



# Effects of transition metal ions on the catalytic activity of carbonic anhydrase mimics



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## ARTICLE INFO

### Article history:

Received 13 April 2015

Received in revised form 3 July 2015

Accepted 4 July 2015

Available online 19 July 2015

### Keywords:

Carbonic anhydrase

Transition metal

Zinc

Cobalt

Nickel

Cadmium

## ABSTRACT

Many carbonic anhydrase (CA)-mimetic catalysts have active sites composed of a Zn ion coordinated with multiple nitrogens of azacyclic compounds. In this study, some divalent transition metal ions ( $\text{Co}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Ni}^{2+}$ ) were examined as potential substitutes for the Zn cofactor when preparing CA-mimetic catalysts by means of self-assembly of histidyl bolaamphiphiles. The self-assembly of histidyl bolaamphiphiles yielded a densely packed histidine imidazole sites offering metal complexation places similar to the triple-histidine imidazoles of natural CA active sites. In the catalytic hydrolysis of *p*-nitrophenyl acetate, the catalytic efficiency of the mimetic catalysts agreed with the order of catalytic activities of metal-substituted carbonic anhydrases:  $\text{Zn} > \text{Co} > \text{Cd} \approx \text{Ni}$ ; activation energy of the CA-mimetic catalysts and practical  $\text{CO}_2$  hydration efficiency also had the same order. However, Co showed better catalytic activity than Zn when the concentrations of metal salts were higher than 0.1 mM because of weakened inhibition by the counter anion. This study elucidated the effect of transition metal ions on the catalytic efficiency of a CA-mimetic catalyst, with emphasis on the coordination of transition metal ions.

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## 1. Introduction

Carbonic anhydrase (CA) is a  $\text{CO}_2$ -hydrating metalloenzyme found in most animal and plant cells. CA catalyzes the hydration of  $\text{CO}_2$  to convert it into bicarbonate anions in aqueous solution, enabling the removal of  $\text{CO}_2$  from the cell [1]. To understand the catalytic function of CA, the structure of CA active sites was explored, and was identified to be composed of triple histidine imidazoles coordinated with a Zn ion. The Zn ion undergoes tetrahedral coordination, associating with the triple histidine imidazoles and a single water molecule. The Zn ion functions as a key catalytic cofactor at which the binding of hydroxide ion, nucleophilic attack on  $\text{CO}_2$ , formation of a temporary resonance structure with the bicarbonate intermediate, and release of the bicarbonate anion take place [2,3]. The extraordinary catalytic property of CA is known to be induced by the nature of zinc, a transition metal ion, which enables tetrahedral coordination with neighboring organic/inorganic ligands and a hydroxide.

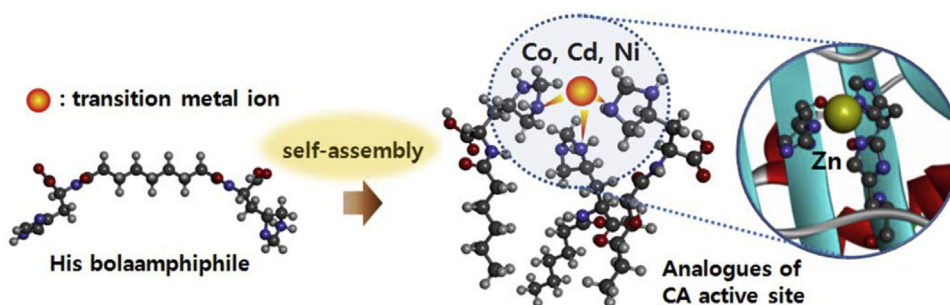
Based on the characteristics of the zinc ion, several divalent transition metal ions have been examined as alternatives of the catalytic zinc cofactor. Some of the transition metal ions were found to be effective in catalyzing conversions of organic substances.

For example, Kazlauskas and coworkers reported that the native CA whose zinc ion was substituted with  $\text{Mn}^{2+}$  showed catalytic oxidative activity toward *o*-dianisidine [4], and  $\text{Rh}^{2+}$ -substituted CA catalyzed the hydrogenation of olefin and the hydroformylation of styrene [5,6]. Substituted CAs do not include only fabricated enzymes; a natural CA of deep sea diatoms was reported to use  $\text{Cd}^{2+}$  instead of  $\text{Zn}^{2+}$  [7]. These studies on the replacement of zinc ion have indicated that some transition metal ions could have  $\text{CO}_2$ -converting catalytic activity. Furthermore, analogues of transition metal-coordinated ligand complexes mimicking CA have been explored to examine their coordination characteristics and catalytic activity, although only a small number of studies have been reported. Reports on the catalytic activity of an imidazole ligand complex coordinated with  $\text{Co}^{2+}$  ion have proposed the formation of an catalytic active site by means of  $\text{Co}^{2+}$ –OH bonding [8,9].

Studies on CA and its transition metal ion-substituted analogues suggest that the transition metal ion significantly influences the catalytic performance; accordingly, selection of an appropriate metal ion is of great importance. The effects of transition metal ions on the catalytic activity of transition metal ion–ligand complexes should be evaluated further to guide the design principles of biomimetic catalysts. Recently, we reported a CA-mimetic catalyst formed by means of histidyl bolaamphiphile self-assembly with a zinc ion cofactor [10]. By means of the self-assembly, nanoscale CA analogues with densely packed surface-exposed histidine imidazoles were created in an aqueous solution, and then zinc ions were

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**Scheme 1.** Schematic illustration of a carbonic anhydrase-mimetic catalyst prepared by self-assembly of histidyl bolaamphiphile and subsequent coordination of a transition metal cofactor (the carbonic anhydrase structure was drawn by using 1CA2.pdb [11]).

associated with the imidazoles to create catalytic analogues over the surface of the self-assembly (Scheme 1). Therefore, the self-assembly of histidyl bolaamphiphiles is a good substitute of the CA apoenzyme for investigating the effects of various transition metal ions as cofactors.

In the present study, we examined the performances of CA-mimetic catalysts prepared by using various transition metal chloride salts. The transition metal ions of cobalt, nickel, and cadmium were applied to construct CA-mimetic catalysts by forming complexes with imidazoles of histidyl bolaamphiphile self-assemblies. Kinetics parameters and activation energies of the prepared CA-mimetic catalysts were evaluated, and were found to be representative of the nature of the transition metal ions, such as their coordination structures and the equilibrium constants of association with their pairing ligands. The catalytic function of  $\text{CO}_2$  hydrolysis was compared further by means of experiments testing the practical sequestration of  $\text{CO}_2$  in  $\text{CaCO}_3$  precipitates.

## 2. Experimental

### 2.1. Preparation of the histidyl bolaamphiphile self-assembly platforms

For the creation of a CA-mimetic catalyst, histidyl bolaamphiphile molecules were synthesized according to a reported procedure [10,12]. Briefly, the histidyl bolaamphiphile was synthesized by conjugating histidine benzylester *p*-toluenesulfonate with azelaic acid (98%). Bioconjugating reagents of *N,N*-diisopropylethylamine (DIEA, 99%), *N*-hydroxysuccinimide (NHS, 98%), and *N,N,N',N'*-tetramethyl-*o*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 98%) were used to conjugate the compounds to produce the histidyl bolaamphiphile molecule of bis(*N*- $\alpha$ -amido-histidine)-1,7-heptane dicarboxylate (hereafter, His-C7). The produced His-C7 powder was dissolved in deionized water (pH  $6.8 \pm 0.1$ ) to self-assemble creating spherical structures. After formation of the spherical self-assemblies, transition metal salt was added to be of a controlled concentration. Cobalt chloride ( $\text{CoCl}_2$ , 99%), nickel chloride ( $\text{NiCl}_2$ , 99%), and cadmium chloride ( $\text{CdCl}_2$ , 99%) were used as transition metal cation sources. Before beginning the catalysis experiment, the pH of each catalyst suspension was adjusted to  $7.0 \pm 0.1$  by addition of citric acid (10 mM). All chemicals were purchased from Sigma-Aldrich unless specified otherwise.

### 2.2. Characterization of catalysts and measurement of catalytic activity

The morphology of the prepared catalysts was observed by scanning electron microscopy (SEM). Association of the transition metal ions with the histidine imidazoles was investigated by means of far-infrared (far-IR) spectroscopy (Vertex 70 spectrom-

eter, Bruker). For the far-IR analyses, catalysts were prepared from 4 mM His-C7 and 1 mM metal chloride salts. Catalytic activity of the prepared CA-mimetic catalysts was examined by monitoring the hydrolysis of *p*-nitrophenyl acetate (*p*-NPA, 99%, Fluka) to produce *p*-nitrophenol (*p*-NP) at 25 °C [13]. Generation of *p*-NP was monitored by means of UV-vis spectroscopy (S-3100, Scinco), which clearly showed the characteristic absorbance peak of *p*-NP at 400 nm. The kinetics parameters of the catalytic hydrolysis reaction were estimated based on Lineweaver-Burk plots. The activation energy of each catalyst was measured by using the *p*-NP concentration profile obtained during the early stage of the reaction, during which the concentration of the substrate (i.e., *p*-NPA) is sufficiently high that the hydrolysis can be considered as a pseudo-first-order reaction. To test the catalysts for practical applicability to  $\text{CO}_2$  conversion, the hydrolysis of  $\text{CO}_2$  was tracked by monitoring the change in pH over time by using a custom-made reactor equipped with a real-time pH monitoring system. Sequestration of  $\text{CO}_2$  to form  $\text{CaCO}_3$  precipitate was performed at room temperature by using a 10 mM  $\text{CaCl}_2$  solution as a  $\text{Ca}^{2+}$  source. The  $\text{CaCO}_3$  precipitates were weighed after drying in a vacuum oven at 100 °C to fully remove water.

## 3. Results and discussion

### 3.1. Characterization of transition metal-associated bolaamphiphile self-assemblies

Before measuring their catalytic performance, the morphologies of the prepared catalysts were observed. The cobalt-associated His-C7 self-assemblies had non-uniform, blob-like shapes (Fig. 1a). The self-assemblies were 85 nm in diameter on average. The non-uniformity of the bolaamphiphile self-assembly implied that the self-organization of the His-C7 was not strongly regulated by intermolecular forces, presumably because of the rather weak polarity of histidine. The histidine imidazole is less polar and its driving force of self-assembly is not strong compared to other peptidic bolaamphiphiles that form well-organized structures [14]. It is also noticeable that the presence of other transition metal ion (i.e.,  $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$ ) did not alter the shape of self-assembly, although another transition metal ion of  $\text{Cu}^{2+}$  is known to bind histidine imidazoles, thereby influencing the morphology of histidyl bolaamphiphile self-assembly [15]. This implies that the coordination of the transition metal ions with surface-exposed histidine imidazoles on the surface of the self-assembly is not strong enough to disrupt the organization of the histidyl bolaamphiphile.

Coordination of the transition metal ions with imidazole moieties was verified by means of the far-IR spectroscopy. Characteristic IR absorption peaks of metal ion-imidazole coordination complexes were observed for every metal ion. The Co-imidazole complex had a strong absorption peak at  $282\text{ cm}^{-1}$  and a weak shoulder at  $293\text{ cm}^{-1}$  (Fig. 1b). A strong peak observed at  $282\text{ cm}^{-1}$

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