USA). Analytical grade solvents were used. Methanol (CH₃OH) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Isopropyl alcohol (C₃H₇OH) was from J. T. Baker (Phillipsburg, NJ, USA). Ultra-pure deionized water was obtained from a water system manufactured by Millipore (Molsheim, France). Standard small-molecule positive and negative ion tuning mixtures were obtained from Hewlett Packard (Polo Alto, CA, USA).

Sample preparation

Chelator standards were weighed using a Cahn-30 microbalance (Cahn Instrument, Cerritos, California, USA). All chelator solutions were prepared at a concentration of 0.2 mg/mL. The solutions of chelator standards were prepared by dissolving weighed chelator solids in either 50:50 methanol/water or 50:50 isopropanol/water solutions. Approximately 1% v/v of ammonium hydroxide was added as a modifier in s-EDDA and U-EDDA standard solutions. The pH of the solution was measured with a pH meter (Orion Research Inc., Cambridge, Mass, USA) or pH paper.

Instrumentation

All ESI-MS data were obtained using a HP 59987A/5989B electrospray ionization mass spectrometer (Hewlett Packard, Palo Alto, California, USA). The instrument was tuned with the standard small molecule positive and negative ion mode tuning mixtures. Instrumental conditions are listed in Table 1. Solutions of chelators were infused into an atmospheric pressure ionization (API) electrospray source at a flow rate of 30 μL/min via a Harvard Apparatus 22 syringe pump (Harvard Apparatus, USA). Nitrogen was used as drying and nebulizing gas for positive ion mode ESI-MS; pure air was used for negative ion mode ESI-MS. A pressure of 80 psi was used for nebulizing gas. The pressure of drying gas was 35 mm Hg. Instrumental control, data acquisition, and data analysis were achieved with a personal computer operating HP ChemStation under a Microsoft Windows 3.11 environment. ESI-MS spectra were obtained via a full scan of the mass in the range of 100–600 Dalton.