



# Toward understanding the activity of cobalt carbonic anhydrase: A comparative study of zinc- and cobalt-cyclen

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## ARTICLE INFO

### Article history:

Received 9 October 2014

Received in revised form 8 December 2014

Accepted 10 December 2014

Available online 22 December 2014

### Keywords:

Carbonic anhydrase  
Density functional theory  
Microkinetic modeling  
Biomimetic catalysis  
Cobalt cyclen

## ABSTRACT

Density functional theory (DFT) calculations were used to study the mechanism of CO<sub>2</sub> hydrolysis by Co-(1,4,7,10-tetrazacyclododecane), also referred to as cobalt-cyclen, and evaluate the associated thermodynamic and kinetic parameters. A microkinetic model was then built based on the kinetics and thermodynamics derived from first principles. The intrinsic reaction rate constant was calculated to be 10572 M<sup>-1</sup> s<sup>-1</sup>, three times larger than that of zinc-cyclen. The high activity was ascribed to a very large pK<sub>a</sub> value of 13.3. The monodentate structure of a key intermediate along the reaction coordinate, [Cyclen-Co-HCO<sub>3</sub>]<sup>+</sup>, was more stable than the bidentate isomer, due to a hydrogen bond formed between bicarbonate and the ligand. The reaction rate constant decreased significantly over 0–12 ms, which was attributed to the fast decrease of the concentration of the catalytic form, Co–OH<sup>-</sup>. The conversion of CO<sub>2</sub> at 1000 ms as a function of pH was calculated to compare the relative activity of zinc-cyclen and cobalt-cyclen, and zinc-cyclen was found to be a much better catalyst. Through calculating the ratio of the net rate to the forward rate of each elementary reaction, the rate-limiting step of the catalytic cycle was identified as the adsorption of CO<sub>2</sub>, which was the same as that for zinc-cyclen, though the product-releasing step was regarded as more difficult in cobalt-cyclen, compared to zinc-cyclen.

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## 1. Introduction

Carbon dioxide, a major greenhouse gas (GHG) associated with human activities in the atmosphere, has drawn significant attention recently as it plays a significant role in global climate change [1]. The atmospheric concentration of CO<sub>2</sub> has increased to a current level of over 400 ppm from its pre-industrial level of ca. 280 ppm, and is expected to continue growth with a rate of >2 ppm/year in the near future [2]. Anthropogenic CO<sub>2</sub> emissions are mainly due to the burning of fossil fuels and various chemical processes, e.g., in the U.S., over 94% of the CO<sub>2</sub> emission is from the combustion of fossil fuels [3]. A great many approaches, including solvents, cryogenic techniques, membrane separation, and solid sorbents, have been pursued to capture CO<sub>2</sub>. Chemical absorption and stripping using alkanolamine solvents, e.g., 30 wt% monoethanolamine (MEA), is the state-of-the-art technology for separating CO<sub>2</sub> from flue gas. However, this process presents several disadvantages, including intensive energy consumption, the corrosion of equipment, and evaporative losses of the solvent. For example, in the

power plant, the energy consumption of MEA-based scrubbing can reach up to 20–30% of the total energy produced [4].

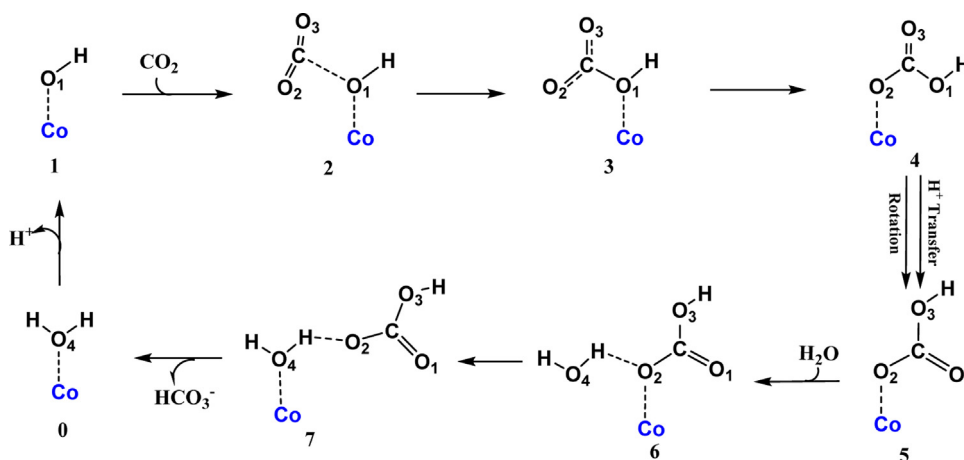
One possible remedy is to convert carbon dioxide catalytically to HCO<sub>3</sub><sup>-</sup> along with amine scrubbing. In nature, HCA II, a member of the human carbonic anhydrase (CA) family of enzymes, is one of the fastest known enzymes with a turnover number for reaction (1) on the order of 10<sup>6</sup> s<sup>-1</sup>, which approaches the diffusion limit [5].



The mechanism depicted in Scheme 1 is widely accepted as an appropriate description of how the catalysis takes place in CA, with the typical zinc center of CA replaced by cobalt in the scheme to be compatible with subsequent discussions [6,7]. In step one, the metal-hydroxide of the active site nucleophilically attacks CO<sub>2</sub> to form a metal-bicarbonate intermediate. Once the metal-bicarbonate intermediate is formed, it will rearrange to form a better leaving group. Then a water molecule will displace the bicarbonate to form metal-H<sub>2</sub>O. The final step, which is regarded as rate-limiting in CA, is the deprotonation of metal-H<sub>2</sub>O. The X-ray crystal structures of HCA II have been studied intensively. The active site includes: a zinc atom tetrahedrally coordinated to three histidines (His-94, His-96, and His-119) and a catalytic water molecule (Wat-263). In addition, an adjacent hydrophobic pocket (Leu-198,

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Scheme 1. Proposed mechanism for CO<sub>2</sub> hydrolysis catalyzed by cobalt-cyclen.

Trp-209, Val-143, Thr-199, Val-207, and Val-121), and a hydrophilic half (Tyr-7, Asn-62, His-64, Asn-67, Thr-199, and Thr-200) also play important roles in the catalytic cycle [8,9].

Metallo variants of CA present an interesting subject of study due to the lack of spectroscopic signatures by the Zn<sup>II</sup> ion. More importantly, studying metallo variants will help rationalize why zinc is preferable for the catalytic function of CA. The Zn<sup>II</sup> ion can be readily extracted from CA without denaturation of the enzyme, while many divalent metal ions can easily bind to the apo-CA (CA without metal in the active site). There is minimal conformational change upon removing the Zn<sup>II</sup> ion or adding the non-zinc metal ions. To date, the Zn<sup>II</sup> ion can be replaced by the following divalent metal ions: Cd<sup>II</sup>, Co<sup>II</sup>, Cu<sup>II</sup>, Fe<sup>II</sup>, Hg<sup>II</sup>, Mn<sup>II</sup>, and Ni<sup>II</sup> [5]. In addition, In<sup>III</sup> can also bind to the active site of apo-BCA II (bovine carbonic anhydrase II) [10]. A cadmium carbonic anhydrase was found to be expressed in marine diatoms where the zinc concentration was low [11]. Cd-CA continues to show activity but at a higher pH value compared to Zn-CA. Cd-CA also produces esterase activity, but the peptidase activity is largely lost [12]. Among these metallo variants of CA, only the Co<sup>II</sup> variant shows high enzymatic activity, which can reach up to 50% of the activity of the wild type CA. In addition, high-spin cobalt(II) is a powerful spectroscopic probe in biological systems [13]. For these reasons, Co-CA was an ideal metallo variant for studying CA.

Enzyme mimics, small molecules that resemble the structural and active sites of enzymes, are often studied to obtain insight into the structures and mechanisms of action of enzymes [14]. Such studies are important due to the fact that enzyme mimics may be more suitable to structural, spectroscopic, and mechanistic studies than enzymes themselves. With an activity of 3300 M<sup>-1</sup>s<sup>-1</sup>, the highest activity among all CA mimics to the best of our knowledge, zinc-cyclen is regarded as the best model catalyst for CA [15]. The kinetics of CO<sub>2</sub> hydrolysis catalyzed by zinc-cyclen have been studied experimentally and theoretically [15–18]. Zhang and van Eldik have measured reaction rates of CO<sub>2</sub> catalytic hydrolysis by Zn([12]aneN<sub>3</sub>)OH<sup>+</sup> and Zn([12]aneN<sub>4</sub>)OH<sup>+</sup> via stopped-flow spectrometry. They suggest that in neutral and acidic solution replacement of bicarbonate is the rate-determining step for dehydration of HCO<sub>3</sub><sup>-</sup>, while in basic solution CO<sub>2</sub> binding is the rate-determining step for CO<sub>2</sub> hydrolysis. However, in a recent study of five different zinc-chelated aza-macrocycle complexes, including Zn([12]aneN<sub>3</sub>)OH<sup>+</sup> and Zn([12]aneN<sub>4</sub>)OH<sup>+</sup>, Koziol et al. found that the trend of activation energies for the bicarbonate release step agreed well with the trend of the rate constants. Based on a combination of quantum-mechanical calculations and results from stopped-flow experiments, they proposed that bicarbonate

release, rather than CO<sub>2</sub> addition or deprotonation of Zn–H<sub>2</sub>O, may be the rate-limiting step. Lau and coworkers have studied the reaction coordinate for CO<sub>2</sub> hydrolysis catalyzed by both Zn-cyclen and Co-cyclen [17]. They found that Co-cyclen has a lower barrier for nucleophilic attack on CO<sub>2</sub> but at the expense of releasing bicarbonate in the reaction.

Recently, our group studied CO<sub>2</sub> catalytic hydrolysis by zinc-cyclen with a combination of DFT calculations and microkinetic modeling [18]. Our microkinetic model successfully matched experimental data of how the reaction rate constant varies with pH. The sigmoidal-shaped curve was ascribed to only the Zn–OH<sup>-</sup> form being active and catalyzing the reaction. Our model also predicted that the initial reaction rate constant decreased over the period 0–12 ms due to conversion of Zn–OH<sup>-</sup> to Zn–HCO<sub>3</sub><sup>-</sup>, where Zn–HCO<sub>3</sub><sup>-</sup> is the most stable point along the potential energy surface, acting as an energy well to trap the active form of the catalyst and retard the catalytic cycle. Given these interesting findings, it would also be fruitful to model a cobalt-cyclen-catalyzed system, compare it with zinc-cyclen, and determine the cause for the difference in activity with CA-catalyzed systems.

## 2. Computational details

### 2.1. Computational methods

Quantum chemical calculations were performed using the Gaussian 09 package of programs [19]. The initial geometries for species involving the catalyst were fully optimized in solvent at the BMK level of theory using the PCM solvation model [20–22]. Based on a diverse and balanced parameterization set including transition metal complexes and hydrogen-bonded systems, the BMK functional was designed to be superior in describing transition state properties as well as atomization energies, geometries, and harmonic frequencies of molecules in the ground state [20,23]. For example, Goel and Masunov tested the BLYP, TPSS, B3LYP, and BMK functionals for hydrides and hydride cations of the 3d-series and found that BMK gave the most accurate bond energies with MUEs in ionization potential of 3 kcal/mol [24]. In addition, we performed extensive benchmarking of different method (i.e., MP2, M06, M06L, and B3LYP) and basis set (i.e., 6-31G(d), 6-311+g(2d,p), and aug-cc-pv-TZ) combinations by comparing with experimental pK<sub>a</sub> values of various carbonic anhydrase mimics. The method that we selected among the numerous ones tested was inexpensive and able to produce accurate results for the Zn-cyclen system that we reported earlier as well as for a wide variety of other catalysts [18].

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