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Synthesis, crystal structure and investigation of mononuclear copper(II) and zinc(II) complexes of a new carboxylate rich tripodal ligand and their interaction with carbohydrates in alkaline aqueous solution

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ABSTRACT

A new carboxylate rich asymmetric tripodal ligand, *N*-[2-carboxybenzomethyl]-*N*-[carboxymethyl]-β-alanine (**H_3camb**), and its *di*-copper(II), $(NH_4)_2[\mathbf{1}]_2$, and *di*-zinc(II), $((CH_3)_4N)_2[\mathbf{2}]_2$, complexes have been synthesized as carbohydrate binding models in aqueous solutions. The ligand and complexes have been fully characterized using several techniques, including single crystal X-ray diffraction. The interactions of $(NH_4)_2[\mathbf{1}]_2$ and $((CH_3)_4N)_2[\mathbf{2}]_2$ with D-glucose, D-mannose, D-xylose and xylitol in aqueous alkaline media were investigated using UV–Vis and ^{13}C-NMR spectroscopic techniques, respectively. The molar conductance, NMR and ESI–MS studies indicate that the complexes dissociate in solution to produce the respective complex anions, $\mathbf{1}^-$ and $\mathbf{2}^-$. Complexes $\mathbf{1}^-$ and $\mathbf{2}^-$ showed chelating ability towards the naturally abundant and biologically relevant sugars, D-glucose, D-mannose, D-xylose, and xylitol. The complex ions bind to one molar equivalent of the sugars, even in the presence of stoichiometric excess of the substrates, in solution. Experimentally obtained spectroscopic data and computational results suggest that the substrates bind to the metal center in a bidentate fashion. Apparent binding constant values, pK_{app}, between the complexes and the substrates were determined and a specific mode of substrate binding is proposed. The pK_{app} and relativistic density functional theory (DFT) calculated Gibbs free energy values indicate that D-mannose displayed the strongest interaction with the complexes. Syntheses, characterizations, detailed substrate binding studies using spectroscopic techniques, single crystal X-ray diffraction and geometry optimizations of the complex-substrates with DFT calculations are also reported.

1. Introduction

Sugar–metal ion interactions are not only ubiquitous in biological processes but are also vital in cell functions [1–5]. Although some effort has been directed towards understanding carbohydrate recognition, unlike various other metalloenzymes, the study of carbohydrates in biological systems using synthetic model complexes is largely unexplored. One of the contributing factors for the absence of such studies has been the lack of technological advances in the realm of high magnetic field NMR and other quantitative spectroscopic methods to handle the complicated equilibrium and polyfunctional nature of carbohydrates and other natural products in solution. However, NMR spectroscopy and several other techniques have developed significantly in recent times. As the consequence of the developments, the study of complicated systems including carbohydrates has been attracting considerable interest. Recently, some synthetic receptors have been used to investigate carbohydrate recognition in relation to the important roles they play in biological processes [3,4]. One of such recognition which is being investigated with ever increasing interest includes simple metal ions in carbohydrate interactions [6–13]. This particular interaction has important implications in a variety of biological systems such as support in membrane systems, cell–cell adhesion, intercellular recognition, signal transduction, fertilization, and as targets of bacterial or viral infections of cells [1–5]. Although understanding carbohydrate–metal ion coordination chemistry is of fundamental importance to these systems, structural and functional investigations of metal complexes with carbohydrates has been limited to complexes derived from amino sugars [14–16]. Aside from the biological relevance, carbohydrate interaction with metal ions has also been a subject of intense research in the field of enantioselective catalysis of organic reactions [17–24].

To understand the carbohydrate–metal ion interactions, some model complexes have been prepared and their interactions reported

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in the literature [25–28]. In the past several years, the study of carbohydrate–transition metal ion interactions in chemistry and biology has been pursued by several research groups [2,14–16,24, 29–32]. For example, synthetic strategies for VO^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and MoO_2^{2+} carbohydrate complexes have been developed [6,7,11–14,28,31–36]. Furthermore, the biologically relevant aspects of carbohydrate complexes of Fe^{3+}, Cr^{3+}, VO^{2+}, and Zn^{2+}, have also been reported [32–35,37–43]. It has also been elucidated that carboxylato-bridged dinuclear complexes of Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}, are involved in non-redox active enzymatic processes [33,44–51].

In an attempt to contribute to the understanding of carbohydrate–metal complex chemistry, recently, we reported new *polynuclear* iron(III) and copper(II) complexes and their interaction with several monosaccharides in aqueous media [34]. Thus, the main focus of the current manuscript is on the interaction of newly synthesized *mononuclear* copper(II) and zinc(II) complexes of a carboxylate rich asymmetric tripodal ligand, *N*-[2-carboxybenzomethyl]-*N*-[carboxymethyl]-β-alanine (H_3camb) with the monosaccharides in alkaline aqueous media. Herein, we report the syntheses and characterizations of H_3camb, $(NH_4)_2[\mathbf{1}]_2$ and $((CH_3)_4N)_2[\mathbf{2}]_2$ complexes,solution behavior and the level and mode of their interaction with D-glucose, D-mannose, D-xylose and xylitol in alkaline aqueous media. Also reported is relativistic density functional theory (DFT) modeling study on the complexes and their interaction with the substrates.

2. Experimental

2.1. General remarks

All starting materials were purchased from commercial sources and were used without further purification. Elemental analyses were determined by Atlantic Microlab, 6180 Atlantic Blvd. Suite M, Norcross, GA. FTIR spectra were recorded on solid samples using a Bruker Vector 22 FTIR–ATR spectrometer. Molar conductivities were measured in aqueous solution of the complexes (10^{-3} M) using a Corning Model 441 conductivity meter.

2.2. UV–Vis spectroscopy

All experiments were performed on an Agilent 8453 diode array UV–visible (UV–Vis) spectrophotometer with 1 cm quartz cell at room temperature over a range of 200–900 nm. An Eppendorf Research micropipette was used to measure volumes. All experiments were carried out in degassed nanopure water, in which pH of the solutions was adjusted using NaOH solution. Each concentration was made and measured three times and the data points were averaged. Standard deviation was applied to these averages. The detailed experimental procedure used for the sugar titrations was adopted from our earlier published work [34].

2.3. NMR spectroscopy

^{1}H and ^{13}C NMR spectra of H_3camb and $((CH_3)_4N)_2[\mathbf{2}]$ were obtained in D_2O solution with a Varian Inova 500 NMR spectrophotometer. All ^{13}C NMR experiments for the determination of binding interactions were performed on the same NMR spectrophotometer at room temperature. The experiments were done in D_2O solution, for which pH was adjusted to pH 12.5 with NaOH prior to the use for each set of NMR titration. The pH of the resulting solution was measured immediately after mixing.

2.4. Mass spectrometry

Electrospray ionization time-of-flight mass-spectrometry (ESI–TOF–MS) spectrometry data was collected using a Bruker Daltonics micrOTOF instrument at the RCMI program. Data was collected for a m/z range of 100–2000 in negative ion mode. Samples were delivered as either dilute (1–2 mg/mL) aqueous or methanol solutions with a relatively moderate flow rate of 0.90 mL/h. In all the measurements, the setting of the nebulization gas, N_2, was 45.0 psi, the capillary potential was 4 kV, the drying gas was 5.0 L/min, the skimmer was set to −33 V, and the hexapole RF was set to 300 Vpp. Data was analyzed and the spectra plots were generated using the Mass software package [52,53]. Simulations of the stable isotope patterns were made using Molecular Weight Calculator (Matthew Monroe, PNNL, Richland WA, U.S.A.) software.

2.5. Potentiometric titration

The potentiometric studies were carried out in doubly distilled and boiled water solution with a Metrohm 848 Titrino plus automatic titrator combined with an Ag/AgCl electrode calibrated to read $-\log[H^+]$ directly, designated as pH. The electrode was calibrated using the data obtained from a potentiometric titration of a known volume of a standard 0.0100 mol L^{-1} HCl solution with standard 0.100 M KOH. The experiments were carried out in a 50 mL sealed thermostated cell at 25 °C and bubbled with argon to ensure an inert atmosphere and the ionic strength adjusted to 0.100 M by addition of KCl. In a typical experiment the mass equivalent to 0.05 mmol of the H_3camb ligand was weighted in an analytical balance and dissolved in 30 mL of water and 20 mL of 0.0100 M HCl solution to ensure the complete protonation and an initial pH value near 2. The titration was carried out by the addition of 0.05 mL aliquots of a standard CO_2-free 0.100 M KOH solution pH 12 was reached. Computation of results was carried out with BEST7 program and species distribution calculations with SPECIES program [54].

2.6. Computational studies

The theoretical calculations were carried out using the Gaussian 09 implementation of B3LYP DFT [55–57]. The geometry optimization was carried out using a 6–31G(d) basis set on C, N, O and H atoms where LANL's double zeta basis set (LANL2DZ) with effective core potentials (ECP) were used for metals [58–60]. All structures were fully optimized with polarizable continuum model (PCM) and frequency analyses were performed to ensure a minimum state was achieved [61,62]. To test the robustness and reliability of the B3LYP functional used in the simulations, all geometries were also optimized under parameterized DFT functional M06-2X, which have shown promise for noncovalent interactions [63]. The thermodynamic functions, including enthalpies, entropies and free energies, were calculated at 298.15 K and 1 atm within the harmonic potential approximation at optimized structures. In order to examine basis set effect, Dunning's correlation consistent triple zeta basis set cc-pVTZ for all atoms was implemented with B3LYP functions to perform the single point energy (SPE) calculation upon the aforementioned optimized geometries [64].

2.7. Syntheses

2.7.1. Synthesis of N-[2-carboxybenzomethyl]-β-alanine, H_2cbal

The ligand has been prepared according to our previously published procedure [65]. The product was collected by filtration, washed with water, methanol and dried at 80 °C. The product was confirmed by elemental analysis and ^{1}H NMR spectroscopy. Yield: 8.5 g (76%). *Anal. Calcd.* for $C_{11}H_{13}NO_4$: C, 59.19%; H, 5.87%; N, 6.27%. *Found*: C, 59.08%; H, 5.93%; N, 6.20%. ^{1}H NMR for the sodium salt of the compound (500 MHz, D_2O, 25 °C, δ): 7.66 (d, 1H, $J = 7.0$ Hz), 7.48 (m, 2H), 7.45 (t, 1H, $J = 7.0$ Hz), 4.11 (s, 2H), 3.05 (t, 2H, $J = 7.5$ Hz), 2.52 (t, 2H, $J = 7.5$ Hz).